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A Contribution to the Physical Chemistry of Colloid Metaphosphates II

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In earlier papers [1–3] some problems of the physical chemistry of the colloid metaphosphates have been treated. During the last years, however, the polymetaphosphates have acquired new interest. They have been used as substrates for enzyme studies [4–7], and metaphosphates of high molecular weight have also been isolated from a microorganism, *Aspergillus niger* [8–10]. Accordingly, some further investigations have been carried out in order to get further elucidations of these substances.

The colloid metaphosphates investigated are the salts of Kurol [11], insoluble in water but soluble in solutions of sodium and ammonium salts. They are prepared by heating primary potassium phosphate:

$$n\ KH_2PO_4 \rightarrow (KPO_3)_n + n\ H_2O$$

If the heating is performed at constant temperature, polymetaphosphates are obtained of which the molecular weight and other related properties correspond to the temperature in question. As shown in a previous paper [3] the molecular weights seem to increase to a limiting value with the temperature of synthesis. A new series of syntheses, the data for which have been drawn up in Table 1, confirms that conclusion. It has not been possible to determine the molecular weights for all preparations but it may be permissable to regard the value of the intrinsic viscosity $(\eta_{sp}/c)_{c \rightarrow 0}$ as a qualitative measure of the molecular weight. Van Wazer [12] reports a linear relationship between intrinsic viscosity and degree of polymerisation. His investigation, however, was carried out on preparations of rather low molecular weights, the degree of polymerisation being less than 300. As is well known, $\eta_{sp} = \eta_r - 1$. η_r = the relative viscosity, c is expressed in no. of grams of substance per 100 gram of solution.

Table 1. Viscosity and molecular weight of some preparations.

Preparation	Temperature of synthesis	$(\eta_{sp}/c)_{c \to 0}$	Molecular weight
K 17	310°	0.31	
K 18	335	0.33	300 000
K 16	425	0.55	
K 15	500	0.92	1 100 000
K 22	535	1.10	
K 25	585	1.13	
K 24	700	1.18	
K 23	725	1.34	

The measurements have been made in a capillary viscosimeter (Ostwald type) and at 25° C; the medium used is 0.4 *M* NaCl.

The behavior of these polymetaphosphates in solution is determined by two main factors: the charge of the anion and the solvation.

Being colloid electrolytes, the colloid nature of which is due to the anion, these polymetaphosphates are greatly influenced by all factors determining charge and solvation, e.g. the concentration and species of ions present, the presence of dehydrating substances other than salts, as alcohol and glycol.

DISSOCIATION

In a previous paper [3] the net charge for certain preparations has been reported (medium 0.4 *M* NaCl). The calculations were performed according to Tiselius' formula [13], which is derived on the assumption that the frictional force acting upon the molecule during sedimentation equals that during electrophoretic motion. The values thus determined are rather low, corresponding only to about 0.1—1 per cent dissociation. However, it is possible to determine the charge of a colloid ion by means of the Donnan membrane equilibrium. From measurements of the membrane potential at Donnan equilibrium Rinde [14] determined the adsorption of ions on colloidal particles; Adair and Adair [15] calculated the charge of protein molecules. The charge or the degree of dissociation can also be calculated from analytical data. Let us assume the following system: inside the membrane: $(KPO_3)_n$, dissolved in sodium chloride solution; outside: sodium chloride solution. Simplifying the calculations by assuming the same activity coefficients on both sides of the membrane the following equation can be derived for equilibrium conditions:

$$\alpha = 100 \cdot \frac{m}{a} \frac{(c^2 - c_1^2)}{c_1} \quad (1)$$

α = per cent apparent dissociation; m = molecular weight of the base molecule ($KPO_3 = 118$); a = no. of grams of $(KPO_3)_n$ per liter of solution; c = concentration of chloride ions outside of the membrane and c_1 inside of the membrane.

As c_1 differs from c by not more than a few per cent, eq. (1) can be approximated

$$\alpha = 200 \frac{m}{a_i} (c - c_1) \quad (2)$$

Certainly it is a very rough approximation to assume the same activity coefficients on both sides of the membrane, but no theory has yet been developed for calculation of activity coefficients of systems involving macro-ions surrounded by clouds of small ions of opposite charge.

A 50 ml sample of polymetaphosphate solution (preparation K 15, dissolved in sodium chloride) was transferred into a cellophane bag and put into a closed cylinder containing about 100 ml of the same sodium chloride solution. The cylinder was then placed in a thermostat. After 3—4 days — the time required for etablishment of equilibrium — the chloride concentrations of the solutions were determined by potentiometric titrations with silver nitrate solution. The results are collected in Table 2.

Table 2. Apparent dissociation under various conditions.

Temperature	Medium	a g/lit.	$(c-c_1)10^4$ mol/lit.	α
5° C	0.10 *M* NaCl	5.00	26	12
20°	0.40 »	5.00	30	14
	0.20 »	6.00	34	13
	0.10 »	5.97	36	14
	0.05 »	4.77	32	16
25°	0.40 »	8.77	60	16
	0.20 »	6.30	38	14
	0.10 »	5.07	34	16
	0.05 »	4.32	29	16
35°	0.40 »	7.20	83	27

As seen from the table the dissociation calculated according to eq. (2) seems to be rather constant within the NaCl concentration 0.05—0.40 *M*. However, the values of the intrinsic viscosity of preparation K 15 at the same NaCl concentrations, vary over a wide range, see Table 4. This might indicate that the charge effect of the anion on the viscosity is largerly eliminated at 0.05 *M*-concentration and that further addition of salt removes the water of solvation thus decreasing the viscosity. A theory of electroviscous effects in polymer solutions has been given by Hermans and Overbeck [16], but their theory does not take into consideration the solvation, which is possibly the most important property.

The dissociation increases with increasing temperature, but it has not been possible to extend the measurements over a wide range of temperature since the polymetaphosphate is not very stable in solution at high temperature and rather slightly soluble at low temperature.

As has already been discussed in a previous paper [7], the polymetaphosphates form complexes with bivalent metal ions. The equilibrium

$$\text{polymetaphosphate} + \text{bivalent metal ion} \rightleftharpoons \text{complex anion}$$

is displaced to the right. However, the ability of the polymetaphosphate to form complex is limited by the fact that a permanent precipitate is formed if the atomic ratio of bivalent metal to phosphorus (Me/P) exceeds a certain value; for most metals this limiting value is $^1/_3$ *.

Many physico-chemical properties such as viscosity, sedimentation and diffusion constants, specific volume etc. are changed on complex formation. This is probably due to a change of molecular shape, but the apparent dissociation is not influenced.

Measurements of the dissociation of cations from the mangano-polymetaphosphate complex show, within the experimental error, that the dissociation is independent of the presence of Mn^{++} ions. The experimental condition were as follows. Inside the membrane: $(KPO_3)_n$, preparation K 15 dissolved in 0.4 *M* NaCl plus a given amount of $MnCl_2$ solution; outside of the membrane: 0.4 *M* NaCl; temperature 25° C. If c_2 denotes the concentration of free manganous ions inside the membrane, equilibrium conditions give the following expression for the degree of dissociation:

$$\alpha = 200 \cdot \frac{m}{a} (c - c_1) \left(1 + \frac{c_2}{c}\right) \qquad (3)$$

* A review of the literature dealing with complex-formation between bivalent metals and metaphosphate is given by Karbe and Jander [17]. Investigations on this complex-formation has also been published recently by Van Wazer and Campanella [18].

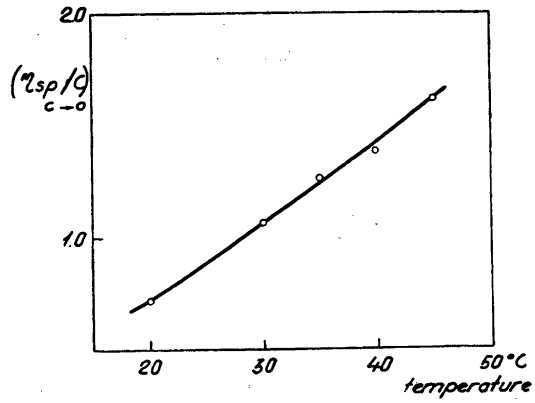


Fig. 1. The intrinsic viscosity as a function of the temperature.

Table 3. Apparent dissociation in the presence of manganese.

a g/lit.	Mn/P	$(c-c_1)\cdot 10^4$ mol/lit.	α
8.33	1 : 64	56	16
8.33	1 : 26	65	18
8.33	1 : 13	47	13
8.33	1 : 7	55	16

where the symbols have the meaning stated before. As c_2 is very small, probably less than 10^{-4}, the correction term is omitted. The results of these measurements, carried out at 25° C, have been collected in Table 3.

VISCOSITY

A condensation product like polymetaphosphate might be expected to be a chain molecule and the work by Van Wazer [19] confirms this assumption. Owing to the fact that these polymetaphosphates show no streaming birefringence in salt solutions of moderate concentration, *i. e.* 0.4 *M* NaCl, (Snellman unpublished) the molecules probably may be assumed to be wrinkled. However, because of the repulsive forces between the negative charges on the big anion it must change shape with increasing or decreasing dissociation. In fact the temperature effect on dissociation and hence on viscosity is very pronounced. For preparation K 15 the intrinsic viscosity has been determined in 0.4 *M* NaCl at different temperatures. The results are shown in Fig. 1 where $(\eta_{sp}/c)_{c\to 0}$ is plotted against temperature. However, as already mentioned, substances other than electrolytes have a great in-

Table 4. Intrinsic viscosity in various media.

Medium	$(\eta_{sp}/c)_{c \to 0}$
0.4 *M* NaCl (water)	0.92
0.3 » »	2.30
0.2 » »	3.40
0.1 » »	5.20
0.1 » (5 % ethanol)	2.95
0.1 » (8 » »)	2.00

fluence on the viscosity of a polymetaphosphate solution. If the salt concentration of such a solution is kept constant but part of the water is substituted with alcohol or ethyleneglycol the viscosity is decreased just as if salts of low molecular weight were added, see Table 4. Preparation used: K 15.

However, it must be remembered that addition of an organic liquid such as alcohol means a change of the dielectric constant of the medium. According to Smoluchowski [20] and Krasny-Ergen [21] the equation for the viscosity of solutions of spherical non solvated particles contains the factor $\frac{\varepsilon^2}{\eta_0}$ in the correction term. η_0 = the viscosity, ε = the dielectric constant of the solvent. The quantity $\frac{\varepsilon^2}{\eta_0}$ is diminished by about 30 per cent when the medium changes from 0.1 *M* NaCl in water to 0.1 *M* NaCl in 10 per cent (by volume) alcohol. This 30 per cent change evidently does not account for the effect of alcohol on the viscosity as shown in Table 3. However, it must be emphasized that the use of Smoluchowski's equation is only an approximation.

ELECTROPHORESIS

The electrophoretic properties of the polymetaphosphates are chiefly determined by the charge of the anion, the solvation being of less importance. At rather low ionic strength ($\mu \sim 0.1$) the mobility has reached a value which does not appreciably change on further addition of salts [3]. However, as might be expected from the dependence of dissociation on temperature, the mobility is subject to a similar dependence. A series of electrophoretic measurements were performed at various temperatures according to the well known technique developed by Tiselius [22] and Svensson [23]. 0.25 per cent solutions of preparation

Table 5. Electrophoretic mobility at various temperatures.

Temperature	$u \cdot 10^5$
5° C	16.4
10	16.9
15	17.9
20	19.2
27	21.3
37	31.7

K 15 in 0.4 *M* NaCl were used for the experiments the results of which have been collected in Table 5. The mobilities, u, expressed in cm^2/volt sec., have been calculated by using the conductivity at 20° in order to get comparable values.

The values of the dissociation (*cf.* Table 2) show an analogy to the mobility values. In both cases an increase of the temperature from 5 to 20° C causes only a modest increase of dissociation relative to resp. mobility, whereas another increase to about 35° is accompanished by a more pronounced effect on both dissociation and mobility.

A change of the medium from water to ethanol — water — the concentration of NaCl being the same, 0.1 *M* — decreases the mobility, Table se 6. The variation of the mobility values, however, are of the same order of magnitude as can be expected from the corresponding change of the dielectric constant and viscosity of the medium. If the alcohol is dialyzed off against 0.1 *M* NaCl the mobility is increased almost to its original value. The experiments were performed at + 5° C; concentration of polymetaphosphate 0.30 % (preparation K 15).

Table 6. Electrophoretic mobility in various media.

Medium	$u \cdot 10^5$
0.1 *M* NaCl (water)	17.3
0.1 » (5 % ethanol)	14.4
0.1 » (8 » »)	11.9
0.1 » (8 % ethanol) the ethanol dialyzed off from the polymetaphosphate solution.	15.8

The mobility of the polymetaphosphate anion is but little changed when its constitution is made complex by addition of bivalent metal ions. To 0.30 per cent solutions of preparation K 15 in 0.4 *M* NaCl calcium and mercury were added in the form of 0.15 *M* chloride solutions to the desired quotient, Me/P, and the mixture was dialyzed against 0.4 *M* NaCl before the electrophoresis run. The experiments were carried out at + 5° C. The results have been drawn up in Table 7. The variations of the mobility values are very small, especially when compared with the changes of the intrinsic and specific viscosity values under the same condition (*Cf.* ref. (7)).

Table 7. Electrophoretic mobility of calcium and mercury polymetaphosphate complex.

Metal added	Me/P	$u \cdot 10^5$
Control	—	17.3
Ca	1/9	18.4
Ca	1/4	17.4
Hg	1/9	17.1
Hg	1/6	17.0
Hg	1/3	16.2

SEDIMENTATION

The great dependance of the sedimentation of these polymetaphosphates on the concentration and species of the low molecular weight salts present in the solution has already been discussed in a previous paper[3]. Lamm[24] has theoretically deduced the retarding effect of the charge of a polyvalent ion on the sedimentation. However, the phenomena shown in Fig. 2, where the sedimentation constant S (given in Svedberg units) has been plotted as a function of the salt concentration in various media, cannot be explained by introducing in Lamm's equation the values of the dissociation determined from measurements of the Donnan equilibrium. However, not only low molecular weight electrolytes, but also any substance reducing the solvation is a factor of importance in this case. It must be pointed out that in all experiments the sedimentation curves present only one peak giving an impression of a rather homogenous substance.

The solvation seems to be the dominating factor in this connection. Addition of alcohol or ethyleneglycol to a polymetaphosphate solution has the same

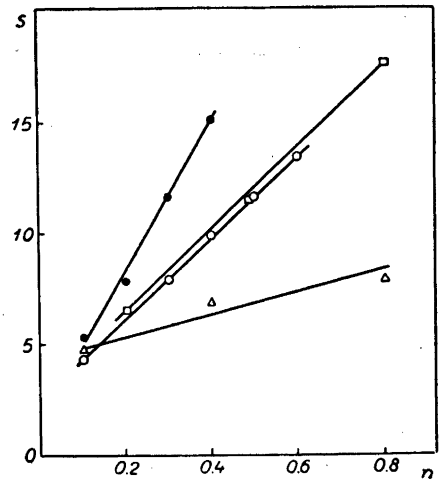


Fig. 2. The variation of s *in electrolyte solution of different normalities.*

● *NaCl* □ Na_2SO_4
○ $Na_3(PO_3)_3$ △ $Na_4P_2O_7$

effect on the sedimentation constant as an increase of the concentration of low molecular salts to the medium, see Table 8. Solutions of preparation K 15 at a concentration of 0.40 % were used for these experiments; solvent: 0.1 *M* NaCl in different mixtures of alcohol or glycol with water.

The sedimentation constant of the polymetaphosphate anion increases if a bivalent metal ion is bound in the form of a complex. If two alkali metal ions (sodium or potassium) are replaced by a heavy metal ion, *e.g.* mercury, one might assume the increase of the sedimentation velocity to be due to the increased anion weight, but the same phenomenon is observed in the case of a light metal, for instance, magnesium.

Solutions, prepared as follows, were used for the centrifuge runs. To a 0,60 % solution of K 15 in 0.4 *M* NaCl, magnesium and mercury were added in the form of 0.15 *M* chloride solutions to the desired quotient, Me/P. Then the concentration of K 15 was adjusted to 0.40 % by addition of 0.4 *M* NaCl, and

Table 8. Dependance of the sedimentation constant on the medium.

Medium	s
0.1 *M* NaCl (water)	5.9
0.1 » (3 % ethanol)	8.0
0.1 » (5 » »)	9.2
0.1 » (8 » »)	11.5
0.1 » (10 » glycol)	7.1

Table 9. Sedimentation constant of magnesium and mercury polymetaphosphate complex.

Metal added	Me/P	s
Control	—	13.0
Mg	1/6	15.3
Mg	1/3	19.9
Hg	1/6	16.4
Hg	1/3	22.6

the solution was dialyzed against 0.4 *M* NaCl. The results have been drawn up in Table 9.

These results very probably are due to a change of the molecular shape on addition of a bivalent metal. Certainly, it is plausible to assume the bivalent metal acting as a bridge between different molecules or different parts of a single molecule thus causing a change of the molecular size and/or shape. It may perhaps also be possible to regard the surface of the polymetaphosphate molecule as a semipermeable membrane. In that case the concentration of bivalent metal ions on both sides of the surface of a molecule is determined by the Donnan equilibrium.

DIFFUSION

The free diffusion of a polymetaphosphate is, like the viscosity and sedimentation, dependent not only on the concentration of low molecular weight electrolytes; it is also influenced by compounds decreasing the solvation. The dependence on electrolytes and charge effects has been treated in an earlier paper [3]. Here there will be mentioned only some measurements in media containing alcohol and some experiments in which the diffusing anion was a complex with magnesium.

The measurements were made at 20° C by Lamm's [25] scale method, using a steel cell after Claesson: [26] preparation K 15, metaphosphate concentration 0.25 %. The solution of the magnesium-metaphosphate complex was prepared as in the electrophoresis experiments described above (p. 7) and diffused against the outer fluid of dialysis. The results have been collected in Table 10. The diffusion coefficient, D_0, has been calculated according to the moment method; extrapolation to zero concentration after Gralén; [27] D_0 is given in 10^{-7} c.g.s.

As seen in Table 10, the diffusion velocity increases on addition of alcohol and magnesium, probably another indication of changed particle shape or size.

Table 10. Dependance of the diffusion coefficient on the medium.

Medium	D_0
0.1 *M* NaCl (water)	1.05
0.4 » »	1.08
0.1 » (5 % ethanol)	1.25
0.1 » (8 » »)	1.36
0.4 » (water); Mg/P = 1/6	1.20
0.4 » » Mg/P = 1/3	1.39

PARTIAL SPECIFIC VOLUME

In a previous paper[2] the partial specific volume, V, was calculated to be 0.39. This value is not correct but at that time only a small amount of substance was available, thus making accurate determinations rather difficult. In solutions the true value is appreciably smaller. In Table 11 are given some values of V determined in different media. Preparation: K 15. The value 0.39, however, is valid for $(KPO_3)_n$ in the solid state, as determined from measurements on suspensions in organic solvents, e.g. benzene.

A correct value of V is of great importance for calculations of molecular weights. The values tabulated above give molecular weights about 10 per cent lower than those obtained with $V = 0.39$. This means that molecular weights previously published[2, 3] are too low by this amount. However, the order of magnitude of these molecular weights is of primary interest. There is little significance to determinations of molecular weights of polydisperse, unfractionated substances to an accuracy better than 10 per cent.

Table 11. Partial specific volume in various media.

Medium	V
0.1 *M* NaCl (water)	0.311
0.2 » »	0.317
0.4 » »	0.316
0.1 $NaNO_3$ »	0.312
0.1 NaCl (5 % ethanol)	0.315
0.1 » (8 % »)	0.313
0.4 » (water) Mg-complex: Mg/P = 1 : 3	0.370

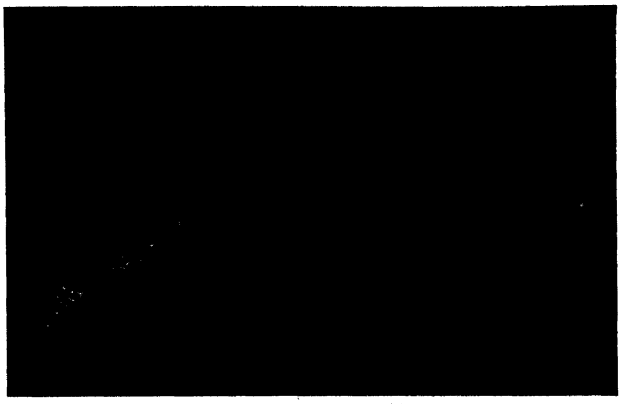


Fig. 3. Electron microscope picture of polymetaphosphate. Magnification: 30.000.

MOLECULAR SHAPE

Earlier attempts to determine the molecular form have not given consistent results [2,3]. The data from measurements of viscosity, sedimentation, diffusion *etc.*, are not sufficient for calculation of the molecular dimensions, as the behavior of these polymetaphosphates in solution is probably governed to a high degree by the solvation, which is very difficult to determine quantitatively. However, the absence of streaming birefringence was a reason for assuming that the polymetaphosphates in solution are "wrinkled molecules". (Kuhn: "geknäuelte Molekeln"). Earlier studies with the electron microscope failed to yield further information, because of agglomeration during the preparation.

Usually a diluted solution of the substance to be investigated is evaporated on a thin film of nitrocellulose and inserted in the focal plane of the microscope. (In the case of polymetaphosphates, the low molecular weight salts must, of course, be removed by dialysis to avoid the formation of a salt crust on the film). The nitrocellulose film is negatively charged and the large anions of a polymetaphosphate solution are repelled causing agglomeration, see Fig. 3. This effect can be eliminated or overcome by a technique developed by E. Ribi and Rånby [28]. The nitrocellulose film is first covered with a thin layer of an oxide of a light metal, *e.g.* beryllium, and thus presents a positively charged surface. In this case the metaphosphate anions are attracted to the film, agglomeration is avoided, and the solution can be removed after a very short "wetting" of the film. An electron micrograph thus obtained is shown in Fig. 4.

The diameter of the smallest particles is about one hundred Å units. Assuming spherical form which is a rough approximation, a particle weight of



Fig. 4. Electron microscope picture of polymetaphosphate. The nitrocellulose film is covered with a thin layer of beryllium oxide. Magnification: 30.000.

1×10^{-18} g. is indicated. The molecular weight determined in 0.4 *M* NaCl is 1 100 000, *i.e.* the weight of a single molecule is $1\ 100\ 000/6.06 \times 10^{23} = 2 \times 10^{-18}$ g. The two values are of the same order of magnitude. The particles of larger dimensions are formations of irregular shape, such as flattened, wrinkled chain molecules or aggregation of molecules might present.

However, it is very probable that the molecular shape changes with the medium. The values of the molecular symmetry factor, f/f_0, are not consistent in different media (f/f_0 equals 1 for a spherical, non solvated particle: molecular assymetry and/or solvation result in values of f/f_0 greater than unity). A summary of some molecular constants of preparation K 15 is given in Table 12.

Table 12. Some molecular constants determined in various media.

Medium	$(\eta_{sp}/c)_{c \to 0}$	S_0	D_0	M	f/f_0
0.1 *M* NaCl (water)	5.20	14	1.05	480 000	5.2
0.4 » »	0.92	32	1.08	1 100 000	3.8
0.1 » (5 % ethanol)	2.95	20	1.25	570 000	4.1
0.1 » (8 » »)	2.00	25	1.36	650 000	3.6
0.4 » Mg-complex Mg/P = 1/3	0.94	38	1.39	1 000 000	2.8

Assuming spherical molecules the values of f/f_0 can be used for calculation of the solvation. The values thus obtained, 15—40 g of water per gram of polymetaphosphate, are not consistent with the values calculated from the specific hydrodynamic volume, the latter beeing about 5 times higher. As the polymetaphosphate molecules are not perfect spheres but wrinkled chains, both methods of calculation probably give values that are too high but there is no doubt that the molecules are very largely solvated.

SUMMARY

High molecular potassium metaphosphates, the so-called salts of Kurol, have been investigated in solution. The colloid properties are due to the highly charged anion, the influence of which seems to be eliminated at low concentrations of low molecular weight salts ($\mu \sim 0.1$). The solvation has a dominating influence on the physico-chemical properties in media of higher ionic strength.

The molecular constants are dependent on the medium: they are also changed on complex formation between the anion and bivalent metals.

Electron microscope pictures support the view that the colloid polymetaphosphates in solution are wrinkled chain molecules.

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Enzymatic Breakdown of Polymetaphosphate

V. Purification and Specificity of the Enzyme

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Phosphatase activity on a great number of phosphorus compounds has been found in extracts from different species of *Aspergilli*, and there exists a rather rich literature in this field of research. Bamann and Salzer [1] have studied the phospho-esterase activity of *A. oryzae* (substrate: α- and β-glycerophosphate), Fleury and Courtois [2] reported pyrophosphatase activity; Neuberg and Fischer [3] and recently Neuberg, Grauer and Mandl [4] investigated its activity on tripolyphosphate $Na_5P_3O_{10}$. Kitasato [5] found in 1928 that "taka-phosphatase" from *A. oryzae* was also capable of liberating orthophosphate from metaphosphate. During his investigation on phosphorus metabolism in mould Mann [6] observed metaphosphatase activity in extracts from *A. niger*. Mann also made the observation that extracts from *A. niger* exhibit activity on a great number of phosphorus compounds, *e.g.* pyrophosphate, metaphosphate, α- and β-phosphoglycerol, 1-phosphoglucose, 6-phosphoglucose, aneurin diphosphate and adenosine triphosphate (all three atoms of phosphorus). Also Krishnan [7] recently reported apyrase activity in mycelial mats of *A. niger*. The polymetaphosphatase-activity (substrate: potassium metaphosphate of molecular weight more than one million) in extracts from *A. niger* has been investigated by the author and Ingelman [8–11], who also succeded in isolating a metaphosphate of high molecular weight from the mycelium of *A. niger* [12–14]. The question arose, however, as to whether *A. niger* possesses a specific polymetaphosphatase or if the activity towards all substrates used by Mann and the polymetaphosphate used by the author was the result of one or a few non-specific enzymes. The purpose of the work presented in this paper was to decide whether there exists a specific polymetaphosphatase or not and — in the former case — to purify the enzyme as much as possible.

MEASUREMENTS OF THE ENZYME ACTIVITY

As the substrate for measurements of the polymetaphosphatase activity a polymetaphosphate $(KPO_3)_n$ — designated K15 — was used the molecular weight of which is more than one million. Colloids of this type have been described in previous papers [15, 16]. The enzyme activity was determined by means of viscosity measurements at 25° C. As a relative measure of the enzyme activity in comparison experiments (the same substrate and substrate concentration) a quantity z, defined by

$$(z = \eta_{sp})_{t=0} \times \frac{d\left(\frac{1}{\eta_{sp}}\right)}{dt}$$

is used, where η_{sp} = specific viscosity and t = time. To 5 ml 0.5 % substrate solution in acetate buffer of ionic strength 0.3 and pH = 5.4 was added 1 ml enzyme solution. For a more detailed discussion of this method of calculation see Ingelman and Malmgren [9].

The enzyme activity on substrates of low molecular weight was measured by determining the amount of orthophosphate liberated in a certain time of incubation (orthophosphorus in percent of total phosphorus). The orthophosphate was determined by the method of Lowry and Lopez [17] (based upon reduction of phospho-molybdate by ascorbic acid at pH = 4). The substrate solutions were always 0.01 *M* with regard to total phosphorus. The proportion of the substrate solution to the enzyme solution was 5 : 1 and the temperature 25° C. As a blank the corresponding substrate solution plus water was always used instead of the enzyme solution. The enzyme solutions were always made free from orthophosphate by dialysis before being used.

CULTIVATION OF THE MOULD

The mould used for the enzyme production was, as described in previous works [8, 10], *A. niger* van Tieghem no. 594 from the National Collection of Type Cultures maintained in Britain by the Medical Research Council. In order to get an optimal production of enzyme a systematic investigation on the relationship between the polymetaphosphatase production and the composition of the nutrient solution was carried out by Lindeberg and the author [18]. According to the results of that work it seemed most suitable to cultivate the mould on the following medium: 5 g $(NH_4)_2\,SO_4$, 100 g glucose, 2 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7$ aq. and distilled water to 1 liter. Fe and Cu were added at a concentration of 1 mg/liter, Zn at 0.1 mg/liter and Mn at 3 mg/liter.

The solutions were inocculated with a suspension of conidia and tapped into Petri dishes. After 4—6 days at about 30° C most of the polymetaphosphatase produced by the mould has gone into the nutrient solution. The advantage of using $(NH_4)_2SO_4$ as a source of nitrogen is that most of the enzyme is found in the nutrient solution. Extraction of the mycelia is avoided which facilitates the concentration and purification of the enzyme. From this point of view $(NH_4)_2SO_4$ is the best source of nitrogen even if the same *total* enzyme production can be achieved by using other nitrogen compounds, *e. g.* nitrates. The pH-value of the final nutrient solution was generally very low, about 1.5—2, and the pH was adjusted to $\sim$ 5 before working up the solution.

PURIFICATION

Adsorption. Mann [6] tried to purify the enzyme by precipitation with acetone in the cold, adsorption on Al-C-γ gel and subsequent elution. In that way Mann achieved a purification from notatin, which — according to Mann — always is present in the mycelium and the medium of *A. niger*. The present author made attempts to follow Mann's preparation scheme. However, the precipitation with acetone causes denaturation of the enzyme proteins to a high degree even when working in the cold. After dissolution of the precipitate only a minor part of the activity is recovered. Also on absorption on Al-C-γ or $Ca_3(PO_4)_2$-gel and elution, which is rather difficult to perform, there is a great loss of enzyme activity. Further, there is no separation effected between the different phosphatase activities. This is shown by the following experiment: 15 ml of the crude enzyme solution (A) + 5 ml $Ca_3(PO_4)_2$ gel (5 mg Ca/ml) were shaken for 10 minutes, and the adsorbent was centrifuged off. The sediment was dissolved in 5 ml of ethylenediamine tetra-acetate * solution (25 mg/ml), dialyzed and the volume made up to 15 ml (B). The two solutions were tested for the activity on several substrates. The activity measurements were carried out in veronal-acetate buffers of optimal activity for the substrate in question (see page 22); time of incubation, 20 hours. The results have been collected in Table 1.

As seen from Table 1 the calcium phosphate gel is no selectiv adsorbent for any of the enzymes acting on these phosphorus compounds. Nor is it possible to get a better separation by washing the gel with a suitable eluent. In order to remove the adsorbed polymetaphosphatase from the calcium phosphate gel it is necessary to use rather concentrated salt solutions, 50 % saturated ammo-

Table 1. Data of adsorption experiments.

Substrate	Activity (per cent liberated ortho P.)		Activity of (B) / Activity of (A)
	(A)	(B)	
$Na_4P_2O_7$	4.12	1.44	0.35
$Na_5P_3O_{10}$	3.84	1.45	0.37
$Na_3(PO_3)_3$	5.29	2.08	0.39
β-Phospho-glycerol	19.8	7.22	0.36
Adenosine-monophosphate	14.9	5.28	0.35
K 15	$z = 5\,600$	$z = 1\,860$	0.33

* Kindly supplied by Leo AB, Helsingborg.

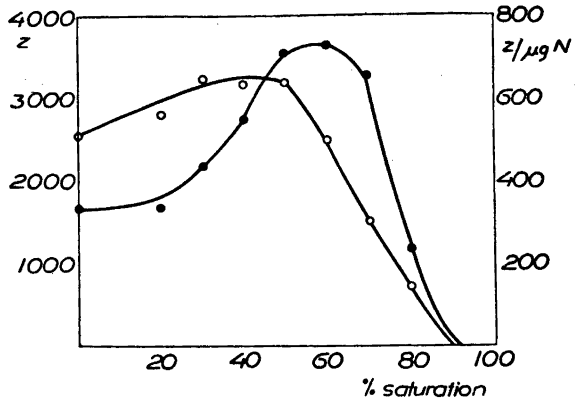


Fig. 1. Remaining activity of supernatants (ordinate to the left; ○) resp. activity / µg N (ordinata to the right; ●) as a function of various degree of ammonium sulphate saturation.

nium sulphate solution, for instance, and only a small amount of the polymetaphosphatase is recovered — less than what is obtained when using the technic of dissolving the adsorbent. Attempts to adsorb the polymetaphosphatase on the substrate, K 15, and on a gel of the corresponding calcium salt were not successful; these compounds do not act as adsorbents.

Fractionation with ammonium sulphate. The wellknown method of fractional precipitation with ammonium sulphate has also been utilised. Equal volumes of dialyzed und buffered crude enzyme solution ($\mu \sim 0.1$) were saturated to various degrees by addition of solid ammonium sulphate. The solutions were left over night in a refringerator, the precipitates were centrifuged off, the ammonium sulphate was dialyzed off and the solutions were made up to the same volume before the determination of the polymetaphosphatase activity and analysis of the nitrogen content. The results of such a series at pH = 3.1 (isoelectric point) is shown in Fig. 1.

As seen from the Figure there is an optimum of the z/N-value at about 60 per cent saturation and even at 70 per cent there is still a high z/N-value. A higher pH-values the enzyme is more soluble at high salt concentrations than at the isoelectric point. The z-values increase a little when passing from the blank to about 40 per cent saturation. Probably there is some protein in the crude enzyme solution acting as inhibitor that is precipitated at the addition of ammonium sulphate. If egg albumin is dissolved in the 70 per cent fraction to a concentration of 0.2 % the z-value is diminished by about 4 % and at a concentration of 0.5 % by about 20 %, which is probably a consequence of the property of proteins to interfere in a system involving metaphosphate.

PREPARATION

For preparative work it has proved most convenient to add ammonium-sulphate to 70 per cent saturation, discard the precipitate and work up the remaining solution with an electrophoretic purification as the last step. The following preparation scheme proved to be the best. The activity and nitrogen values refer to a preparation of average yield.

1. About 20 liters nutrient solution (after 6 days of cultivation) buffered to pH = 5	z = 4,000; z/mg N = 50,000 (non dialysable nitrogen)
2. Evaporation in vacuo to 3 liters volume; temperature not above 25° C	z = 20,000
3. Dialysis against calcium-free water *	
4. Evaporation in vacuo to 200 ml; pH adjusted to ~ 3—4 (acetate buffer)	z = 190,000
5. $(NH_4)_2SO_4$ to 70 % saturation; precipitate discarded	
6. $(NH_4)_2SO_4$ dialyzed off against calcium-free water *	
7. Evaporation to about 20 ml volume (in cellophane sac in streaming air of room temperature in order to avoid local super-heating)	z = 770,000 z/mg N = 650,000
8. Solution run in electrophoresis at the isoelectric point pH = 3.1 for 15 hours; afterwards dialyzed against distilled water	z = 600,000 z/mg N ~ 10^6

This preparation yields are enzyme solution purified to about twenty times the activity of the crude material. The z-value 600,000 means that even at a dilution of 1 : 10,000 (corresponding to 0.00006 mg N/ml) there is a measurable activity.

The preparation described above proved to be rather homogenous in the ultracentrifuge. In acetate buffer of pH = 5.4 and ionic strength 0.3 the value S_0 = 3.4 S was obtained; this is in agreement with the value 3.2 obtained from activity measurement after centrifugation in a separation cell [8]. The value of the diffusion constant determined by the usual optical method (Lamms' scale method) is 5.1 × 10^{-7} c.g.s. which is not in agreement with that

* It is of importance to use calcium free water when dialysing off the ammonium sulphate else the polymetaphosphatase in adsorbed on the calcium sulphate precipitate.

obtained by the porous disc method [8], 8.8. Probably the latter value is to be regarded as the best, because it refers to a certain enzyme activity and not to an impure enzyme.

However, even after the purification previously described the resulting enzyme solutions acts on the same phosphorus compounds as the original solution. It is probably very difficult — if possible — to separate the polymetaphosphatase from the other phosphatases by means of fractionation with ammonium sulphate or other salts. In fact, all these phosphatases seem to have the same solubility properties. If the supernatants from a series of enzyme solutions which are more and more saturated with ammonium sulphate, are tested for enzyme activity on different phosphorus compounds, it is found that the activity decreases on increasing degree of saturation in almost the same proportion for every substrate. The solutions from the salting out experiments at pH = 3.1 — previously described — were also tested for activity on other phosphorus compounds. The experiments were carried out in veronal-acetate buffers at the pH-optima of the substrates; time of incubation: 20 hours. The activity was put arbitrarity at 10 for the blank (no ammonium sulphate added). The results are collected in Table 2. For comparison the z-values have been reduced to the same scale.

As is seen from the table there is too small a difference in solubility between the polymetaphosphatase and the other phosphatases to permit a separation.

Further, the increase of activity in the supernatants up to 50 % saturation might indicate the presence of an inhibitor in the crude enzyme solution from which it is precipitated at rather moderate concentration of ammonium sulphate.

Table 2. Relative solubility of the phosphatases of A. niger.

Substrate / percent saturation	Blank	20	30	40	50	60	70	80	100
	Relative enzyme activity of supernatants								
K 15	10	11	12	12	12	10	8	2	0
$Na_4P_2O_7$	10	11	12	12	12	9	9	3	0
$Na_5P_3O_{10}$	10	10	13	13	13	10	6	3	0
$Na_3(PO_3)_3$	10	11	13	11	13	8	7	4	0
$(NaPO_3)_{\sim 50}$	10	10	12	11	12	10	8	2	0
Glucose-1-phosphate	10	11	11	10	11	10	7	2	0
β-Glycero-phosphate	10	11	12	11	10	10	9	3	0
Adenosine-monophosphate	10	11	11	11	11	10	8	3	0

It is also very difficult to perform an electrophoretic separation. All proteins remaining in the enzyme solution obtained according to the preparation scheme previously described have almost the same electrophoretic properties. Preliminary attempts to get a separation by means of paper electrophoresis were not successful; the moving boundary is not sharp but there is no separation effected — quite in analogy to electrophoresis when using the usual optical methods of observation.

SPECIFICITY

Even if it has not been possible to get the polymetaphosphate degrading enzyme in a pure state, there is full evidence for the existence of such a specific enzyme. A comparison between the pH-optima and the conditions of activation and inhibition leads to this conclusion.

The enzymatic breakdown caused by the polymetaphosphates seems to stop when the scission products have reached an average particle weight of $\sim$ 480, *i. e.* a tetra-or pentaphosphate depending on the dissociation. This result was obtained from equilibrium centrifugations of a polymetaphosphate solution incubated for two days at pH = 5.7 with a strong enzyme solution ($z = 70{,}000$). After this length of time, less than 0.2 per cent of the total phosphorus had been converted to orthophosphate.

pH-optima. As already mentioned Mann [6] showed that the crude extracts of the mycelia (and the remaining nutrient solution) exhibit phosphatase activity towards a great number of substrates; Mann reports an optimal activity in the pH-range 3.7—4.2 without giving the pH-value for every substrate used. In order to get a better picture of the specificity of the phosphatases of *A. niger*, the crude enzyme solution was investigated regarding the pH of optimal activity for the various substrates such as salts of condensed phosphoric acids of various degree of polymerisation and some organic esters of phosphoric acid. The buffers used in these experiments were of the veronal-acetate system. The results are drawn up in Table 3.

Table 3. pH-optima of different phosphatase activities.

Substrate	pH-optimum
$Na_4P_2O_7$	2.2
$Na_5P_3O_{10}$	2.3
$Na_3(PO_3)_3$	2.0
$(NaPO_3)_{\sim 50}$	2.6
K 15	5.7
Glucose-1-phosphate	2.2
β-Glycero-phosphate	3.0
Adenosine-monophosphate	4.0

All pH-optima — except that of K 15 — are in a very acid range. Fleury and Courtois [2] report an optimal activity of pyrophosphatase from *A. oryzae* at pH = 7. Neuberg and Fischer [3] found optimal tripolyphosphatase activity in extracts from *A. oryzae* at pH = 2.9; Bamann and Salzer [1] found optimal phosphoesterase activity (α- and β-glycero phosphate) at pH = 4.2 (*A. oryzae*). The values of *A. niger* given in Table 3 are somewhat lower they are also lower than the values of *A. niger* reported by Mann [6]. However the *A. niger* of the present investigation has been cultivated on a very acid medium which may account for the difference. The most interesting thing of Table 3 is the position of the pH-optimum of the polymetaphosphatase activity, pH = 5.7, which is considerably higher than the other values. It must be pointed out that in measurements of the activity by the analysis of the orthophosphate liberated, the rate determining factor may be the final reaction of a pyrophosphate if the substrate contains a chain of three or more atoms of phosphorus.

Activation and inhibition. As reported in a previous paper [10] the polymetaphosphatase is activated by many bivalent metals of which manganese and zinc seem to be most effective; also magnesium and calcium are activators. Under proper conditions the presence of manganese can activate the enzyme about 30 times. However, on no other substrate than polymetaphosphates of high molecular weight can such an effect be recorded. On the contrary, the phosphatases acting on substrates of low molecular weights are rather a little inhibited if the substrate solutions contain $MnCl_2$, see Table 4. However, not only with respect to the activation but also to the inhibition there exists a difference between the polymetaphosphatase and the other phosphatases of *A. niger*. Already Inouye [19] found that the acid phosphatases of *A. oryzae* are inhibited by fluoride ions. This is also the case with all the phosphatases of *A. niger* except that acting on metaphosphates of high molecular weight. Two other substances, *viz.* phloretin phosphate * and citric acid have the same effect as sodium fluoride, see Table 4. This property of citric acid is somewhat surprising as — according to Bamann and Salzer [1] — citrate ions do not inhibit the phospho-esterases of *A. oryzae* but cancel out the phosphatase inhibitor of *A. oryzae* (Taka diastase) investigated by — among others — Inouye [20].

The experiments were carried out in veronal-acetate buffers at the pH of optimal activity of the substrate in question The substrate solutions were made 0.0025 *M* in NaF, $MnCl_2$ and citric acid and 0.1 % in phloretin phosphate respectively. Time of incubation: 20 hours.

* Phloretin phosphate was supplied by *Leo AB*, Hälsingborg. It is a condensation product of phosphorylated phloretin. The substance used by the author has a molecular weight of about 15,000. For further details is referred to a coming paper in *Acta Chem. Scand.* by the scientific staff of *Leo*.

Table 4. Inhibition of the phosphatases of A. niger.

Substrate \ Inhibitor	NaF	$MnCl_2$	Citric acid	Phloretin phosphate
	percent inhibition			
K 15	2	600 *activation*	10	33
$Na_4P_2O_7$	98	14	96	83
$Na_5P_3O_{10}$	87	4	100	96
$Na_3(PO_3)_3$	100	14	100	91
$(NaPO_3)_{\sim 50}$	100	8	91	97
β-Phosphoglycerol	99	0	100	94
Glucose-1-phosphate	74	9	100	75
Adenosine-monophosphate	98	2	68	75

There exists a difference between the polymetaphosphate degrading enzyme and the other phosphatases not only with regard to the chemical activation and inhibition but also to the heat inactivation; the polymetaphosphatase is more sensitive to heat than the other enzymes. The heat inactivation is, however, not only a question of the heat resistivity of the enzyme as such but also of its purity. A crude enzyme solution is not so sensitive to heat as a purified enzyme probably because the crude solution contains some proteins or lipo-proteins acting as protective colloids.

A series of heat inactivations was carried out by keeping portions of crude enzyme solution at different temperatures (in a water bath) for ten minutes. The solutions were rapidly cooled down and tested for activity on different substrates. The results are seen in the Fig. 2, where the percentage of liberated ortho-phosphate after 4 hours' incubation is plotted against the temperature, the buffers used were veronal-acetate buffers of pH of optimal activities.

As seen from the Fig. 2 there is a sudden drop in the polymetaphosphatase activity at 50° C whereas the other curves show an activity decrease when passing 60° C. After purification the activity decreases already at 35—40° C, but also in this case the polymetaphosphatase is the most heat labile of the phosphatases.

OCCURRENCE OF METAPHOSPHATASES IN HIGHER ORGANISMS

Hitherto polymetaphosphatase has only been found in fungi and the bacteria *Proteus vulgaris* [10]. But in tissue extracts from higher animals metaphosphatase activity on substrates of low molecular weight can be demonstrated. The author found metaphosphatase activity in extracts from bovin

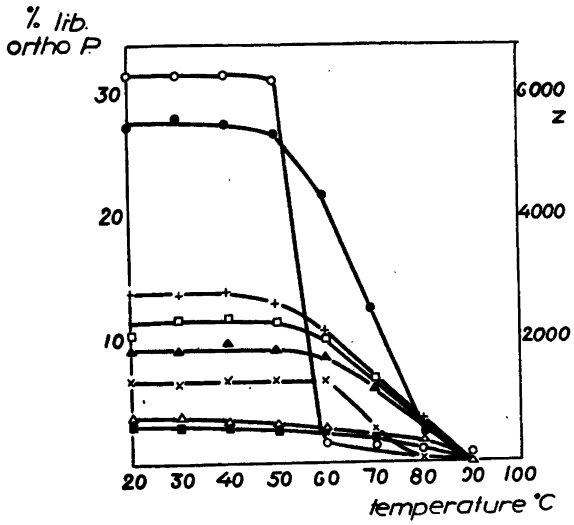


Fig. 2. Activity of heat inactivated enzyme solution as a function of temperature. Ordinata to the left: per cent liberated ortho phosphate; ordinata to the right: z*-values.*

○ = *K15*; × = $Na_4P_2O_7$; ■ = $(NaPO_3)_3$
\+ = $Na_5P_3O_{10}$; □ = $(NaPO_3)_{\sim 50}$; ● = *glucose-1-phosphate* ▲ = *β-phosphoglycerol*; △ = *adenosine-monophosphate.*

liver. (Water extract fractionated with ammoniumsulphate; the fraction 30—60 % saturation was taken, dissolved in distilled water and dialyzed.) This solution was tested for activity on a series of phosphates of various degree of polymerisation, see Table 5. Medium: acetate buffer of pH = 5.2; time of incubation: 4 hours.

The liver enzyme does not split off any orthophosphate from the polymetaphosphate of very high molecular weight, nor does it reduce the viscosity

Table 5. Activity of liver enzyme on various substrates.

Substrate	Per cent liberated ortho-phosphate
$Na_4P_2O_7$	19.7
$Na_3(PO_3)_3$	19.3
$(NaPO_3)_{\sim 50}$	4.8
K 15	0

of the solutions of this colloid (K 15). It is very likely that the metaphosphates of lower molecular weights are broken down by some non specific phosphatase *e.g.* a pyrophosphatase. Metaphosphate has not yet been isolated from tissues of higher animals and there is no reason why specific metaphosphatases should be found in such tissues.

SUMMARY

1. It has been proved that *A. niger* possesses an enzyme specifically directed on polymetaphosphate of high molecular weight. The final scission product is a tetra-or pentaphosphate.

2. The turn over number of this polymetaphosphatase is high. There is still good activity in a solution containing only 0.00006 mg *total* nitrogen per ml of which probably the less part belonging to the polymetaphosphatase.

3. The polymetaphosphatase has been purified about twenty times, but it has not been possible to prepare the enzyme in a pure state. The physicochemical properties of all the phosphatases of *A. niger* are very alike, thus making a separation very difficult; besides it would be necessary to cultivate the mould on a bigger scale, perhaps ten fold or hundred fold that used at present.

The author wishes to express his sincere thanks to Prof. A. Tiselius for the privilege of carrying out this work in his laboratory and for his kind interest. He is also indebted to Miss I. Karlsson for valuable technical assistance. The work has been supported financially by the Swedish Natural Science Research Council.

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Enzymatic Breakdown of Polymetaphosphate

VI. Influence of Nutritional Factors on the Polymetaphosphatase Production of *Aspergillus niger*

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During earlier investigations on polymetaphosphatase in *Aspergillus niger* one of the authors [1] (M.) found it desirable to obtain the enzyme in as large quantities as possible. Therefore, an investigation was begun in order to elucidate the optimum conditions of polymetaphosphatase production. Special emphasis was placed on a study of the following factors: carbon and nitrogen sources, trace elements and pH.

METHODS

Aspergillus niger van Tieghem (no. 594 from the National Collection of Type Cultures maintained in Britain by the Medical Research Council) was used as a test organism. The stock culture was grown on malt extract agar (2.5 % malt extract; 2 % agar-agar). The chemicals used in the nutrient solution were of the following qualities: crude and refined commercial sucrose; saccharose, purum; the other chemicals of reagent grade. The trace element content of the chemicals used was determined colorimetrically *. The following amounts of Fe, Zn, Mn and Cu were found (μg/g).

	Fe	Zn	Mn	Cu
Glucose	0.0	0.1	0.2	0.4
K_2HPO_4	8.0	0.0	0.0	1.1
$MgSO_4 \cdot 7$ aq.	0.7	0.0	0.1	0.2
$(NH_4)_2SO_4$	1.0	0.0	0.2	0.3
NH_4-tartrate	2.0	2.2	0.9	0.6
$NaNO_3$	3.4	0.1	0.0	0.3
Asparagine	0.0	1.5	0.0	0.4
Glycine	0.3	8.8	0.0	8.2
Urea	0.0	1.1	0.0	1.0

Thus, in most experiments the trace element content of the chemicals used is small in comparison with the addition of the same elements.

* The determinations were carried out according to the official methods of Kungl. Lantbruksstyrelsens Kungörelser, Nr 9, 1950 and Sandell: Colorimetric determination of traces of metals. New York (1950).

The water was distilled first in a stainless steel apparatus and then in a pyrex glass apparatus. 300 ml Erlenmeyer flasks (Pyrex) were used as culture vessels. They were carefully cleaned with dichromate-sulphuric acid, hot water, and finally streaming vapor before use. Each flask contained 40 ml of sterile nutrient solution. Suspensions of conidia in sterile water were prepared for the inoculation. These suspensions were filtered through thin layers of sterile cotton before use. Each flask was inoculated with 1 ml suspension. The flasks were incubated at 30° C. Eight parallels were harvested from each series each time. Four of them were used for the determination of the mycelium dry weight and — in some cases — analysed for nitrogen and phosphorus. These mycelia were rinsed for about half an hour in running tap water and finally with distilled water, care being taken that the conidia-bearing surface was not wetted. The remaining four flasks were used in determining the polymetaphosphatase activity of the mycelia and the nutrient solution.

The polymetaphosphatase activity was determined viscosimetrically according to a method described by Ingelman and Malmgren [2]. As a relative measure of the enzyme activity (using the same substrate and the same substrate concentration) a quantity z is used. This quantity is defined by:

$$z = (\eta_{sp})_{t=0} \times \frac{d\left(\frac{1}{\eta_{sp}}\right)}{dt}$$

where η_{sp} = the specific viscosity, t = time after incubation with enzyme of the substrate solution. As substrate was used a potassium metaphosphate $(KPO_3)_n$, n being of the order 10 000. z is expressed in 10^{-4} min.$^{-1}$.

The relative amount of polymetaphosphatase secreted into the nutrient solution, A_s, was calculated as the product of the z-value and the volume (ml) of the solution. The corresponding value for the quantity of enzyme in the mycelium, A_m, was calculated analogously after extracting the mycelia in the following way: After addition of acetate buffer (pH = 5.4) to a total volume of 25 ml per mycelium, the mycelia were ground up in a Waring blendor. After about one hour the suspension was centrifuged and the activity of the supernatant was determined. The total polymetaphosphatase production, *i. e.* the sum of A_s and A_m, is designated ΣA. All these quantities are given in units of $z \cdot 10^{-4}$.

Control experiments were made in order to check that too much enzyme was not lost by extracting the mycelia with only one portion of buffer. The sediment was washed repeatedly until the washings did not show any activity. It was found that the losses of polymetaphosphatase depending on incomplete extraction were small, less than 5 %. In only one case, where asparagine was used as a nitrogen source, did the loss of enzyme amount to 10 %.

The determinations of the nitrogen content of the mycelia were made according to Kjeldahl, the phosphorus determinations following the method of Lowry and Lopez [3].

EXPERIMENTS AND RESULTS

Influence of the nature of the carbon source

In some earlier experiments a mixture of crude and refined sucrose was used as carbon source (2.5 % and 7.5 % resp.). In experiment no. 1 the growth

Table 1. Influence of the carbon source on enzyme production. Time of incubation: 6 days.

Carbohydrate	Mycelium dry weight (ω) in g	pH	ΣA	$\frac{\Sigma A}{\omega}$
Crude sucrose + refined sucrose	0.93	1.7	6	6
Refined sucrose	0.94	1.6	6	6
Saccharose	0.95	1.6	7	7
Glucose	0.95	1.7	5	5

and the enzyme production of the mould was compared when using pure glucose and different qualities of saccharose as carbon source.

Experiment no. 1. Basal medium: 5 g $(NH_4)_2SO_4$, 2 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7$ aq., 100 ml tap water, distilled water to 1 000 ml (including the sugars). Four series according to Table 1.

The growth and enzyme production of the mould was almost the same in all four cases. Owing to the necessity of using as well-defined chemicals as possible glucose was preferred as carbon source during the continued work.

Influence of trace elements

Preliminary experiments showed that an addition of tap water to the nutrient solution had a considerable influence on the enzyme production. Hence tap water must contain some metal ions of importance in this connection.

In the next experiment the influence of different metals on the enzyme production of the mould was investigated.

The following metals, which have earlier been shown to be essential for *A. niger* (for references see Steinberg [4]), were tested: iron, zinc, manganese and copper.

Experiment no. 2. Basal medium: 100 g glucose, 5 g $(NH_4)_2SO_4$, 2 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7$ aq., redistilled water to 1 000 ml (including the trace element additions). The following salts of the trace elements were used: Fe-citrate (ferric), $MnSO_4 \cdot 4$ aq., $CuSO_4 \cdot 5$ aq. and $ZnSO_4 \cdot 7$ aq. The final series contained the trace elements in the following concentrations: Fe, Cu and Zn 1 p.p.m., Mn 3 p.p.m. Twelve series according to Table 2.

Enzyme production in the control series was found to be rather low. Fe and Cu had a stimulating effect both on growth and enzyme production.

Table 2. Influence of different trace elements on the enzyme production. Time of incubation: 6 days.

Metals added	Mycelium dry weight (ω) in g	pH	ΣA	$\frac{\Sigma A}{w}$
Control	0.25	2.2	11	44
Fe	0.44	1.8	39	89
Cu	0.34	1.9	31	91
Mn	0.45	2.1	21	47
Zn	0.65	1.8	1	2
Zn, Fe	0.72	1.7	2	3
Zn, Cu	0.46	1.7	1	2
Zn, Mn	0.46	1.8	1	2
Fe, Mn	0.39	2.2	25	64
Fe, Cu	0.30	2.0	32	107
Cu, Mn	0.25	2.0	17	68
Fe, Mn, Cu	0.48	1.8	36	75

Mn alone stimulated growth but caused no increase in the relative enzyme production, while in combination with Fe or Cu, it seemed to limit slightly the stimulating effect of these metals. This result is difficult to explain without further experiments. An addition of 1 mg Zn per liter almost completely inhibited enzyme production, and also caused a marked inhibition of conidial formation (cf Roberg [6]). Since high enzyme production and the most uniform growth of the mould occurred on addition of Fe, Mn and Cu, these metals were added in most of the following experiments.

In an additional experiment, the influence of calcium, which under certain conditions affects growth of several fungi (cf Lindeberg [5]), was also tested. However, at a concentration of 20 p.p.m. this metal had no action either on growth or on polymetaphosphatase production.

Although zinc at a concentration of 1 p.p.m. inhibits the enzyme production, it cannot be presumed that this metal should also have an inhibitory effect at lower concentrations. The influence of different concentrations of zinc on the enzyme production was therefore studied.

Experiment no. 3. Basal medium: 100 g glucose, 5 g $(NH_4)_2SO_4$, 2 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7$ aq, Fe and Cu 1 p.p.m. Mn 3 p.p.m., redistilled water to 1 000 ml. The results of 5 series are given in Table 3.

The mycelial weight increased with increasing Zn-concentration until a maximum was reached at 100 μg Zn per liter. At 1 000 μg per liter the mycelial weight was somewhat lower. At concentrations of 1—100 μg Zn per liter no

Table 3. Influence of different concentrations of zinc on enzyme production. Time of incubation: 7 days.

µg Zn added per liter	Mycelium dry weight (ω) in g	pH	ΣA	$\frac{\Sigma A}{w}$
0	0.25	1.8	54	220
10	0.57	1.7	53	93
100	1.04	1.6	59	57
1 000	0.81	1.6	3	4

pronounced effect on the total enzyme production was observed, whereas at 1 000 µg Zn per liter it was strongly inhibited. The enzyme production per gram mycelium $\frac{\Sigma A}{w}$ decreased on increasing additions of Zn.

Influence of the nature of the nitrogen source and of hydrogen ion concentration

At an early stage of the investigation it was found that the nature of the nitrogen source was of great importance for the polymetaphosphatase production. One experiment which also demonstrates the role of the incubation time will be described here.

Experiment no. 4. Basal medium: 25 g crude and 75 g refined sucrose, 2 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7$ aq, 100 ml tap water; distilled water to 1000 ml. Two series containing 5 g $(NH_4)_2SO_4$ resp. 7.7 g KNO_3 per liter. Fig. 1.

In the ammonium sulphate series the mycelial dry weight reached a (maximum) value of about 0.85 g.; simultaneously the pH decreased from 6.7 to 1.7. Subsequently both mycelial weight and pH remained practically unchanged. Autolysis of the mycelium was not observed. The total amount of enzyme ΣA reached a value of about 3 after 6 days, and remained virtually the same. The activity of the mycelial extracts was low, only $\sim 1/6$ of the total amount of enzyme being found in the mycelium. Thus, in this case most of the polymetaphosphatase was secreted into the medium.

In the nitrate series the mycelial dry weight passed a maximum of $\sim$ 1.20 g. after 6 days after which a rather pronounced autolysis began. After 2 days the pH had decreased to 3.8 but when the autolysis set in the pH increased again, reaching the value of 8.5 after 10 days and the value of 9.0 after 13 days. The total enzyme production was somewhat higher than in the former series,

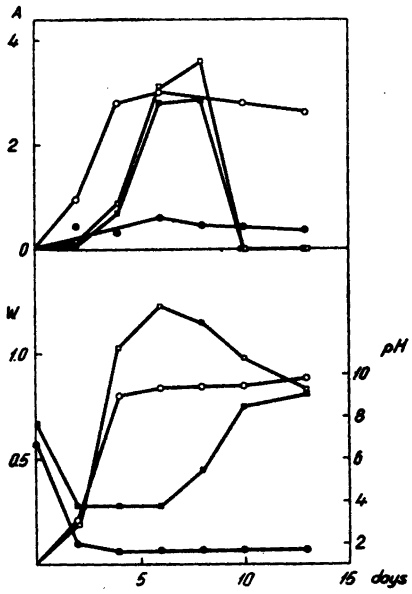


Fig. 1. Abscissa: time of growth in days. Ordinata to the left, lower part of the figure: mycelium dry weight in g □ = KNO_3; ○ = $(NH_4)_2SO_4$. *Ordinata to the right, lower part of the figure: pH of the medium;* ■ = KNO_3; ● = $(NH_4)_2SO_4$. *Ordinata, upper part of the figure: relative amount of enzyme;* □ = Σ A KNO_3; ■ = A_m KNO_3; ○ = ΣA $(NH_4)_2SO_4$; ● = A_m $(NH_4)_2SO_4$.

but most of the enzyme was found in the mycelium, only a slight amount being secreted into the nutrient solution.

The large secretion of enzyme in the ammonium sulphate series is due probably to the increased concentration of hydrogen ions in this series. Hence, one might expect an adjustment of the pH to a lower value than the "normal" one of, for instance, the nitrate medium, to result in an increased secretion. Furthermore, if a certain minimum amount of enzyme is needed inside the mycelial cells, an increase of the secretion might result in an increased total enzyme production.

The effect of the hydrogen ion concentration of the medium on the production of certain enzymes of microorganisms has earlier been shown. In different bacteria Gale [7,8] observed an increased production of catalase, urease, fumarase, and certain dehydrogenases when the pH of the medium was lowered. Lindeberg and Fåhraeus [9] (unpublished) found an increased production of laccase in the hymenomycetes *Polyporus zonatus* and *P. versicolor* when the hydrogen ion concentration was increased.

As demonstrated above the polymetaphosphatase production is dependent on the nitrogen source, and it was suggested that this fact might possibly be related to a variation of the amount of assimilated nitrogen in the mycelium. The question also arises if there is any relation between the polymetaphosphatase production and the phosphorus content of the mycelium. It could

be assumed that a high assimilation of phosphorus would facilitate the enzyme production. On the other hand it might be expected that the presence of more enzyme in the cells would cause a more intense synthesis of polymetaphosphate, the occurrence of which in the mycelium has been demonstrated by Ingelman and Malmgren [10]. In the following experiments the factors discussed above have been taken into consideration. The nitrogen sources used were: equivalent amounts of $(NH_4)_2SO_4$, NH_4-tartrate, $NaNO_3$, asparagine, glycine and urea, i.e. ammonium salts of one strong and one weak acid, anionic inorganic nitrogen, amido-amino-, pure amino- and pure amido-nitrogen.

Experiment no. 5. Basal medium: 100 g glucose, 2 g K_2HPO_4, 0.5 g $MgSO_4 \cdot 7$ aq, Fe and Cu 1 p.p.m., Mn 3 p.p.m., Zn 0.1 p.p.m., redistilled water to 1000 ml (including the nitrogen compounds). In different series were added: 5 g $(NH_4)_2SO_4$, 7 g NH_4-tartrate, 6.4 g $NaNO_3$, 5 g asparagine, 5.7 g glycine and 2.3 g urea resp.

Each nitrogen source (except $(NH_4)_2SO_4$) was added to two series, one of which was used for pH-adjustment. In this series the pH was adjusted to $\sim$ 2 by sterile addition of 1 *M* H_2SO_4 after 2 days' incubation when a continuous mat had been formed by the fungus. The mycelia were analysed for nitrogen and total phosphorus.

The results are presented in Tables 4 and 5.

As seen in Table 4 there was a distinct difference between enzyme production on different nitrogen sources. Regarding the „normal" conditions of growth (no pH-adjustment), it is found that ammonium sulphate and sodium nitrate gave the highest total enzyme production, followed by ammonium tartrate, glycine and urea. With asparagine as nitrogen source the polymetaphosphatase production was very low. The secretion of enzyme into the nutrient solution also varied. In the ammonium sulphate series (the lowest pH-value) only 25 per cent of the total activity remained in the mycelium, in the asparagine series 80—90 per cent.

The effect of pH-adjustment was as expected. The most pronounced effect was observed in the asparagine series in which the total enzyme production was increased 4—5 times. The pH-adjustment also resulted in a decreased formation of conidia and a mycelium dry weight lower than in the corresponding reference series.

The $\frac{A_m}{w}$-values were highest for the sodium nitrate series and lowest for the asparagine series. It may be mentioned here that in several other experiments — not described in this paper — the lowest $\frac{A_m}{w}$-value, 3, was also found in the asparagine series.

The analytical data of Table 5 show no correlation between the assimilation of nitrogen and phosphorus on the one hand and the enzyme production on the other. As shown by Behr [11], the percentage of nitrogen in the mycelium

Table 4. Influence of the nitrogen source on growth and enzyme production.

Nitrogen source	Time of incubation in days	Mycelium dry weight (ω) g	pH	ΣA	$\frac{A_m}{\Sigma A}$	$\frac{A_m}{w}$
$(NH_4)_2SO_4$	4	0.76	1.5	44	0.24	14
	7	0.95	1.9	43	0.25	11
NH_4-tartrate	4	0.95	2.2	16	0.45	8
	7	1.07	3.0	28	0.25	7
pH adjusted	4	0.85	1.5	56	0.36	24
	7	1.03	1.9	45	0.19	8
$NaNO_3$	4	0.81	3.6	47	0.79	46
	7	0.97	4.5	43	0.55	24
pH adjusted	4	0.78	2.5	57	0.78	57
	7	0.97	2.2	96	0.46	46
Asparagine	4	0.90	2.3	4	0.84	4
	7	1.13	3.0	4	0.91	3
pH adjusted	4	0.69	1.7	21	0.40	12
	7	0.92	2.0	15	0.17	3
Glycine	4	0.77	2.3	21	0.40	11
	7	1.03	2.7	13	0.58	7
pH adjusted	4	0.62	1.5	30	0.20	10
	7	0.85	1.7	36	0.12	5
Urea	4	0.53	2.5	13	0.30	7
	7	0.73	2.7	12	0.28	5
pH adjusted	4	0.48	1.9	16	0.31	10
	7	0.66	2.0	30	0.13	6

decreases with time. The total nitrogen uptake from the medium varied between about 28 and 38 mg per flask, corresponding to 70—90 % of the available nitrogen. In all cases maximum nitrogen uptake seems to have been reached after four days. On the contrary, the phosphorus uptake in most series increased from the fourth to the seventh day, causing in several series an

Table 5. Analytical data of mycelia grown on media with different nitrogen sources.

Nitrogen source	Time of incubation in days	% N	% P	mg N per mycelium *	mg P per mycelium *
$(NH_4)_2SO_4$	4	4.4	0.74	33	5.6
	7	2.7	0.47	26	4.5
NH_4-tartrate	4	3.5	0.71	33	6.7
	7	3.1	1.07	33	11
pH adjusted	4	3.7	0.87	32	7.4
	7	3.2	0.79	33	8.1
$NaNO_3$	4	4.7	0.79	38	6.4
	7	3.5	1.06	34	10
pH adjusted	4	4.9	0.79	38	6.2
	7	3.9	1.07	38	10
Asparagine	4	3.4	0.64	31	5.8
	7	2.8	1.25	31	14
pH adjusted	4	4.1	0.74	29	5.1
	7	3.0	0.95	28	8.8
Glycine	4	4.2	0.70	32	5.4
	7	3.4	1.21	35	12
pH adjusted	4	5.3	0.76	33	4.7
	7	3.5	0.67	30	5.7
Urea	4	5.2	0.82	28	4.3
	7	3.7	0.81	27	5.9
pH adjusted	4	5.9	0.86	28	4.1
	7	2.3	0.89	15	5.8

* In all series the medium contained 42 mg N and 14 mg P per flask (40 ml)

increase in the relative phosphorus content of the mycelium also. Of course, it would be very desirable to know also the polymetaphosphate content of the mycelium. However, owing to the great difficulties implied in the quantitative determination of polymetaphosphate, the question of the amount of this compound present in mycelia from different media must be left open in this work.

Influence of an addition of polymetaphosphate

The polymetaphosphatase of *A. niger* is a constitutive enzyme in the sense that it is synthesized by the organism even if the nutrient medium does not contain the specific substrate of the enzyme (Karström [12]). The difference between constitutive and adaptive enzymes is, however, not sharp as shown by Karström and others. An organism may exhibit a certain degree of adaptation even with regard to constitutive enzymes. Among the nitrogen compounds tested asparagine gives the lowest polymetaphosphatase production and an adaptive feature in the character of the enzyme might be expected to be more easily revealed with asparagine as a nitrogen source than with the other compounds. In order to investigate whether an addition of polymetaphosphate to the medium stimulates the formation of polymetaphosphatase the following experiment was carried out.

Experiment no. 6. Basal medium 100 g glucose, 2 g K_2HPO_4, 0.2 g $MgSO_4 \cdot 7$ aq, 5 g asparagine, Fe and Cu 1 p.p.m., Mn 3 p.p.m., Zn 0.1 p.p.m., redistilled water to 875 ml. To this medium 125 ml 0.4 % polymetaphosphate in 0.1 *M* NaCl, resp. 125 ml 0.1 *M* NaCl were added in different series.

The results are presented in Table 6.

Table 6. Influence of polymetaphosphate on enzyme production.

	Time of incubation in days	Mycelium dry weight (ω) in g	pH	ΣA	$\frac{A_m}{\Sigma A}$
Control	4	1.05	2.8	27	0.88
	7	1.06	6.7	14	0.73
$(KPO_3)_n$ added	4	1.02	2.8	21	0.74
	7	1.13	5.5	15	0.85

As seen from the Table the addition of polymetaphosphate had no influence on the enzyme production.

SUMMARY

1. The influence of certain factors on the polymetaphosphatase production of *A. niger* has been studied.

2. The enzyme production was about the same on media containing glucose or saccharose as carbon sources.

3. An increased enzyme production was achieved upon addition of Fe, Cu (1 p.p.m.) and Mn (3 p.p.m.), Fe being the most effective of these metals. Ca had no effect.

4. The enzyme production per gram mycelium decreased on addition of zinc (10—1 000 μg/liter). The total enzyme production was strongly inhibited in the presence of 1 mg Zn per liter.

5. The nature of the nitrogen source greatly affected the enzyme production. Highest production was found with nitrates and ammonium salts, definitely lower production with organic nitrogen sources, *e. g.* glycine, urea and, especially, asparagine.

6. With increasing concentration of hydrogen ions the secretion of enzyme into the medium was increased. By lowering the pH of the nutrient solution to about 1.5—2 the enzyme production was greatly stimulated.

7. No relation could be observed between the total nitrogen and phosphorus content of the mycelium and the enzyme production.

8. Addition of polymetaphosphate to the medium did not increase the enzyme production.

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Studies on Lignin *

III. Oxidation of Wood from *Picea abies* (L.) Karst. (Norway Spruce) with Nitrobenzene and Alkali

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In their important paper on the oxidation of spruce lignin with nitrobenzene and alkali, Freudenberg, Lautsch, and Engler[1] reported the occurrence among the oxidation products of a number of phenolic compounds. Vanillin, vanillic acid, 5-carboxyvanillin and guaiacol were identified, but the remaining substances were present in amounts too small to permit isolation and identification in the usual way.

Since then, however, the method of paper partition chromatography introduced by Consden, Gordon, and Martin [2] has made it possible to detect very small amounts of compounds, and the present paper describes a reinvestigation of the nitrobenzene oxidation products from spruce, utilising chromatographic methods. Similar methods have been used by Bland [3,4] who studied such oxidation products from *Eucalyptus* species and isolated small amounts of *p*-hydroxybenzaldehyde, in addition to vanillin and syringaldehyde.**

As a result of the present work a comparatively large number of phenolic oxidation products have been detected and, in most cases, isolated and identified. Recently Pearl [5] has isolated some of the same compounds from the mixture obtained by oxidation with cupric hydroxide of sulphite spent liquor from spruce.

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* Replacing the general title Studies on the Sulphonation of Lignin — Part II. *Acta Chem. Scand.* 4 (1950) 971.

** Note added in proof: Bland, Ho, and Cohen [19] have recently studied the aldehyde mixtures obtained from some Conifer species, and demonstrated (paper chromatographically) that they contain *p*-hydroxybenzaldehyde and, for some species probably also syringaldehyde (*cf.* following paper).

In Table 1 the compounds found are listed, the yields being calculated on the basis of the lignin content of the wood employed. The yields within brackets were estimated visually from the intensities of the corresponding spots on the paper chromatograms and are consequently only approximate.

Table 1. Nitrobenzene oxidation products from spruce wood.

Compound	Yield %
Vanillin *	27.5
p-Hydroxybenzaldehyde	0.25
Syringaldehyde	0.06
5-Formylvanillin	0.23
Dehydrodivanillin **	0.80
Vanillic acid *	4.8
(*p*-Hydroxybenzoic acid)***	—
Syringic acid	(0.02)
5-Formylvanillic acid	(0.1)
5-Carboxyvanillin *	1.2
Dehydrodivanillic acid **	0.03
Guaiacol *	—
Acetoguaiacone **	0.05

* Previously reported by Freudenberg [1].
** Previously reported by Pearl [5].
*** Observed only in two cases.

METHODS

The oxidation was carried out essentially as described by Freudenberg *et al.*, but reaction conditions more close to optimum [6] were employed (180°, 2 hrs).

After the removal of nitrogenous compounds and material insoluble in alkali, the oxidation mixture was extracted with trichloroethylene and the extract divided into aldehydic, phenolic and acidic fractions as described on p. 44. In addition, an ether extraction was made, yielding 9 % of oxalic and 2 % of fumaric acid, calculated on the lignin content of the wood. The latter acid has not previously been isolated from the oxidation products of spruce wood.

The three fractions soluble in trichloroethylene were examined by means of paper chromatography, employing the procedures outlined below, each fraction being treated separately.

Aldehyde fraction

The use of paper partition chromatography for the separation of phenolic aldehydes has been described previously by Bland [3] who separated a mixture of vanillin, syringaldehyde, and *p*-hydroxybenzaldehyde, using ligroin as the mobile phase. He also separated *p*-hydroxybenzaldehyde from vanillin and syringaldehyde by means of a "chromatopile"[4]. Recently Pearl and Dickey [7] described the quantitative separation of vanillin and syringaldehyde by adsorption chromatography on a column of "Magnesol".

In the case of spruce, Bland's simple procedure had to be modified, owing to the difficulty of detecting small amounts of syringaldehyde, which gives a much more diffuse spot than does *p*-hydroxybenzaldehyde. It was found necessary to use larger amounts of material than is usually employed for a paper chromatogram and this, in turn, resulted in strong trailing effects. Several methods of overcoming this trailing were tried, the most effective being to use papers previously impregnated with a buffer solution of suitable pH. (For details, see experimental part.)

The results of a typical separation experiment using this method are given in Table 2.

The subsequent quantitative separation of the mixture was complicated by the fact that *p*-hydroxybenzaldehyde, syringaldehyde, and 5-formylvanillin constitute only a very small part of the aldehyde mixture (about 2 %), the bulk of which consists of vanillin.

Table 2. Paper chromatographic analysis of aldehyde fraction. Paper: Munktell OB, impregnated with a phosphate buffer of pH 6.8. Solvent: Ligroin (b.p. 85—110°)-benzene (2 : 1). Developer: 2,4-dinitrophenylhydrazine (DNF).

Compound	R_F	Appearance of spot		Remarks
		in UV	with DNF	
Unidentified	0.02	Greenish fluoresc.	Yellow	
p-Hydroxybenzaldehyde	0.06	Dark blue	Red	Spots circular, sharp contours
Unidentified	0.16	Invisible	Yellow	
Syringaldehyde	0.31	Invisible	Brownish	Oblong, diffuse spots, fade rapidly
5-Formylvanillin	0.43	Greenish fluoresc.	Yellow	R_F very sensitive to pH
Vanillin	0.53	Dark blue	Brick red	

To increase the proportion of the desired aldehydes, the crude mixture was recrystallized from aqueous ethanol. The material from the mother liquor from this recrystallization contained less than 20 % of the vanillin and almost all of the other aldehydes, and this mixture, dissolved in benzene, was adsorbed on top of a column of cellulose. A large volume of ligroin-benzene (2:1) eluted most of the vanillin and part of the 5-formylvanillin, leaving all the *p*-hydroxybenzaldehyde and syringaldehyde and a large part of the 5-formylvanillin on the column. The latter aldehydes could then be eluted with ethanol. The final separation of this aldehyde mixture was achieved using a column of "Magnesol" similar to that described by Pearl and Dickey [7], and the "liquid chromatogram" technique with light petroleum-ethanol mixtures. By increasing the percentage of ethanol from 0.8 to 2 when no more vanillin and 5-formylvanillin could be detected in the eluate, a quantitative separation was obtained, the only overlapping fractions being those of vanillin and 5-formylvanillin (*cf.* Fig. 1, p. 45).

From the water-insoluble portion of the aldehyde fraction a further amount of 5-formylvanillin could be isolated. Some dehydrodivanillin was also obtained and this compound is probably the "acid" described by Freudenberg *et al* [1]., of m.p. 301°. The elementary composition reported by them agrees well with that of dehydrodivanillin.

Acid fraction

Methods for separation of phenol carboxylic acid by paper chromatography, using butanol with different admixtures [8,9] or benzene-acetic acid-water [10] as solvents have been described by several authors.

For the present purpose a technique using buffered paper proved to be very effective. With butanol-water as solvent very good results were obtained when the pH of the impregnating buffer solution was a little over 7.

In Table 3, the results of a typical separation experiment using this method are given.

The fraction was then divided into bisulphite-soluble and bisulphite-insoluble portions and each was examined separately. These experiments, combined with the use of 2,4-dinitrophenylhydrazine as a developing agent, confirmed the results given in Table 3 and indicated that the two last mentioned unidentified compounds were aldehydic in nature.

Of the acids detected above, only vanillic acid and 5-carboxyvanillin were isolated.

Table 3. Paper chromatographic analysis of acid fraction. Paper: Munktell OB, impregnated with a phosphate buffer of pH 7.5. Solvent: Butanol. Developer: Bis-diazotized benzidine [11] *(DB).*

Compound	R_F	Appearance of spot		Remarks
		in UV	with DB	
Unidentified	0.06	Blue fluoresc.	Invisible	Possibly dehydrodivanillic acid
5-Formylvanillic acid	0.31	Greenish fluoresc.	Reddish brown	
Syringic acid	0.35	Invisible	Claret red	Colour soon fading to brown
Vanillic acid	0.48	Dark blue	Light brown	
p-Hydroxybenzoic acid	0.53	Invisible	Pale yellow	Colour develops very slowly. Observed only in 2 cases
Unidentified *	0.59	Greenish fluoresc.	Almost invisible	
5-Carboxyvanillin	0.67	Blue fluoresc.	Brown	
Unidentified *	0.81	Greenish fluoresc.	Invisible	

* Present in very small amount.

Phenolic fraction

Several paper chromatographic procedures for the separation of mixtures of this kind have been devised [8,12,13]. However, only a few experiments were carried out with this fraction and the method described above was employed. Guaiacol ($R_F = 0.70$), acetoguaiacone ($R_F = 0.47$) and *p*-hydroxyazobenzene ($R_F = 0.75$) were detected, using ligroin-benzene (2:1) as solvent, and paper buffered to pH 9.

DISCUSSION

Undoubtedly the most interesting compounds isolated are syringaldehyde and *p*-hydroxybenzaldehyde, as this is the first time these substances have

been obtained from spruce. The only analogous compound previously isolated is trimethyl gallic acid obtained in very small yield by permanganate oxidation of methylated spruce wood [14].

The occurrence of *p*-hydroxyphenyl and syringyl elements in spruce lignin is of great interest in relation to the "methoxyl deficiency" of this lignin. Thus, the methoxyl content of spruce lignin is somewhat lower than the theoretical value for a lignin containing only guaiacyl nuclei with three-carbon side-chains, the number of methoxyl groups per ten carbon atoms being about 0.95 instead of the expected 1.0.

This "deficiency" could obviously be explained by the occurrence in spruce lignin of methoxyl-free elements, such as *p*-hydroxyphenyl nuclei. However, *p*-hydroxybenzaldehyde accounts for only about 1 % of the aldehyde mixture which, furthermore, contains about 0.2 % of syringaldehyde which influences the methoxyl value in the opposite direction. The proportion of *p*-hydroxyphenyl elements in the lignin itself would therefore have to be much higher than is indicated by the percentage of *p*-hydroxybenzaldehyde in the aldehyde mixture, *i.e.* the yield of *p*-hydroxybenzaldehyde from the *p*-hydroxyphenyl elements would have to be much lower than the yield of vanillin from the guaiacyl elements. This is, in fact, not at all unlikely, as the *p*-hydroxyphenyl element would be expected to contain a larger proportion of "condensed" elements, *i.e.* containing an extra carbon-carbon linkage in the position *ortho* to the hydroxyl group, than the guaiacyl residues. Completely analogous is the well-known fact that the yield of syringaldehyde from the syringyl elements in hardwood lignin is much higher than the yield of vanillin from the guaiacyl elements.

Whether or not the isolation of dehydrodivanillin indicates the presence of diphenyl elements in spruce lignin is an open question. Freudenberg, Meister and Flickinger [15] found dehydrodiveratric acid among the permanganate oxidation products from methylated spruce and considered it possible that the diphenyl link was formed during the reaction.

Such a dehydrogenation has, however, never been observed to take place during the oxidation of various model compounds, either with nitrobenzene and alkali or with potassium permanganate. Further, since lignin is probably formed in Nature by a dehydrogenative coupling of aromatic precursors it would not be unexpected if diphenyl linkages do occur to a small extent in genuine spruce lignin.

The isolation of 5-formylvanillin does not call for special comment, especially in view of the results previously reported [16] on the oxidation of model compounds.

EXPERIMENTAL

An amount of air-dry powdered spruce wood corresponding to 150 g dry weight (4.92 % OCH_3, 27.5 % Klason lignin) which had previously been extracted with benzene-alcohol (1 : 1) for 24 hours, was mixed with 2 *N* sodium hydroxide (1650 ml) and nitrobenzene (165 ml). The mixture was heated to 180° for two hours in autoclaves rotating in an oil bath, then steam-distilled to remove nitrogenous compounds and, after cooling, centrifugated and the clear centrifugate neutralized with concentrated sulphuric acid. More conc. sulphuric acid (20 ml) was added and the pH of the solution was adjusted to 7.3 by the addition of trisodium phosphate. The pH then remained fairly constant throughout the whole extraction and never exceeded 8.0.

The mixture was continuously extracted with trichloroethylene for about 24 hours, (extract A), then acidified to pH of ca. 2, extracted again with trichloroethylene (extract B) and finally extracted with ether (extract C).

Extract A was shaken with 5 % sodium bisulphite (extract Ab) and then with 2 % sodium hydroxide (extract Ah). The bisulphite solution Ab was acidified with sulphuric acid and the sulphur dioxide was expelled by heating the mixture on the water bath and passing in carbon dioxide. The precipitate (Ab_1) (2.2 g) obtained was collected from the cold solution, and the filtrate was exhaustively extracted with ether. The ether extract was dried (Na_2SO_4) and evaporated, giving a yellowish-red crystalline residue (11.5 g) (Ab_2).

This was recrystallized from dilute ethanol (15 %), yielding yellowish crystals (m.p. 77—79°, 9.55 g) of vanillin contaminated with a small amount of 5-formylvanillin and possibly a trace of syringaldehyde. The mother liquor was extracted with ether and gave a mixture of aldehydes (Ab_2'), (1.83 g), which were further separated on a column of cellulose pretreated with boiling dilute nitric acid as described by Burstall, Davies, and Wells [17].

The aldehyde mixture Ab_2' (1.5 g), dissolved in benzene (25 ml), was applied to the top of the column (4.5 × 50 cm) and elution was commenced with water-saturated ligroin (85—110°)-benzene (2 : 1) at a flow rate of ca. 1 l per hour. When only traces of aldehydic material could be detected in the eluate, the solvent was replaced by ethanol. The first 100 ml of ethanol were collected and evaporated, yielding an oil weighing 282 mg.

The final separation was carried out by means of chromatography on a column (16 × 165 mm) of acid-washed "Magnesol"-"Celite" (5 : 1) (*cf.* Pearl and Dickey [7]).

Light petroleum (b.p. 40—60°)-ethanol (125 : 1) was forced through the column by means of compressed air and 20 ml fractions were collected until no more vanillin or 5-formylvanillin could be detected in the eluate. The ethanol content of the solvent was then increased to 2 %, and elution continued until the eluate was free from aldehydes (Fig. 1).

Evaporation of the solvent from the *p*-hydroxybenzaldehyde fraction, gave a product melting at 107—110° which after recrystallization from light petroleum—benzene formed almost colourless crystals, m.p. 114—115°, undepressed by authentic *p-hydroxybenzaldehyde.*

The crude syringaldehyde was contaminated with ca. 25 % of a coloured impurity, which could, however, be removed by filtration through a small column of acid-washed alumina. Evaporation of the solvent yielded a crystalline residue, which after recrystallization from light petroleum—benzene formed almost colourless needles, m.p. 109—110°, undepressed on admixture with authentic *syringaldehyde.*

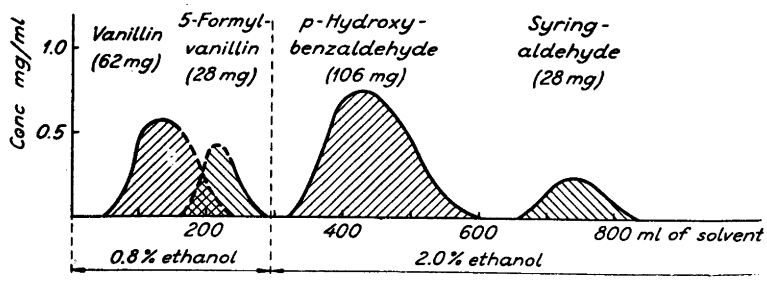


Fig. 1. Chromatographic separation of aldehyde mixture obtained from spruce.

The water-insoluble fraction Ab_1 was repeatedly extracted with boiling water (filtrate Ab_1'), then dissolved in hot nitrobenzene and filtered. The pale brown needles which separated on cooling (m.p. 300°, 0.32 g) were recrystallized from nitrobenzene yielding colourless needles, m.p. 300—301°, undepressed on admixture with authentic *dehydrodivanillin.*

$C_{16}H_{14}O_6$	Calc.	C	63.6	H	4.67	OCH_3	20.6
	Found	»	63.6	»	4.72	»	21.3

On cooling, the aqueous filtrate Ab_1' (200 ml) yielded an amorphous precipitate (1.2 g), from which no crystalline compound could be obtained. The remaining solution was extracted with ether, and the ether extract dried (Na_2SO_4) and evaporated. The oily residue (0.36 g) was extracted with boiling light petroleum and then dissolved in boiling dilute ethanol (15 %) and the solution treated with charcoal and filtered hot. On cooling, a crystalline compound separated. This material, together with the undissolved residue obtained by extraction of the vanillin fraction from the adsorption column with boiling light petroleum, was then recrystallized from dilute ethanol, yielding yellowish crystals (75 mg), m.p. 118—120°, undepressed on admixture with authentic *5-formylvanillin.* From the light petroleum solutions vanillin (0.22 g) could be recovered.

The alkaline fraction Ah was acidified and extracted with ether, and the extract dried (Na_2SO_4) and evaporated. The residue (2.6 g) — a red crystalline mass with a strong smell of guaiacol — was repeatedly extracted with boiling water and the resulting aqueous solution (Ah_2) was filtered hot. The residue was dissolved in very dilute, boiling alcohol, and the solution filtered hot. On cooling, brownish red, crystalline material (1.1 g) precipitated and this, after repeated recrystallization from dilute alcohol, gave brick red crystals of p-hydroxyazobenzene [1] (m.p. 150—151°).

From the aqueous solution Ah_2, there separated a yellow oil which slowly partially crystallised. The crystals were collected, washed with a little dilute methanol and sublimed at 1 mm giving colourless crystals (22 mg), m.p. 114—115°, undepressed on admixture with authentic *acetoguaiacone.*

The oily material probably consisted largely of *guaiacol.*

Of the trichloroethylene solution B, an aliquot was used for paper chromatographic purpose, and the remainder was extracted with 5 % sodium bicarbonate solution. Acidification of the solution gave a brown precipitate (0.8 g) which was filtered off (filtrate B_2)

and repeatedly extracted with hot dioxan which on cooling and dilution with water, deposited 0.4 g of material which was stirred with cold dioxan. Part of the material dissolved, the residue being a crystalline product which after recrystallization from dioxan formed colourless plates, m.p. 252—254°, undepressed by authentic *5-carboxy-vanillin*, although examination by paper chromatography revealed the presence of a small amount of *5-formylvanillic acid*.

The almost colourless material undissolved by the hot dioxan was dissolved in boiling nitrobenzene. On cooling, *dehydrodivanillic acid* (14 mg) separated as fine needles, m.p. 293—295°, undepressed by an authentic sample.

The aqueous solution B_2 was extracted with ether and the extract dried (Na_2SO_4) and evaporated. A sample of the crystalline material obtained was sublimed at 1 mm, yielding *vanillic acid* in amount corresponding to a yield of 1.95 g for the total batch (2.75 g).

For the paper chromatographic experiments a few drops of the aliquot referred to above were used directly, while the remainder was extracted first with 5 % sodium bisulphite and then with 5 % sodium bicarbonate. These two fractions were examined separately as mentioned above.

Evaporation of the ether from extract C (*cf.* p. 44), gave a syrupy residue which soon deposited crystals. On treatment with cold water, the syrup dissolved (C_2), leaving an almost colourless material which was collected, washed with a little water and recrystallized from water (charcoal), giving 0.8 g of crystalline material. The melting point determination was complicated by the volatility of the compound, sublimation commencing at about 230°. The substance was therefore placed in a melting point apparatus (aluminium block type), preheated to about 270° and in this way the melting point was found to be 285—288°. The melting point and sublimation were indicative of *fumaric acid* but a mixed melting point would not be very reliable under these conditions. Consequently the fumaric acid was identified by conversion to N-phenylasparaginanil [18], m.p. 210—211°, undepressed by an authentic specimen.

The aqueous solution C_2 was buffered with sodium acetate and treated with calcium acetate. The cream-coloured precipitate (6.6 g) which formed was collected, washed thoroughly with water and dissolved in dilute hydrochloric acid. The resulting solution was continuously extracted with ether and the ether evaporated to give a crystalline residue (5.8 g) which after repeated recrystallization from xylene, yielded anhydrous *oxalic acid* (3.6 g), m.p. and mixed m.p. 189—190°.

Paper chromatography

For all the experiments the descending method was used. The paper used was Munktell OB (Whatman no. 1 paper was tried but gave rise to serious trailing effects). The paper was cut in strips 10 × 48 cm and the strips were soaked in a 0.1 *N* phosphate buffer solution of the desired pH and then dried at 105° for about 15 minutes before use.

The examination of the aldehyde fraction offered some special problems, owing to the low concentration of, in particular, syringaldehyde and the relative difficulty of detecting the corresponding spots after development. Thus, ten drops of a 5 % alcoholic solution of the aldehyde mixture had to be applied to the paper successively. Several solvent mixtures were tried, using benzene and ligroin as well as butanol. A mixture of 1 part (by volume) of benzene and 2 parts of ligroin (b.p. 85—110°) proved to be very effective. The pH of the impregnating buffer solution was also varied, the most distinct syring-

aldehyde spot being obtained with a buffer of pH ca. 7. The R_F values of the aldehydes other than 5-formylvanillin were virtually independent of pH in the range 6—8 but with 5-formylvanillin, the R_F varied from 0.60 on unimpregnated paper to 0.19 at pH 7.8. A pH-value of 6.8 was chosen, as at this pH the formylvanillin spot appeared in a position where it did not interfere with other spots.

Of the developers tried, only 2,4-dinitrophenylhydrazine proved sufficiently sensitive. The sensitivity seems to increase if the solution used contains more hydrochloric acid than usually. The most satisfactory reagent was prepared by dissolving dinitrophenylhydrazine (1 g) in concentrated hydrochloric acid (300 ml) and diluting to one litre.

Owing to the slow formation of a yellow background, the weaker spots gradually became indistinguishable. Attempts to prevent this formation of a background by rinsing the paper in water after spraying only resulted in blurred spots. The spots were therefore always inspected within a few minutes of spraying.

For the chromatography of the acid fraction, smaller amounts of material gave better results and usually one or two drops of a 1 % alcoholic solution were applied to the paper. The influence of the pH of the buffer solution was studied also with this fraction, and it was found that the best separation was obtained at a pH a little over 7. The only solvent tried was water-saturated butanol. A satisfactory method of detection was inspection in UV light and subsequent spraying with bis-diazotized benzidine solution [11]. Attempts to separate the acids as their ammonium salts, using propanol-ammonia-water as solvent and ninhydrin as developer were not successful.

SUMMARY

The oxidation of spruce wood with nitrobenzene and alkali has been thoroughly studied by means of chromatographic methods.

In addition to vanillin, dehydrodivanillin, vanillic acid, 5-carboxyvanillin, dehydrodivanillic acid, acetoguaiacone, oxalic acid and *p*-hydroxyazobenzene, which have been isolated previously by other authors, the following compounds were detected on paper chromatograms or isolated:

Syringaldehyde, *p*-hydroxybenzaldehyde, 5-formylvanillin, syringic acid, *p*-hydroxybenzoic acid, 5-formylvanillic acid, and fumaric acid.

The significance of the presence of *p*-hydroxybenzaldehyde, syringaldehyde and dehydrodivanillin in connection with the structure of spruce lignin is discussed.

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Studies on Lignin

IV. * Investigation on the Nitrobenzene Oxidation Products of Lignin from Different Woods by Paper Partition Chromatography

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In general, there are characteristic differences between lignin from plants belonging to different botanical groups, although there is relative uniformity within each group. Thus, the Mäule [1] colour test is, with some exception, negative with gymnosperms but positive with dicotyledons and monocotyledons [2, 3].

In 1934 Holmberg [4] published an important paper on the study of lignins extracted from over fifty plant species, using thioglycolic acid as the extraction agent. He clearly demonstrated that the carbon contents of lignins from gymnosperms are generally 3—5 % higher than those of lignins from angiosperms, and at the same time the latter lignins often show a higher methoxyl content.

Of the more recent investigations in this field, one of the most important, dealing with the oxidation products from numerous species of gymnosperms, dicotyledons and monocotyledons, was carried out by Hibbert *et al.*[5]. These workers used the nitrobenzene oxidation technique introduced by Freudenberg *et al.*[6] and found that with a few exceptions, the gymnosperms yielded vanillin as the only aldehydic oxidation product, while the angiosperms yielded both vanillin and syringaldehyde, usually in the ratio 1 : 3. In addition, some of the monocotyledons yielded considerable amounts of *p*-hydroxybenzaldehyde [7]. They also showed that woods which gave a positive Mäule test, always yielded syringaldehyde [8].

The main differences in the structure of lignin from plants of different botanical groups should therefore consist in variations of the amounts of guaia-

* Part III. *Acta Chem. Scand.* 6 (1952) 38.

Table 1. Nitrobenzene oxidation of gymnosperm lignin.

Order	Family	Species	OCH_3 %	Klason lignin %	Total aldehydes based on OCH_3 %	Total aldehydes based on lignin %	*p*-Hydroxybenzaldehyde % *	Syringaldehyde % *	Remarks
Araucariales	*Araucariaceae*	*Araucaria araucana* (Mol.) Koch	4.92	27.6	31.1	27.2	+	—	
		Agathis alba (Rumph.) Warburg	5.70	—	32.5	—	++	+	
Podocarpales	*Podocarpaceae*	*Dacrydium cupressinum* Sol.	5.20	—	31.6	—	(+)	++	
		Podocarpus spicatus R. Br.	5.68	—	31.1	—	++	—	
		Podocarpus Totara D. Don.	6.33	35.6	31.0	27.1	+	3.0	
		Phyllocladus romboidalis Rich.	5.64	32.3	31.1	26.6	++	+	
Pinales	*Pinaceae*	*Pseudotsuga taxifolia* (Poir.) Britt.	4.47	26.0	31.9	26.9	++	++	Syringic acid detected
		Tsuga heterophylla (R.) Sarg.	5.05	—	31.2	—	(+)	—	No syringic acid
		Tsuga canadensis (L.) Carr.	5.30	31.2	32.0	26.6	3.5	++	
		Picea abies (L.) Karst.	4.92	27.5	31.6	28.0	+++	+	Syringic acid detected
		Larix sibirica Ledeb.	4.66	—	32.4	—	++	++	Possibly also *p*-hydroxy benzoic acid
		Cedrus Deodara (Roxb.) Loud	5.50	31.1	30.5	26.4	+++	++	
		Pinus sylvestris L.	4.71	26.9	31.7	27.3	+++	+	Syringic acid detected
Cupressales	*Taxodiaceae*	*Sciadopitys verticillata* (Thunb.) Sieb. et Zucc.	5.74	—	31.3	—	2.5	(+)	
		Sequoiadendron giganteum (Lindl.) Buch	6.10	35.4	32.3	27.3	3.5	(+)	
		Taxodium distichum Rich.	6.20	37.0	31.6	26.0	2.0	(+)	
		Cryptomeria japonica (L. fil) Don.	5.20	—	32.6	—	2.5	+	
		Athrotaxis selaginoides Don.	5.78	—	32.2	—	2.5	++	
	Cupressaceae	*Tetraclinis articulata* (Wahl) Mast.	6.72	31.8	—	36.8**	+	~50	
		Widdringtonia juniperoides (L.) Endl.	5.44	—	31.0	—	(+)	—	No syringic acid
		Thuja plicata D. Don	6.09	33.2	30.3	27.4	+	(+)	
		Libocedrus chilensis (D. Don) Endl.	5.55	31.2	30.0	26.2	3.5	(+)	
		Cupressus Macnabiana Murr.	5.55	—	32.0	—	3.0	(+)	
		Chamaecyparis nootkatensis (Lamb.) Spach	5.30	—	32.4	—	+	+	
		Juniperus communis L.	5.33	30.0	31.4	27.4	2.5	—	
Cephalotaxales	*Cephalotaxaceae*	*Cephalotaxus drupacea* Sieb. et Zucc.	6.13	36.1	25.0	21.4	+	—	
Taxales	*Taxaceae*	*Taxus baccata* L.	5.69	32.5	27.4	23.6	3.5	—	

* — no spot
(+) identification uncertain
\+ 0.5 % or less of tot. aldeh.
++ 0.5—1.0 % —"—
+++ 1.0—2.0 % —"—
In general, amounts corresponding to 0.1—0.2 % of total aldehydes give spots which are barely visible.
** Calculated from the weight of the total aldehydes extracted with ether.

cyl, syringyl and *p*-hydroxyphenyl elements present, thus giving rise to the variations in carbon and methoxyl content.

The methods used by Hibbert to isolate and separate the different aldehydes made it very difficult to detect small amounts of for example syringaldehyde, in mixture with a large amount of vanillin. Therefore, when one of the present authors succeeded in identifying small amounts of *p*-hydroxybenzaldehyde and syringaldehyde among the nitrobenzene oxidation products from spruce wood (Part III), using chromatographic methods, it seemed to be of interest to extend this study to some other species of wood.

In an investigation of a large group of plants such as the Conifers, the coverage must necessarily be very incomplete, but we have tried to select species available in this department and which at the same time formed a representative cross-section of the whole group.

The oxidations and isolation of the aldehyde mixtures (in a few cases also the acid fractions) and the paper chromatographic analyses were carried out as described in Part III. The aldehydes were estimated by the bisulphite titration method described previously [9], giving values which were generally 3—4 % lower than the corresponding weights of the aldehyde mixtures obtained by ether extraction. The total yields of aldehydes were calculated (as vanillin) on the basis of the methoxyl content and, in many cases, also of the lignin content of the extracted dry wood.

Table 1 lists the results obtained, with the different species. The yields of *p*-hydroxybenzaldehyde and syringaldehyde indicated by + signs or figures are of course very approximative, since they have been determined visually by comparison of the intensities of the spots on the paper chromatograms, with spots obtained from standard solutions (*cf.* experimental part).

In addition, the oxidation products from three common hardwoods were studied (Table 2).

Table 2. Nitrobenzene oxidation of hardwood lignin.

Species	OCH_3 %	Klason lignin %	Total aldehydes based on lignin %	*p*-Hydroxybenzaldehyde %	Syringaldehyde %
Acer platanoides	6.15	24.2	44.6	(+)	~ 75
Betula papyrifera	5.40	20.0	47.0	(+)	~ 75
Populus sp.	5.60	25.0	40.0	+	~ 75

The results listed in Table 1 indicate that most Conifers yield *p*-hydroxybenzaldehyde but that the occurrence of syringaldehyde is not nearly so general. The highest yields of *p*-hydroxybenzaldehyde are given by members of the families *Taxodiaceae* and *Pinaceae*, although some representatives of the family *Cupressaceae* also give significant amounts.

Syringaldehyde seems to occur most frequently in the *Pinaceae*. In the *Podocarpaceae*, the genus *Podocarpus* is of especial interest as Hibbert showed that some species give high yields of syringaldehyde while others (*Podocarpus neriifolius* and *P. acutifolium*) give none. The latter two species together with *P. macrophyllus v. maki* (presence of syringaldehyde uncertain) would of course have been of interest for the present investigation, but they were unfortunately not available. The study of two other *Podocarpus* species, however, tends to give support to the view that the composition of lignin from *Podocarpus* varies considerably, the various species giving syringaldehyde in yields varying from 0 to 50 % of the total aldehydes.

In the *Cupressaceae*, the yield of syringaldehyde in general is low or nil with the peculiar exception of *Tetraclinis articulata* which Hibbert found gave an aldehyde mixture containing about 45 % of syringaldehyde.

The most surprising inconsistency in Table 1 is the great difference between *Tsuga canadensis* and *Tsuga heterophylla*, the latter giving very little or no *p*-hydroxybenzaldehyde and syringaldehyde and thus being perhaps an exceptional member of the family *Pinaceae*.

Before drawing any definite conclusions, however, knowledge must be gained about the individual variations within each species by investigating a large number of wood samples. This, however, is entirely outside the scope of the present investigation. (In the case of *Picea abies*, three samples were studied, and gave almost identical results.)

The few acid fractions investigated always contained syringic acid if the corresponding aldehyde fraction contained syringaldehyde, but no *p*-hydroxybenzoic acid could be detected except possibly in the case of *Picea abies* (*cf.* Part III).

The total yields of aldehydes were very uniform. With three exceptions they varied between 30.0 and 32.6 % (average 31.6 %) based on the methoxyl content, and between 26.0 and 28.0 % (average 27.0 %) based on the lignin content of the wood. This variation is insignificant as three different samples of the same species (*Picea abies*) gave yields of 32.1, 31.6 and 30.8 % (28.0, 28.0 and 27.0 %) respectively.

Cephalotaxus drupacea and *Taxus baccata* gave yields of aldehydes lower than the average and it is interesting to note that *Cephalotaxus* has been considered to be related to *Taxus*, although the modern trend seems to be to

place *Taxus* outside the Conifer group. The high yield of aldehydes from *Tetraclinis articulata* is, of course, due to the large amount of syringaldehyde present.

It may be mentioned that the total yields of aldehydes reported by Hibbert were much lower than those reported here, and in addition, varied considerably from species to species. This discrepancy is probably due to the fact that Hibbert did not employ optimal oxidation conditions. He states that the time of reaction may be reduced from 3 hours to 2 hours at 160° when only small amounts of material are oxidized, but this seems to be quite improbable and is, in fact, not in agreement with recent experimental findings [10].

EXPERIMENTAL

In each experiment powdered, air-dried wood (3—5 g previously extracted with benzene-ethanol (1:1)), was oxidized by the method described in Part III. Only the bisulphite-soluble fractions of the oxidation products were investigated, except in five cases (see Table 1), when in addition the acid fractions were recovered and examined. (*Cf.* Part III.)

After removal of the sulphur dioxide, cooling and filtering, an aliquot of the bisulphite extract was titrated to determine the total aldehyde content [9], and the remainder of the solution was extracted with ether to recover the aldehydes. The ether solutions were dried over sodium sulphate, the ether evaporated, and the crystalline residue analysed by means of paper chromatography as described in Part III.

The semi-quantitative estimation of the relative amounts of *p*-hydroxybenzaldehyde and syringaldehyde was carried out in the following way:

50 Mg of the sample to be investigated were dissolved in ethanol (1 ml) and part of this solution was sucked into a capillary pipet to a predetermined height. The solution was then applied to the paper dropwise (10 drops), with drying between each drop. The same procedure with the same pipet was used for the standard solutions, containing amounts of *p*-hydroxybenzaldehyde and syringaldehyde corresponding to 0.2, 0.5, 0.7, 1.0, 1.5, 2.0, 3.0, 5.0 and 25 % of the total aldehydes. Chromatograms were run as described in Part III.

By comparing visually the spots from the unknown samples with those from the standard solutions, a comparatively good estimate of the relative amounts could be obtained, especially if the concentrations were not too high.

SUMMARY

The nitrobenzene oxidation products from the wood of 27 Conifers and 3 hardwoods have been studied by means of paper partition chromatography.

The wood from almost all coniferous genera gives small and varying amounts of *p*-hydroxybenzaldehyde. The families *Taxodiaceae* and *Pinaceae* give comparatively high and uniform yields. Many Conifers, belonging mainly to the family *Pinaceae*, give small amounts of syringaldehyde. The results

obtained with *Podocarpus* species and *Tetraclinis articulata* confirm the earlier results of Hibbert.

The hardwoods investigated gave, in addition to vanillin and syringaldehyde, only very little or no *p*-hydroxybenzaldehyde.

On the whole, the total yields of aldehydes showed very small deviations from the average within the whole class *Coniferae*.

The authors wish to express their thanks to *Statens Tekniska Forskningsråd* for financial support.

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Studies on Lignin

VI *. Nitrobenzene Oxidation of Lignin Sulphonic Acids

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Lignin sulphonic acids from sulphite waste liquors are of considerable interest as raw material for the manufacture of vanillin, and many papers dealing with this subject have been published [1]. Of the numerous oxidation procedures suggested, the method employing nitrobenzene and alkali seems to be one of the most promising because of the high yields obtainable.

Little has been done, however, to correlate these yields with the method of preparation, sulphur content, pretreatment *etc.* of the sulphonic acids employed. In their original paper [2], Freudenberg, Lautsch and Engler stated that the yield of vanillin from lignin in ordinary sulphite waste liquor was 10—20 % lower than that obtainable directly from the lignin in the wood. Later, Lautsch and Piazolo [3] isolated several fractions of lignin sulphonic acids from sulphite waste liquor by fractional precipitation with benzacridine and showed that these fractions gave yields which varied from 15 to 27 % (on the basis of the weight of lignin), the yield increasing with the sulphur content.

During the course of a normal sulphite cook, however, the lignin is subjected to rather drastic treatment, and some of the results previously obtained may be due to the occurrence of secondary reactions during this process, thus making it difficult to draw any definite conclusions about the influence of different variables on the yield of vanillin.

In the present paper, therefore, so-called "low sulphonated lignin" [4] has been employed for a study of the nitrobenzene oxidation of lignin sulphonic acids, since this material is prepared under very mild conditions.

The "low sulphonated lignin" was heated with sulphite solutions of various pH, for different lengths of time, and the products were analyzed and sub-

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* Part V. *Svensk Kem. Tid.* **63** (1951) 195.

jected to oxidation with nitrobenzene and alkali. The results are shown in Table 1 and Fig. 1.

The analytical values for sulphur, barium, and methoxyl are based on the weight of dry material, while the yields of sulphonation products and vanillin are based on the amount of methoxyl recovered. The sulphite solutions (75 ml for 4 g of material) all contained 6 % of total sulphur dioxide and sufficient sodium hydroxide to give the desired pH. The reaction temperature was 135°.

Table 1. Sulphonation of "low sulphonated lignin".

pH of sulphite soln. *	Time h	Yield %	Content of			2Ba/S	Yield of vanillin %
			sulphur %	barium %	methoxyl %		
—	—	—	4.3	9.8	12.0	1.06	31.9
1.4	2	71	5.6	12.3	11.7	1.03	—
1.4	6	74	6.9	14.9	10.7	1.01	—
1.4	12	77	7.9	17.9	9.5	1.06	32.1
1.4	24	70	8.0	18.6	9.0	1.08	31.6
3.4	4	76	4.7	10.6	11.9	1.04	—
3.4	10	72	5.2	11.3	11.8	1.02	—
3.4	24	68	6.4	14.5	11.0	1.06	—
3.4	48	76	7.7	17.3	10.6	1.05	31.2
5.8	8	70	4.7	11.1	12.1	1.09	—
5.8	24	70	5.2	11.5	11.8	1.03	31.6
5.8	72	75	6.4	15.8	10.2	1.14	32.3

* Measured at room temperature. The initial and final pH's of the solutions were the same.

Fig. 1 shows the ratios S/OCH_3 for different pH values as functions of the time of heating.

In strongly acid solutions the uptake of sulphur is rather rapid, in agreement with the findings of Erdtman [4], and Erdtman, Hägglund and Lindgren [5] who showed further that this is not so for lignin sulphonic acids from an ordinary acid sulphite waste liquor, which are sulphonated only to a small extent. The rate of sulphonation decreases with time and the ratio S/OCH_3 finally approaches unity.

At higher pH values, sulphonation takes place to an appreciable extent, although at a much lower rate. However, the rate seems to decrease only

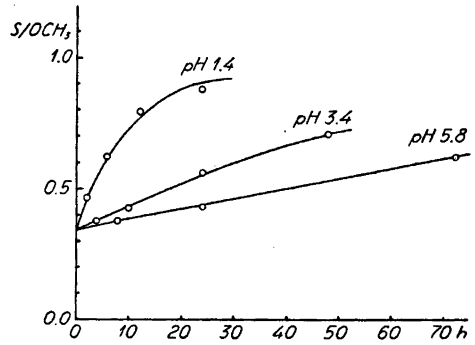


Fig. 1. Sulphonation of "low sulphonated lignin" at different pH-values.

slowly with time and this indicates that most of the reactive groups in "low sulphonated lignin" can be sulphonated in almost neutral media. In the wood, on the other hand, lignin cannot be sulphonated to more than 0.33 sulphur atom per methoxyl group at pH 6, even on prolonged heating [6]. On dissolution of the lignin sulphonic acids from the sulphonated wood, the unsulphonated groups (Group B [7]) are apparently converted into groups showing an increased reactivity towards sulphite, probably by hydrolytic fission of an acetal [8] or benzyl ether [9,10] linkage with formation of a reactive hydroxyl group [11] (Group B' [8]).

The yield of vanillin is completely independent of the degree of sulphonation, in agreement with previous model experiments [12]. The average yield of vanillin, 31.8 %, is somewhat higher than that obtained from spruce wood directly (for the wood employed here, 30.5 %). However, the latter yield is based on the methoxyl content of the wood, and probably almost 10 % of the methoxyl groups originate from non-lignin material [13]. When allowance is made for this, the vanillin yield from the wood becomes about 33.5 %, somewhat higher than the figure for "low sulphonated lignin". The latter represents only part of the total lignin in the wood, however, and it will be shown in a later communication that the β-lignin sulphonic acids contain fractions which give vanillin in yields of almost 40 %. It is therefore justifiable to say that sulphonation in itself does not change the yield of vanillin at any stage.

It is well known that when lignin sulphonic acids are heated with alkali, some vanillin is obtained, the amount generally being 4—7 % of the weight of the lignin [14–16]. We have now studied the effect of alkaline pretreatment of "low sulphonated lignin" on the yield of vanillin obtained on oxidation with nitrobenzene and alkali. The heating with alkali was carried out both in the presence and absence of sulphite (*cf.* experimental part) at a temperature of 135° for various lengths of time. The pH employed was 11.4 (measured at

Table 2. Alkaline treatment of "low sulphonated lignin" and subsequent nitrobenzene oxidation.

Time hours	Sulphite present	Yield %	Content of			2Ba/S	S/OCH_3	Yield of vanillin %	
			sulphur %	barium %	methoxyl %			During alkali treatment	On subsequent nitrobenzene oxidation
0	—	—	4.3	9.8	12.0	1.06	0.34	—	31.9
2	+	63	4.7	11.3	11.5	1.11	0.40	—	31.3
6	+	52	5.3	13.6	10.9	1.18	0.48	—	28.0
12	+	41	6.3	14.8	10.6	1.21	0.55	4.2	27.0
2	—	63	4.0	9.4	12.0	1.09	0.32	—	31.1
6	—	50	3.6	9.2	12.5	1.18	0.28	—	28.8
12	—	40	3.0	7.5	12.9	1.20	0.23	3.9	27.0

room temperature). The results obtained are listed in Table 2. (Analytical figures calculated as in Table 1.)

It is evident that the decrease in the yield of vanillin obtained after oxidation corresponds approximately to the amount of vanillin obtained on alkaline hydrolysis of the lignin sulphonic acids, and thus the total amount of vanillin obtained from the lignin remains constant.

However, serious changes do occur during the alkaline treatment. Thus, unlike untreated "low sulphonated lignin", the products obtained give barium salts which are soluble in ethanol and precipitate only on the addition of ether. The material is dark grey in colour, and gives solutions which are almost black. The barium/sulphur ratios increase with time of heating, indicating the formation of carboxyl groups. Further, the yields of products decrease with time of heating to rather low values, and, finally the ultra-violet spectra become progressively distorted with flattening of the maxima and increased absorption in the 300—350 mμ region. This is not the case when the sulphonation is carried out in neutral or acid media [17] (Fig. 2).

When lignin is heated with acids, particularly in the presence of reactive phenols, the reactivity towards sulphite is greatly reduced [7,18]. This effect is believed to involve the condensation of reactive groups, at least partly of benzyl alcoholic character. As a result of this condensation, the α-carbon atoms of some of the elements would become attached to two aromatic nuclei.

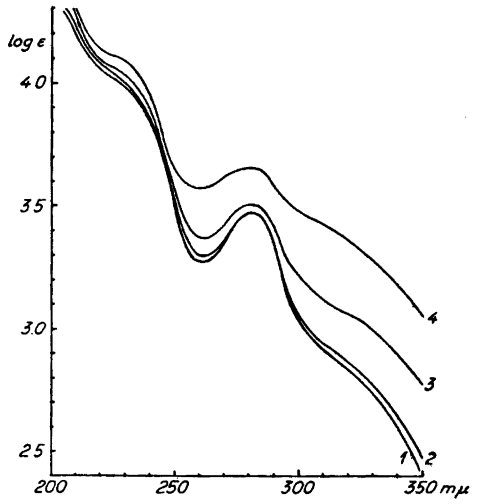


Fig. 2. Ultra-violet absorption curves of

(1) "low sulphonated lignin"
(2) "low sulphonated lignin" sulphonated at pH 5.8 for 72 hours
(3) "low sulphonated lignin" sulphonated at pH 11.4 for 6 hours
(4) "low sulphonated lignin" sulphonated at pH 11.4 for 12 hours

The ε-values are based on the equivalent weights, calculated from the methoxyl content.

This would make them incapable of yielding vanillin [19], and thus the yield of vanillin would be reduced. To study this effect, lignin sulphonic acids of different degrees of sulphonation (obtained by sulphonation of "low sulphonated lignin") were subjected to heating with sulphuric acid solutions of pH 1.4 and pH 0.5, respectively, in the presence of resorcinol (1 g/g of lignin

Table 3. Treatment of "low sulphonated lignin" with resorcinol in acid solution, and subsequent nitrobenzene oxidation.

Time hours	Yield %		S/OCH_3		Yield of vanillin %	
	pH 0.5	pH 1.4	pH 0.5	pH 1.4	pH 0.5	pH 1.4
0	—	—	0.34	0.34	31.9	31.9
2	60	65	—	0.35	16.5	22.0
6	60	62	—	—	12.7	17.5
12	62	57	0.35	0.34	13.5	17.1
0	—	—	0.49	0.49	32.3	32.3
2	69	66	—	—	—	23.9
6	68	60	—	—	19.5	24.6
12	62	64	0.45	0.51	18.6	24.1
0	—	—	—	0.62	—	31.6
6	—	70	—	—	—	27.0
12	—	68	—	0.60	—	26.3

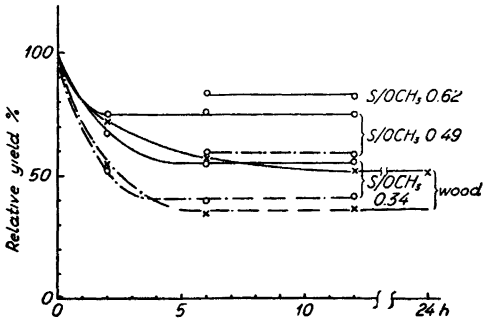


Fig. 3. Relative yields of vanillin from lignin sulphonic acids of different degrees of sulphonation and from wood, previously heated with resorcinol in sulphuric acid solutions of pH 0.5 (·—·—·) and pH 1.4 (————).

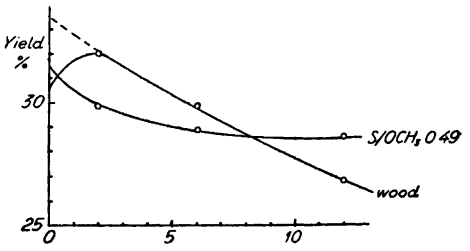


Fig. 4. Yields of vanillin from a lignin sulphonic acid and from wood, previously heated with sulphuric acid.

sulphonic acid), and subsequently oxidized with nitrobenzene and alkali. The temperature employed in the acid treatment was 135°. The results obtained are given in Table 3 and Fig. 3.

In Fig. 3, the yields of vanillin from the different preparations described in Table 3, (expressed as per cent of the yield obtained from untreated "low sulphonated lignin") are plotted against the time of heating with resorcinol and acid. For comparison, the corresponding yields from extracted spruce, heated under the same conditions, are given.

Fig. 4 shows the yields of vanillin for wood and for a lignin sulphonic acid (See Table 3, $S/OCH_3 = 0.49$), heated with a sulphuric acid solution of pH 1.4, without the addition of resorcinol.

Fig. 3 shows that the condensation responsible for the decrease in the yield of vanillin is a fairly rapid reaction, and that only some of the vanillin-yielding elements in the lignin sulphonic acids contain reactive groups available for condensation. The number of such available groups decreases with increasing degree of sulphonation, the reactive groups being blocked by sulphonic acid residues [7,18]. The experiments with wood show, however, that part of the vanillin-yielding elements of the lignin contain groups which are "blocked" even in the wood. Some of these groups (ca. 0.15 per methoxyl group) remain unaffected at pH 1.4 but react with resorcinol at pH 0.5. The same effect is exhibited by the lignin sulphonic acids, and in this case also the proportion of groups behaving in this way is ca. 0.15 per methoxyl group.

It is further seen that the introduction of sulphonic acid groups to form "low sulphonated lignin" (sulphonation of group A[7]) has very little influence

on the decrease in the yield of vanillin caused by the condensation of the lignin with resorcinol. Thus, the major part of group A probably belongs to elements which are not originally capable of yielding vanillin, *i. e.* "condensed elements"[12] or, possibly elements of the pinoresinol type[19]. On further introduction of sulphonic acid groups (sulphonation of group B), the effect on the vanillin yield is considerable, although decreasing in the later stages of the sulphonation. Thus group B probably belongs largely to elements of the open type[12], of which at least 25 % are rather stable towards acids and reactive phenols.

Fig. 4 shows that even in the case of treatment with acids in the absence of added phenol, sulphonation renders the lignin more stable toward condensing reagents, although the effect is less pronounced, because of the lower rate of condensation.

The maximum in the curve for acid-treated wood (in Fig. 4) may be caused by a rapid dissolution of non-lignin methoxyl-containing components present in the wood[13], thus causing an apparent increase in the yield of vanillin based on the methoxyl content. The dashed line indicates the yield of vanillin based on methoxyl, corrected for this "extra" methoxyl.

EXPERIMENTAL

The "low sulphonated lignin" was prepared essentially as described by Erdtman[4]. The further sulphonation of this material was carried out as follows:

A solution of "low sulphonated lignin" (barium salt) was passed through a column of Amberlite IR 120. The effluent was neutralized with sodium hydroxide, and sulphur dioxide corresponding to 6 % of the final amount of solution was absorbed. The solution was then divided into samples, each containing 4 g of the lignin sulphonic acid salt. Sodium hydroxide was added to the samples until the desired pH was reached, and the volumes were adjusted to 75 ml.

After heating in an autoclave of stainless steel at 135° for a predetermined length of time, each sample was cooled and freed from excess sulphur dioxide by a stream of carbon dioxide. The sodium ions were exchanged for hydrogen ions, and the solution was neutralized with barium carbonate, filtered, evaporated *in vacuo* to ca. 35 ml, and centrifuged. The clear solution was then poured, with vigorous stirring, into absolute ethanol (150 ml) containing, if necessary, ether (50 ml). The precipitate was separated by centrifugation and then washed twice with ethanol and three times with ether. The product was obtained as a fine powder of very light cream colour (except in the case of alkaline sulphonation).

The acid and alkaline treatments were carried out in essentially the same way. The acid solutions were prepared by adding dilute sulphuric acid to a solution, containing the sulphonic acid and 1.4 % of sodium hydroxide, until the desired pH was reached. Samples corresponding to 4 g of sulphonic acid were diluted to 75 ml, and resorcinol (4 g) added if desired. The alkaline solutions containing no sulphite were prepared in the same way as the corresponding sulphite solutions, but instead of sulphur dioxide, sulphuric acid was added until a pH of 11.4 was reached.

The products obtained on heating with resorcinol were of a red colour and rather soluble in ethanol, while those obtained on heating with acid were brownish and, in some cases, difficultly soluble in water.

In the experiments with wood, 5 g samples of extracted spruce wood flour (*Picea abies* (L.) Karst.) were heated with 40 ml each of the acid solution described above. The amount of resorcinol employed was 0.3 g/g of wood.

The undissolved residues were filtered off, washed thoroughly with water and acetone and dried at 105° for 4 hours.

The analyses were carried out on samples dried *in vacuo* over phosphorous pentoxide and paraffin at 70° for about one week. Sulphur was determined by a modified Grote-Krekeler [20] method and methoxyl by the Vieböck-Schwappach [21] method. The nitrobenzene oxidations and vanillin determinations were carried out as described previously [12, 22]. The UV-spectra were determined with a Beckman spectrophotometer, model DU.

SUMMARY

"Low sulphonated lignin" has been heated with sulphite solutions of various pH, and with alkali, for different lengths of time. It has also been heated in acid solution either alone or in the presence of resorcinol. The products obtained were analyzed and oxidized by means of nitrobenzene and alkali.

The following conclusions may be drawn from the results:

The major part of the reactive groups in "low sulphonated lignin" (Group B') may be sulphonated in almost neutral media, although at a low rate.

The yield of vanillin obtained on oxidation with nitrobenzene and alkali is not affected by sulphonation.

Alkaline treatment of "low sulphonated lignin" causes deep-seated changes in the structure, even in the presence of sulphite, but the total yield of vanillin seems to be relatively constant.

Sulphonation renders the lignin more stable toward condensing agents, sulphonic acid residues blocking reactive groups.

The major part of the sulphonated groups in "low sulphonated lignin" (Group A) probably belong to "condensed elements", or, possibly elements of the pinoresinol type, while the remainder of the groups (Group B) belong largely to elements of the open type, of which at least 25 % are rather stable toward acids and reactive phenols.

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Studies on Lignin

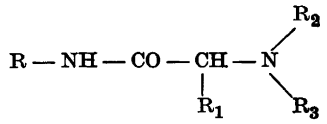
VII *. β-Lignin Sulphonic Acids

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While the preparation of so-called α-lignin sulphonic acids is comparatively simple, the isolation of the low molecular weight β-lignin sulphonic acids in a "pure" state offers considerable difficulties [1–3], since they are accompanied by carbohydrate material of acidic character which is very difficult to remove. Consequently, the β-fractions have not been so extensively studied as the α-fractions. However, the chemistry of the low molecular lignin fractions is a matter of great interest, and renewed attempts have now been made to find methods for the isolation of β-lignin sulphonic acids in the purest possible condition without excessive loss.

Erdtman [4–6] studied the step-wise precipitation of lignin sulphonic acids from waste liquors by means of various amines and found that compounds of the general structure



showed considerable promise and that 1-(N-piperidinoacetylamino)-naphthalene ("PAN", $C_{10}H_7—NH—CO—CH_2—NC_5H_{10}$) is a powerful precipitating agent. This compound and two new analogues derived from anthraquinone, 1-(N-piperidinoacetylamino)-anthraquinone ("PAA") and 1,5-bis(N-piperidinoacetylamino)-anthraquinone ("BPA"), together with S-1-naphthylmethylthiuronium chloride [7] ("NMT") have now been employed for precipitation experiments. For comparison, bis-(4-dimethylaminophenyl)-

* Part VI. *Acta Chem. Scand.* 6 (1952) 55.

methane ("BIS") was studied, since this reagent is generally considered to leave the β-lignin sulphonic acids in solution [5].

The experiments were carried out with "liquors" obtained by heating spruce wood flour with a sulphite solution of pH 5.3 at 135° for about 15 hours [8] (*cf.* experimental part). Under these conditions, ca. 30 % of the lignin in the wood dissolves and, since the lignin sulphonic acids are formed under very mild conditions, they provide excellent material for the study of the β-fraction.

Two different liquors, one freshly prepared (A) and one about a year old (B) were employed. Liquor B was also precipitated after concentration to about one third of its original volume. All the precipitating agents, except "NMT", were used in the form of their sulphates in aqueous solution.

The methoxyl content of the liquor after precipitation was determined, and thus the percentage of lignin sulphonic acids precipitated estimated roughly. The results obtained are listed in Table 1.

Table 1. Methoxyl precipitated with different reagents.

Reagent	Methoxyl precipitated %		
	A	B directly	B conc.
BIS	22	27	28
BPA	67	65	70
NMT	63	68	—
PAN	80	73	79
PAA	86	84	87

It is evident from the table that the age and concentration of the liquor has only a small influence on the values.

Only about 25 % of the lignin sulphonic acids are precipitated with BIS, the remainder being of rather low molecular weight.

PAA is clearly the most powerful precipitant of the reagents studied, and furthermore, as it is strongly coloured (yellow) it is easier to ensure that the final product is free from precipitating agent. Consequently, PAA was employed for the isolation of a large amount of lignin sulphonic acids from the liquor A. The product obtained was then divided into three fractions by consecutive precipitations with BIS (fraction a_1), BPA (fraction a_2), and PAA (fraction a_3). In a separate experiment, a lignin sulphonic acid preparation (fraction b) was isolated by means of NMT from liquor B, previously precipitated with BIS.

The sulphonic acids were converted into barium salts and these preparations were analyzed and oxidized with nitrobenzene and alkali.

The results are listed in Table 2, where the yields of vanillin are based on the methoxyl content of the lignin sulphonic acids, while the yields of products are calculated as methoxyl recovered as a percentage of the total methoxyl in the liquor. For comparison, the yields of the different fractions, calculated from Table 1, are given.

Table 2. Composition of lignin sulphonic acid fractions.

Fraction	Yield %		Analyses							2Ba/S	S/OCH_3	Yield of vanillin %
	Found	Calc.	S %	Ba %	OCH_3 %	C %	H %	Cu_I*	Cu_{II}**			
a_1	15	22	5.8	13.9	11.2	47.1	5.0	7.1	34.1	1.13	0.50	33.0
a_2	31	45	5.8	12.3	11.6	49.0	5.3	2.9	10.6	0.99	0.48	36.0
a_3	14	19	7.2	15.4	10.4	43.9	4.9	0	7.1	1.00	0.66	38.3
b	26	41	6.1	14.0	10.3	—	—	2.2	5.0	1.07	0.57	36.8

* Copper number prior to hydrolysis.
** Copper number after hydrolysis.

The amount of methoxyl recovered is quite low compared with the figures in Table 1, due to losses in different stages of the preparation. The "purity" of the fractions a_2 and a_3, which may be considered as β-lignin sulphonic acids, is very high, as judged by their low reducing power and their barium/sulphur ratio.

From the analyses in Table 2, the following empirical formulae are derived.

a_1: $C_9H_{9.91}O_{3.54}(OCH_3)_{0.91}S_{0.46}Ba_{0.26}$
a_2: $C_9H_{9.93}O_{3.30}(OCH_3)_{0.91}S_{0.44}Ba_{0.21}$
a_3: $C_9H_{10.41}O_{3.96}(OCH_3)_{0.91}S_{0.60}Ba_{0.30}$

The following formulae are obtained on a sulphur- and barium-free basis, by substituting $SO_3\frac{Ba}{2}$ for OH:

a_1: $C_9H_{10.45}O_{2.62}(OCH_3)_{0.91}$
a_2: $C_9H_{10.37}O_{2.42}(OCH_3)_{0.91}$
a_3: $C_9H_{11.01}O_{2.76}(OCH_3)_{0.91}$

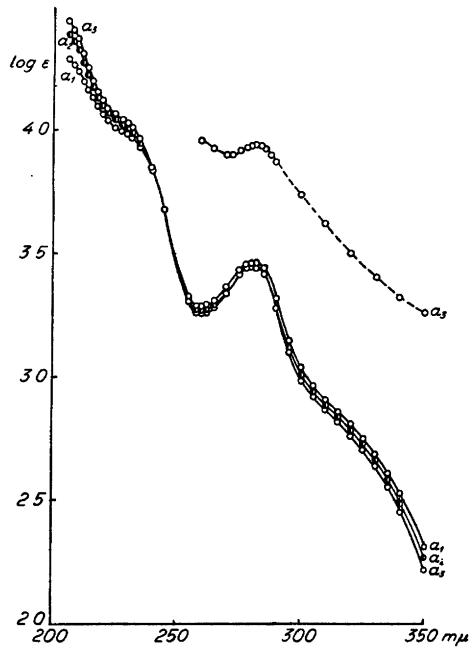


Fig. 1. Ultra-violet absorption spectra of fractions a_1, a_2, and a_3 in water (————) and of fraction a_3 in 0.1 N NaOH (— — — —). The ordinates of the latter curve have been increased by 0.5. The ε-values are based on the equivalent weight, calculated from the methoxyl content.

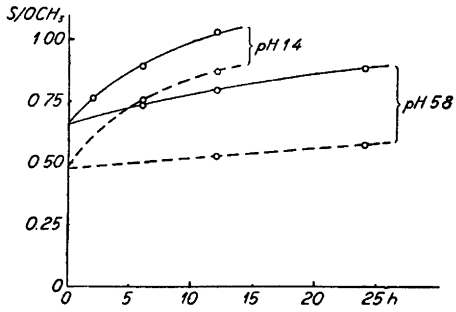


Fig. 2. Sulphonation of fractions a_2 (— — — —) and a_3 (————) at pH 1.4 and 5.8.

When these formulae are compared to those given by Erdtman, Lindgren, and Pettersson [9] for "low sulphonated lignin", the only significant difference is the appreciably higher hydrogen content of the β-lignin sulphonic acids. As the oxygen content is "normal", this cannot imply that the two types of material differ only by the addition or removal of water. The methoxyl content is only slightly lower than the average of the recorded values for α-lignin sulphonic acids and the very low methoxyl values found previously for β-fractions [1] must be ascribed to contamination with carbohydrate material.

The ultra-violet absorption spectra of fractions a_1, a_2, and a_3 are shown in Fig. 1.

No appreciable differences seem to exist between the different fractions, and the curves are very similar to those of "low sulphonated lignin".

The most interesting figures in Table 2 relate to the yields of vanillin, which increase with decreasing molecular weight to values almost 20 % higher than the average value for spruce lignin. Thus, low molecular lignin probably contains fewer "condensed elements" [10], *i.e.* elements which are connected by carbon-carbon linkages.

Examination of the aldehydic oxidation products, by means of paper chromatography, as described in Part III [11], revealed that, in addition to vanillin, *p*-hydroxybenzaldehyde, syringaldehyde and 5-formylvanillin were present in approximately the same amounts as are obtained by direct oxidation of the wood. This also applies to "low sulphonated lignin" (*cf.* Part VI). In this connection it may be mentioned that the aldehydic mixture obtained on "anaerobic" alkaline hydrolysis of lignin sulphonic acids * contained *p*-hydroxybenzaldehyde in addition to vanillin, but no trace of syringaldehyde or 5-formylvanillin.

To estimate the content of phenolic hydroxyl groups in the β-lignin sulphonic acids, the ultra-violet absorption spectrum of fraction a_3 in 0.1 *N* sodium hydroxide solution was determined. (Dashed line in Fig. 1.) The displacement of the 280 mμ maximum is very small (ca. 2 mμ), indicating a

Table 3. Sulphonation of β-lignin sulphonic acids.

Fraction	pH	Time hours	Yield %	Analyses					2Ba/S	Yield of vanillin %
				S %	Ba %	OCH_3 %	Cu_I	Cu_{II}		
a_2	1.4	6	68	7.5	16.3	9.4	—	—	1.01	31.3
»	1.4	12	65	7.8	17.6	8.7	0	1.0	1.05	30.1
»	5.8	12	72	6.3	13.4	11.5	—	—	0.99	—
»	5.8	24	68	6.8	13.9	11.4	0	2.8	0.96	36.4
a_3	1.4	2	65	7.5	16.8	9.6	—	—	1.04	—
»	1.4	6	66	7.9	18.1	8.7	—	—	1.07	34.2
»	1.4	12	70	9.1	20.5	8.5	0	0	1.04	33.5
»	5.8	6	71	7.4	16.7	9.8	—	—	1.05	—
»	5.8	12	64	7.7	17.3	9.4	—	—	1.04	37.6
»	5.8	24	67	8.0	17.8	8.7	0	3.9	1.04	38.5

* Kindly supplied by Dr. E. Adler.

phenolic hydroxyl content of the same order of magnitude as in "low sulphonated lignin"[12].

In Table 3 the results of sulphonating fractions a_2 and a_3 at pH 1.4 and 5.8 are shown, the yields of products and of vanillin being based on the amount of methoxyl recovered. The sulphite solutions (75 ml for 4 g of material) all contained 6 % of total sulphur dioxide and sufficient sodium hydroxide to give the desired pH, and the reaction temperature used was 135°.

Fig. 2 shows the S/OCH_3 ratio as function of the time of heating for different pH-values.

The results show that fractions a_2 and a_3 are sulphonated at about the same rate as "low sulphonated lignin" at pH 1.4 (*cf.* Part VI), but fraction a_3 (the fraction of lowest molecular weight) may be sulphonated to S/OCH_3 ratios exceeding unity. Further, at pH 5.8, fraction a_3 is sulphonated much faster than "low sulphonated lignin" or fraction a_2, which sulphonate at exactly the same rate. Thus fraction a_3 differs from the bulk of the lignin, not only in the content of groups reactive towards sulphite, but also in the character of those groups.

When β-lignin sulphonic acids are heated with a sulphite solution of pH 1.4, the yield of vanillin subsequently obtained decreases rather rapidly initially and appears to reach a constant value. This phenomenon is not exhibited by "low sulphonated lignin", does not take place if the sulphite solution has pH 5.8, and appears to involve 15—20% of the vanillin-yielding elements initially present. This percentage agrees well with the amount of "extra" vanillin obtained from the β-sulphonic acids and it seems reasonable to assume that acid-sensitive groups are the ones responsible for this "extra" vanillin. This may be thought to imply that the bulk of the lignin contains such acid-sensitive groups, but that these are destroyed in the preparation of "low sulphonated lignin". This is, however, unlikely as the only acid treatment in the preparation of the latter involves heating at pH about 2 at a temperature of 80—90° for three hours; that is to say much milder acid treatment than was employed in the sulphonation of the β-lignin sulphonic acids. Furthermore, the yield of vanillin obtained directly from the lignin in the wood only exceeds that obtained from "low sulphonated lignin" by a small amount, just enough to cover the "extra" amount formed from the lowest molecular weight portion (*cf.* Part VI).

EXPERIMENTAL

1-(N-Piperidinoacetylamino)-naphthalene. 1-Chloroacetylamino-naphthalene[13] (35 g) was suspended in dry toluene (300 ml) and piperidine (36 ml) added portionwise. The temperature soon rose, all the material dissolved, and when almost all the piperidine had been added, piperidine hydrochloride commenced to separate. When the reaction

was over, the mixture was cooled and filtered, and the solid material washed with toluene. The filtrate and washings were extracted with dilute sulphuric acid, and the acid solution was made alkaline with sodium hydroxide. The colourless material (36.8 g, 86%) which precipitated was collected by filtration, washed and dried and after two recrystallizations from ethanol formed colourless plates, m.p. 116—116.5°.

$C_{17}H_{20}ON_2$	Calc.	N	10.4	Equivalent weight		268.4
	Found	»	10.5	»	»	267.2

1-(N-Piperidinoacetylamino)-antraquinone. A mixture of 1-chloroacetylaminoantraquinone[14] (30 g), piperidine (50 ml) and dry xylene (500 ml) was refluxed for one hour, then cooled and extracted with dilute sulphuric acid. The dark red acid solution was made alkaline with sodium hydroxide, and the yellowish-green precipitate formed (34.5 g, 99%) was collected by filtration, washed with water, dried and repeatedly recrystallized from dioxan, giving yellow needles, m.p. 240—242° (decomp.).

$C_{21}H_{20}O_3N_2$	Calc.	N	8.0	Equivalent weight		348.4
	Found	»	7.7	»	»	350.4

1,5-Bis-(chloroacetylamino)-antraquinone. A mixture of 1,5-diaminoantraquinone[15] (40 g) and chloroacetyl chloride (52 g) in nitrobenzene (400 ml) was heated at 130° until hydrogen chloride ceased to be evolved (ca. 15 minutes). The dark green material (53 g, 81 %) which separated from the solution on cooling was collected by filtration and washed with nitrobenzene and ether and dried. Recrystallization from nitrobenzene gave dark green plates which decomposed at 315°.

$C_{18}H_{12}O_4N_2Cl_2$	Calc.	Cl	18.2	N	7.2
	Found	»	17.8	»	7.2

1,5-Bis-(N-piperidinoacetylamino)-antraquinone. A mixture of the above material (30 g), piperidine (75 ml) and dry xylene (500 ml) was refluxed for one hour, then cooled and extracted with dilute sulphuric acid. The brownish-red acid solution was made alkaline with sodium hydroxide and the red precipitate (36.5 g, 97 %) collected by filtration, washed with water and dried. Repeated recrystallization from dioxan yielded orange needles, m.p. 261—262° (decomp.).

$C_{28}H_{32}O_4N_4$	Calc.	N	11.5	Equivalent weight		244.3
	Found	»	11.0	»	»	241.9

Precipitation experiments

Two different liquors (A and B) were employed. Liquor A was freshly prepared from extracted spruce wood flour (*Picea abies* (L) Karst.) (corresponding to 1.68 kg dry weight) and a solution of pH 5.3 (8.4 l) containing crystallized sodium sulphite (335 g) and sodium pyrosulphite (845 g). The mixture was heated in an electrically-heated rotating autoclave to 130° in the course of 4 hours, then to 135° in a further 2 hours and maintained at that temperature for 14 hours. The autoclave was kept rotating until it was cool, then the contents were filtered. The solid was washed with lukewarm water and the washings

combined with the filtrate which was brownish yellow in colour and had pH 5.25. The total volume was 11.5 litres, the content of solids was 130 g/l and the methoxyl content of the solids 1.87 %.

Liquor B had been prepared about one year previously, in essentially the same way as A. The content of solids was 133 g/l and the methoxyl content of the solids 1.94 %.

The precipitating power of the different reagents was determined in the following way:

To the liquor to be precipitated (100 ml) was added an excess of the reagent dissolved in the equivalent amount of dilute sulphuric acid (in the case of naphthylmethylthiuronium chloride (NMT) dissolved in hot water). The mixture was stirred vigorously and the volume adjusted to 150 ml. The pH after precipitation varied between 4.5 and 5.3. The mixture was set aside overnight, then the precipitate was collected by centrifugation and washed thoroughly with water. The mother liquor and washings were combined, the volume adjusted to 250 ml, and aliquots taken for the determination of the methoxyl and solid contents.

In the methoxyl determinations [16], an extra absorption vessel containing cadmium sulphate solution [17] (to remove hydrogen sulphide) was used in order to avoid the difficulties encountered by Abrahamsson, Lindgren and Hägglund [3]. It was later found, however, that no extra precautions were necessary if the amount of bromine in the absorption vessel was large compared with the amount of sulphate and sulphite in the sample, at least if the gas was washed with bicarbonate solution before absorption in the bromine reagent.

After determining the minimum amount of the reagents BIS, PAA and BPA necessary for complete precipitation of liquor A, a larger amount of lignin sulphonic acid was isolated.

PAA (125 g), dissolved in the equivalent amount of dilute sulphuric acid was added to liquor A (5.6 l). The mixture was set aside overnight, then the supernatant liquid was decanted and the precipitate was squeezed to remove adhering solution as completely as possible. The solid was then converted portionwise to sodium salt by treatment with 1 % sodium hydroxide in a mortar, care being taken to avoid excess of alkali and to keep pH as close to 7 as possible. The mixture was centrifugated and the precipitate washed with water.

The combined solutions (1 l), containing the sodium salts of the precipitated lignin sulphonic acids, were then fractionated as follows:

BIS (35 g), dissolved in sulphuric acid, was added. The precipitate obtained was separated and washed and the sulphonic acids recovered as the sodium salts as described above, then converted to the barium salts (fraction a_1) by means of an ion-exchanger as described in Part VI. The original mother liquor was made slightly alkaline (pH 10) and the excess of BIS was centrifugated off. The solution was neutralized and a solution of BPA (55 g) in dilute sulphuric acid was added. The precipitate was converted into the barium salt as described above (fraction a_2) and the original solution was treated with PAA (45 g), dissolved in sulphuric acid. Conversion of the precipitate into the barium salt gave fraction a_3.

In a similar way liquor B was first precipitated with BIS and then with NMT. The product obtained from the latter was purified by reprecipitation with NMT (fraction b).

Fractions a_2 and a_3 were further sulphonated by the method described in Part VI.

The oxidations with nitrobenzene and alkali and the vanillin determinations were carried out as described previously [10, 11].

SUMMARY

A method for the isolation of so-called β-lignin sulphonic acids in a "pure" state by precipitation with basic reagents has been devised.

The products, which were isolated from a solution obtained by heating wood with a sulphite solution of pH 5.3, had a higher sulphur content than "low sulphonated lignin". The yields of vanillin from the β-lignin sulphonic acids were higher than for the average lignin, and increased with decreasing molecular weight.

Further sulphonation of the β-acids showed, that at low pH-values (1.4) the sulphonation proceeds at about the same rate as that of "low sulphonated lignin", but that the fraction of lowest molecular weight contains more groups reactive towards sulphite than the average lignin. At higher pH-values (5.8) the rate of sulphonation is much higher in the case of the fraction of lowest molecular weight than of the remainder of the lignin.

The β-lignin sulphonic acids seem to contain about the same amount of phenolic hydroxyl groups as the remainder of the lignin.

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Problems of an International Chemical Nomenclature

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Interest in chemical nomenclature has been increasing in recent years. The marked activity on all fronts of chemistry has increased the demand for effective communication, for clearness and precision in information, and has thus stimulated interest in chemical nomenclature problems. International co-operation on chemical problems has at the same time emphasized the need for more conformity in the chemical nomenclature of different languages.

The Geneva conference of 1892 initiated chemical nomenclature work on an international scale, and this was later continued under the auspices of the International Union of Chemistry, but was unfortunately interrupted and severely hampered by two world wars and their depressing influence on international co-operation and confidence. Nevertheless the International Union of Chemistry succeeded in accomplishing a nomenclature work which has already exercised a far-reaching influence on chemical literature.

On several points, however, the committees of the International Union of Chemistry have refrained from attempts to create a truly international chemical nomenclature, leaving it to national committees to modify the adopted names in conformity with "the genius of each language". By doing this problems of a purely linguistic nature have been highly overestimated. Actually there is, from a modern philological point of view, no fundamental reason for not introducing a much more radical unification of chemical terms, at least among the Indo-European languages. Among these languages there is a unanimity of major pattern which in principle makes it possible to formulate an idea in much the same way. There is no real reason that the French should not say ferrochloride instead of chlorure ferreux, the Italian ferro-clorido instead of cloruro ferroso, the Czech ferro chlorid instead of chlorid železnatý, and so on. Only habit prevents the use of essentially the same name in all these languages. Similarly no rule of language forbids the Italians to write *e.g.* helio and oxido instead of elio and ossido, the Spanish to write scandio and

yttrio instead of escandio and itrio, the Germans to write Citronensäure instead of Zitronensäure. There is no more reason for some languages to continue to use names such as benzol, kolesterin, dioxyacetone *etc.*, than there was to use irrational names such as carbolic acid and spirit of wine.

In the following, some of the most stubborn obstacles for obtaining an international chemical nomenclature are considered.

I. THE ABANDONMENT OF NATIONAL NAMES

It is now commonly agreed that a scientific nomenclature should be as international as possible. Formerly, however, it was thought to be a point of national self-assertion to introduce national terms. In some languages (Finnish, Icelandic, Czech etc.) almost all foreign words have been replaced by purely national words, even such words as the names of the months, electricity, cigar, etc., which otherwise pass almost unchanged from language to language. In many languages the names hydrogen, nitrogen, oxygen and carbon have been translated (German: Wasserstoff etc.) or replaced by new-created national words, *e.g.* Danish brint (hydrogen), ilt (oxygen) and Polish tlen (oxygen). The Slavic languages also have national names for silicon, aluminium, magnesium and calcium. This again gives national names to the inorganic compounds. From Danish "ilt" (oxygen) is derived "ilte" = oxide; we thus obtain names as "brintoverilte", "kvælstofilte" and so on for compounds for which just as well the names hydrogenperoxid and nitrogenoxid could be used, as has now actually been proposed by the Danish nomenclature committee. Czech analogously has a special name for oxide, "kysličnik", and thus names as for instance kysličnik vápenatý = calcium oxide and kysličnik uhličitý = carbon dioxide are obtained.

By unification of the names of the chemical elements as mentioned under II it would in principle be possible to do away with all these inconsistencies.

In older times most of the chemical terms were taken from words of daily life and thus differed from language to language: "slaked lime", "sugar of lead", "butter of antimony", "heavy spar", "massicot", and so on. By the introduction of rational terms the old names have almost disappeared from scientific literature. In several languages, however, names of some organic compounds, particularly organic acids, still are reminiscent of the names of early chemistry. For succinic acid for instance, names such as Bernsteinsäure (German), ravsyre (Danish), yantarnaya kislota (Russian) are used. In English and French latinized names of these acids are used, and these could as well be applied in other languages, *e.g.* Succinsäure instead of Bernsteinsäure. Even if it is difficult now to change these old names, new names should at least not be

formed in the old-fashioned way. When the name folic acid had to be used in other languages some authors translated it into "Blättersäure" (German) and "bladsyre" (Danish, Norwegian). Pretty rapidly, however, the names Folinsäure and folinsyre gained ground. *It would be very important if the International Union of Chemistry would state as a principle that new chemical names should always be formed from Greek or Latin roots. When introducing new names due consideration as to their applicability in other languages should be taken.*

These rules should also apply to chemical terms that are not names of compounds. Terms as "lone pair", "shared electrons", „core", "Zwitterion", "Eigenfunktion", "Bindigkeit", "Ionen-Beziehung", "Oktett-Lücke" are particularly difficult to translate and often are taken over as such in other languages, a practice which can only be considered as make-shift. By the way "Zwitterion" is rendered in Danish as "ampho ion", a word of real international form which ought to be introduced in other languages.

For most of the organic functional groups, terms of international character are used. This, however, is not the case with the name acid, for which very different names are used (Säure, syre, happo, kwas, kislota, kyselina etc.). It would be preferable to introduce a term of Latin origin, *e.g.* acid (as has actually been done in Turkish asid), but the difficulty is that in several languages it will not be possible to distinguish in pronunciation between acid and azid.

Words of a national character should not be connected with international names. In Polish for instance the national numerals are used instead of mono, di, tri, etc. resulting in names as dwunitrotoluen, czterohydropirano for dinitrotoluene and tetrahydropyran. The inconvenience of this is obvious, but no less inconvenient is the use of national names as iron, lead, Wasserstoff, Bernstein in connection with international terms as oxid(e), sulfat(e), amid(e) etc.

II. THE NAMES OF THE ELEMENTS

One of the most needed improvements in inorganic chemical nomenclature is the unification of the names of the chemical elements. If essentially the same names of the elements could be introduced in different languages, the names of inorganic compounds in these languages would automatically become very similar.

The problem of the unification of the names of the elements has been considered in detail elsewhere *. Here only the main points will be mentioned:

* In a memorandum presented to the committee on inorganic chemical nomenclature of The International Union of Chemistry. Copies of this paper may be obtained from the author.

The differences in the names of the elements in different languages are of the following types:

1. The elements known from antiquity (gold, silver, copper, iron, mercury, lead, tin and sulfur) have quite different names in different languages.

2. For some of the more recently discovered elements (H, C, N and O) several languages employ translations of the original names of international character.

3. In some cases two names of international form have been introduced for the same element:

beryllium and glucinium	lutecium and cassiopeium
sodium and natrium	hafnium and celtium
potassium and kalium	antimon and stibium
columbium and niobium	tungsten and wolfram

4. In several languages the spelling of the names of the elements has been changed by applying a more or less phonetical spelling. As the pronunciation of the names of the elements differs from language to language and the phonetical value of the letters is not the same in different languages, these changes have made the names look rather different not only from the latinized names but also from each other:

k replaces c: kalcium, kadmium, skandium, aktinium, kobalt (Scandinavian, German, Slavic languages).

k replaces ch: klor, krom (Scandinavian, Croatian, Hungarian, Turkish).

z replaces c or vice versa: Kalzium, Zäsium (German), cynk, cyrkon (Polish).

s replaces c or z: kalsium, sink (Norwegian, Finnish), seziyom (often in Turkish instead of cesium).

h is eliminated: litium, renium, tallium, torium, tulium, rodium, rutenium, lantan (several languages); and Italian elio (He) and afnio (Hf).

i replaces y or vice versa: berillio, kripto, itrio, iterbio (Italian and Spanish), tytan, iryd, cyrkon, cynk (Polish).

the ending -ium is altered into -io (Italian, Spanish), -iu (Roumanian) or -ij (Russian) or completely omitted: Cer, Niob, Zirkon (German), nioob (Dutch), lit, skand, gal, osm, ren, rad (Polish).

To obtain practically the same names in different languages the following changes are necessary:

ad 1. The Latin names of the elements of the first category (aurum, argentum, cuprum, ferrum, hydrargyrum, plumbum, stannum and sulfur) should be introduced as scientific names into the literature of all countries. These should not necessarily replace the national names in common usage. The use

of Latin names may be of importance in distinguishing between for example the element ferrum and common iron, but especially it will be an advantage to use these names when forming names of compounds (see below).

In English the Latin names sulfur and mercury have practically superseded the names brimstone and quicksilver even in common usage. Unfortunately, however, the name mercury is at variance with the chemical symbol.

ad 2. The names of this group are artificial names, that have not become part of every day language to the same extent as the names of the first group. It should without doubt be possible to replace the national names Wasserstoff etc. by the names hydrogen, oxygen, nitrogen and carbon.

ad 3. The problems concerning the names of this group are in some cases mingled with a conflict of priority and therefore involve national prestige. In many cases, however, the question of priority cannot be settled unambiguously. Further a chemical name should not be accepted on grounds of priority alone: its adequacy, applicability and present usage in different languages should also be considered.

At the Amsterdam meeting of the IUC (September 1949) it was decided to recommend the names beryllium, niobium, lutetium, hafnium and wolfram. The cases where there are two different names would thus be reduced to three Unfortunately opposition to the name wolfram, especially from the English side. caused the committee to leave this name optional again.

The reasons for choosing the above mentioned names are the following:

Beryllium: The name glucinium has been little used outside France and is not particularly characteristic for this element. The name beryllium was proposed by Wöhler, who first prepared metallic beryllium. The priority of Vauquelin for being the first to prove a new "earth" in beryl is not contested by this decision.

Niobium: The name columbium has only been used by American and some British authors. The name niobium, proposed by H. Rose who was the first to give definite proof of the existence of this element, is used in all other languages. On grounds of priority there is no reason for preferring the name columbium (*cf.* A. Larsson [1]).

Lutetium: The name cassiopeium is only used in German and (in part) Dutch. Both for this reason and because the name is difficult to adapt to some languages the name lutetium is preferred. It is not intended to settle the question of priority (*cf.* Paneth [2]) by this decision. The hitherto used spelling lutecium should be replaced by lutetium, because the name should be derived from the Latin form Lutetia rather than from its French equivalent Lutèce.

Hafnium: The name hafnium is used in all other languages than French. It is also to be preferred on grounds of priority.

Wolfram: Wolfram is the name proposed by the discoverers of this element, the brothers de Elhuyar, it is in accordance with the chemical symbol and is used in 12—13 languages out of 17 considered. On ground of his historical studies Moles [3] has strongly advocated the general introduction of the name wolfram, and even in England this name has been

recommended (Hadfield[4]). Further tungsten in Scandinavian languages means "heavy stone" and could not well be applied to an element; it is the original and still used name for the wolfram mineral scheelite, $CaWO_4$. Thus the arguments for abandoning the name tungsten for the element are certainly very strong.

In the remaining cases where there are two names for the same element consideration of the accepted symbols favours the names natrium, kalium and stibium instead of sodium, potassium and antimony.

ad. 4. The spelling should be regulated on the lines mentioned under VI. In the main languages only minor changes in spelling are necessary:

The spelling baryum is only used in French and Czech, where it should be changed to barium. In English sulfur should not be spelled with *ph*. In American aluminum should be replaced by aluminium.

In recent German there has been a tendency to use *k* and *z* instead of *c* (as for instance Kalzium, Zäsium, Kadmium). After the war, however, the Latin forms are gaining ground again. Vanadin should be changed into Vanadium, Cer into Cerium, Niob into Niobium and Wismut into Bismuth.

In other languages rather more extensive changes in the spelling are necessary to arrive at true international names (*cf.* VI).

The introduction of the names mentioned under 1 and 2 would bring us a major step forwards toward an international chemical nomenclature. Thus sulfurtrioxid(e) would replace Schwefeltrioxyd, zwaveltrioxyd, svovltrioxyd, kysličnik sirový, trekh-okis sery. Hydrogenperoxid(e) would replace Wasserstoffperoxyd, brintoverilte, vätesuperoxid, perekis vodoroda. Nitrogenoxid(e) would replace Stickstoffoxyd, kväveoxid, oxyde d'azote, kvælstofilte, kysličnik dusnatý, and so on. Other examples are to be found under III.

III. THE STOCK NOMENCLATURE

According to the IUC rules for naming inorganic compounds »indication of the electrochemical valency in the names of compounds should be made only by Stock's method» and »the system of valency indication by terminations such as -ous, -ic .... should now be avoided not only in scientific but also in technical writing».

This report was published in 1940, when work in the International Union was suspended because of the war, and could not therefore be thoroughly discussed and criticized before an international forum. In the following years the Stock system became rather widespread, especially in Germany and the United States.

From an international point of view, however, the Stock nomenclature suffers from the serious drawback of being much more national in character than the older nomenclature. This may be sufficiently substantiated by giving the names of $FeSO_4$ in different languages according to the older nomenclature and the Stock system:

	Older name	Stock name
Danish	Ferrosulfat	Jern-to-sulfat
Dutch	Ferrosulfaat	Ijzer-twee-sulfaat
English	Ferrous sulfate	Iron-two-sulfate
Finnish	Ferrosulfaatti	Rauta-kaksi-sulfaatti
French	Sulfate ferreux	Sulfate de fer-deux
German	Ferrosulfat	Eisen-zwei-sulfat
Hungarian	Ferroszulfát	Vas-kettö-szulfát
Italian	Solfato ferroso	Solfato di ferro-due
Spanish	Sulfato ferroso	Sulfato de hierro-dos
Swedish	Ferrosulfat	Järn-två-sulfat
Turkish	Ferrosulfat	Demir-ikki-sulfat

Accordingly the failure of the Stock system is that it uses the national names of the elements and, in spoken language, the national numerals. In written language the numerals will be designated by Roman figures, but even then the Stock nomenclature is much more national than the one hitherto used. The joint Scandinavian (Danish, Finnish, Norwegian and Swedish) nomenclature committee writes on this matter to the International Union of Chemistry: "We find this objection so significant that we question the advisability of recommending the Stock nomenclature without radical alteration of it. At any rate we find it objectionable that the international rules mention Stock's system as the only one allowed for indicating the electrochemical valency."

In the written language the designation by Stock's method can be made international by the use of the Latin names of the elements, *e. g.*: ferrum(II)-sulfate, cuprum(I)-chloride, stannum(IV)-chloride, aurum(III)-chloride. Contrary to the English version of the international rules it is considered advisable to use a hyphen after the parenthesis.

Fernelius [5] says that there is a definite tendency to shift to English names throughout; for example to use silver(I) ion instead of argentous ion and iron(II) ion instead of ferrous ion. This is certainly a step backward. According to Fernelius: "the percentage of such names is so small that any benefit a foreign reader may be expected to gain is negligible". In a chemical text, however, the chemical terms are so important that often little more than the

understanding of the chemical terms is necessary to get the meaning of a chemical text in a foreign language. Furthermore it is an advantage to the chemists of the small countries, who are compelled to *write* in a main language that the chemical terms look similar in different languages.

The proposal to use the Latin names of the elements seems to be the only satisfactory solution of this intricate matter. It is true that it does not do away with the difficulty that the national numerals have to be used, but as chemical knowledge is mainly communicated in written language this objection is of minor importance.

The names ferrum(II)-chloride etc. resemble very much the hitherto used names, and indeed the endings *-ous* and *-ic* may be used when they leave no uncertainty as to the valence. The circumstance that the ending -ous sometimes refers to a univalent, sometimes to a bivalent and sometimes to a tervalent state is no serious objection. But often it is necessary to indicate more than two valencies, and there the ous-ic system fails. As is well known Alfred Werner proposed an alteration and extension of the *o-i*-system, using the endings *a-o-i-e-an-on-in-en*, thereby being able to indicate 8 different valencies. This ingenious system failed because the vowels in some languages, particularly English, are not pronounced distinctly enough. In Czechoslovakia a system is in use in which the name of the metal is used in adjective form with different endings, indicating different valencies. Thus the oxides MnO, Mn_2O_3, MnO_2, MnO_3 and Mn_2O_7 in Czech are called kysličnik manganatý, manganitý, manganičitý, manganový and manganistý. This nomenclature represents a purely national solution of the problem and indeed is an example of the way in which a scientific nomenclature should not be developed.

Thus it seems to be most satisfactory to use the Latin names of the elements with the Stock numbering system.

IV. THE NAMES OF SALTS

In the Stock nomenclature the acid residue is indicated in the same way as in the *o-i*-system. The terminations *-at*(-e, -o) and *-it* (*-e*, *-o*) are now universally adopted and the names formed in this way have almost completely superseded the names of the types "schwefelsaures Magnesium" or "schwefelsaures Magnesia", formerly used in German, Russian and Scandinavian languages. The termination *-id*(*-e*, *-o*), however, is not universally adopted. The Romance languages (and Turkish) use instead the termination *-ur*(*-e*, *-o*). This was formerly also used in English and German, where it, however, was used to designate a lower oxidation state (Eisenchlorür=$FeCl_2$; Eisenchlorid=$FeCl_3$). When by the introduction of the o-i-system the valency state was

indicated by terminations of the name of the metal, one of the terminations -ur and -id became superfluous, and unfortunately in most languages the termination *-id(-e, -o)* was chosen. Actually it is difficult to distinguish in pronunciation between *-id(-e, -o)* and *it(-e, -o)*. When the d is not followed by a vowel it is generally pronounced as t and recourse has therefore to be taken to modify the length of the *i* to distinguish between -id and -it. In some languages, however, a certain vowel can have only one length, and in these languages it is absolutely impossible to distinguish between chlorid and chlorit, sulfid and sulfit etc. Even when the d is followed by a vowel (as in English) the difference between *-ide(-o)* and *-ite(-o)* is not very distinct.

Therefore the re-introduction of the termination *-ur(-e, -o)* would offer certain advantages. Unfortunately, however, the termination *-id(-e, -o)* is much more widespread and even in the Romance languages the name oxyde(-o), not oxure(-o), is used.

Perhaps the best solution of this problem would be to choose a new termination. By analogy with the name oxyd(e), which is used in French, German and some other languages one might write chloryde, sulfyde etc. In the languages where the above mentioned difficulty of distinguishing arises, the termination *-yd* could be pronounced, and perhaps written (although this would violate the principles set forth under VI) as *-üd*.

It would of course be an advantage if all compounds of this type were designated in an uniform manner in all languages. But the difficulty in attaining this is unusually great, since the changes will be great, whatever decision be taken:

a. If the termination *id(-e, -o)* is retained the termination *ur(-e, -o)* in Romance languages should be changed and oxyd(e) should be changed into oxid(e). The problem of distinguishing clearly between *-id* and *-it* is not solved in this manner.

b. The introduction of the termination *-ur(-e, -o)* means a change in all other languages than the Romance ones, and also for these oxur*(-e, -o)* is a a new name.

c. The introduction of the termination *-yd(-e, -o)* means a change in all languages, but this termination is not very different from *-id(-e, -o)* and may be pronounced as this. In the languages where a different pronunciation is desirable it may be pronounced as -üd.

The names indicating the anion can be combined with the name of the metal in two ways, illustrated by magnesium sulfate and sulfate of magnesium. In French exclusively names of the latter type are used (sulfate de magnesium). The names of the first type are most convenient when the acid has a short name, and they could well be employed in French also. When, however, the acid has a very long name the distance between the name of the metal and the ending -ate becomes unduly long, and in such cases it seems more con-

venient to use names of the French type, *e.g.* 3,5-dinitro-4-dimethylaminobenzoate of sodium(in German Natriumsalz der 3,5-Dinitro-4-dimethylaminobenzoesäure or 3,5-dinitro-4-dimethylaminobenzoesaures Natrium).

The same applies to esters where names of the type benzoic ethylester (in German: Benzoesäureäthylester) are to be preferred to names of the type ethyl benzoate when the acid has a long name.

V. THE TERMINATIONS OF NAMES OF ORGANIC COMPOUNDS

Building on the famous Geneva rules the International Union of Chemistry in its Definitive Report of the Commission on the Reform of the Nomenclature of Organic Chemistry (the "Liége Report") has created a consistent system of terminations expressing the functions of organic compounds. This system was first adopted in the English literature and was then also introduced in French and (in part) in Dutch, Spanish and Polish. Quite recently it is also being introduced (as far as possible) in the Scandinavian languages. Although the Geneva rules were created also on the initiative of German chemists German literature continues to use the names benzol, toluol, carotin, cholesterin, glycerin, resorcin *etc.* It is true that the Liége rules cannot be applied so consistently to the German language as to English, but the names mentioned could at least be altered as proposed already in the Geneva rules. The difficulty of a consistent application of the Liége rules is connected with the termination *-e*, which in German indicates the plural. Therefore it is not possible to use the endings *-an* and *-ane*, *-in* and *-ine*, *-ol* and *-ole* as designation of different functions. Oxazole in German is simply the plural form of Oxazol. Similar difficulties, however, are encountered in Scandinavian and other languages, where one nevertheless has found it advantageous to introduce the system as far as possible.

One of the most urgent problems for the creation of an international chemical nomenclature is the universal adoption of the endings *-en*(*-e*, *-o*) for unsaturated hydrocarbons (benzen, toluen, styren, caroten etc.) and the ending *-ol*(*-o*) for hydroxy compounds (glycerol, resorcinol, cholesterol *etc.*). On the other hand it is not possible in several languages to avoid giving heterocyclic compounds the same ending as hydroxy compounds (pyrrol-glycerol) or saturated hydrocarbons (pyran-methan) or to give some non-nitrogeneous compounds the same ending as nitrogen bases (dextrin-pyridin). Even with this limitation the Liége rules offer such great advantages that it is unwise to neglect them.

In this connection it may be pointed out that the prefix hydroxy- should be universally adopted to express the alcohol- or phenol-function. In German

and several other languages (presumably under the influence of the Beilstein nomenclature) the prefix oxy- is used (Oxysäuren = hydroxyacids, Oxybenzole = hydroxybenzenes). This is a relic of the old addition nomenclature, *oxy-* meaning an oxygen atom (*cf.* trioxymethylene, phosphorus oxychloride), but organic chemical nomenclature now generally follows the principle of substitution.

The prefix hydroxy- (oxy-) should be spelled thus and not hydroxi- (oxi-).

VI. THE SPELLING OF CHEMICAL TERMS

a. Importance of agreement on spelling of the beginning of words

The international committees of chemical nomenclature have hitherto concentrated their efforts on attaining uniformity in the endings of chemical names. In this respect they have been quite successful. The beginnings of the names on the contrary have been left aside as a problem of orthography which cannot be dealt with by chemists, at least on an international level. For the perception of the names, however, the beginnings of the words are as least as significant as the endings, simply because we read the words from left to right. When we read the words there is certainly as much difference between cholesterol and kolesterol as between cholesterol and cholesterin, between caroten and karoten as between carotene and carotin, between phloroglucinol and floroglucinol as between phloroglucinol and phloroglucin. It is particularly inconvenient, both as to recognizability and to mnemonics, when chemical names of plant substances are spelled in another way than the botanical names from which they are derived. In some languages for instance the names of compounds isolated from *Colchicum*, *Crocus*, *Coca*, *Citrus*, *Rhamnus*, *Rheum*, *Quercus*, *Physalis*, *Thymus*, *Haematoxylon*, *Hedera*, *Chelidonium etc.* are spelled: Kolkicin, krozetin, kokain, zitral or sitral, ramnose, rein, kversetin, fysalien, tymol, ematossilina, ederagenina, kelidonin. For chymosine one finds the spellings chimosina, quimosina, kymosin, for chlorophyll the spellings clorofilla, klorofyl, and so on.

Another important point is that conformity in spelling gives the same alphabetical order of chemical terms irrespective of the language. Each chemist frequently uses compilations of chemical data and subject indices of journals and books of reference in other languages than his own. It is inconvenient to search quinoline under *q* in an English journal, but under *ch* (Chinolin) in a German and under *k* (kinolin) in a Scandinavian journal. Even such small differences as between thorium and torium, rhodium and rodium,

rhamnose and ramnose etc. are inconvenient and may even cause one to miss important references.

Finally the chemists of the small countries, who are obliged to write their papers in a main language are hampered by their national spelling. The Scandinavian Chemical literature, written in English, sometimes contain such misspellings as bensoic acid, bensyl, styfninic acid, tiazole, metyl *etc.*, and even if such errors are corrected in the proofs great care is necessary to avoid them. Influenced by the chemical symbols and by papers written in foreign languages Scandinavian students of chemistry are inclined to use spellings such as calcium, chlor, chrom, phenthiazin *etc.*; but hitherto these have been corrected to kalcium, klor, krom, fentiazin *etc.* When these spellings finally have been impressed on the students, they add to confusing when the students have to read or write in a foreign language. From the point of view of mental effort the unification of the names of the chemical compounds accordingly offers great advantages. Because the chemical symbols are international, the efforts should primarily aim at introducing names of the elements which are in accordance with their symbols.

b. Etymological versus phonetical spelling

The divergent spellings are particularly numerous in the smaller European languages where the written language has no old tradition, but has mainly been established in the 19th century. At that time the predominant opinion of philologists was that a written language should, as far as possible, be a phonetic rendition of the spoken language. But as the pronunciation differs from language to language, and the phonetical value of a certain letter is not the same in different languages (*c* is *e. g.* pronounced as *s* or *k* in western languages, but as *ts* in Slavic languages; *z* is pronounced as *ts* in German, but as voiced *s* in Slavic languages) the introduction of phonetic spelling has isolated these languages from each other and from the classical languages. The newer theory of language, however, considers the spoken and written language as two different coordinated modes of expression and does not approve of efforts to bring them as close together as possible. Moreover the process of reading is not, as was formerly thought, simply a process of spelling. In reading we do not spell the words but perceive them as entities, as symbols. It is not absolutely necessary that these symbols have any connection at all with the sounds for which they stand — as is well known, for instance, from the Chinese ideographs and from the chemical symbols or the numeral figures. A certain approach to a phonetical rendition has some value as to mnemonics, but an exact phonetical rendition of the spoken language is impossible because the

pronunciation of a certain word may vary with place, time and even the connection, in which it is used. Because we read the words as symbols it is of great importance that the same thing is always described by the same symbol whenever we come across it. A written language has as a rule only one accepted spelling of each word even if there may be several pronunciations. The advantage of this is uncontested when a single language is concerned, but it also applies to international words occurring in different languages. The pronunciation of "lieutenant" for example is quite different in French and English, but it is obviously an advantage to understanding that the "symbol" is the same. Nevertheless many languages have nationalized the spelling of such international words. An alternative to the phonetical spelling of words is an etymological spelling, in accordance with the derivation from the parent language *. The phonetical spelling will vary from language to language, the etymological spelling may be constant. Accordingly an etymological spelling is the only spelling on which there is the possibility of an international agreement. A difficulty, however, arises with terms derived from languages that are not written with the Latin alphabet: Arabic, Chinese, Greek, Russian. The etymological spelling of words derived from these languages will only be constant if there is agreement concerning their transliteration. Now the foundation of the sciences was laid in a literature written in Latin and in this literature words of Greek (and Arabic) origin were transcribed by a consistent and universally accepted system. As will be discussed in more detail below it is the most rational to continue to use this system, even if it in some cases does not give the simplest spelling possible.

Fortunately English and French have to a large extent retained the classic spelling of words of Latin and Greek origin, even if the pronunciation has changed much from the original one. Also German had formerly mainly the classic spelling of words of Latin and Greek origin, but in more recent times the spelling has been nationalized by introducing *z* and *k* instead of *c*, for instance Krozetin, Kalzium, Zäsium, Kapronsäure, Karbazol, Karboxyl, Kozymase, Kumarin, Zellobiose, Zetylalkohol, Zitronensäure, Zitral, Zyan, Zyklohexan, Zystein. This spelling has also spread to a great extent in Swiss literature. The big German handbooks, Beilstein, Gmelin and Richter-Anschütz, however, use the older spelling and it seems that after the war this spelling is gaining ground again, so there should be a fair chance of bringing the German spelling of chemical terms very close to the English and French one.

* As a matter of principle scientific terms should as far as possible be derived directly from the classic languages and not from modern languages (*cf.* I). In some instances terms have been formed from words of modern languages and should retain the spelling of the language of origin (*e. g.* ytterbium, named after the Swedish town Ytterby).

The main difference between the English-French and older German spelling is the rendition of Greek *kappa*, which will be discussed in more detail below, and the spelling of names related to quinine (quinoline, quinic acid, quinone, quinhydrone, quinuclidine, quinoxaline, *inter alia*), which in German are spelled with ch. The common origin of these words is a Peruvian word *kina-* (bark), but as this has come into European languages via Spanish, where it is spelled *quina*, it seems most logical to use the spelling with *qu* and not that with *ch*.

c. The problem of transliteration of words of Greek origin

The peculiarities of the different spellings of chemical terms are in most cases connected with a different transliteration of words of Greek origin. Thus *zeta* may be reproduced as *z*, *s* or *c; theta* as *th* or *t; kappa* as *c*, *k*, *s* or *z; ksi* as *x* or *ks; rho* as *r* or *rh; upsilon* as *y* or *i; phi* as *ph* or *f;* and *chi* as *ch* or *k*. Further in words of Latin origin *c* may be reproduced as *k* and *qu* as *kv*.

It would be of great importance if the International Chemical Union could recommend only one system to be used, when forming chemical names from Greek roots. Without doubt the most rational would be only to use the Latin transliteration, *i. e. kappa* = *c*, *phi* = *ph*, *chi* = *ch* etc.

German, in contrast to English and French, often reproduce *kappa* as *k*, when it is pronounced as *k:* Pikrinsäure, Kresol, Kakodyl, often also Galaktose, Oktan etc. There is certainly something to be said in favour of this practice, but the following reasons make it difficult for the Greek *kappa* to be generally reproduced as *k:*

α. In chemical names introduced in older times the Latin transliteration of Greek words was used exclusively, and accordingly the pronunciation of *kappa* before front vowels (*e*, *i*, *y*) changed to *s (ts)*, for instance cetyl, decyl, cinnabar, glycin, cyan, cyclo-, cystin, cytochrom. It certainly does not seem advisable to change these spellings to ketyl, dekyl, kinnabar, glykin, kyan, kyklo-, kystin, kytokhrom. In the words where *c* is pronounced as *k* the spelling could in many cases be changed, as has been done in German and several other languages, but in some words the *c* has been stabilized by becoming part of a chemical symbol. It is unwise to introduce spellings as Kadmium and Aktinium as long as their chemical symbols are Cd and Ac.

β. In the international, latinized names of zoological and botanical species the Latin transliteration for Greek names are, of course, still used. When the name of a chemical compound is derived from these names it is important

that the spelling should not be altered. For instance crocetin should be spelled thus, because its name is derived from the botanical name *Crocus*, although this again is derived from Greek κρόκος. The same argument applies to colchicin, coniin, corydalin and many others.

Thus it is not possible to reproduce *kappa* as *k* consistently. Therefore the most rational procedure is undoubtly to use the classic Latin transliteration of Greek words, *i.e.* practically not to use the letter *k* in chemical names. This is in accordance with French, other Romance languages and, for the most part, with English. In modern English chemical nomenclature there is, however, a tendency to reproduce *kappa* as *k*, for instance katalase, hexokinase, kathepsin, keratin, kynurenine, kyanmethine, kephalin. *To avoid further confusion it is very important that a rule should be established concerning the formation of chemical names from Greek roots.*

Something might also be said in favour of the simplified reproduction of *phi* as *f*. However *ph* will presumably be used for ever in the names of botanical and zoological species and in medical terms, and it is desirable that chemical names derived from these terms should conserve the same spelling (*e. g.* physostigmine, physaliene, phthiocol). Further it is unwise to spell phosphorus with *f* as long as the chemical symbol is P. It therefore seems most rational to adhere strictly to the Latin transliteration of Greek words and to spell words of Latin origin in the same way as the words from which they are derived.

It should, however, be emphasized that a system of transliteration should not be considered as an end but as a means, and exceptions to it may therefore be admitted, if they are agreed to internationally. For instance the spellings krypton, barium, ethyl, kynurenin are found in more languages than the more orthodox spellings crypton, baryum, aethyl, cynurenin, and it would therefore be easier to obtain international agreement concerning the first spellings. Such exceptions should, however, not be too numerous.

d. Languages with phonetical spelling

Next to English, French and German the languages of greatest importance to chemistry are Czech, Dutch, Italian, Polish Russian and Spanish. Russian need not be considered here, because the use of another alphabet makes almost any approach to an international spelling impossible. In Dutch almost no changes are necessary. Polish has strictly phonetical spelling with *k* instead of *c*, *ks* instead of *x* (even when initial sound: ksenon, ksantogen) and *f* instead of *ph*. A peculiarity of Polish is that *i* has been replaced by *y* and vice versa; thus pyridine for instance becomes pirydyna. Thus rather extensive changes are

necessary. Czech on the other hand has a spelling of international words which is to a great extent in accordance with the international spelling, though for many compounds purely national names have been introduced (see under I).

In Italian the most serious deviations from the international spelling are the disuse of the letter *h* and the replacement of *x* by *s* or *ss* and of *y* by *i*. Thus names as esaossicicloesane (hexahydroxycyclohexane) arise. *H* is not pronounced in Italian, but nevertheless is retained in some purely Italian words (*ho*, *ha*), so it should be possible to replace names such as elio, afnio, emina, emoglobina, eptacosano by helio, hafnio, hemina, hemoglobina, heptacosano. By reintroducing *x* and *y* such names as esaidropirano, esosi, ossiemoglobina, ialuronidasi would be hexahydropyrano, hexosi, oxyhemoglobina, hyaluronidasi and thus much more easily recognizable.

In Spanish the most serious deviations from international spelling are caused by the use of *i* instead of *y*, by insertion of an *e* before *s* followed by another consonant, and by sometimes reproducing *ch* as *qu*. Thus names as itrio (yttrium), escandio, estroncio, escopolamina and quimosina (chymosine) arise.

In the languages having a phonetical spelling the introduction of an etymological spelling is often rejected with the argument, that it is not in accordance with the "genius of each language". This argument has, however, been grossly abused in discussions of chemical nomenclature. The latinized botanical and zoological names are used unchanged in different languages. In Italian for example the botanical names Oxalis and Helenium are accepted unhesitatingly; why, then, not oxigeno and helio? In all languages proper names exist which do not conform with a phonetical spelling. But the names of the chemical elements and compounds are in some sense to be considered as proper names.

To introduce the changes necessary to obtain an international spelling of chemical terms it is necessary to consider these as latinized scientific terms similar to the names of botanical and zoological species. From this point of view it should be possible to change the spelling of chemical terms without changing the spelling of words of every day life of Greek or Latin origin, and conversely, when first established the international spelling of chemical terms should not follow changes in the spelling of common words.

VII. PRONUNCIATION

A real unification of the pronunciation of chemical terms is not attainable, nor is it of any great importance because most chemical communication is given in written language. A few words should, however, be said about English as a congress language. French or German are in some sense better suited as

international languages because they are pronounced more distinctly. Without doubt, however, English is becoming more and more important as a congress language, and if this is to be for the good, English and American chemists should make efforts to articulate more distinctly.

The importance of English as an international language is also severely hampered by the fact that often international words which are pronounced in much the same way in several languages are pronounced quite different in English. Names as amid(e, -o), methyl, cyanid(-e) etc. sound very similar in French, German, Italian, Russian, Danish *etc.*, but quite different in English. The cause of this is that *i* and *y* in English may be pronounced as *ai*, a pronunciation which is different from that of all other languages, and by the way from the original Anglo-Saxon one. From this point of view the American pronunciation of methyl, phenyl *etc.* is much to be preferred to the English one. The American Chemical Society has issued a list of recommended pronunciations, and in many cases two possible pronunciations have been placed in order of preference. *In almost all cases, from an international point of view, the order of preference should be reversed,* because the specific English pronunciation has been placed first. Further some pronunciations have been described as Germanisms. As a matter of fact these pronunciations resemble not only the German pronunciation, but also that of other languages and therefore their use should be encouraged rather than discouraged. A term particularly difficult to understand for foreigners is thio-. It would be an advantage if it could be decided to pronounce it teeo- as in most other languages.

CONCLUSION

Above some of the difficulties encountered in the establishment of an international chemical nomenclature have been discussed. These difficulties will perhaps appear insuperable to those who are strongly dependent on habit and to those who consider the language as a growing organism which cannot be influenced arbitrarily. The latter opinion is quite erroneous. Which word should be used for a certain notion is almost exclusively a question of convention, but it must be admitted that habit will strongly oppose all attempts at radical changes of the language. Notwithstanding this difficulty the nomenclature committees of the International Union of Chemistry should seriously consider the possibilities of unification of chemical terms. Otherwise their influence will be mainly limited to a few languages only.

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Pinoresinol as a Lignin Model *

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A great deal of work has been carried out to see if substituted benzyl alcohols are appropriate lignin models, and it has been shown that in several respects these alcohols do indeed resemble the sulphite-reacting groups present in lignin [1–5]. However, the benzyl alcohols hitherto investigated react too rapidly with neutral sulphite solutions to be close analogues of the less reactive of the sulphonatable groups in lignin — the groups B [6].

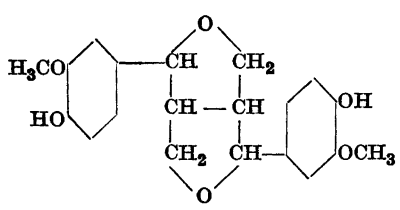
Recently, therefore, we investigated the reactivity of a model substance (diveratryl ether) containing benzyl alkyl ether residues towards, *inter alia*, sulphite solutions of various degrees of acidity [5]. It appeared from the results obtained with this model that the groups B might possibly be of the *p*-alkoxybenzyl alkyl ether type [7].

It then occurred to us that the more reactive of the sulphite-reacting groups — groups X and Z [8] — might be some other type of benzyl alkyl ether groups. Both groups X and Z are sulphonated in neutral solution (X rapidly and Z slowly) so that it was necessary to search for a benzyl alkyl ether that is sulphonated under these conditions.

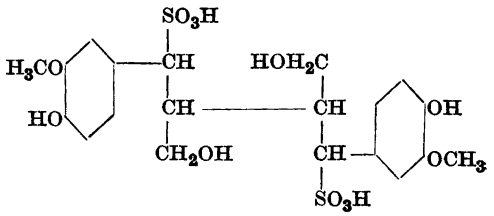
Sulphonation experiments with benzyl alcohols have shown that *p*-alkoxybenzyl alcohols are sulphonated considerably more readily than *p*-alkoxybenzyl alcohols [2], especially by neutral or faintly alkaline sulphite solutions, so that it appeared that a *p*-hydroxybenzyl alkyl ether structure would have the desired properties.

Groups of this type occur in several of the lignans (natural products built up from two phenyl propane units) such as, for example, pinoresinol (I). Erdtman [9] has studied the action of acidic sulphite solutions on this substance, and showed that pinoresinol does not dissolve in such solutions (pH 1.5—2)

* Part 51 on the chemistry of the sulphite process by E. Hägglund and coworkers. Part 50, *Svensk Papperstidn.* **54** (1951) 795.



I



II

at 135°, but is converted into an insoluble condensation product which is almost free of sulphur. Consequently the major portion of the benzyl ether groups in the pinoresinol had not been sulphonated. Similar experiments with dimethyl and diethyl ethers of pinoresinol showed that these structures are not sulphonated under the same conditions [9].

Earlier researches have shown that the sulphonation of benzyl alcohols [2,3,5] and of diveratryl ether [5] is supressed by phenols at pH 1—2 and that this effect diminishes with increasing pH. As pinoresinol itself contains phenolic hydroxyl groups which may hinder the sulphonation, it seemed possible that pinoresinol could be sulphonated at pH's higher than those employed by Erdtman. We have, therefore, investigated the reaction between pinoresinol and sulphite solutions of various degrees of acidity.

When pinoresinol was heated with sulphite solutions of various pH at 135° for 45 minutes we obtained the following results (Table 1):

At pH 2, we found, as did Erdtman, that most of the pinoresinol was converted into an insoluble condensation product.

At pH 4, approximately 60 % of the pinoresinol dissolved, the remainder being converted into an insoluble condensation product.

At pH 6, 8, and 10, the pinoresinol dissolved almost completely in the sulphite solutions.

From the sulphite solution obtained from an experiment using neutral sulphite solution heated at 135° for 1.5 hours we isolated an amorphous

Table 1. Pinoresinol dissolved after treatment with sulphite solutions of varying pH for 45 minutes at 135°.

pH	2	4	6	8	10
Pinoresinol dissolved, %	18	64	97	99	93

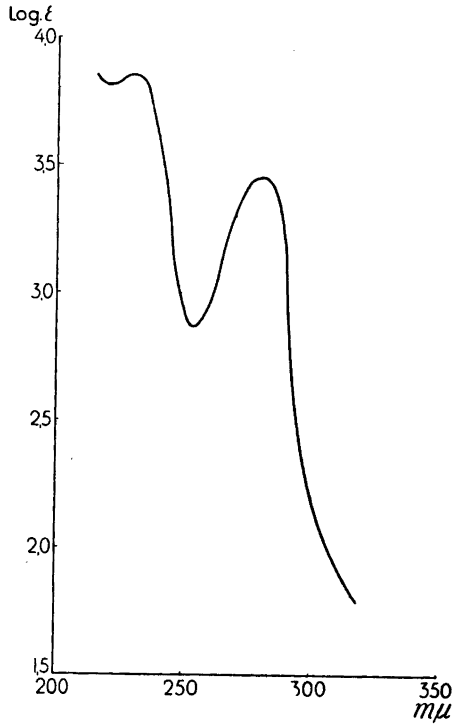


Fig. 1. The ultra-violet absorption curve of sulphonated pinoresinol.

product in a yield which accounted for 57 % of the methoxyl content of the starting material. The ultra-violet absorption curve (Fig. 1) exhibited the characteristics expected for an aromatic compound with no double bond conjugated with the benzene ring.

The water-solubility, sulphur content and ash content showed that the product contained sulphonic acid groups and, on analogy with the sulphonation of diveratryl ether, these must have been formed by the sulphonation of the benzyl ether groups. However the product contained appreciably less sulphur and more methoxyl than the structure II which would be the expected product from the sulphonation of pinoresinol. It appears that not all of the benzyl ether groups of the pinoresinol had been sulphonated and as even prolongation of the time of heating to 3 hours (compared with 1.5 hours) gave a product which showed the same deviation from the expected structure II, the remainder of the benzyl ether groups must have undergone condensation under the influence of the sulphite solution.

After this work was completed, Leopold (private communication) sulphonated pinoresinol and obtained a product, the composition of which agreed

Table 2. Pinoresinol dimethyl ether dissolved (in percent) after treatment with sulphite solutions of varying pH, for various lengths of time at 135°.

Time, hours	pH 2	3	4	5	6	11
2	15	5				
13		15				
20			8*	9*	8*	0*

* The dimethyl ether was recovered in crystalline form.

much better with the structure II than that of the material described above. Leopold used a larger ratio of sulphite solution to pinoresinol than we employed, and his material (the barium-salt) contained 8.37 % S and 9.07 % methoxyl (calculated for barium-salt of II, 9.74 % S and 9.43 % methoxyl).

Sulphite cooking of pinoresinol has shown, therefore, that *p*-hydroxybenzyl ether groups which are sulphonated in neutral solution as rapidly as the groups X in lignin do indeed exist. In a preliminary note in Part 48 of this series [8] we pointed out that the groups X might be of this type. At the same time, however, we also pointed out that there is another possibility, namely that these lignin groups may be *p*-hydroxybenzyl alcohol groups.

We have also investigated the reaction of pinoresinol dimethyl ether with sulphite solutions of various pH, and confirmed the findings of Erdtman [9] that at low pH the dimethyl ether is not sulphonated to any greater extent (Table 2). As the ether is transformed into an amorphous product in this pH-region it has probably condensed. At pH 4 and above the ether neither condenses nor sulphonates, but is recovered unchanged.

That the *p*-alkoxybenzyl ether groups in pinoresinol ethers, unlike those in diveratryl ether, are not sulphonated but undergo condensation under the action of acid sulphite solutions, is probably due to the sparing solubility in water of the former. Because of the insolubility it is more difficult for the sulphite ions to react with the model substances. The condensation reaction, however, is not hindered to the same extent because of the mobility of the hydrogen ions.

EXPERIMENTAL

A mixture of pinoresinol (1 g) and a solution of sodium sulphite (10 ml, 5 % total SO_2 adjusted to pH 7 with NaOH) was heated in a rotating glass tube at 135° for 1.5 hours, during which time the pinoresinol dissolved almost completely. Sodium ions were remov-

ed by passage through a cation exchanger and the sulphur dioxide expelled by a stream of nitrogen. The solution was neutralised with barium hydroxide, filtered, and evaporated *in vacuo* and the residue (1.47 g) dissolved in a little water. An equal volume of isopropyl alcohol was added, the precipitated barium sulphite filtered off and the product precipitated by the addition of acetone and ether. This material (1.05 g) contained neither sulphate nor sulphite ions.

$C_{20}H_{24}O_{12}S_2Ba$ (formula II)	Calc.	OCH_3	9.4	S	9.7
	Found	»	11.2	»	6.4

The ultra-violet absorption curve of the material is shown in Fig. 1.

The results listed in Tables 1 and 2 were obtained by heating the model substances with sulphite solutions in the same way as was used for the corresponding determinations on diveratryl ether and veratryl alcohol [5].

SUMMARY

Pinoresinol has been sulphonated by sulphite solutions at pH 6—9.

We wish to thank Professor H. Erdtman and Dr. B. Lindberg for the gift of pinoresinol. The investigation was supported by a grant from Swedish Natural Science Research Council.

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Butyric Acid and Lactic Acid in Aqueous Solutions as Solubilizers for Carcinogenic Hydrocarbons

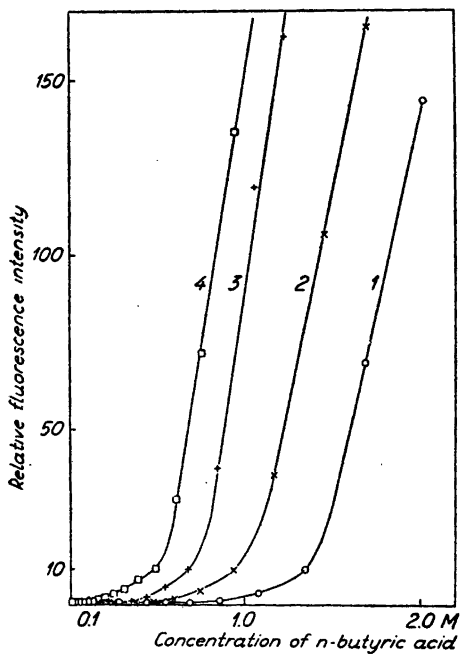
PER EKWALL and LARS SJÖBLOM

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Bury and coworkers [1,2] have shown by measuring the specific volumes and freezing point lowerings that an association leading to micelle formation takes place in aqueous solutions of *n*-butyric acid. As in soap solutions micelles begin to form at a definite concentration, "the critical concentration for micelle formation", which in this case is about 1.1 *M* at 35° C. Since soaps and other association colloids are able to solubilize water-insoluble carcinogenic hydrocarbons [3–5], it was of interest to investigate whether these hydrocarbons are solubilized also by the butyric acid micelles. Owing to the fact that butyric acid has a very short carbon chain, it would be expected that also the micelles it forms are quite small and hence have a poor solubilizing power.

n-Butyric acid solutions of increasing concentration were shaken 48 hours at 40° C with an excess of finely powdered 3,4-benzpyrene (BP). In pure water and in dilute butyric acid solution the polycyclic hydrocarbon is practically insoluble, but when the butyric acid concentration attains a certain value the solubility begins to increase as shown by the fluorescence in ultraviolet light (Fig. 1, curve 1). Already in a 0.85 *M* butyric acid solution an extremly weak fluorescence is noted, which becomes gradually stronger with increasing butyric acid concentration. A marked fluorescence is first evident in the 1.08 *M* solution and above 1.35 *M* the intensity of the fluorescence increases rapidly. This latter values agrees well with the value for the critical concentration which is evident from Bury's data [1,2]. It is clear that the actual micelle formation begins at this concentration, but it may be that in the presence of benzpyrene micelle formation is preceded by an association of lesser degree which begins at a concentration near 0.85 *M*.

The behaviour is much the same in butyric acid solutions containing hydrochloric acid or sodium chloride (0.1 *N*), or both (0.1 *N* + 0.1 *N*). The abrupt



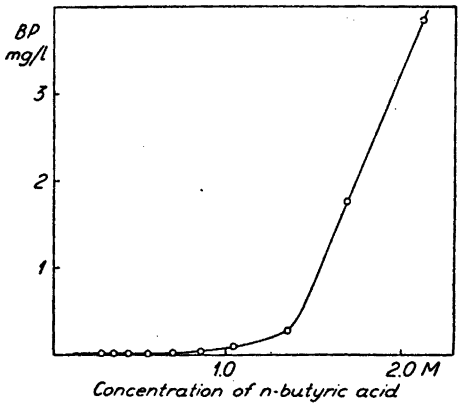


Fig. 2. The solubility of 3,4-benzpyrene in n-butyric acid solutions.

Fig. 1. The fluorescence intensity curves of solutions saturated with 3,4-benzpyrene.
Curve 1. n-Butyric acid alone.
» 2. n-Butyric acid and d,l-lactic acid, molar ratio unity.
» 3. n-Butyric acid and d,l-lactic acid molar ratio two.
» 4. n-Butyric acid, d,l-lactic acid and sodium taurocholate, molar ratios 1: 2: 0.19.

rise in the fluorescence occurs in all these cases when the butyric acid concentration is approximately 1.1 *M*; the first weak fluorescence is observed at a somewhat lower concentration (about 0.7 *M*) in the presence of the electrolytes.

The solubility of benzpyrene in a 1.24 *M* butyric acid solution was found to be 0.18 mg per litre. With this value as a basis we have estimated from the fluorescence intensities the solubility of benzpyrene at the other butyric acid concentrations (Fig. 2). The values obtained in this manner are only approximate, but will serve for orientation. In the 2 *M* solution the solubility has increased to about 2.7 mg benzpyrene per litre.

Several other fatty acids were studied to determine whether their aqueous solution are also able to solubilize benzpyrene. Saturated solutions of caproic, caprylic, nonylic, capric and oleic acids were prepared and saturated with benzpyrene in the manner described above. No fluorescence, however, was

observed in any of these solutions which shows that no benzpyrene was solubilized. This is probably due to the fact that the solubilities of these acids in water are lower than their respective critical concentrations.

In the literature we have not found any data which would indicate that lactic acid undergoes micelle formation. Despite this we have studied whether aqueous solutions of this acid are able to effect solution of benzpyrene. The results were positive. A marked fluorescence was observed in *d,l*-lactic acid solutions saturated with benzpyrene beginning with a lactic acid concentration of about 4.3 *M* (Fig. 3). The fluorescence increased rapidly above this concentration. A weak fluorescence is noted when the lactic acid concentration is about 2.8 *M*. The measurements were to some extent rendered difficult by the weak greenish fluorescence of the pure lactic acid solutions. An approximate determination of the solubility of benzpyrene based on fluorescence measurements gave a value of about 2.3 mg per litre of 6.8 *M* lactic acid (38 weight per cent lactic acid). — As long as micelle formation by lactic acid has not been proved by other methods, and since very high concentrations of the acid are involved, we prefer to attribute the solubility of benzpyrene to a hydrotropic effect on the part of lactic acid.

The ability of butyric acid to solubilize benzpyrene is greatly increased in the presence of lactic acid. When the molar ratio of lactic acid to butyric acid is unity the fluorescence begins to increase rapidly when the butyric acid concentration exceeds 0.85 *M*; when the molar ratio is two the fluorescence increases already when the butyric acid concentration exceeds 0.65 *M*. A weak fluorescence is observed in these cases at butyric acid concentrations of 0.5 and 0.3 *M* respectively. In none of these solutions was the butyric acid concentration high enough for it alone to be able to solubilize benzpyrene. Thus it appears that mixed micelle formation is involved, *i. e.*, butyric acid micelles containing lactic acid molecules are formed.

The ability of butyric and lactic acids to solubilize carcinogenic hydrocarbons is of biological interest since in certain conditions these acids are found free in the stomach. When the hydrochloric acid content of the stomach is low, lactic acid fermentation can take place in the stomach and according to the literature this is often followed by butyric acid fermentation [6]. On the other hand, if under extreme conditions (lack of HCl) the lipase of the stomach is able to split fatty substances, it is the lower fatty acids in the glycerides that will be liberated first [7]; it is possible that this may lead to the formation of some free butyric acid in the stomach. Furthermore, appreciable amounts of free lactic acid enter into the stomach with food (sour whole milk, bread). In this connection it may also be noted that lactic acid is produced by oxidative metabolism in the cells of the gastric mucosa [12].

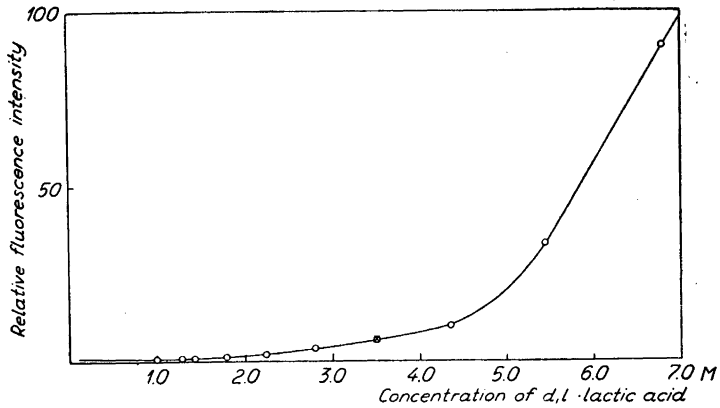


Fig. 3. The fluorescence intensities of d,l-lactic acid solutions saturated with 3,4-benzpyrene.

The investigations of Setälä, Ekwall and their coworkers [5,8,10] have shown that benzpyrene solubilized by association colloids is able to penetrate into the glandular tissue of the stomach in mice and cats despite the presence of the protective mucous layer of the stomach. It therefore appears probable that also butyric acid solutions are able to induce a penetration of carcinogens solubilized in them.

It has previously been pointed out [5,8,11] that the bile is an association colloid solution consisting primarily of bile acid and fatty acid salts, lecithin and cholesterol, which is able to solubilize carcinogens and that this property is not lost even in acid solutions (pH 1). When bile enters the stomach, the conditions become such that carcinogenic hydrocarbons can penetrate into the tissues of the stomach wall [5,8,11].

The present investigation thus shows that in the stomach in addition to bile there can occur also other association colloid systems capable of effecting the solubilization of carcinogenic hydrocarbons, *viz.* solutions of butyric and lactic acids. It is of interest to know how these micelle systems affect each other's ability to solubilize carcinogenic hydrocarbons. With taurocholate present in the ratio of one mole to 70 moles butyric acid a definite solubilization is noted already in an aqueous solution that is 0.3 *M* in butyric acid (a weak fluorescence begins at a concentration of 0.2 *M*). In these solutions the critical concentration of taurocholate has not yet been exceeded. On the other hand, the critical concentration of taurocholate is lowered when butyric and lactic acids are present in concentrations that are alone insufficient to effect the solubilization of benzpyrene. Thus, when bile enters the stomach when the latter contains butyric and lactic acids the conditions

for the solubilization of carcinogenic hydrocarbons and hence for their penetration into the walls of the stomach are improved.

Since, in order to have any effect, the carcinogen must first penetrate into the tissues, our investigations suggest a new way by which gastric cancer may be effected by chemical carcinogenesis. This possibility possesses considerable interest since statistics show that in humans gastric cancers and low stomach acidities are frequently encountered together.

It is proposed to continue the investigations to determine the power of butyric acid — lactic acid solutions and of butyric acid — lactic acid — bile solutions to promote the penetration of carcinogens into the glandular tissue of the stomachs of animals, to determine the amounts of butyric and lactic acids present in the human stomach in different conditions and to study the ability of the stomach content to solubilize carcinogenic hydrocarbons.

SUMMARY

Aqueous solutions of *n*-butyric acid of concentrations above 1.1 *M* solubilize the fluorescent carcinogenic hydrocarbon 3,4-benzpyrene. Benzpyrene dissolves also in aqueous solutions of *d,l*-lactic acid when the concentration of the acid exceeds 4.3 *M*.

In the presence of lactic acid the ability of butyric acid to solubilize benzpyrene is increased greatly. Sodium taurocholate in concentrations below its critical concentration has the same effect.

The results are discussed from the point of view of chemical carcinogenesis in the stomach.

A grant from the Sigrid Juselius' Stiftelse is gratefully acknowledged.

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The Mercerisation of Cellulose

I. A Thermodynamic Discussion

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The cellulose occurring in nature contains a crystalline lattice of *Cellulose I* (native cellulose)*. After an intermediate swelling, for instance, with strong lye or sulphuric acid, and removal of the swelling agent at room temperature, the native cellulose is transformed into mercerised cellulose, earlier called "hydrate cellulose", a new modification with changed properties and containing a new crystalline lattice, *Cellulose II*. The process is called mercerisation or mercerising after John Mercer, who discovered and patented the process (1851). The industrial importance of Mercer's discovery was much increased by applying tension to the fibres during the treatment, a process developed by Horace Lowe (1890). In this process, shrinkage was prevented and the cotton acquired a silk-like luster and greater strength.

Mercerising has been the object of innumerable investigations, chiefly of technical or commercial aspects, and several monographs on mercerising have been published. The most comprehensive is that of Marsh [1], dealing mainly with the treatment of cotton with caustic soda. Mercerising with other agents (concentrated acids or salts, organic bases), and of other fibres is described in other more general handbooks or reviews on cellulose, especially those of Valko [2], Marsh and Wood [3], Ott [4], and Heuser [5], which give a general description and references to the literature. Only facts of special importance for this paper will be outlined here with references to the original papers. Attention is focused on the mercerising of cotton and ramie with caustic soda, because comprehensive and reliable data on this reaction have been published. The aim is to give an interpretation of the mercerisation process, mainly from a thermodynamic point of view.

* There is in fact only one exception known, the sea algae *Halicystis*, reported by Sisson (*Science* **87** (1938) 350, *Contr. Boyce Thompson Inst.* **12** (1941) 31) to have cell membranes containing mercerised cellulose. These peculiar observations require further study.

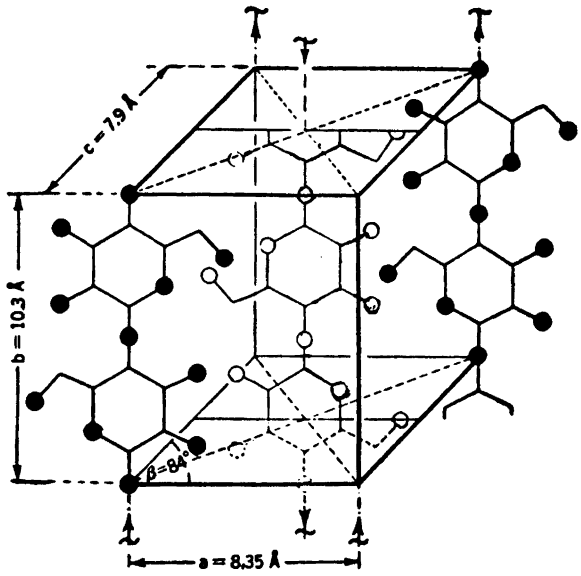


Fig. 1. The unit cell of native cellulose (Cellulose I) after Meyer, Mark, and Misch [8,15]. *Two edge chains are omitted for clarity.*

I. CRYSTAL STRUCTURE CHANGES ON MERCERISING

The lattice transition on mercerising has been studied by many authors and surveyed in an excellent way by Mark [6], in 1932. The structure conception described there has been further treated and frequently discussed (*cf.* Hermans [7] for a review and for references).

A diagrammatic representation — after Meyer, Mark and Misch [8] — of the monoclinic unit cell of native cellulose containing four glucose units is shown in Fig. 1. The transition of *Cellulose I* to *Cellulose II* (also monoclinic) caused by mercerising — implying $\sim 30°$ rotation of the planes of the cellulose chains, related to the diagonal planes 101 and $10\bar{1}$ — is demonstrated in a projection perpendicular to the b-axis (Fig. 2) according to Andress [9]. The main features of these structure models may still be accepted [10–12] but the need for a detailed crystallographic investigation of the two lattices represents a serious lack. However, this work has now been started [13,14].

Published data of the unit cell dimensions have been collected in Table 1.

As pointed out by Hermans [17] the cellulose chains in the lattice form long, flat cylinders (Fig. 3) with the hydrophilic OH-groups in limited regions at the edges. The lattice forces of *Cellulose I* and *II* are then of different origin in

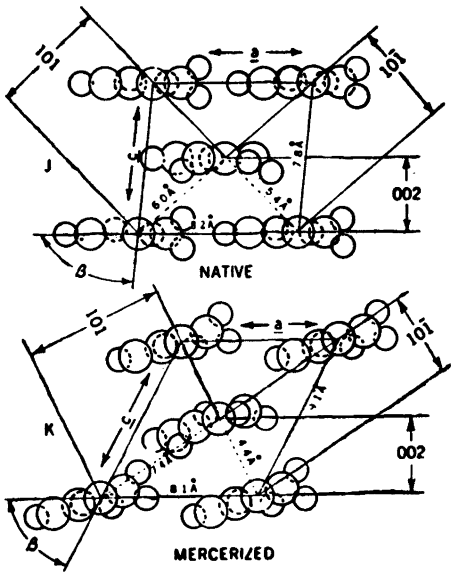


Fig. 2. Projections after Andress [9] of the lower half of the unit cells of native and mercerised cellulose (Celluloses I and II) on the lower ac-plane of the cell.

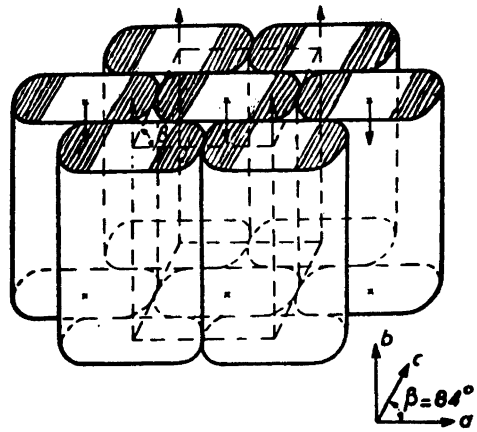


Fig. 3. Diagram of latticed molecules of native cellulose after Hermans [17]. The unit cell is indicated by broken lines. The regions containing the hydroxyl groups are shaded.

the directions of the three axes, as discussed in detail by Mark [18]. Most of the hydroxyl groups take part in formation of hydrogen bonds, as indicated by infrared absorption spectra [19]. The lattice forces hold the structure together so firmly, that is has recently been found possible to isolate crystalline cellulose particles, micelles, as well-defined rod-like morphological units [20]. The hydroxyl groups of free cellulose chains have a considerable affinity for water, reacting exothermically with it. Inside the micelles, however, the hydroxyl groups are mainly saturated by inter- and intra-chain bond formation, making

Table 1. Lattice parameters of Cellulose I and II.

Lattice	Unit cell dimensions				Ref.
	Axes in Å			Angle	
	a	*b*	*c*	*β*	
Cellulose I	8.30	10.30	7.96	84°	Meyer and Mark [8,15]
»	8.23	10.28	7.84	84°	Andress [16]
Cellulose II	8.14	10.30	9.14	62°	Andress [9]

penetration of water molecules into the lattice impossible *(Cellulose I)* or possible to only a limited extent *(Cellulose II)* cf. [21], and giving the cellulose micelles in aqueous colloidal solution a *hydrophobic* character [22]. This means, that the cellulose particles forming colloidal sols, although wetted by water, are coagulated by the addition of small amounts of electrolytes. In fact, penetration of water molecules into the lattice is not assumed for *Cellulose I*.* But when alkali-cellulose (Na-cell.) is washed with cold water, two cellulose hydrates (water celluloses) are found, containing a lattice of *Cellulose II* into which water molecules have penetrated between the cellulose chains [23].

Unfortunately it is not possible to follow the mercerisation of cellulose as a pure lattice transformation of *Cellulose I* → *II*. All measurements to be used for this interpretation were made for fibres, assumed to contain both crystalline and non-crystalline cellulose.

II. ABSOLUTE CRYSTALLINITY

Mercerising of native cellulose causes a decrease in absolute crystallinity or lateral order according to measurements by physical, as well as chemical, methods. Representative figures from physical methods are those of Hermans *et al.* obtained by their own X-ray method [24]. They measured a decrease in crystallinity from 70 to 47 per cent for ramie cellulose [24], and about the same values for wood pulp [25].

Representative crystallinity determinations, using chemical methods, are those of Nickerson [26] with boiling HCl—$FeCl_3$-solution, with the original values, corrected for a calculated recrystallization by Howsmon [27]. This method gives a crystalline portion of 96 per cent (corrected 86 per cent) for cotton and 89 per cent (corrected 70 per cent) for mercerised cotton cellulose.

Chemical methods (hydrolysis, oxidation, formation of derivatives) generally give a lower percentage amount of noncrystalline cellulose than physical methods (X-ray diffraction, water sorption, deuterium exchange).

III. MICELLE DIMENSIONS

Closely associated with the decrease in crystallinity are changes in the micelle dimensions on mercerising. The micelles have been isolated by hydrolyzing with boiling 2.5 *N* sulphuric acid and washing the hydrocellulose with

* *Note added in proof:* In a recent paper, however, Legrand (*Compt. rend.* **226** (1948) **1983**) concludes, from the relative intensity of the 002 and 101 X-ray reflections, that water molecules do penetrate the native cellulose lattice without affecting the unit cell dimensions.

distilled water. Measurements on electron micrographs showed that the micelle widths were unchanged after mercerisation (~ 70 Å) and identical with the width of the micelle strings in the native and mercerised cellulose before hydrolysis. The weight averages of the micelle lengths decreased on mercerising from about 500 Å to 350—400 Å for cotton cellulose, and to 250—300 Å for chemical grade wood cellulose [28]. The measurements were made for micelles isolated through peptisation after hydrolysis. Due to degradation and a possible recrystallisation, these micelles cannot be considered as identical with the micelles of the solid state, *Cf.* [27].

IV. DENSITY CHANGES

Density measurements of cellulose in water are misleading because of the interaction of cellulose and water, which results in a contraction of the system and a too high apparent density of cellulose or water. Density measurements in helium and in organic liquids, considered to be the most reliable, are collected in Table 2.

From the figures of Table 2 it is obvious that mercerising causes only a small density change, a net decrease in density (increase in volume) of about 1 per cent.

Table 2. Density (ϱ) of cotton and ramie cellulose.

Sample	Medium	ϱ	Medium	ϱ	Ref.
Cotton, native	Helium	1.567	Toluene	1.550	Davidson [29]
» merc.	»	1.550	»	1.536	Davidson [29]
Ramie, native	Carbontetrachloride	1.553			Hermans [30]
» merc.	»	1.543	(merc. under tension)		Hermans [30]
» »	»	1.526	» without »		Hermans [30]

From the unit cell dimensions of Table 1 the corresponding densities of the crystalline cellulose have been calculated to 1.60 (average) for native and 1.58 for mercerised cellulose. These calculated density values are ~2 per cent higher than those measured in helium. The comparison shows that the decrease in density on mercerising can at least partly be related to the lattice transition.

V. CHEMICAL CHANGES

So far as it is known, mercerising does not cause any change in the chemical composition of the cellulose *per se*. The glucose residues and the chains remain

unchanged with a possible slight depolymerisation. Impurities, oxycellulose, so-called hemicelluloses, and low molecular weight cellulose are partly extracted from the highly swollen alkali cellulose upon mercerising.

VI. HEAT EFFECTS

When cellulose is soaked in caustic soda, heat is evolved, an early observation in technical mercerising. Quantitative measurements of the amounts of heat have been made by Barratt and Lewis [31], D'Ans and Jäger [32], Neale [33], Tschilikin [34], Okamura [35], and Morrison, Boyd Campbell and Maass [36]. The last two investigations are complementary and they will be discussed together here. The measurements were made for dried cellulose with adiabatic calorimeters.

The following thermodynamic symbols, according to Guggenheim [37], will be used:

U Energy content
H Heat content, enthalpy
G Gibbs free energy
P, V, S, and T are the general accepted symbols.

ΔH is negative for a process, where heat is evolved (exothermic). ΔG is negative for all natural (spontaneous) reactions.

The heat effect (ΔH) in the reaction between cellulose and caustic soda can be separated into three parts according to its origin:

(*a*) heat of wetting the dry cellulose,
(*b*) heat of reaction of the alkali with the wet cellulose, and
(c) heat of a possible lattice transition.

$$\text{We have } \Delta H_T = \Delta H_a + \Delta H_b + \Delta H_c \qquad (1)$$

Neale [33] has separated the ΔH_T into parts in another way, to suit his discussion.

Okamura [35] used purified ramie, dried to constant weight over P_2O_5, one sample native and one sample mercerised (21.6 g NaOH pro 100 ml). For the reaction of 0.5 g dry cellulose with water or caustic soda of different concentrations, ΔH_T cal/g cellulose is represented in the diagram of Fig. 4 (curve 1 for native and curve 2 for mercerised cellulose). If the heat of wetting (ΔH_a) is subtracted (−3.66 and −6.09 cal/g for native and mercerised cellulose, resp.) the remainder represents $\Delta H_b + \Delta H_c$ (curve 3 and 4). For a concentration >20 g NaOH/100 cc the difference between the ΔH-values of curve 3 and 4

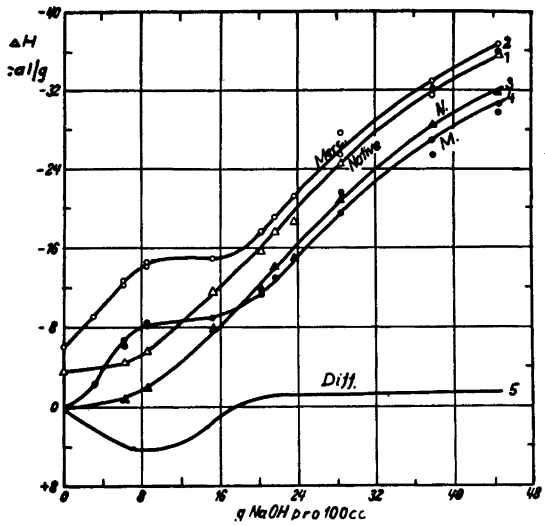


Fig. 4. The enthalpy effects (ΔH) of the reaction of native [1] and mercerised [2] ramie with caustic soda of different concentrations after Okamura [35].

seems to be constant (curve 5) and equal to the heat of transition ($\Delta H_c = -1.4$ cal/g) of wet native to wet mercerised cellulose, *i.e.*, the transition should be an exothermic reaction. This conclusion is not definite, however, as the standard error of the ΔH_c-values is of the order of magnitude of 0.4—0.8 cal/g (*cf.* the measurements indicated in Fig. 4). The ΔH-differences for concentrations <20 g NaOH/100 cc indicate, that mercerised cellulose reacts more easily than native with diluted caustic soda, in conformity with other known facts.

The measurements of Morrison, Boyd Campbell, and Maass [36] are more accurate than those of Okamura [35]. They were made for samples of cotton cellulose (standard cotton) in the native state and after immersion in sodium hydroxide solutions of concentrations from 5 to 18 %, washing and air-drying. All samples were finally oven-dried at 100° C. The heat of wetting with water was measured for all samples.

The heats of reaction for the pretreated samples were measured with a sodium hydroxide solution of a concentration the same as that employed in the pretreatment in each case. The results are summarized in Table 3.

The ΔH_T-values of col. 2 diminished by ΔH_a for the original cellulose (10.18 cal/g) and the ΔH_T-values of col. 3 by the corresponding ΔH_a-values of col. 4 give $\Delta H_b + \Delta H_c$ for original and pretreated cellulose, resp. The differences between these two series of ΔH-values represent the heat of trans-

Table 3. Heats of reaction (ΔH) of water and of sodium solutions with standard cellulose (cotton) and pretreated s.c. [36]. *The notations are according to equ. (1).*

NaOH conc. in weight-%	Heat of reaction with standard cellulose, cal/g cotton	(ΔH_T) of NaOH sol. with s.c. pretreated with col. 1, cal/g cotton	Heat of wetting (ΔH_s) of pretreated s.c. by water, cal/g cotton
0	—	—	— 10.18
5.04	— 12.96	— 13.19	— 10.34
8.00	— 14.41	— 15.02	— 10.49
11.00	— 17.30	— 18.63	— 11.87
13.18	— 22.57	— 27.69	— 15.48
15.69	— 28.80	— 35.22	— 18.15
18.14	— 30.56	— 37.19	— 18.79

formation (ΔH_c) of wet native to wet mercerised cellulose, analogous to the interpretation of Okamura's measurements [35]. In the diagram of Fig. 5 the ΔH_c-values are plotted as a function of the NaOH-concentration (curve 1). This quantity is close to zero until at 12 % NaOH; after mercerisation it is about -2 cal/g (Fig. 5), with a standard error of 0.1—0.3 cal/g. Morrison, Boyd Campbell, and Maass [36] have defined the quantity $-(\Delta H_{T\,\text{merc.}} - \Delta H_{T\,\text{native}})$ as "heat of mercerisation", plotted in curve 2 of Fig. 5. This quantity is denoted ΔH_{dry}, as it apparently corresponds to the heat of reaction in the hypothetical transition of dry native to dry mercerised cellulose. Such an interpretation is inconvenient and misleading. It is preferable to treat the wet native cellulose as the initial state and define the ΔH_c referring to wet cellulose as the heat of mercerisation for the following reasons:

1. cellulose is deposited in nature as wet native cellulose,
2. wet cellulose is better defined than dry cellulose as the last traces of wetting agents are very difficult to remove and it is difficult to keep the cellulose free from the wetting agent during treatment, and
3. mercerisation is reported only for systems, where the cellulose is wetted, with water or an organic solvent.

The changes in enthalpy (ΔH) in soaking native (A) and mercerised (B) cellulose in 18.14 % NaOH [36] are represented in the diagram of Fig. 6. Wet native cellulose has been assigned a $\Delta H = 0$. The lowest enthalpy level ($\Delta H = -20.38$ cal/g) is reached for cellulose soaked in lye, apparently identical, if native or mercerised cellulose are the starting materials. Dry cellulose has the highest enthalpy (+ 10.18 and + 16.81 cal/g for native and mercerised,

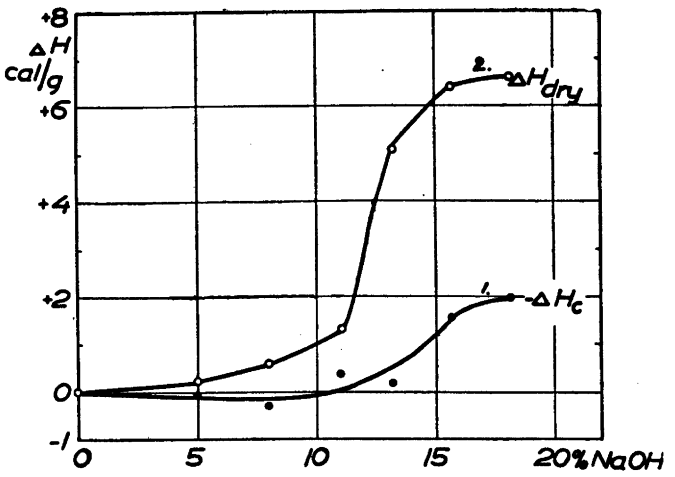


Fig. 5. The heat of transformation of native to mercerised cellulose, curve 1 for wet samples and curve 2 for dry samples plotted after measurements of Morrison, Boyd Campbell and Maass [36]

resp.). The wet celluloses are on an intermediate level with $\Delta H_{Cell.\ II\ wet} = -1.98$ cal/g in fairly good agreement with Okamura's measurements. This enthalpy effect (ΔH_c) is small compared to the total effects (ΔH_T) for the cellulose-lye-reaction. We define ΔH_c as composed of two contributions, one ΔH_{c1} from the lattice transition *per se* (an interchain reaction) and one ΔH_{c2} from the reaction of the phases with water, but for our purpose it is not even necessary to know the sign of these two contributions. We have

$$\Delta H_c = \Delta H_{c1} + \Delta H_{c2} \tag{2}$$

From the measurements discussed it is concluded that the transformation of wet native to wet mercerised cellulose is an exothermic process with a small enthalpy change ($\Delta H \sim -2$ cal/g cellulose).

VII. ENTROPY EFFECTS

No absolute entropy values of cellulose have been measured or calculated so far. The entropy effects in the reaction of cellulose with water and other vapors have been studied, reviews have been made by Valko [38], and Rees [39], and papers have been published recently by Wahba [40], and Guthrie [41]. Nothing seems to be written on the entropy effects of mercerisation.

If a more accurate picture of the morphology and structure of native and mercerised cellulose, than at present, were available, it would be possible to calculate the entropy of mercerisation theoretically using statistical thermodynamics. Such calculations could be performed by analogy with those for high polymers (*cf.* Bawn [42] for a review). In our case, however, it is sufficient to find out, if the entropy of cellulose increases or decreases on mercerising.

As reviewed in sections 3, 4, and 5, mercerising definitely causes a lower absolute crystallinity, *i.e.* a decrease in the lateral order of the chains, as indicated from measurements with physical and chemical methods. The density is lowered and the crystalline regions (the micelles) are partly divided into fragments. The orientation of the micelles parallel to the fibre axis decreases upon mercerisation without tension [43]. All these measured effects indicate a transformation of the cellulose into a state of greater disorder and consequently of a higher probability, *i.e.*, an increase in the entropy function most probably occurs at room temperature ($\Delta S_{merc.} > 0$). This must be the case both for dry and wet cellulose, according to the crystallinity and accessibility measurements, made for these two states.*

VIII. THERMODYNAMIC INTERPRETATION

Mercerisation of cellulose with caustic soda is possible to perform as an adiabatic process, if the temperature does not exceed 50—60° C [*Cf.* 4, p. 274]. The ΔH-measurements are carried out under such conditions [35, 36] within a small temperature interval. As the entropy function increases in the reaction (ΔS positive according to Sect. 7), it follows that the adiabatic mercerisation is a spontaneous (natural) process.

For an isothermal reaction the following relation holds according to the definitions of the symbols:

$$\Delta G = \Delta H - T\Delta S \tag{3}$$

To calculate the ΔG for the isothermal transformation of wet native to wet mercerised cellulose fibres it is necessary to follow a reversible way from the initial (native) to the final (mercerised) state. Such a calculation is not possible from the data presented in Sects. 6 and 7, as the values of ΔH and ΔS

* The entropy discussion is strictly valid only if the mercerisation leaves the cellulose chemically unchanged. A slight depolymerisation of the chains (comp. sect. 6) is the only chemical effect known. As entropy is primarily a function of the order of the chain segments (glucose units) it cannot be affected very much by the breaking of a few bonds (the order of magnitude is one broken bond per 500—1 000 glucose units).

have been determined for the spontaneous, adiabatic process only. Additional information, for instance of the heat capacity for the wet phases, which is necessary for the calculation of ΔG according to equ. (3), is not yet available.

From this discussion it is concluded, that the transformation of wet native to wet mercerised cellulose is a natural process at room temperature and atmospheric pressure and that wet native cellulose is in a metastable state under these conditions.

Mercerisation causes a decrease in the total energy (ΔU) of the cellulose, too. Because of the small volume change upon mercerisation (Sect. 4), ΔU — at atmospheric pressure — is practically identical with the decrease in enthalpy (ΔH).

As already pointed out (Sect. 2) this thermodynamic discussion is valid only for the mercerisation of wet cellulose fibres assumed to contain both crystalline and non-crystalline substances. So far it is not possible to treat the transition of the pure lattices of *Celluse I→II*.

IX. STABILITY OF NATIVE AND MERCERISED CELLULOSES

The conclusion that wet native cellulose is in a metastable and wet mercerised in a stable state at room temperature is already verified by experiment. Hess, Kiessig, and Gundermann [44] observed that bone-dry cellulose fibres, treated in a ball-mill for several hours, lost their lattice order completely. When these fibres were treated with water at room temperature, they re-formed a lattice, which was invariably *Cellulose II*, even if the original cellulose was native. These findings are quite analogous to the well established fact that an intramicellar swollen cellulose (with caustic soda solutions, organic bases, cuprammonium, strong sulphuric acid or zinc chloride solutions) on deswelling at room temperature always recrystallises with the lattice of *Cellulose II*. Mercerised cellulose, however, doesn't seem to be stable at higher temperatures. It has been shown [45], that high temperature treatment (250° C) of regenerated cellulose fibres (containing the lattice of *Cellulose II*) with glycerine, glycol, formamid or other liquids of strong polarity causes a transition to *Cellulose I*. The transition was more complete, if the fibres were pre-swollen as alkali-cellulose. Kubo's results are supported by Hermans' observations [46], that saponification of cellulose acetate in 2 *N* ammonia solution at 100° C is accompanied by a partial transformation into native cellulose. No transformation into mercerised cellulose at high temperature is described in the literature. Some confusion exists on the true nature of the cellulose lattice regenerated from *Cellulose II* at high temperature. Hess, Kiessig and Gundermann [44]

hold the view that a new lattice, *Cellulose IV*, high temperature (HT) cellulose, and not *Cellulose I* is formed by heating in glycerine. The lattice of *Cellulose IV* is reported to resemble that of *Cellulose I* very closely, the a and c axes are vertical and the unit cell accordingly rhombic, but the X-ray diagrams published to support the two views show too low a resolving power for a definite conclusion. Further experiments should be made on this point, as the problem is also of thermodynamic interest. From his experiments, Kubo [45] assumes that the stability of *Celluloses I and II* at different temperatures follows approximately the diagram of Fig. 7 (the ordinate should be the Gibbs free energy (G), not the "stability" of Kubo). If native cellulose is really stable and mercerised cellulose really metastable at high temperature, the curves ought to intersect at an intermediate temperature, at which $\Delta G = 0$ and $\Delta H = T \Delta S$ for a lattice transition.

Conversion experiments of mercerised to native cellulose have also been performed by Meyer and Badenhuizen [48]. They obtained a partial conversion through heating to 200—300° C with water, too. Their conclusion was that "at least over the range of temperatures covered by these experiments native cellulose is the stable form and hydrate cellulose the non-stable". The velocity of transformation, however, was assumed to be very low (not measureable) at low temperatures. These views have obviously no support from later measurements (Sect. 2—7) and their thermodynamic interpretation (Sect. 8, 9) and are then to be abandoned.

X. DISCUSSION

Work on the lattice transformation of cellulose is of direct importance as the change in fibre properties on mercerising, for example, the penetration and regain of water, accessibility to dyestuffs, and chemical reagents, are due to the phenomenon. It is of an indirect importance in many respects, too. Without a doubt, investigations of the lattice transition *per se* has increased the general knowledge of cellulose, its properties and reactions. Mercerisation is still the best evidence of an intra-micellar swelling of native cellulose. Its thermodynamic interpretation as an exothermic, natural process explains among other things the negative temperature coefficient of the alkali swelling of cellulose.

Furthermore, the thermodynamic conditions of lattice stability seem of great importance for a proper understanding of the formation of cellulose in nature. As already pointed out, cellulose is always formed with the lattice of *Cellulose I*, *i.e.*, in a metastable state. This is true for fibre cellulose in plants and for animal cellulose (tunicin) as well as bacterial cellulose, formed extracellularly as water-swollen gels. Only one exception is known (comp. a note to Sect. 1). Many authors have assumed that cellulose is synthesised as chains,

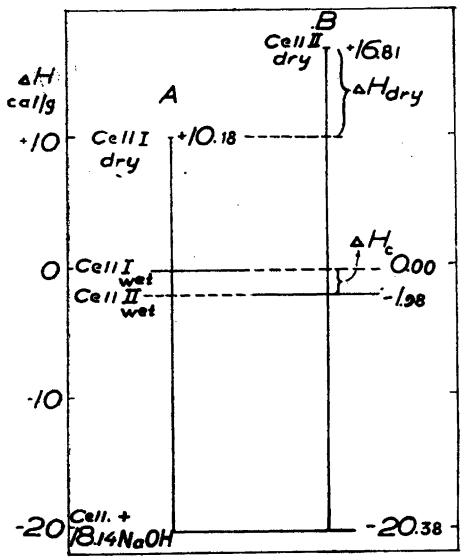


Fig. 6. The enthalpy effects (ΔH) on wetting and soaking native (A) and mercerised (B) cellulose in 18.14 NaOH; after measurements of Morrison, Boyd Campbell and Maass [36].

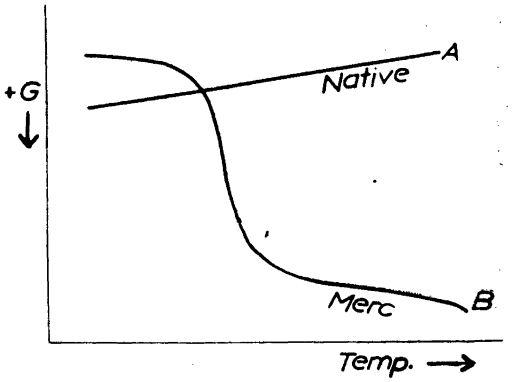


Fig. 7. Hypothetical diagram after Kubo [45] *of the Gibbs free energy of native and mercerised cellulose at different temperatures.*

which should aggregate and partly crystallize, forming a micellar framework [47]. Such a conception is doubtful for the following reasons. A crystallization of free chains or a deswelling of intra-micellar swollen cellulose at room temperature in the presence of water *in vitro* invariably and rapidly leads to a cellulose containing the lattice *Cellulose II*. This is quite reasonable from a thermodynamic point of view (Sect. 10). On the contrary, an assumed crystallization of chains *in vivo* must proceed in quite another way as it apparently leads to a cellulose, containing the lattice *Cellulose I*, *i.e.*, to a metastable state, never observed to be formed *in vitro* in the presence of water at room temperature. This fact makes the assumption of a crystallization of free chains in the water-rich system of the living cell very improbable. It is much more reasonable, then, to assume that the lattice of the native micelles (*Cellulose I*) is formed from chains under influence of a specific enzyme system or by a direct synthesis in the cell [28,49]. These assumptions on the formation of cellulose are also made probable by the fact that the micelles and the micelle strings have a uniform width (70 ± 15 Å) according to measurements on electron micrographs *Cf.* [20, 28].

SUMMARY

1. Based on published data, the enthalpy (ΔH) and entropy (ΔS) effects upon mercerisation of cellulose have been discussed and a thermodynamic interpretation of the mercerising has been proposed.

2. If the author's views (p. 110) are accepted and applied on available data, the conversion of wet native to wet mercerised cellulose is an exothermic (ΔH negative) and natural (ΔS positive) process.

3. The stability of native and mercerised cellulose at different temperatures, conversion experiments *in vitro* and the synthesis of cellulose *in vivo* have been discussed in connexion with the thermodynamic interpretation.

This investigation is part of a research programme on cellulose carried out at the Institute of Physical Chemistry, University of Uppsala, under supervision of Professor The Svedberg and in collaboration with the Swedish cellulose firms *Billeruds AB*, *Mo och Domsjö AB*, *Stora Kopparbergs Bergslags AB*, *Svenska Cellulosa AB* and *Uddeholms AB*.

The author is indebted to Professor The Svedberg for his interest in the work and to Professor Stig Claesson, Docent Ingvar Hedlund, Fil.mag. Gunnar Jacobsson, Docent Ingvar Jullander, and Docent Olle Snellman for many helpful suggestions in the preparation of the manuscript.

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The Mercerisation of Cellulose

II. A Phase Transition Study with X-Ray Diffraction

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Reference has already been made to earlier work on the mercerisation of cellulose with sodium hydroxide solutions in a preceding paper [1]. The process as a phase transition problem — the transition of *Cellulose I* to *Cellulose II* — has been investigated mainly with gravimetric, titrimetric, and X-ray methods. The gravimetric and titrimetric methods include measurements of the swelling of the fibres or the caustic soda uptake by the cellulose and the sorption of water on the cellulose before and after its transition (*cf.* a review by Valko [2]). Methods of this kind are indirect since they are based on the assumption that the quantities measured are related to the phase transition. X-ray diffraction, however, is a direct and absolute method. It is used throughout in this investigation. In a following paper (Part III) an analogous study using electron diffraction will be described.

The mercerisation of cellulose is of interest as a well-defined phase transition. A thermodynamic interpretation of the process has been proposed in a preceding paper [1]. The reaction of cellulose with lye has been studied extensively, not only in connexion with mercerisation [3]. Furthermore, it is well known that cellulose samples of different origin require different minimum caustic soda concentrations for mercerisation [4], but the reason has not been explained.

The main purpose of this investigation is to study the mercerisation of cotton and wood cellulose and thus obtain information on the transition mechanism and the possible difference in fine structure between these celluloses. This study is based mainly on the results obtained by Calkin [5], Sisson and Saner [6], and Katz and Seiberlich [4]. Parts of Katz and Seiberlich's results were supported by Jörgensen and Ribi [7]. During the course of the work, papers by Woods *et al.*[8] on the mercerisation of bast fibres were published.

EXPERIMENTAL

Cellulose samples. For this study three cotton and three wood cellulose samples were used.

A. *Egyptian cotton* (supplied by Borås Väfweri AB., Borås, Sweden, as raw cotton, free from dark particles), boiled for 6 hours with 2 per cent caustic soda in an atmosphere of nitrogen, washed free from alkali and extracted in a Soxhlet apparatus with ethanol, benzene, and ether (for 6 hours each). Total loss in weight 9 per cent.

B. *Linters cellulose* (from British Bemberg Ltd., Doncaster, England), used for cuprammonium rayon production, bleached, α-cellulose 98.9 per cent, pentosan 0.5 per cent, viscosity (1 per cent cellulose in cuprammonium, Tappi 206 m) 62 cp.

C. *Linters cellulose* (from AKU, Arnhem, Holland), used for viscose rayon production, bleached, α-cellulose 98.3 per cent, pentosan 0.4 per cent, viscosity 17.1 cp (Tappi 206 m).

D. *Holocellulose* prepared from spruce chips with sodium chlorite solution (pH = 4.2, HAc—NaAc-buffer, 60° C, three treatments, each followed by washing with warm and cold water), yield 67.0 per cent of the wood (extracted with alcohol and benzene), pentosan 8.6 per cent, practically no lignin *).

E. *Sulphate pulp* (produced from pine), prehydrolysed and bleached, used for viscose rayon production, α-cellulose 93.4 per cent, pentosan 3.2 per cent, viscosity 14.4 cp (Tappi 206 m).

F. *Sulphite pulp* (SSS, produced from spruce by Billeruds AB., Säffle, Sweden), bleached and hot alkali refined acetylation grade pulp, α-cellulose 95.5 per cent, pentosan 1.2 per cent, viscosity 20.5 cp (Tappi 206 m).

These samples were used both in their original state and after hydrolysis with boiling dilute sulphuric acid (2.5 N H_2SO_4, 11.4 per cent). The purpose of the hydrolysis was to depolymerise the cellulose and break up the fibers into a powder. In the first experiments, hydrolysis was continued for only short lengths of time (2 min. and 30 min.), followed by treatment with caustic soda and washing out the lye on glass filters in the usual way. This method proved impractical since 50 per cent or more of the hydrolysed cellulose was lost in the alkali treatment. When the sodium hydroxide was removed by dialysis through cellophane against distilled water, only a few per cent were lost even after prolonged hydrolysis. Only the experiments with samples hydrolysed for 4 hours and dialysed after the caustic soda treatment have been described in this paper. The hydrolysed samples have been denoted AH, BH, CH, EH, and FH. No hydrolysed sample was prepared from the holocellulose (D) as the amounts of the substance available were too small.

The degree of polymerisation of nitrates of these samples has been determined viscosimetrically in acetone solution using a capillary viscosimeter. The nitrogen contents were determined by the Dumas' method. The K_m-constants at the measured N-contents are according to Jullander (ref. 9, Table 36, p. 101). The results have been collected in Table 1.

Alkali treatment. Solutions of sodium hydroxide (EKA, analytical grade) were prepared in concentrations ranging from 4 to 16 per cent by weight, in increments of about one half per cent (determined by titration with methyl red — methylene blue as indicator).

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* The author is indebted to Mr. H. W. Giertz, Swedish Forest Products Research Laboratory, Stockholm, Sweden, for his kindness in supplying the holocellulose sample.

Table 1. Intrinsic viscosity [η], nitrogen content, and degree of polymerisation (Z) for the nitrates of samples A—F.

Sample	[η]	N-content per cent	$K_m \cdot 10^3$	Z
A	32.1	13.55	9.4	3 410
AH	1.45	13.35	8.9	163
B	16.3	13.82	10.5	1 550
BH	1.49	13.92	11.4	131
C	9.1	13.45	9.1	1 000
CH	1.45	13.45	9.1	159
D	17.7	12.87	7.7	2 300
E	8.0	13.79	10.4	770
EH	1.19	13.70	10.0	119
F	10.5	13.82	10.5	1 000
FH	1.38	13.71	10.0	138

Data of viscometer: length of capillary 9.0 cm, volume of bulb 1.0 ml, and time of flow for pure acetone 119 sec. The [η]-values have been extrapolated from ($\log\eta_{sp}$/c,c)-diagrams by straight lines. c is in g/100 ml.

As Sørensen lye (50 %) and fresh-boiled distilled water were used, the carbonate concentrations were lower than 0.5 per cent of the caustic soda. The treatments were performed in Erlenmeyer flasks in an ice bath for 1 hour with 1 g (or 0.5 g) of air-dry cellulose in about 30 ml of lye. When equilibrium was reached (10—15 min.), the cellulose-lye suspension had a temperature of 0.1—0.2 ° C. The treatment was stopped by introducing double the amount of ice-cold water, sucking off the dilute lye on glass filter (Jena G 2) and washing with distilled water, 2 per cent acetic acid, and again with distilled water until neutral reaction.

In concentrations at which mercerisation occurs the hydrolysed celluloses form fairly homogeneous and clear, viscous suspensions. It has already been mentioned that it was not possible in these cases to remove the lye by filtration. When the samples had been diluted with pre-cooled, distilled water to a concentration lower than 4 per cent, most of the lye was removed by dialysis through cellophane membranes into distilled water (one day), and the residue removed by centrifugation with distilled water, 2 per cent acetic acid and distilled water until neutral reaction. The water was then removed by freeze-drying to prevent lump formation.

Sorption measurements. The sorption of water was measured for all samples in an atmosphere of 65 % relative humidity (in a desiccator over 36.3 per cent sulphuric acid by weight, according to [10]). The measurements were always made on the adsorption isotherm, starting with samples in equilibrium with the laboratory air (about 40 per cent R. H.). When equilibrium at 65 per cent R. H. was reached (3—5 days), the samples were dried at 105° C to constant weight (3—6 hours).

X-Ray diffraction. The air-dried samples were analysed by X-ray diffraction (Copper Kα-radiation, filtered through Nickel folium, $\lambda = 1.54$ Å, from a Philips' fused tube) using a Debye-Scherrer powder camera (radius = 6.5 cm) with pressed rods of the samples (without glass capillary tubes) and of a diameter of 0.75 mm and containing 0.40 ± 0.05 mg cellulose per mm length. The exposures were all two hours with the same

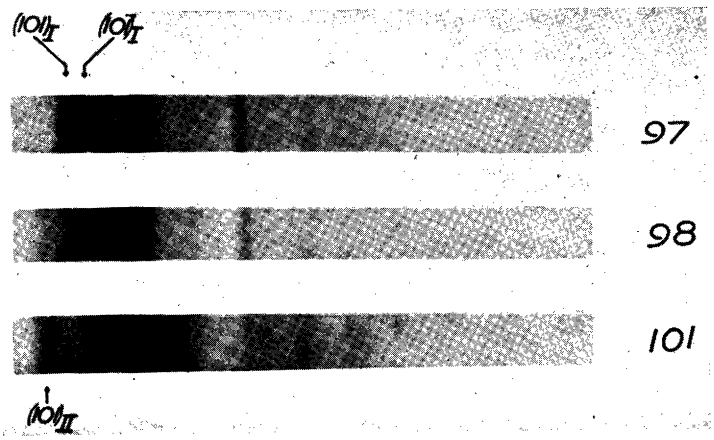


Fig. 1. X-ray diffraction diagrams (Debye-Scherrer) of cotton cellulose (sample C), treated with 7.74 (97), 8.85 (98), and 11.75 (101) per cent NaOH. No. 98 contains both patterns (I and II).

X-ray intensity (~ 18 mA, 40 kV) and the film (Kodak Ultra-Speed, Code 3) developed for 5 mins. at 20—22° C in the same developer (Kodak, X-Ray). To demonstrate the resolving power obtained, the diffraction diagrams of native, partly mercerised and mercerised cotton linters (sample C) are shown as positive prints in Fig. 1 and the corresponding photometer curves in Fig. 2. Due to the strong back-ground of the Debye-Scherrer diagrams, it was found necessary to use a wide slit in the photometer, giving poor resolution in the curves. In this paper the term diffraction "pattern" refers to a particular pure component (*Cellulose I* or *II*) while the term diffraction "diagram" refers to a given specimen, containing one or more components. Neither the high tension nor the emission current of the X-ray tube were stabilized. Adjustments of the heating current were made occassionally during the exposures to maintain the emission current at 18 ± 1 mA. Variations as high as 2 mA were sometimes observed. The high tension transformer was kept at the same setting (40 kV) during the experiments.

Control experiments for some samples (comp. Figs. 7 and 8) were made using Guinier powder cameras, built by Prof. G. Hägg (Cu Kα-radiation, $\lambda = 1.5387$ Å, made monochromatic and focussed with a bent quartz crystal). The cameras were calibrated using sodium chloride.

RESULTS

Yield after the alkali treatment. Part of the substance is dissolved in the sodium hydroxide solutions. The yield has been determined at three different lye concentrations (below mercerising, partly mercerising, and mercerising) with the samples B, C, E, and F. The results calculated for samples dried at 105° C have been collected in Table 2.

The hydrolysed samples, from which the lye was removed by dialysis through cellophane, gave yield values which are not comparable to those of

Table 2. Yield after treatment with sodium hydroxide solutions.

Sample	Caustic conc. per cent	Yield per cent	Sample	Caustic conc. per cent	Yield per cent
	7.0	96.4		5.0	97.4
B	9.0	96.2	E	7.0	91.1
	12.0	96.6		10.0	93.7
	7.0	96.0		5.0	94.7
C	9.0	88.9	F	7.0	91.2
	12.0	91.0		10.0	86.2

the original samples (Table 2). The values were quite high — 95—98 per cent for sample BH and CH and 93—97 per cent for EH with 10 and 7 per cent lye respectively — and rather insignificant because losses were made due to adsorption on the cellophane.

The samples were used for the further sorption and X-ray studies without introducing any corrections for the losses (Table 2). Such corrections are doubtful since both low molecular cellulose and impurities as other carbohydrates are dissolved in the alkali treatment.

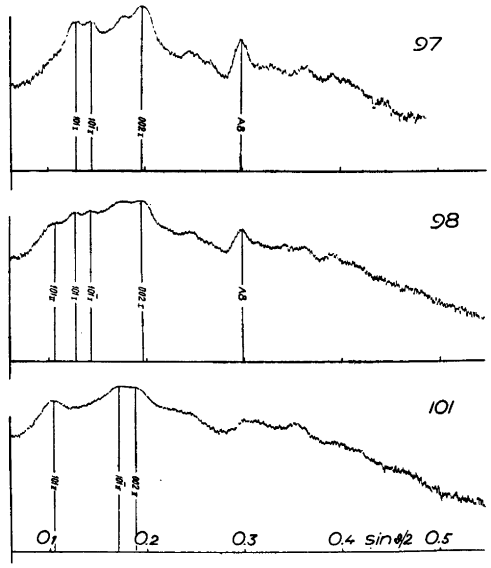
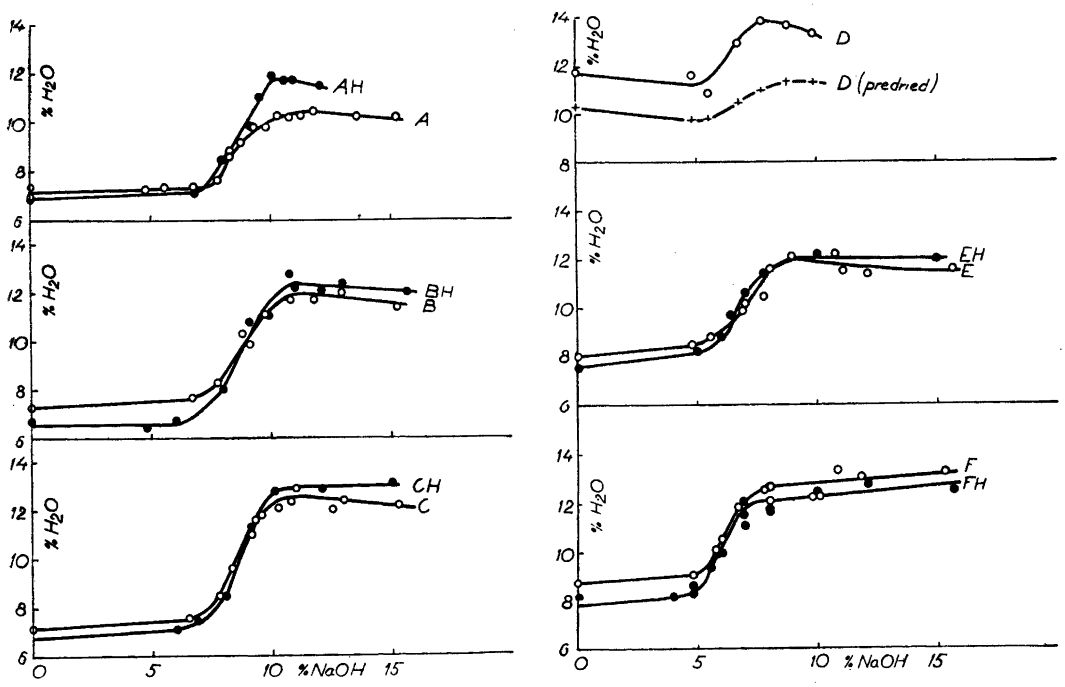


Fig. 2. Photometer curves of the diagrams of Fig. 1.



Figs. 3 and 4. Sorption of water on cellulose samples (in per cent of the dry cellulose), treated with different NaOH-concentrations. The holocellulose sample (D) was investigated also after drying at 105° C.

Sorption measurements. The sorption of water at 65 per cent relative humidity (over 36.3 per cent sulphuric acid) for the different samples has been plotted in Figs. 3 and 4. Such sorption curves have a typical shape with one sharp sorption increase within a limited NaOH-range, starting at 7 and ending at 10—11 per cent sodium hydroxide for cotton celluloses (Fig. 3) and at about 2 per cent lower NaOH-values for the corresponding wood celluloses (Fig. 4).

Sample D (holocellulose) has an abnormal water sorption curve, because of its high content of hemicellulose, partly extracted with increasing NaOH-concentration. It is characteristic that the rapid sorption increase starts at 7.0 $\pm$ 0.5 per cent NaOH for the cotton cellulose and at 5.0 $\pm$ 0.5 per cent NaOH for the wood cellulose, both original and hydrolysed samples.

X-Ray diffraction analysis. In the X-ray analysis, diffraction diagrams were obtained, the general type of which is shown in Figs. 1 and 2. The hydrolysed samples showed similar diffraction diagrams during the transition. Photometer curves of the diagrams for a hydrolysed wood cellulose (sample FH)

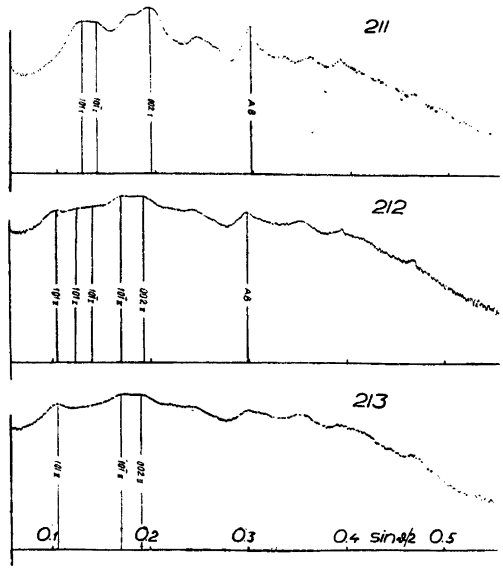


Fig. 5. Photometer curves of diffraction diagrams (Debye-Scherrer). Hydrolysed wood cellulose (sample FH), treated with 5.50 (211), 6.92 (212), and 8.00 (213) per cent NaOH.

are plotted in Fig. 5. As a general rule, mercerised samples give diagrams with stronger back-ground and more diffuse reflections than native samples.

A quantitative estimate of the amount of *Cellulose I* and *II* in the diffraction diagrams was obtained by visual comparison of the intensity of the reflections 101 and $10\bar{1}$ of pattern I with that of 101 of pattern II (Figs. 2 and 5). For pure native and mercerised cellulose respectively, the ratio of the intensities is 2 : 1. It was possible, after some practice, to estimate the relative intensities of these three reflections using a scale with ten intervals. When the reflection 101 of pattern II first appeared visible, its intensity was estimated to one tenth or one twentieth of the 101 and $10\bar{1}$ of I.

It is impossible, without the help of a separate investigation of the phase transition using an independent method, to assume a simple proportionality between the intensity of the X-ray patterns and the relative amounts of the phases. In this case a relative intensity number I_r was defined

$$I_r = \frac{2\,I_{II}}{I_I + 2\,I_{II}}$$

where I_I and I_{II} ($I_I + I_{II} = 1.0$) are the estimated intensities of the reflections mentioned. I_r is 0.0 for a pure native cellulose sample and 1.0 for a pure mercerised sample. Intermediate I_r-values indicate partial mercerisation.

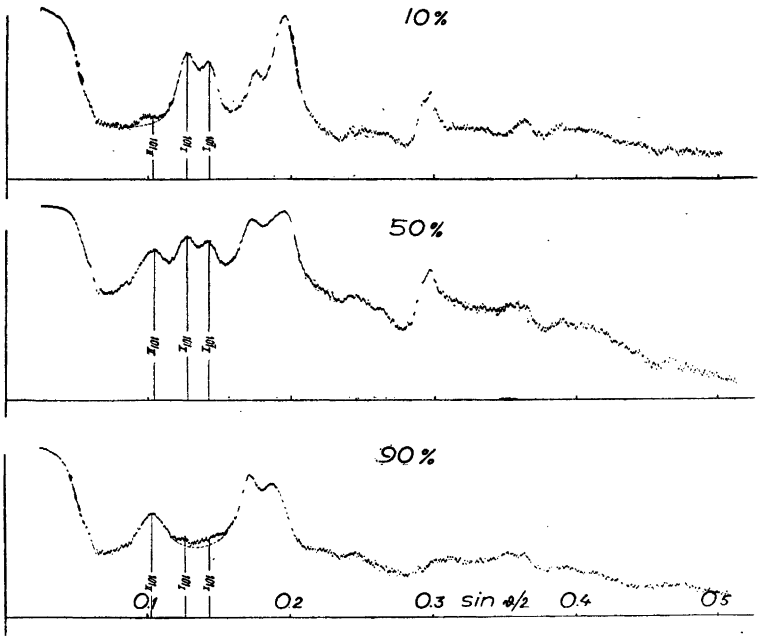
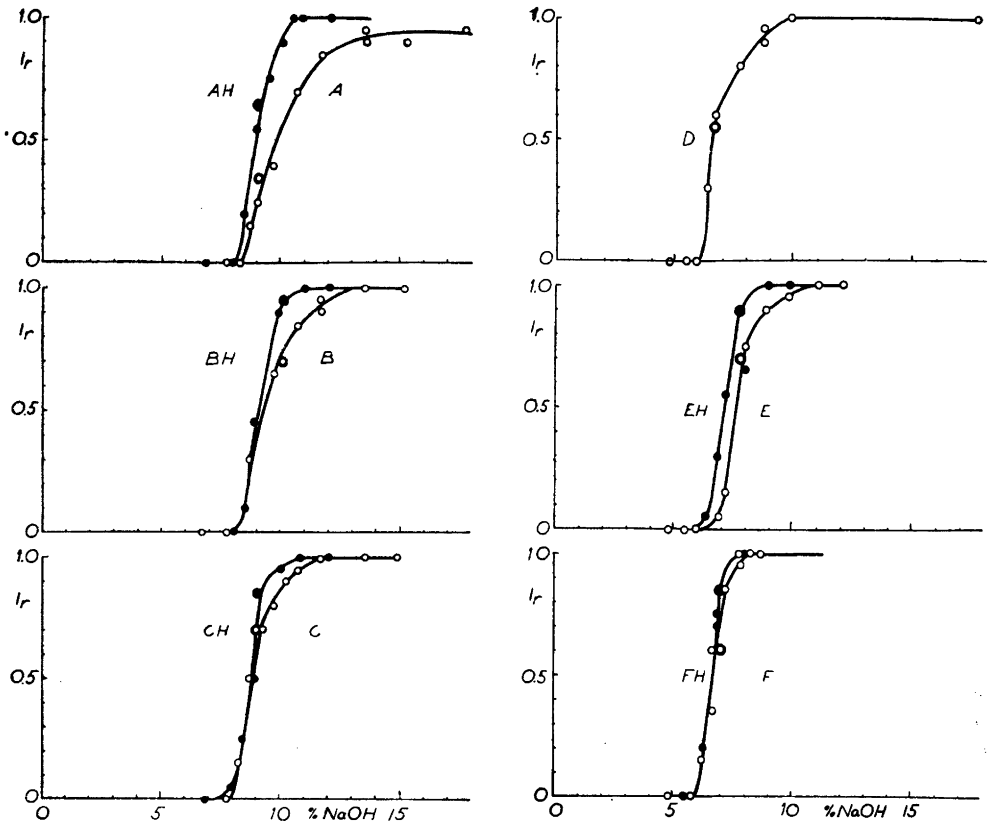


Fig. 6. Photometer curves of diffraction diagrams for hydrolysed cotton (CH), containing 10, 50, and 90 per cent mercerised cellulose respectively (mixed samples). Guinier powder camera G 2, exposed for 6 hours (45 kV, 25 mA), total amounts of cellulose 95.2, 109.8, and 108.1 mg resp.

Test experiments were made using samples containing known amounts of native and mercerised cellulose (mixtures of sample CH, original and treated with 12.0 per cent NaOH). The photometer curves of Guinier diagrams for samples with 10, 50 and 90 per cent mercerised cellulose are shown in Fig. 6. The estimated I_r-values were 0.1, 0.55 and 0.90—0.95 respectively. Analogous results were obtained using the Debye-Scherrer powder camera.

The relative intensity numbers I_r as a function of the NaOH-concentration have been plotted in the diagrams of Figs. 7 and 8. The phase transition starts at 8.0—8.5 per cent for the cotton celluloses (A, B, C) and at 6.0—6.5 for the wood celluloses (D and F). The influence of hydrolysis is very slight upon the NaOH-range which initiates transition (comp. the H-samples), but promotes the completion of the transition within a short interval. This effect is very marked for sample A, which is not completely mercerised even with 17.8 per cent NaOH and is still considerable for sample B and E, but small for sample C and F.



Figs. 7 and 8. The transition of Cellulose I → II for cotton and wood cellulose samples as I_r-values vs. concentration of NaOH in per cent. Double points refer to Guinier exposures (control).

The transition properties are given in Table 3. The I_r-value 0.1 is reached at 8.3—8.7 per cent NaOH for the cotton and 6.3—6.6 per cent for the wood celluloses with the exception of sample E (not hydrolysed) with 7.1 per cent. The transition intervals are in general agreement with earlier measurements [4,6,7].

DISCUSSION

The increase in water sorption is not a simple function of the phase transition as can be concluded from the X-ray diagrams. The rapid increase starts at about 1 per cent lower NaOH-concentration than the mercerisation. In fact the increase in water sorption is about 25 per cent of the total increase

Table 3. Transition intervals (in per cent NaOH) for the cotton and wood celluloses.

Sample	Concentration of caustic soda in per cent giving the I_r-values		
	0.10	0.50	0.90
A	8.7	9.8	12.6
AH	8.4	9.0	10.0
B	8.4	9.4	11.3
BH	8.5	9.2	9.9
C	8.3	8.9	10.3
CH	8.3	8.9	9.5
D	6.3	6.6	8.5
E	7.1	7.6	9.0
EH	6.6	7.2	7.8
F	6.3	6.8	7.4
FH	6.3	6.7	7.1

when mercerisation is first observed in the X-ray diagrams. Furthermore 50 per cent increase does not correspond to an I_r-value 0.5; the difference is 0.5 —1.5 per cent NaOH according to the diagrams of Figs. 3, 4, 7, and 8.

It is not possible to interpret the sorption phenomenon quantitatively. For native cellulose, the sorption is probably a surface phenomenon, as no effect on the lattice parameters is observed. It is not known, if the increase in water sorption, before transition is observed, is caused by a swelling of the fibres which results in larger surfaces of the micelle strings becoming available, or by a reaction on these surfaces. For a mercerised cellulose, water is both adsorbed on the surfaces of the micelle strings and absorbed into the lattice. Unbleached cotton (sample A), which is not completely mercerised, has a low water sorption.

The difference between the original and the hydrolysed celluloses is of the same kind for all samples: it does not affect the start, but it favours the completion of the mercerisation within a short range of NaOH-concentration. The sulphate pulp, sample E, is an exception. Its transition starts with 0.5—1.0 per cent higher caustic concentration. After hydrolysis, which breaks the fibres down into a powder, the transition interval is very similar to the other wood cellulose samples.

The transition mechanism is now assumed to be the following: the caustic penetrates (swells) the native cellulose lattice partially or completely, forming alkali-cellulose, which gives the mercerised lattice, when the alkali is washed out at room temperature [Cf. 5 and 11]. If the formation of alkali-cellulose is incomplete, due to bad penetration of the lye or restricted swelling of the fibres, the mercerisation is partial. If these views are accepted, it seems rea-

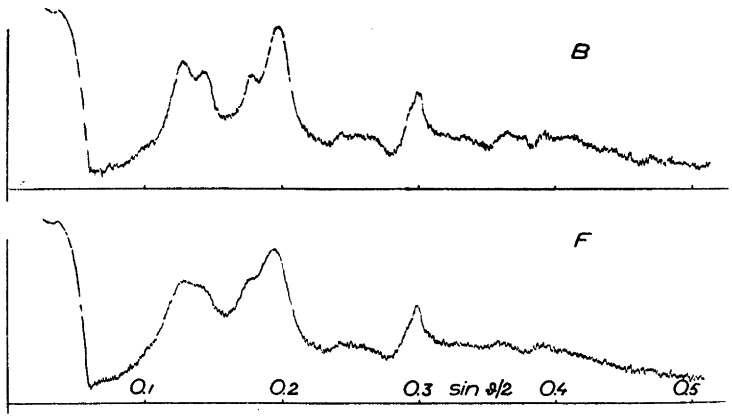


Fig. 9. Photometer curves of diffraction diagrams (Guinier) for cotton (B) and wood (F) celluloses. Powder camera G 1, exposed for 6 hours, amounts of cellulose 13.9, and 15.8 mg resp.

sonable to assume that the start of the transition is related mainly to the reacting structure elements, *i.e.* the micelles and the micelle strings, while the completion can be hindered by the packing of the elements, *i.e.* the fibre structure. An interpretation along these lines is in agreement with the experiments described in this paper.

The difference between the reacting elements of wood and cotton cellulose is considerable and of fundamental nature: the transition intervals of the samples C and F hardly overlap. It seems reasonable to relate the NaOH-concentration necessary for transition with the water sorption of the original native fibres: both the initiation of the transition and the sorption of water implies a reaction with the hydroxyl groups on the surface of the micelle strings. A cellulose with a transition interval at low NaOH-concentrations is then expected to have a high water sorption. That is true in this case, in agreement with earlier sorption measurements [12]. The three cotton samples (A, B, and C) have a water sorption (in equilibrium with air of 65 per cent relative humidity) of 7.50, 7.35 and 7.16 per cent respectively and the wood cellulose samples (E and F) 8.30 and 8.24 per cent. In connexion with other investigations, it is found that the micelle strings of wood cellulose are 10—20 Å thinner than those of cotton cellulose as measured on electron micrographs.[13] Furthermore, the X-ray diffraction pattern of pure native cotton cellulose (sample B) has sharper and more narrow reflections than pure native wood cellulose (sample F, Fig. 9). All these facts indicate that native wood cellulose has a larger active surface than cotton cellulose. So far it is not possible to decide whether this is an effect of both the dimension and the lattice order of the micelle strings.

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The author is much indebted to Professor The Svedberg for his stimulating interest in this work and to Professor Stig Claesson, the present head of the Institute, for facilities put at the author's disposal.

Most of the Debye-Scherrer X-ray exposures and the sorption measurements were performed by Mrs. Els Pärkma and Mr. Sigvard Eriksson.

The Guinier X-ray photographs were made in the Chemical Institute of the University with the kind permission of Professor Gunnar Hägg, to whom the author is very grateful.

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The Mercerisation of Cellulose

III. A Phase Transition Study using Electron Diffraction

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Electron diffraction has been applied as a tool for structure investigation mainly in three fields: a) the molecular structure of gases and vapours, b) the structure of thin films and surfaces and c) the structure of polycrystalline materials.

Brockway [1] and Glasstone [2] have reviewed the first field, and Finch, Quarrell, and Wilman [3] the second. Applications in the third field are scarcer, since most of the work on the structure of small crystals is done using X-rays (powder diagrams). However, fast electron beams offer important possibilities, which have as yet not been fully realized. The scattering of fast electrons is due chiefly to the interaction of the electrons and the electrostatic fields of the atomic nuclei. X-rays are scattered mainly by the extra-nuclear electrons of the atoms. Furthermore, the angle of incidence tolerance which is only a few minutes for X-rays, is several degrees for the Bragg reflections of the electrons. The resulting diffracted electron beams are intense even from a crystalline layer containing few lattice planes. The diffracted intensity is usually sufficient to produce visible patterns on a fluorescent screen, allowing consequently short exposures (generally less than one second) on normal photographic plates. The significant factor in the study of surface structures using fast electrons is the tremendous power of even small amounts of material in scattering the electrons. This is also important in the analysis of small crystals containing light atoms. The strong scattering decreases the penetration, however, which restricts the use of crystals of larger dimensions and aggregates from study with fast electrons.

I. CALIBRATION EXPERIMENTS

According to de Broglie's wave theory, the wave length λ of charged particles is related to the accelerating potential V by

$$\lambda = h / \sqrt{2\ m\ e\ V} \tag{1}$$

For electrons, with the numerical values of charge (e), and mass (m_0) according to Birge [4], the relation is (λ in Å, and V in Volts)

$$V = 150.0/\lambda^2 \tag{2}$$

No corrections according to relativity mechanics are introduced in equ. (2).

According to the diffraction theory the conditions for ordinary Bragg reflections are

$$n\lambda = 2\ d\ \sin\ \Theta/2 \tag{3}$$

where n is an integer, d the distance between the lattice planes, and $\Theta/2$ the angle which the incident or diffracted beam makes with the lattice planes. The electron diffraction diagram of a polycrystalline substance forms a system of concentric rings on the screen or the plate. The mean radius R of the rings corresponds to the angle Θ between the primary (incident) and the diffracted beam. For $n = 1$ (first order) and L the distance from specimen to plate equ. (3) is transformed and approximated into

$$\lambda = 2\ d\ \sin\ \Theta/2 = 2\ d \cdot \mathrm{tg}\ \Theta/2 \cdot \cos\ \Theta/2 \simeq$$

$$\simeq 2\ d\ \frac{R}{2\ L} \cdot \cos\ \Theta/2 \simeq d \cdot \frac{R}{L} \tag{4}$$

As the distance L is about 60 cm and the ring radius never exceeds 2 cm in the following experiments, the maximum approximation in equ. (4) is 0.015 per cent.

The wave length λ of the electron beam was determined from the diffraction diagrams of substances containing known lattices.

The electron diffraction exposures were made using a Siemens electron microscope (Type 1939 Ü M 100, hot cathode) with the diaphragm of the objective lens and the pole pieces of the projection lens removed. The high tension transformer was kept at a constant setting (P 3, $\sim$ 100 kV) and the emission current as low as possible ($\sim$ 0.03 mA). The distance between specimen and photographic plate was 58.20 cm within 0.02—0.03

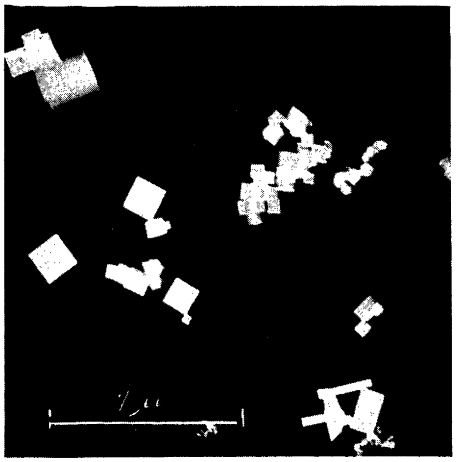


Fig. 1. Electron micrograph of magnesium oxide smoke, shadowed with Gold-Manganese (Magnification 25 500 ×). Exp. by G. Eklund.

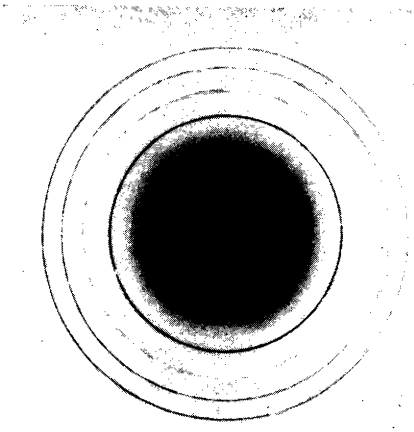
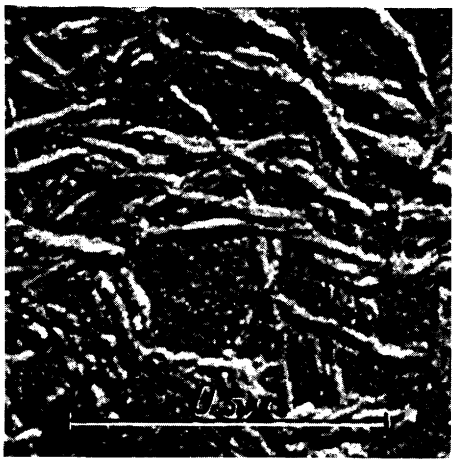
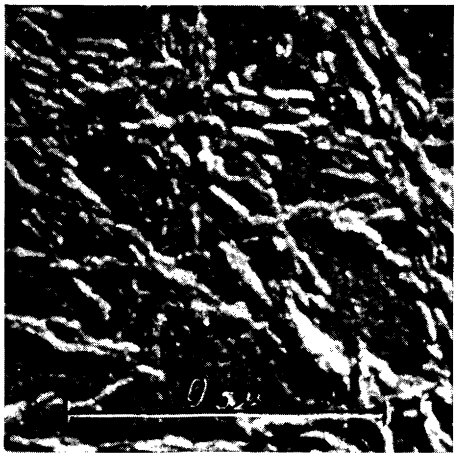


Fig. 2. Electron diffraction diagram of magnesium oxide (original size). The (220) — ring has the diameter 3.1 cm.

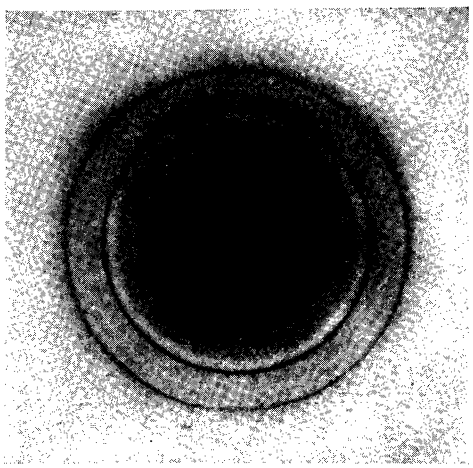
cm. A special type of specimen holder was used: circular discs of Platinum foil (0.10 or 0.15 mm) with one hole (diameter 0.15—0.20 mm) drilled in the center. The specimen holders were covered with a thin carrier membrane (zapon laquer) cast on a water surface in the usual way.

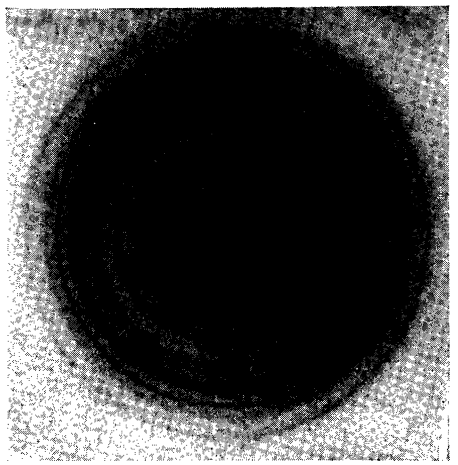
Two reference substances were used: *magnesium oxide* (MgO, prepared directly on the carrier membranes as a fine-grain powder from magnesium oxide smoke), and *graphite*





Figs. 3 and 4. Electron micrographs of diffraction specimens (micelles) from native and mercerised wood cellulose, shadowed. (Magnification 85 000 ×).





Figs. 5 and 6. Electron diffraction diagrams of cellulose micelles from native (P) and mercerised (MP) wood cellulose (original plate magnified 2 ×).

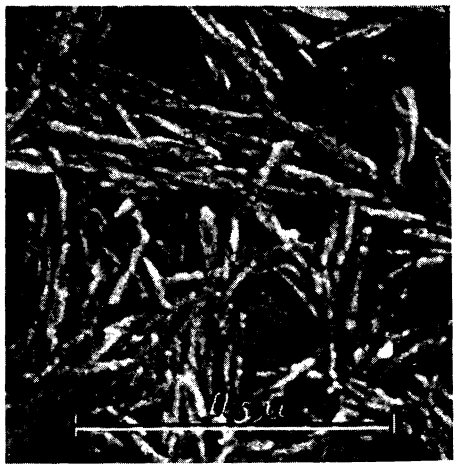


Fig. 7. Electron micrograph of diffraction specimen (micelles) from native cotton cellulose (Sample B), shadowed. (Magnification 85 000 ×).

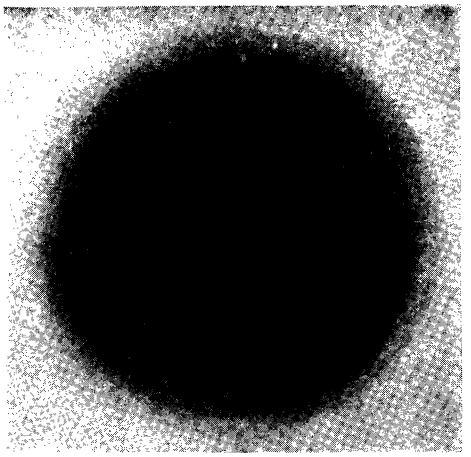


Fig. 8. Electron diffraction diagram of cellulose micelles from wood cellulose (E), treated with 6.53 per cent sodium hydroxide, partly mercerised. (Magnified 2 ×). Note that the strong inner reflections are of about equal intensity.

(powdered pure graphite, chemical grade, prepared on the carrier membranes from a water surface). The cubic, face-centered lattice of MgO is known with high precision [5]. X-ray diffraction has given the parameter a = 4.204 ± 0.004 Å and electron diffraction 4.190 ± 0.010 Å. This difference may be due to a contraction effect in ionic crystals of small dimensions predicted by Lennard-Jones [6] and discussed by Finch and Wilman [3,p.418]. The electron diffraction value d_{220} = 1.481 Å was used for the calibration. The hexagonal lattice of graphite has the axes a = 2.458 Å and c = 6.701 Å determined with electron diffraction, in close agreement with X-ray determinations [3,p.418]. The length of the a-axis, referred to the C—C distance in the hexagon layers (d_{200} = 1.229 Å), was used for the calibration.

An electron micrograph and a diffraction diagram of magnesium oxide are shown in Figs. 1 and 2. The ring diameters were measured directly on the plates using a microcomparator with an accuracy of 0.001 cm or better. As the rings were slightly distorted (length ratio of extreme diameters 1.002—1.004), it was necessary to measure along four directions (45°) and use the average value. The wave lengths of the electron beam were calculated from the calibration exposures according to equ. (4). The average wave length λ during the investigation was = $(3.926 \pm 0.008) \cdot 10^{-2}$ Å which corresponds to the high tension V = 107 600 ± 500 Volt (equ. (2)).* The variation in λ during the course of one day was usually smaller. The maximal variation observed during the whole period was $\pm 0.014 \cdot 10^{-2}$ Å.

II. NATIVE AND MERCERISED CELLULOSE

There are a few references to electron diffraction studies of cellulose in the literature, describing investigations of thin cellulose membranes, precipitated from cuprammonium [7a] or prepared from nitrocellulose by denitration [7b].

When cellulose particles of colloidal dimensions (micelles) were isolated as sols [8] it was easy to obtain useful specimens for electron diffraction. The first diffraction experiments using cellulose sols were referred to by Svedberg [9] in a lecture. Investigations on cellulose particles from viscose [10] and cuprammonium rayon [11] have also been reported.

The test experiments with pure native and mercerised cellulose were made using the wood cellulose sols (samples P and MP), the colloidal properties of which recently were described by Rånby [12].

The cellulose sols were centrifuged (15 min., field 1 000 g) and treated with ultrasonic waves (90 min., 22 kc, ca. 1 W/cmsq) to decrease the aggregation. The electron diffrac-

* This value is calculated with the relativistic correction in the electron mass $m = m_0/\sqrt{1-\beta^2}$ introduced, where m_0 is the mass at rest, $\beta = v/c$, v the velocity of the electron and c the velocity of light (*cf.* Finch and Wilman [3, p. 393]).

tion specimens were prepared from the aqueous sols directly on the specimen holders covered with carrier membranes. Diffraction diagrams with resolved lines and a minimum of background scattering were obtained from specimens containing only a thin ("monoparticle") layer of cellulose micelles, aggregated as little as possible (Figs. 3 and 4). The amounts of substance giving diffraction diagrams clearly visible on the fluorescent screen are of the order of magnitude $5 \cdot 10^{-10}$ g as calculated both from the volume and the concentration of the cellulose sols and directly from the structure of the specimen observed in the electron microscope. The specimens were exposed for 0.5—1 sec. and the plates (Ilford Process) developed for 2—3 min. in a dilute metol-hydroquinone developer (Ilford ID—2).

As a comparison, X-ray powder diagrams were obtained from specimens containing 1—10 mg cellulose and exposed for one hour or more (the X-ray diffraction diagrams are invisible on the fluorescent screen). This demonstrates the very high intensity of the diffracted electron beam compared to that of the X-ray.

A serious drawback of electron diffraction is the rapid destruction of the cellulose lattice, probably due to heat. After exposure in the beam for 5—10 sec., the diffraction rings gradually become fainter and generally they disappear after 15—20 sec. To avoid long exposures of the specimen in the electron beam, the high tension was switched on first and the cathode heating current 1—2 min. later, when the dark current (leak) was constant. After about 2 sec., when the emission current was constant, the plate was exposed.

Electron diffraction diagrams of micelles from the samples P (native) and MP (mercerised cellulose) are reproduced in Figs. 5 and 6. The main reflections were measured, the corresponding d-values (= distances between the lattice planes) calculated, and the results collected in Table 1. The d-values are compared with recent X-ray determinations by Kiessig [13] and electron diffraction values by Ribi [10]. The accuracy in these determinations is better than 0.5 per cent.

The relative intensity of the cellulose reflections is not the same for X-ray and electron diffraction. This is partly due to the orientation of the micelles in electron diffraction exposures (most of the micelles form a right angle to the beam). This effect will be treated in a forthcoming publication.

III. PHASE TRANSITION OF COLLOIDAL CELLULOSE

In a previous paper [14] the phase transition of cotton and wood cellulose (as fibres and as powder after hydrolysis) was studied using X-ray diffraction. Because of the influence of the fibre morphology, it was of interest to perform an analogous investigation on colloidal cellulose particles (micelles) using electron diffraction.

Table 1. Lattice plane distances (d in Å) for native and mercerised cellulose micelles.

Native cellulose Index / Plate	(101)	(10$\bar{1}$)	(021) (120)	(002)	(103) (311)	(110) (011) (004)
132	—	—	4.349	3.939	2.584	2.008
137	5.975	5.290	4.362	3.937	2.584	2.005
144	5.905	5.209	4.338	3.925	2.580	2.002
Average	(5.940)	(5.250)	4.350	3.934	2.583	2.005
Kiessig [13]	5.941	5.358	4.32	3.901	—	—

Mercerised cellulose Index / Plate	(10$\bar{1}$)	(002)	(130) (031)	(040) (12$\bar{2}$) (312) (311)	(10$\bar{3}$) (14$\bar{1}$) (20$\bar{2}$)	(061)
126	4.431	4.023	3.116	2.575	2.218	1.683
127	4.452	4.036	3.126	2.582	2.230	1.695
128	4.423	4.021	3.112	2.570	2.207	1.682
Average	4.435	4.027	3.118	2.576	2.218	1.687
Kiessig [13]	4.432	4.052	—	—	—	—
Ribi [10]	4.42	4.04	3.12	2.57	2.20	—

The indices are according to H. Mark, *Physik und Chemie der Cellulose*, pp. 154—155, 166—167, Verlag Springer, Berlin (1932).

The electron diffraction plates have been measured by Miss Anna-Lisa Norling and Mrs Els Pärkma.

A. Samples prepared

Cellulose sols from three samples (B, E, and F, ref. 14) were investigated:

B. Bleached cotton linters (chemical grade).

E. Prehydrolysed, bleached, sulphate pulp (rayon grade).

F. Bleached, hot alkali refined sulphite pulp (acetylation grade).

Sample F is identical with sample P in sect. 3 of this paper *Cf.* [12].

The aqueous cellulose sols were prepared according to Rånby [12] by peptization after hydrolysis of cellulose in a boiling 2.5 *N* sulphuric acid (11.4 per cent) for 16 hours (fresh acid after 6 hours). The concentration of the peptized sols was brought to 0.01 g/100 ml by dilution. The sols were treated with ultrasonic waves (22 kc, ca. 1 W/cmsq.) for 90 min. Sol portions of 15.0 ml in centrifuge tubes were flocculated by adding 0.5 ml 0.1 *N* sodium chloride. After half an hour the precipitates (voluminous clouds) were centrifuged down and the supernatant removed. The cellulose gel had a volume of 0.25—0.5 ml in each tube, containing less than 0.5 per cent cellulose. The same series of sodium

hydroxide solutions with increasing concentrations, as described in the previous paper [14], were used for the alkali treatments. 15.0 ml of caustic soda was added to each tube at room temperature (20—22° C) and the gel was dispersed in the lye by stirring. The amount of gel was weighed before adding the lye and the exact alkali concentration calculated. The suspensions were poured into Erlenmeyer flasks, which were then stoppered and cooled in an ice-bath for 1 hour. The minimum caustic concentration to form a clear (or slightly turbid) suspension was observed. The treatment was stopped by introducing 30 ml ice-cold water and the alkali was removed by dialysis through cellophane bags against distilled water over-night, against 0.5 per cent acetic acid until the cellulose flocculated (4—5 hours), and against water again over-night. The flocculated cellulose was collected and washed with water by centrifuging, and suspended in 15 ml water. To obtain homogeneous sols for diffraction specimens, the suspensions were treated with ultrasonic waves for 1—2 hours. A typical specimen of sample B (cotton) is shown in Fig. 7. Its diffraction diagram is very similar to that of wood cellulose micelles.

In this method of preparation the cellulose particles in a gel are dispersed in a lye of a certain concentration, which is lowered 0.2—0.4 per cent by dilution by the water of the gel. This dilution does not influence the phase transition of the cellulose particles, however, as the cooling from 20° to 0° C is equivalent to a 2 per cent increase in the caustic concentration according to observations of Katz and Seiberlich [15].

B. Phase analysis

Electron diffraction diagrams of the alkali-treated samples were obtained in the same way as described in Sect. 3, and the phase transition followed by a visual comparison of the diagrams. In the corresponding X-ray diffraction diagrams [14], this comparison was done using the relative intensity of the reflections (101) and ($10\bar{1}$) of the native pattern (I) and (101) of the mercerised (II). This method was not applicable for electron diffraction because of the strong background scattering close to the primary beam, and it was necessary to use other reflections. In pattern I the intense reflection (002) with d = 3.93 Å and the weaker, mixed reflection (120) and (021) with d = 4.35 Å form a resolved pair. In pattern II the corresponding pair of reflections is the intense ($10\bar{1}$) with d = 4.44 Å and the weaker (002) with d = 4.03 Å (comp. Figs. 5 and 6). It is possible to follow the phase transition by a visual comparison of the intensity of these reflections, but the method is not as accurate as that used in the X-ray diffraction work. No relative intensity numbers were defined. The first shift in the relative intensity of the two I-reflections was recorded as the start of the transition. When the relative intensity of the two II-reflections was identical with that of micelles from pure mercerized cellulose (Fig. 6), the transition was recorded as complete. An electron diffraction diagram of partly mercerised micelles from sample E is shown in Fig. 8.

Table 2. Observations on the turbidity of the cellulose suspensions in sodium hydroxide solutions and the composition of the electron diffraction diagram.

Sample E			Sample F		
NaOH per cent	Turbidity	Diffraction diagram	NaOH per cent	Turbidity	Diffraction diagram
4.66	3	I	4.94	3	I
5.33	3	I	5.48	3	I
5.74	3	I	5.91	2	I(+ II)
6.15	2	I(+ II)	6.34	2	I + II
6.53	2	I + II	6.88	1	((I +))II
6.97	1	(I +) II	7.15	1	II
7.29	1	II	7.92	1	II

Sample B

NaOH per cent	Turbidity	Diffraction diagram
7.48	3	I
7.90	3	I
8.35	2	I ((+ II))
8.74	2	I + II
9.42	1	(I +) II
9.66	1	((I +))II
10.40	1	II

Singel brackets, for instance (+ II), mean that pattern II is fainter than I, double brackets mean much fainter. Turbidity 1 means clear or almost clear suspension, 2 means turbid without and 3 with lumps.

From Table 2 containing observations on the alkali suspensions and the diffraction diagrams it is obvious that the cotton cellulose sol (sample B) has its transition interval between 8 and 9.5 per cent NaOH and the two wood cellulose sols (samples E and F) between 6 and 7 per cent. The difference between E and F is 0.2—0.3 per cent, *i.e.* of the order of magnitude of the probable error and thus insignificant.

SUMMARY AND DISCUSSION

The phase transition study of *Cellulose I→II* with X-ray diffraction, described in the preceeding paper [14], made the assumption of a fundamental difference between cotton and wood cellulose very probable. These views are

supported by the result of the present investigation using electron diffraction. The phase transition of cellulose micelles from wood and cotton cellulose starts at the same NaOH-concentration as that of the corresponding hydrocelluloses. The completion of the transition, however, occurs within a shorter interval of NaOH-concentrations (the difference is 0.5 to 1.0 per cent). This is quite reasonable as the micelles are free or in small aggregates, where hard packing, incomplete penetration and restricted swelling that could hinder the complete mercerisation are not expected. The conclusion is that the difference between wood and cotton cellulose is due not only to the fibre morphology or the possible impurities but also to the properties of the submicroscopic structure elements, the micelle strings, from which the cellulose micelles are derived. The latter difference can be interpreted as a greater active surface of wood cellulose micelles, which might be both a surface area and a surface structure question.

This investigation has been performed as part of a research programme on cellulose and related substances under the supervision of Professor The Svedberg. The author is much indebted to Professor Svedberg and Professor Stig Claesson, the present head of the Institute, for their great interest in this work.

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Differences in the Chemical Composition of Some Pea Proteins

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Vicilin and legumin, the two globulins in seeds of the pea, *Pisum sativum*, were shown by Osborne and Campbell [1] to have different elementary composition. Thus especially the sulfur content of legumin is much higher than that of vicilin. The same differences were observed if the two globulins had been prepared from seeds of other plants belonging to the family *Leguminosae*. It is, however, not probable that the proteins analyzed by Osborne were homogeneous because his method of separation of the two globulins [fractionation by precipitation with $(NH_4)_2SO_4$] give protein preparations which are inhomogeneous by ultracentrifugation [2].

Earlier investigations published from this Institute [2,3] have described fractionations of vicilin and legumin by which the two globulins are obtained homogeneous as tested by both ultracentrifugation and electrophoresis. In this paper some determinations on the amino acid composition of the pea proteins are reported. Determinations have been carried out for only a few amino acids, which could be determined by simple methods, because preliminary experiments using paper chromatography showed that the differences in the amino acid contents between the pea proteins were not very large. The main problem was to settle whether or not any differences in the amino acid composition of the different pea proteins could be observed.

A. Preparation of the pea proteins

The homogeneous preparations of vicilin and legumin were prepared according to a method described in a previous paper [3]. The albumin preparation used in the present investigation was prepared according to the following method.

Ground ripe pea seeds (field variety "Torsdagsärt II") are extracted over night at + 4° C with 0.2 *M* NaCl, pH 7. The extract is centrifuged and filtered clear and then

dialysed in a cellophan bag against water (24 hours against running tap water, then distilled water). By this procedure the globulins are precipitated when the salt concentration is lowered, and low-molecular nitrogenous compounds (amino acids, peptides *etc.*) pass through the bag. After centrifugation the supernate is dialysed once more, until no precipitate is obtained by prolonged dialysis. When all globulins have been removed the supernate contains only albumins. The solution is cooled to − 16° C and dried *in vacuo* over $CaSO_4$. In this way the albumin fraction is obtained in the form of a white powder which is easily soluble in water. It is, however, not homogeneous in the ultracentrifuge and in the electrophoresis apparatus.

As can be seen from Fig. 1 d two inhomogeneous components are obtained in the ultracentrifuge diagrams [4] after about 90 minutes at 300 000 g. Below, this albumin fraction will be called "albumin". Fig. 1 a shows an ultracentrifuge diagram of an extract obtained from peas with 0.2 *M* NaCl, pH 7. Three peaks can be seen in the diagram, corresponding to, from the left, 1) albumins + low-molecular material, 2) vicilin and 3) legumin. A similar diagram is obtained even after a few minutes' extraction. Thus, vicilin and legumin are extracted from the seed material in the form of two different protein components, and are not products formed during the purification process. Fig. 1 b shows the composition of the solution after 48 hours' dialysis of the extract against the extraction buffer by which procedure the low-molecular material has been removed, and the solution contains albumin, vicilin and legumin. Fig. 1 c shows the composition of the precipitate which is obtained when the solution containing albumin, vicilin and legumin is dialysed against water. Only the two globulins are precipitated, and the albumin is left in the supernate (Fig. 1 d). If the precipitate containing vicilin and legumin is treated with a solution of 0.2 *M* NaCl, pH 4.7, the vicilin dissolves but legumin does not. After centrifugation, traces of legumin are removed from the supernate by dialysis against a buffer of pH 4.7 and vicilin is obtained free from legumin (Fig. 1 e). The precipitate from the centrifugation, which contains legumin contaminated with small amounts of vicilin, is dissolved in 0.2 *M* NaCl, pH 9.2 and the solution dialysed against 0.2 *M* NaCl, pH 4.7. Legumin precipitates, and by repeated dialysis at pH 4.7 it is completely separated from vicilin, as can be seen in Fig. 1 f.

B. Nitrogen content of the pea proteins

Nitrogen determinations according to Kjeldahl were carried out on the freeze-dried protein preparations. The determinations were carried out on samples containing 0.5—1 mg N with an experimental error of ± 1 %. The values in Table 1 are average values from four determinations. The water content, about 7 %, was determined by heating 0.3—0.5 g of protein to 105° C for 24 hours. The values in Table 1 are corrected for water content but not for ash.

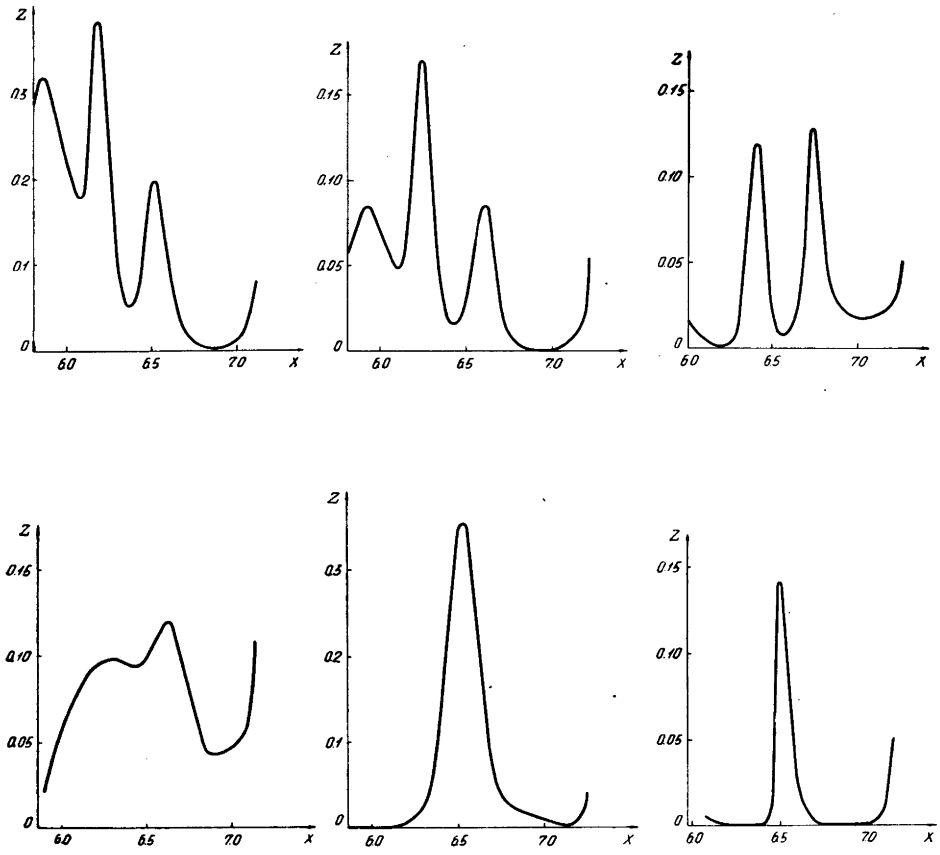


Fig. 1. Ultracentrifuge diagrams of pea proteins at different stages of the purification process. The abscissae represent distance from the axis of rotation (cm.) and the ordinates represent the scale-line displacement (mm.); t = time in minutes after reaching full speed and b = distance between cell and scale in mm.

a) *Top left. Original extract. t = 35′, b = 60 at 60.000 r.p.m.*
b) *Top centre. Original extract after dialysis against 0.2 M NaCl, pH 7. t = 35′, b = 60 at 60.000 r.p.m.*
c) *Top right. Original extract, dialysed against H_2O. The precipitate after dissolving in 0.2 M NaCl, pH 7. t = 25′, b = 60 at 65.000 r.p.m.*
d) *Bottom left. Original extract, dialysed against H_2O. The supernate after concentration (albumin). t = 140′, b = 60 at 60 000 r.p.m.*
e) *Bottom centre. Final preparation of vicilin. t = 160′, b = 40 at 40.000 r.p.m.*
f) *Bottom right. Final preparation of legumin. t = 15′, b = 20 at 65.000 r.p.m.*

Table 1. Nitrogen content of pea proteins.

Preparation	% N	% N Average values
Vicilin no. 10	17.32	
» no. 22	17.60	17.5
» no. 25	17.60	
Legumin no. 8	18.50	
» no. 11	17.35	17.9
» no. 24	17.86	
Albumin no. 1	15.07	15.1

As can be seen from Table 1 the nitrogen content is not the same in the different preparations of the same protein. Osborne and Campbell [1] determined the nitrogen content of vicilin to 17.40 % and of legumin to 18.04 %. Thus, the agreement between their values and the values in Table 1 can be considered satisfactory. The nitrogen content of the albumin used in these investigations is very low, but determinations on other albumin preparations prepared in the same way have shown that the value in Table 1 is correct.

C. Determination of tyrosine and tryptophan

In a previous paper it was preliminary shown that legumin contains more tryptophan than vicilin [5]. The method used for the determination of tyrosine and tryptophan is based on measurement of light absorption in the ultra violet region of a protein solution in 0.1 *N* NaOH [6-8]. Only the aromatic amino acids and cystine have any measurable absorption in the region 250—320 mμ [4]. By selecting the wave-length region 280—320 mμ the absorption of phenylalanine can be neglected, and the absorption measured is that of tyrosine, tryptophan and cystine. The absorption of cystine can not be neglected at 280 mμ when the amounts of tyrosine and tryptophan are calculated, unless the cystine content is very low compared with the amounts of tyrosine and tryptophan. Preliminary determinations with the phosphotungstic acid method according to Schöberl and Rambacher [10] of the cyst(e)ine (cysteine + cystine) content gave the value 0.25 % cyst(e)ine N/total N for vicilin and 0.42 % for legumin. Thus, the cyst(e)ine contribution to the absorption can be neglected.

Experimental: 25 mg dried protein are dissolved in 7.5 ml 0.1 *N* NaOH and the solution is filtered. Two nitrogen determinations are carried out on 1.0 ml each. The solution is then diluted 4 times (in case of albumin 7 times) with 0.1 *N* NaOH and the extinction is measured in a Beckman spectrophotometer with 0.1 *N* NaOH as reference solution. For the calculation of the tyrosine and tryptophan contents the extinction is usually measured at 280, 294.4, 340 and 370 mμ [8,5] but in these experiments the whole absorption curve was measured in the region 250—370 mμ. For the calculation of the tyrosine and tryptophan content see Goodwin and Morton [10] and Danielsson [8]. The results of the measurements are found in Table 2 and Fig. 2.

As can be seen from Table 2, the tyrosine N in % of total N is determined with a maximal deviation from the average of ± 5 %. The corresponding value for tryptophan is ± 15 %.

Table 2. Determinations of the tyrosine and tryptophan contents in pea proteins by measurement of light absorption in the ultra violet region. The extinction values (E) are observed values.

Preparation	Concentration mg N/ml	E_{280}	$E_{294.4}$	E_{340}	E_{370}	Tyrosine N in % of total N	Tryptophan N in % of total N
Vicilin no. 10	0.127	0.389	0.480	0.020	0.003	1.87	0.28
	0.135	0.410	0.500	0.035	0.024	1.79	0.33
Vicilin no. 22	0.132	0.390	0.485	0.020	0.010	1.86	0.27
	0.147	0.445	0.555	0.021	0.010	1.93	0.31
Vicilin no. 25	0.139	0.420	0.518	0.039	0.029	1.82	0.28
	0.151	0.466	0.572	0.029	0.018	1.88	0.33
						Av.: 1.86	0.30
Legumin no. 8	0.132	0.580	0.560	0.035	0.020	1.75	0.98
	0.133	0.690	0.650	0.082	0.060	1.85	1.03
Legumin no. 11	0.140	0.678	0.650	0.050	0.028	1.82	1.14
	0.146	0.705	0.680	0.070	0.048	1.78	0.90
Legumin no. 24	0.131	0.540	0.545	0.028	0.015	1.77	0.92
	0.146	0.680	0.688	0.075	0.056	1.89	0.94
						Av.: 1.81	0.99
Albumin no. 1	0.078	0.628	0.590	0.046	0.031	2.92	2.05
	0.068	0.534	0.490	0.040	0.023	2.77	2.10
	0.074	0.610	0.560	0.051	0.031	2.77	2.07
						Av.: 2.82	2.07

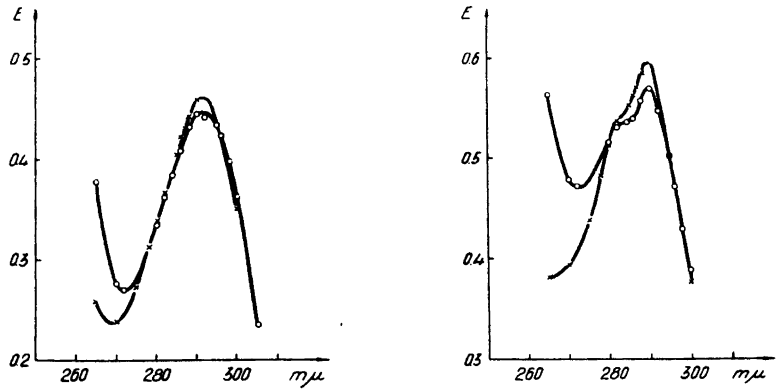


Fig. 2. Experimental (○) *absorption curves in the ultra violet region for vicilin (left) and legumin (right) in 0.1 N NaOH.* (×) *represent theoretical curves obtained by adding the extinctions of tyrosine and tryptophan in the concentrations calculated from the experimental curves.*

Fig. 2 shows the agreement between the experimental curves and the theoretical curves, obtained by adding the extinction of pure tyrosine and tryptophan in the same concentration as was calculated from the experimental curve. The agreement in the wave length region used for the calculation of the tyrosine and tryptophan contents must be considered satisfactory.

D. Determination of acid and basic amino acids

The acid and basic amino acids were separated by electrodialysis according to Theorell and Åkesson[11] with the modifications indicated by Weibull[12].

Experimental. 40 mg of protein were hydrolyzed for 30 hours with 19 % HCl on a boiling water bath under a reflux condenser. After addition of diluted H_2SO_4 in equivalent amount to the total nitrogen present the hydrolysate was evaporated three times to dryness in order to remove HCl, because chlorine may destroy the acid amino acids during the electrodialysis[11]. The amino acid mixture was then dissolved in 25 ml water. No humin formation was observed. During the electrodialysis the ammonia formed in the cathode compartment was taken up in 25 ml 0.1 *N* HCl. The amide nitrogen was determined by distillation according to Theorell and Åkesson[11]. The basic amino acids were determined according to Macpherson[13]. The acid amino acids were determined by separation on an ion exchange column (Dowex 50) combined with automatic, continuous measurement of the electrolytic conductivity of the eluate. By this method the ratio between the concentrations of glutamic acid and aspartic acid is obtained. The method has been worked out by Drake[14] at this Institute.

Table 3. The distribution of acid, neutral and basic amino acids in pea proteins from electrodialysis experiments, and quantitative determinations of some separate amino acids. All values are expressed as specific N in % of total N.

Preparation	Acid amino acids	Neutral amino acids	Basic amino acids	Amide nitrogen	Recovery %	Arginine	Histidine	Lysine	Glutamic acid	Aspartic acid
Vicilin no. 10	21.64	39.47	29.24	10.00	100.35	21.00	3.59	4.65	12.24	9.40
» no. 22	20.21	40.96	30.90	9.96	102.03	20.87	3.71	6.32	11.73	8.48
» no. 25	22.49	38.46	29.54	10.64	101.13	21.62	3.75	4.17	12.48	10.00
Av.:	21.45	39.63	29.89	10.20		21.16	3.68	5.05	12.15	9.29
Legumin no. 8	25.03	34.85	32.46	10.44	102.78	23.42	4.54	4.50	15.95	9.09
» no. 11	24.98	33.96	31.30	10.81	101.05	23.63	4.55	3.12	15.82	9.16
» no. 24	25.70	34.02	31.94	10.57	102.23	23.68	4.60	3.66	15.61	10.09
Av.:	25.24	34.28	31.90	10.61		23.56	4.58	3.76	15.79	9.45
Albumin no. 1	20.23	40.50	29.62	8.58	98.93	10.73	5.59	13.30	10.61	9.61
	20.00	40.84	29.43	8.91	99.18	10.87	5.82	12.74	10.02	9.98
	20.18	40.05	30.37	8.14	98.74	11.14	6.00	13.23	9.88	10.30
Av.:	20.14	40.46	29.81	8.54		10.91	5.80	13.09	10.17	9.96

The results of the measurements are given in Table 3. In the separation of acid, neutral and basic amino acids, the greatest error was obtained when vicilin was analyzed, with a maximal deviation from the average of $\pm$ 6 %. The corresponding value for legumin and albumin is $\pm$ 2 %. It is possible that the different vicilin preparations were not completely homogeneous and this fact caused the greater part of the error in these determinations. A more probable explanation, however, is that the first determinations were carried out on vicilin and the experimental accuracy was higher when legumin and albumin were analyzed. According to Weibull [12] 1.5 % of the acid amino acids remain in the mixture of neutral amino acids in the method used. The values in Table 3 are not corrected for this error, but in Table 4 the values are corrected. The values in Table 3 for amide nitrogen are a little too high depending on the decomposition of serine and threonine [12]. In the determinations of the basic amino acids the maximum deviation from the average between the different preparations is $\pm$ 2 % for arginine, $\pm$ 4 % for histidine and $\pm$ 25 % for lysine. The corresponding values for the acid amino acids are $\pm$ 4 % for glutamic acid and $\pm$ 9 % for aspartic acid. The great error in the determination of lysine probably depends on the fact that lysine is deter-

Table 4. Amino acid content (% of weight) of some seed proteins.

Amino acid	Vicilin	Legumin	Albumin	Edestin[17]	Arachin[18]	Glycinin[18]
Tryptophan	0.4	1.3	2.3	1.48	0.6	0.4
Tyrosine	4.2	4.2	5.5	4.34	4.5	2.7
Arginine	11.5	13.1	5.1	16.7	15.4	11.7
Histidine	2.4	3.0	3.2	2.9	1.9	0
Lysine	4.6	3.5	10.3	2.4	2—4	6
Glutamic acid	22.7	30.1	16.3	20.7	17	19
Aspartic »	15.7	16.3	14.5	12.0	5	2

mined by difference. Thus, when the lysine content is low, the accuracy of the method is comparatively low.

E. A comparison of the compositions of the pea proteins

As to the distribution of acid, neutral and basic amino acids in the pea proteins, it can be seen that vicilin and albumin are very similar. It is evident that legumin has a higher content of acid and basic and a smaller content of neutral amino acids than vicilin. These differences are considerably greater than the experimental errors. Legumin has the same amount of amide nitrogen as vicilin. Albumin contains less amide nitrogen.

Some conclusions concerning the composition of the pea proteins can be drawn if the errors in the determinations of the separate amino acids are taken into consideration. According to Table 4 legumin contains 3 times and albumin 6 times more tryptophan than vicilin does. The tyrosine content is the same in vicilin and in legumin but higher in albumin. The arginine content is considerably higher in legumin than in vicilin but remarkably low in albumin. Legumin and albumin have a higher content of histidine than vicilin. As there are big errors in the determinations of the lysine content, it is impossible to say if vicilin and legumin contain different amounts of this amino acid. The lysine content of albumin, however, is very high and as far as the author knows higher than for any other seed protein investigated. Legumin has more and albumin less glutamic acid than vicilin. The aspartic acid content is the same for the three proteins.

DISCUSSION

A comparison of the amino acid compositions of vicilin and legumin shows that clear differences exist in the tryptophan, arginine, histidine and glutamic acid content of the two proteins. Differences can also be seen in the distribution of acid, neutral and basic amino acids. These results together with those obtained earlier from ultracentrifuge and electrophoresis experiments show clearly that vicilin and legumin are two well-defined and different globulins.

The albumin fraction is quite different from the two globulins both in chemical composition and physical-chemical properties. Characteristic of the albumin fraction is its high tryptophan and lysine contents and low arginine content. The clear character of reserve proteins of the globulins was shown by germination experiments on pea seeds [15]. The albumin fraction probably has a very small importance as a reserve protein, but it contains many enzymes. A proteolytic enzyme has been isolated from this fraction [16], and the albumin fraction also contains a peroxidase and a dipeptidase.

The results of the measurements are found in Table 4, where the compositions of the pea proteins are compared with those of three other important seed proteins [17,18]. Compared with these seed proteins the pea globulins are characterized by their high content of acid amino acids. The high content of tryptophan and lysine in the albumin fraction is very remarkable and is reminiscent of the values which have been found for animal proteins, *e.g.* β-lactoglobulin. Lysine, being one of the essential amino acids for the chicken, and probably for man, is often present in too low a concentration in plant proteins, especially in the cereal proteins, to give these proteins a sufficiently high nutritive value [14]. Table 4 shows that the pea proteins, especially the albumin fraction, can be important from a nutritive point of view.

SUMMARY

1. Quantitative determinations of tyrosine, tryptophane, arginine, histidine, lysine, aspartic acid and glutamic acid in vicilin, legumin and albumin from pea seeds are reported.

2. Vicilin and legumin have different amino acid composition and are two well-defined and different globulins.

3. The amino acid composition of the albumin fraction is quite different from that of the globulins. No relationship has been observed between the globulins and the albumins.

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A Contribution to the Study of the Synthesis of the Reserve Proteins in Ripening Pea Seeds

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The synthesis of the high-molecular reserve proteins in seeds during the ripening process has not been investigated with modern physical methods. The concentrations of various low-molecular nitrogenous substances, in particular amino acids and asparagine, have been determined, however, in the seeds and in other parts of plants during different stages of the ripening process. Emmerling [1–3] studied for 20 years the protein synthesis in ripening seeds of the broad bean, *Vicia faba major*. He showed that nitrogenous products are transported from different parts of the plant to the seed pod where they are stored before they are transported to the ripening seeds. Vassilieff [4] and Pfenninger [5] made the same observation. Emmerling stated that the seed proteins are synthesized from organic nitrogenous compounds (amides and amino acids) synthesized in the leaves. Schulze and Winterstein [6] found that the seed pods from the pea contain much asparagine and little arginine, while the seeds contain very little asparagine and large amounts of arginine. By analyzing the ripening pea seeds at 4 different stages they were able to show that the amount of protein nitrogen increases and the amount of non-protein nitrogen decreases during the ripening process. Most published values of protein concentration in seeds are not very reliable, however, because the determinations were carried out by precipitation of "total protein", in most cases by metals. Such methods precipitate all substances of a protein nature. Thus it is not possible to observe any relative changes of concentration between the different proteins.

In a previous paper [7] dealing with the germination process in pea seeds, the present author has shown that pea seeds contain a few well-defined protein fractions. A fractionating method was worked out by which two globulins, vicilin and legumin, and an inhomogenous albumin fraction could be determined

quantitatively. The amounts of low-molecular nitrogenous substances were determined by difference. This method has been used in the present study of the synthesis of the high-molecular reserve proteins in ripening pea seeds.

A. Treatment of the seed material

In the first experiments, seeds of the field variety of pea "Torsdagsärt II" were harvested in 1950 at different stages of the ripening process. The seeds were separated from the seed pods and were left to dry at room temperature. After about 1 month they were placed in water over night and then macerated in a Waring blendor for 6 minutes. After cooling to $-16°$ C, the product was dried *in vacuo* over $CaSO_4$. Table 1 gives some data on the seed material.

When the unripe seeds are dried in air, most of their water content is released. At stage I, 82 % of the original weight evaporated (in the form of water), and at stage VII 55 %.

B. Determinations of the nitrogen distribution in extracts of unripe, air-dried seeds

The lyophilized material from air-dried, unripe seeds, harvested in 1950, was extracted with 0.2 *M* NaCl, pH 7, as described in a previous paper [7]. At different stages of the ripening process the contents of extractable N, protein N, low-molecular N, albumin N and globulin N were determined. The results of these measurements are given in Table 2 and Figs. 1 and 2. At least five experiments were carried out at each stage of ripening. The values in Table 2

Table 1. Weight of 100 air-dried seeds, harvested in 1950, and nitrogen content of the lyophilized seed material at different stages of ripening.

Stage	Date of harvest	Weight per 100 seeds g	Nitrogen content (Kjeldahl) %
I	11.7	2.97	4.65
II	15.7	3.45	4.62
III	19.7	8.95	4.10
IV	23.7	14.60	4.19
V	27.7	19.00	3.86
VI	31.7	21.59	3.67
VII	4.8	21.47	3.55

Table 2. Nitrogen distribution in NaCl extracts from pea seeds at different stages of the ripening process. In all experiments, 1.50 g of dried seed material were extracted.

Stage	Total N in 1.50 g dried material, mg	Extractable N mg	Nondialysable N (protein N) mg	Dialysable N mg	Globulin N mg	Albumin N mg
I	69.7	52.4	8.2	44.2	2.6	3.5
II	69.3	52.8	13.6	39.2	8.2	3.6
III	61.5	44.0	21.1	22.9	15.1	3.5
IV	62.8	46.6	26.7	19.9	21.1	5.2
V	55.0	40.8	25.6	15.7	18.1	5.0
VI	57.9	38.0	22.5	15.5	17.2	3.7
VII	53.2	42.0	27.0	15.0	20.5	6.2

are average values from these determinations. The errors in the determinations were about the same as in the investigations on the germination process [7], except for stage I, where bigger errors were obtained depending on the very low protein content of the seeds at this stage of ripening. From Table 2 and Fig. 2 it can be seen that all values obtained for seeds from stage V are too low. This fact can probably be explained by assuming that something happened to

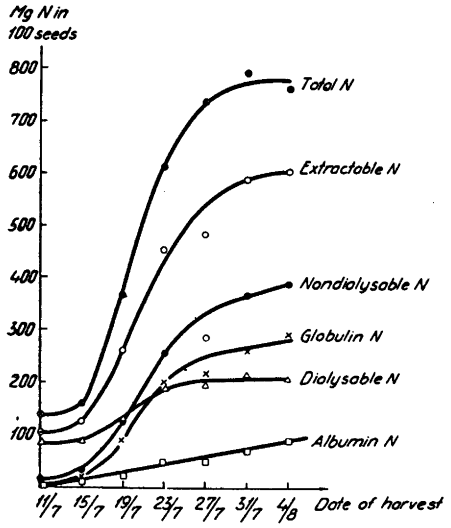


Fig. 1. Nitrogen distribution in pea seeds at different stages of the ripening process.

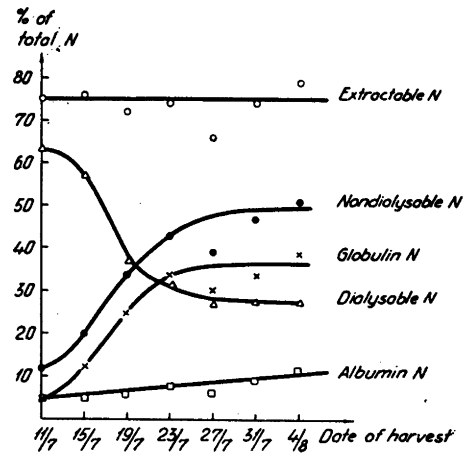


Fig. 2. Specific N as a percentage of the total N in developing pea seeds.

these seeds during the drying process. Five different extractions were carried out on this fraction at different times, and all determinations agree within $\pm$ 6 %. Thus the low values cannot depend on errors in the extraction method used.

C. Ultracentrifuge experiments on extracts from air-dried seeds

Extracts which had been dialyzed against the extraction buffer were analyzed in the ultracentrifuge, as described in a previous paper [7]. The ultracentrifuge diagrams for unripe seeds were very similar to those obtained for ripe seeds. The results are shown in Table 3. The results of some experiments on the garden pea variety are also found in this table [8]. The different stages of ripening for the two varieties are based on seed weight. Stage VII refer in both cases to ripe seeds. As can be seen from Table 3, the unripe seeds have a higher $\frac{\text{vicilin}}{\text{legumin}}$ ratio than the ripe seeds. For the peas of the field variety, the same ratio, 1.5, has been obtained for ripe seeds from three different years.

Table 3. The ratio $\frac{vicilin}{legumin}$ *in extracts from unripe pea seeds as determined from ultracentrifuge diagrams.*

Stage	Field variety $\frac{\text{vicilin}}{\text{legumin}}$	Average	Stage	Garden variety $\frac{\text{vicilin}}{\text{legumin}}$	Average
II	2.54 2.63 2.61 2.91	2.7	III	3.42 3.26 3.62	3.4
III	2.90 2.46 2.96	2.8	IV	2.70 2.70 3.13	2.8
VII	1.40 1.52 1.54	1.5	VII	1.30 1.19 1.28 1.15	1.2

D. Ultracentrifuge investigations on extracts from unripe seeds preserved by freezing

The experiments described above were carried out on seeds which had been dried in air after they had been harvested. It is very probable that some changes in the nitrogen distribution occur during this drying process, however. The main problem is whether or not any protein, especially well-defined high-molecular protein components, is synthesized, after harvesting, from low-molecular nitrogen constituents of the undeveloped seed.

In order to get some information on this point, seeds of the field variety of pea "Torsdagsärt II" were harvested in 1951 at different stages of the ripening process. Immediately after harvesting the seeds were separated from the seed pods, some seeds being preserved by cooling to — 20° C and some being allowed to dry at room temperature. After about two months both fractions were macerated and dried (lyophilized) as described above. A homogeneous green powder was obtained in both cases. Table 4 gives some data on the seed material used. Extractions were carried out with 0.2 M NaCl, pH 7, and after dialysis of the extracts against the extraction buffer the solutions were analyzed in the ultracentrifuge. The results of these investigations are given in Figs. 3 and 4.

As can be seen from Fig. 3, the well-defined globulin components appear rather suddenly in the middle of the ripening process. In the present case these components appear between stage III and stage IV. The amounts of high-molecular substances, as determined from the ultracentrifuge diagrams, were about the same for stage I—III, but for stage IV this value increased by 125 %. In earlier stages traces of well-defined globulin components can be

Table 4. Wet and dry weight of 100 seeds, harvested in 1951, after drying at room temperature.

Stage	Date of harvest	Weight per 100 seeds (g.)		Moisture
		Wet	Dry	%
I	14.7	—	—	85
II	18.7	15.1	2.8	82
III	21.7	19.3	3.6	82
IV	25.7	23.7	5.3	78
V	28.7	31.2	8.6	72
VI	1.8	34.4	10.8	69
VII	4.8	39.8	13.9	65
VIII	8.8	43.2	18.9	56

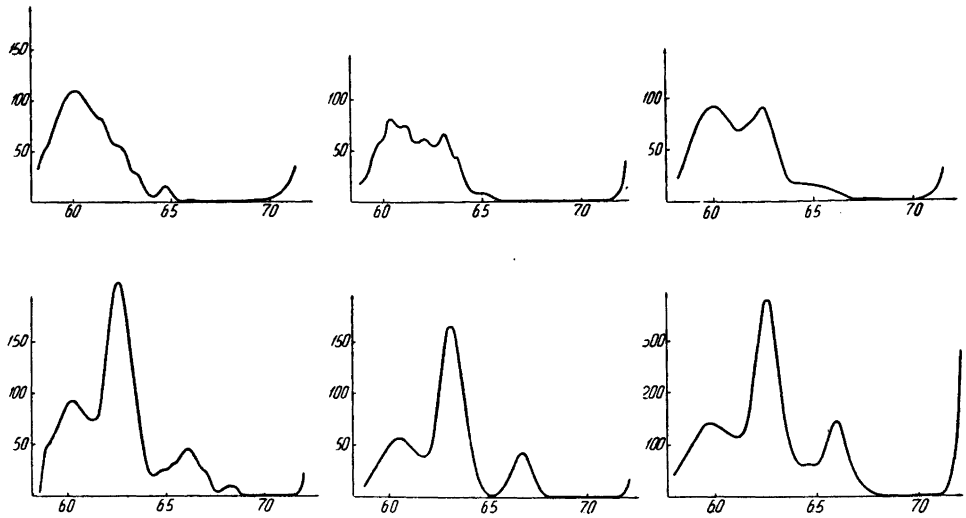


Fig. 3. Ultracentrifuge diagrams from dialyzed extracts of unripe pea seeds preserved by freezing. The abscissae represent distance from the axis of of rotation (cm.), the ordinates represent the scale-line displacement (μ). The exposures were all taken 35 minutes after full speed. Scale distance 120 mm. Centrifugal field 260,000 × gravity.

Top left: stage I.
Top centre: stage II.
Top right: stage III.
Bottom left: stage IV.
Bottom centre: stage V.
Bottom right: stage VI.

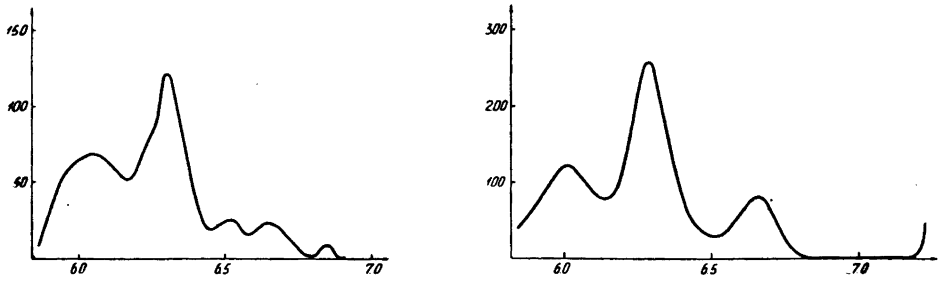


Fig. 4. Ultracentrifuge diagrams for unripe pea seeds which had been dried at room temperature. The experimental conditions were the same as those given in the caption of Fig. 3.

Left: stage I. Right: stage II.

Table 5. The ratios $\frac{vicilin}{legumin}$ *and* $\frac{albumin}{globulin}$ *in extracts from pea seeds, preserved by freezing. The values were obtained from ultracentrifuge diagrams.*

Stage	$\frac{\text{vicilin}}{\text{legumin}}$	$\frac{\text{albumin}}{\text{globulin}}$
II	7.7	6.4
III	5.1	4.8
IV	3.7	2.9
V	3.9	2.7
VI	2.3	2.2
VII	2.5	2.0
VIII	1.7	2.4

Table 6. Content of amino nitrogen in water extracts of lyophilized pea seeds, harvested in 1951.

Stage	Amino nitrogen, g equivalents/ml × 10^5	
	Seeds preserved by freezing	Air-dried seeds
I	9.35	10.58
II	7.16	7.56
III	6.95	6.66
IV	6.00	5.04
V	5.27	3.91
VI	3.73	3.75
VII	3.66	3.41
VIII	3.69	3.52

detected in the diagrams. Fig. 4, however, shows that if the seeds are allowed to dry at room temperature, rather well-defined components are found even at stage I. Table 5 shows the ratios between the different components.

E. Determinations of amino nitrogen

0.1 g lyophilized seed material was extracted with 2 ml H_2O for 24 hours. After centrifugation amino nitrogen was determined by acetone titration according to the Linderstrøm-Lang method [9]. Each titration was carried out with 0.1 ml extract and 1 ml acetone. The results are given in Table 6. Exactly the same experiment was also carried out on pea seeds which had been preserved at $-20°$ C for seven months. The new values were identical with those in Table 6.

DISCUSSION

Table 1 shows that the nitrogen content of the seeds, calculated as percentage of dry weight of the lyophilized material decreases almost linearly during the ripening process, probably depending on the fact that the in-flow of non-nitrogenous substances increases as ripening proceeds. Fig. 1 shows the increase in concentration of the different nitrogenous substances in 100 seeds during the development of the seeds. This figure gives a clear picture of the conditions during the ripening process in a single plant which delivers the nitrogenous substances synthesized in the leaves to a fixed number of seeds.

The absolute amount of all nitrogenous substances increases during the ripening process according to Fig. 1. Of special interest is the fact that the high-molecular nitrogenous substances are formed at an early stage of seed development, *e.g.*, 2/3 of the final amount of globulin is synthesized during the first half of the period of ripening. It is also significant that the amount of albumin increases slowly at a constant rate.

As was mentioned above, the experiments were carried out on seeds which had been harvested in two different years, 1950 and 1951, and the seed material had been treated in different ways. However, a comparison can be made between the two crops if all results obtained are expressed in terms of seed weight. From the seeds harvested in 1950, well-defined globulin components were obtained in the ultracentrifuge even in the first stages of ripening. It can be seen from Fig. 4 that when the seeds are allowed to dry in air at room temperature well-defined globulin components are found even in very small seeds. When the seeds are preserved by freezing, no such components can be detected in these early stages. (Fig. 3) Thus the ripening process continues in seeds which have been separated from the mother plant, and those nitrogenous substances which are delivered to the very small seed from its mother plant have the proper composition to form the well-defined globulin components.

Fig. 2 gives some information about different categories of nitrogen in ripening pea seeds, if the fact that the ripening process has been going on after harvesting is taken into consideration. This figure shows specific N as a percentage of the total N in the seeds. Extractable N is practically constant during the whole of the period studied. Protein N and globulin N increase mainly during the first part of the ripening process. Albumin N increases slowly at a constant rate all the time. Low-molecular N decreases at the same rate as protein N increases. The curves of formation for protein N, globulin N and albumin N during the ripening process are very similar to those obtained for the breakdown of these fractions during germination [7]. The most interesting fact is that the albumin fraction is synthesized and broken down quite independently of the globulin fraction. Together with the finding that the chemical composition of these two high-molecular protein fractions are different [10], this result gives strong evidence that the globulins and albumins are two quite different protein fractions.

The ultracentrifuge experiments which were carried out on unripe seeds harvested in 1951 showed (Fig. 3) that well-defined globulin components could be isolated at stage III—IV, corresponding to stage II for the seeds harvested in 1950. Thus according to Figs. 1 and 2, synthesis of well-defined globulin components begins at the same time as the content of protein N and globulin N begins to increase.

Determinations of the ratio $\frac{\text{vicilin}}{\text{legumin}}$ from ultracentrifuge diagrams show that this ratio decreases during the ripening process. The differences are so large that even the rather considerable errors involved in the methods used cannot have any effect on this result. The changes in the ratio $\frac{\text{vicilin}}{\text{legumin}}$ during the ripening process can be seen most clearly from the experiments on the pea seeds preserved by freezing. Vicilin is formed before legumin during the first part of the ripening process, and the two globulins are formed at different rates as ripening progresses. This fact can be clearly understood if the values from Table 3 are combined with those from Table 2. The absolute amounts of both vicilin and legumin increase during the ripening process, which fact can also be seen directly from the ultracentrifuge diagrams. The alternative that vicilin is synthesized first and than transformed to legumin is thus ruled out. During the germination process, however, the two globulins are broken down at the same rate [7].

The most important question in these investigations is whether or not any high-molecular intermediate products can be observed during the synthesis of the well-defined reserve proteins. There are many conceivable possibilities with regard to protein synthesis. For this discussion we assume that proteins are built up by condensation of amino acids [11] and not by polymerization of some simple unit, *e.g.*, formaldehyde and ammonia [12]. Wood and Petrie [13] found a correlation between amino acid and protein nitrogen and from their experiments they concluded that protein is formed by direct condensation of amino acids. Steward and Preston [14], however, who worked with metabolizing potato discs, thought that the amino acids are first deaminated. Simple amino acids may gradually condense to a long peptide chain which then forms a globular protein by coiling. A second possibility is that many amino acid molecules are arranged in the appropriate way and then condense very rapidly, all at the same time, to a peptide chain, large enough for the formation of one protein molecule. The third possibility is that some rather high-molecular peptide chains are first synthesized, which react with each other through a few active groups and form the protein molecule. It is very difficult to throw any light upon the mechanism of these reactions but some information about intermediate products may be obtained from Fig. 3. During stage I of the ripening process, no well-defined globulin components can be seen in the ultracentrifuge diagrams. The diagrams from stage II and III show very small amounts of the pure components, but the diagrams from stage IV show well-defined components in considerable quantity, and the diagrams are very similar to those obtained from the ripe seeds. The sudden change in the

amounts of high-molecular substances, as determined from the ultracentrifuge diagrams, indicates that the formation of the well-defined components is rather rapid, contrary to what happens during the germination process [7]. It is, however, very difficult to settle definitily from the ultracentrifuge diagrams whether any high-molecular nitrogenous substances serve as intermediate products in the protein synthesis. So a new method had to be used.

Figs. 3 and 4 show clearly that the ripening process is going on in the seeds which have been separated from the mother plant and left to dry at room temperature. The seeds from stage I contained no globulin components at all when the ripening process had been broken by freezing, but when the seeds had been dried at room temperature, all high-molecular nitrogenous substances were transformed to well-defined components. Thus chemical analysis of the two fractions from stage I can give some information about the synthesis of the reserve proteins. In our case amino nitrogen was determined by acetone titration according to the Linderstrøm-Lang method [9]. As can be seen from Table 6, amino nitrogen decreases at a constant rate during the first half of the ripening process until a constant value of the amino nitrogen content is obtained. All experiments indicate rather small differences in the amounts of extractable amino nitrogen compared with the total change during the ripening process between the fractions preserved by freezing and those dried in air. The air-dried fractions contained less amino nitrogen than the frozen fractions as was to be expected. At stage I the amount of amino nitrogen is three times higher than the value for stage VIII. Some conclusions can be drawn from these results. Firstly, the small differences in amino nitrogen between fractions containing no well-defined components and fractions from which well-defined components could be isolated indicate that high-molecular intermediate products are probably involved in protein synthesis, because the formation of large molecules (M = 186 000 and 330 000) from rather large intermediate molecules should give only small changes in amino nitrogen. The fact that amino nitrogen decreases linearly with respect to time also indicates that the intermediate products in the synthesis of proteins are of a high-molecular nature, because the ultracentrifuge diagrams show that the globulin components are formed rather rapidly.

Of course, much work remains to be done in this field. Unripe seeds may be more convenient for the study of protein synthesis than other materials which have been used, such as regenerating liver, because the protein systems in the seeds are rather simple, and large amounts of material can be obtained at different stages of protein synthesis. Furthermore the substances under investigation are all the time in a closed system, which is advantageous in many respects.

SUMMARY

1. Studies have been made of the nitrogen distribution in developing pea seeds.

2. Globulin N increases rapidly during the first part of the ripening process. Albumin N increases at a constant rate. Globulins and albumins are synthesized independently.

3. Vicilin and legumin are synthesized at different rates. The concentration ratio $\frac{\text{vicilin}}{\text{legumin}}$ is diminished as ripening proceeds.

4. Protein synthesis proceeds in unripe seeds which have been harvested. This synthesis is arrested by cooling to $-$ 20° C.

5. The possibility of high-molecular intermediate products in the synthesis of the reserve proteins is discussed.

The author wishes to thank Prof. A. Tiselius for his great interest in this work and for valuable discussions. The investigation was supported by a grant from the Swedish Natural Science Research Council.

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Short Communication

A New Reaction Product from Acetylacetone and *p*-Benzoquinone

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In 1927 Ionescu [1] described a reaction between acetylacetone and *p*-benzoquinone leading to a dihydroxydiacetylindacene. The reaction obviously being base-catalysed, took place in pyridine solution to which the reactants were added in the correct molar ratio.

It has now been found that if *p*-benzoquinone is dissolved in about ten times its own weight of very pure acetylacetone to which 3—4 per cent of pyridine has been added, the reaction takes a different course. In poor yield (5—8 per cent of theory) an intensely red-coloured substance separates as fine needles. The substance (M. p. 212—213° dec.) has the empirical formula $C_{16}H_{16}O_6$.

Calc.	C	63.16	H	5.29
Found	»	63.15	»	5.30

(Actually no molecular weight determinations could be carried out due to the slight solubility of this compound and its derivatives. Classifying it as a C_{16}-compound, however, is in agreement with the number of C-atoms in the starting materials, assuming that no rupture of C—C bonds has occurred during the reaction).

$C_{16}H_{16}O_6$ gives a positive iodoform reaction, is soluble to some extent in water and dissolves readily in aqueous alkali, the solution being dark violet. On addition of mineral acid the original substance separates again. Prolonged action of alkali brings about far-reaching decomposition of the substance.

On catalytic hydrogenation it takes up one mole of hydrogen. The colourless, hydrogenated substance (M. p. ca. 205°) is rather unstable when exposed to the air, especially when in solution but also in the solid, crystalline state. With acetyl chloride it gives a stable diacetate (M. p. 220—221°) with the formula $C_{20}H_{22}O_8$.

Calc.	C	61.53	H	5.69
Found	»	61.50	»	5.92

The substance $C_{16}H_{16}O_6$ is obviously capable of undergoing a reductive acetylation, but as the product is very difficult to purify no reliable analysis of it has so far been obtained.

The red substance is further subject to some interesting transformations when treated with hydrogen chloride in various organic solvents. With methanolic hydrogen chloride it gives a colourless and chlorine-free product $C_{17}H_{18}O_6$ (M. p. 149—150°) containing one methoxyl group.

Calc.	C	64.15	H	5.69	OCH_3	9.74
Found	»	63.93	»	5.62	»	9.71

Most of the above-mentioned facts seem to have a bearing upon the substance $C_{16}H_{16}O_6$ being a quinone. The author, however, will not suggest any structural formula yet, but has nevertheless found it appropriate to publish his results at this stage. The investigation will be continued.

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Tuberculostatic Derivatives of *p*-Aminobenzoic Acid

III. Heterocyclic Derivatives of 4-Aminosalicylic Acid

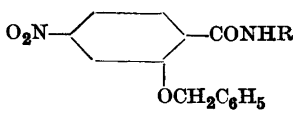
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In a preceding paper [1] some amides of 4-aminosalicylic acid (PAS) were prepared for the purpose of investigating their tuberculostatic activity. The method applied, *viz.* the reaction of ethyl 4-nitrosalicylate with amines and reduction of the nitro compounds formed, is only applicable to amines having not too weak a basic character, *i. e.* mainly aliphatic amines. While the piperidide and morpholide could be prepared in the same way as the aliphatic derivatives, 2-aminopyridine and similar compounds did not react with ethyl 4-nitrosalicylate. As heterocyclic derivatives of this type would be similar to the most strongly bacteriostatically active sulphonamides, their investigation was deemed to be of interest.

The acid chloride of 4-nitrosalicylic acid, like salicylic acid chloride, is rather unstable. By reaction with aniline the corresponding anilide could be prepared, and this could be reduced to 4-aminosalicylanilide. By reaction of the acid chloride with 2-aminopyridine, 2-aminothiazole and other heterocyclic amino compounds, however, a more complex reaction took place, and at first it was not possible to isolate the desired compounds (*cf.* the following paper).

It was then investigated whether the corresponding compounds with the hydroxyl group protected by benzylation would be more easily obtainable. 2-Benzyloxy-4-nitrobenzoic acid gave a quite stable acid chloride, which by reaction with heterocyclic amines in pyridine gave well-defined and beautifully crystalline amides of the type:



R = 2-pyridyl, 2-thiazolyl etc.

By catalytic hydrogenation the nitrogroup was easily reduced, but, contrary to our expectation, the benzyl group was not split off, even by prolonged hydrogenation. This is very remarkable, the more so because it was found that 4-nitro-2-benzyloxybenzoic acid and the corresponding amide and anilide were easily transformed into 4-aminosalicylic acid, 4-aminosalicylamide and 4-aminosalicylanilide respectively. In these cases the benzyl group was split off so easily that a reduction of the nitro group alone by catalytic hydrogenation could only be carried out with some difficulty.

The difficulty of reductive splitting off of the benzyl group is therefore a peculiarity of the heterocyclic derivatives. Even with the use of a considerable amount of platinum as a catalyst or by hydrogenation at 100° and 150 atmospheres no toluene was formed. These findings have been examined further and will be discussed in a subsequent paper.

EXPERIMENTAL

4-Nitro-salicylmorpholide. A mixture of 3 g of ethyl 4-nitrosalicylate and 3 g of morpholine was heated in a sealed tube at 120° for 5 hours. The excess of morpholine was removed in vacuo at 100°. The residue was dissolved in hot water acidified with acetic acid. On cooling crystals separated which were recrystallized from 50 % ethanol after treatment with activated carbon. Yield 50 %. The morpholide forms yellow needles with m.p. 247—248°.

$C_{11}H_{12}O_5N_2$ (252.2) Calc. N 11.11 Found N 10.97

4-Nitro-salicylpiperidide. Prepared in the same way as the morpholide. M. p. 230—232°.

$C_{12}H_{14}O_4N_2$ (250.2) Calc. N 11.20 Found N 11.10

4-Amino-salicylmorpholide. The above mentioned nitro compound was hydrogenated (0.5 g of the nitro compound and 0.01 g PtO_2 in 25 ml of ethanol). The ethanol was removed in vacuo leaving an oil which crystallized by rubbing. The compound was extremely soluble in ethanol and easily soluble also in water. For purification it was dissolved in dry benzene and fractionally precipitated by addition of petroleum ether, the first fractions being discarded. By cooling in ice and rubbing almost colourless crystals separated. Yield 0.2 g. M. p. 161—162°.

$C_{11}H_{14}O_3N_2$ (222.2) Calc. N 12.61 Found N 12.77

4-Amino-salicylpiperidide. Prepared and purified in the same way as the morpholide. M. p. 134—135°.

$C_{12}H_{16}O_2N_2$ (220.2) Calc. N 12.73 Found N 12.83

2-Benzyloxy-4-nitrobenzoic acid. To a suspension of 50 g of 4-nitrosalicylic acid in 100 ml of ethanol were added 35 g of benzyl chloride and 50 ml of 20 % sodium hydroxide. The mixture was heated to boiling and on refluxing the reddish brown colour faded after some time. A 2-N solution of sodium hydroxide was then added until the reddish brown colour reappeared. On addition of the alkali the solution became turbid and ethanol was therefore added, until it was clear. These steps were repeated until the colour remained permanent on continued refluxing. After 1—2 hours the reaction was complete. The ethanol was then partially removed by distillation and replaced by water. Dilute hydrochloric acid was then added, until the precipitation of the benzyl compound was complete. The precipitate was recrystallized from boiling 60 % ethanol. Yield 40 g or 55 %. M. p. 170°.

$C_{14}H_{11}O_5N$ (273.2) Calc. N 5.22 Found N 5.13

2-Benzyloxy-4-amino-benzoic acid. By hydrogenation of the above compound (1 g in 20 ml of ethanol + 0.05 g of PtO_2) toluene and 4-aminosalicylic acid were formed. When the hydrogenation was stopped as soon as the amount of hydrogen calculated for reduction of the nitro group had been absorbed 2-benzyloxy-4-amino-benzoic acid could be isolated. The filtrate from platinum was evaporated in vacuo until crystallization occurred and the product was recrystallized from benzene. M. p. 160°.

$C_{14}H_{13}O_3N$ (243.2)	Calc.	N	5.76	Equiv. wt.	243.2
	Found	»	5.48	» »	245.4

2-Benzyloxy-4-nitrobenzoyl chloride. A mixture of 10 g of 2-benzyloxy-4-nitrobenzoic acid and 10 ml of thionyl chloride was refluxed for 1—1 ½ hour on a water bath. Excess of thionyl chloride was removed in vacuo and the residue was recrystallized from benzene after treatment with dried carbon. The acid chloride separated as large yellow crystals. Yield 9.2 g or 86 %. M. p. 122°.

$C_{14}H_{10}O_4NCl$ (291.7)	Calc.	N	4.80	Cl	12.16
	Found	»	4.71	»	12.12

2-Benzyloxy-4-nitrobenzamide. Two grams of the acid chloride was added to 10 ml of ice-cold, concentrated aqueous ammonia, 30 ml of water was added and the solution neutralized with acetic acid. The yellow crystals which separated were recrystallized from 90 % ethanol acidified with a few drops of concentrated acetic acid. Yield 1.3 g. M. p. 178°.

$C_{14}H_{12}O_4N_2$ (272.3) Calc. N 10.30 Found N 10.14

By catalytic hydrogenation 4-aminosalicylamide was obtained.

2-Benzyloxy-4-nitrobenzanilide. A solution of 1 g of aniline in 5 ml of pyridine was added to 2.9 g of 2-benzyloxy-4-nitrobenzoyl chloride. The mixture became warm, but cooling was unnecessary and the acid chloride dissolved on stirring. After slow cooling, during which some anilide crystallized, the mixture was poured into 300 ml of water. The anilide, which separated, was filtered and recrystallized from acetic acid. Yellow crystals; slightly soluble in ethanol and benzene. Yield 2.2 g. M. p. 201°.

$C_{20}H_{16}O_4N_2$ (348.3) Calc. N 8.04 Found N 8.13

4-Aminosalicylanilide. A hot suspension of 1 g of the preceding compound and 100 mg of PtO_2 in 25 ml of ethanol was hydrogenated. The precipitate gradually dissolved, and after 2 hours the calculated amount of hydrogen had been absorbed. The solution, which had an odour of toluene, was filtered and part of the ethanol was removed *in vacuo*. Water was added and the solution was heated to boiling, so that the precipitate formed was dissolved. Charcoal was added and the hot solution filtered. On cooling the anilide separated as colourless crystals. M. p. 143°. The compound gives a violet colour with ferric chloride.

$C_{20}H_{18}O_2N_2$ (318.3) Calc. N 12.28 Found N 12.18

The following compounds were prepared in the same way as the anilide of 2-benzyloxy-4-nitrobenzoic acid with the exception that the reaction went more slowly, and in the case of the pyrimidine compound slight warming was appropriate. In each case 2.9 g of the acid chloride was employed.

2-(2'-Benzyloxy-4'-nitrobenzoyl)-aminopyridine. Yield 2.2 g. M. p. 144°. Recrystallized from acetic acid (90 %).

$C_{19}H_{15}O_4N_3$ (349.3) Calc. N 12.03 Found N 11.99

2-(2'-Benzyloxy-4'-nitrobenzoyl)-aminothiazole. Yield 2.7 g. M. p. 201°. Recrystallized from acetic acid.

$C_{17}H_{13}O_4N_3S$ (355.3) Calc. N 11.83 Found N 11.95

2-(2'-Benzyloxy-4'-nitrobenzoyl)-amino-5-methyl-1,3,4-thiadiazole. For the preparation 2-amino-1,3,4-thiazole was dissolved in boiling pyridine. Yield 1.7 g. M. p. 196°. Recrystallized from acetic acid (90 %).

$C_{17}H_{14}O_4N_4S$ (370.4) Calc. N 15.13 Found N 15.03

2-(2'-Benzyloxy-4'-nitrobenzoyl)-amino-4,6-dimethylpyrimidine. Yield 1.7 g. M. p. 206°. Recrystallized from 70 % acetic acid.

$C_{20}H_{18}O_4N_4$ (378.4) Calc. N 14.81 Found N 15.05

All these compounds form yellow crystals which are only slightly soluble in benzene and ethanol.

By catalytic hydrogenation the following compounds were prepared:

2-(2'-Benzyloxy-4'-aminobenzoyl)-amino-pyridine. One gram of the nitro compound and 50 mg of PtO_2 were suspended in 40 ml of hot ethanol and hydrogenated. In the course of 2 hours the nitro compound dissolved and the amino derivative was isolated in the same way as the anilide. Almost white crystals with m. p. 183°.

$C_{19}H_{17}O_2N_3$ (319.3) Calc. N 13.16 Found N 13.49

2-(2'-Benzyloxy-4'-aminobenzoyl)-amino-thiazole. This compound is less soluble in ethanol than the preceding compound and crystallized directly from the ethanol solution without addition of water. The compound is light yellow. M. p. 214—215°. Yield 0.50 g.

$C_{17}H_{15}O_2N_3S$ (325.3) Calc. N 12.92 Found N 12.97

2-(2'-Benzyloxy-4'-aminobenzoyl)-amino-5-methyl-1,3,4-thiazole. Prepared in the same way as the thiazole derivative. Slightly soluble in ethanol. Light yellow. Yield 0.58 g. M. p. 110—111°.

$C_{17}H_{16}ONS$ (340.4) Calc. N 16.46 Found N 16.56

By catalytic hydrogenation at 100° and 150 atmospheres the benzyloxy-compounds were reisolated. No toluene was formed and the reacting mixture gave no colour with ferric chloride, showing that no hydroxy compound had been formed. A difference from the hydrogenations at low pressure was, however, that the solutions formed by hydrogenation of the nitro compounds on exposition to air deposited some intensely yellow precipitates, which were almost insoluble in all common solvents. It seems that these compounds are azoxy compounds corresponding to the nitro compounds used.

Thiazole compound:

$C_{34}H_{26}O_5N_6S_2$ (662.7)	Calc.	N 12.68	C 61.60	H 3.95
	Found	» 12.70	» 62.23	» 3.98

SUMMARY

Some heterocyclic derivatives of 4-nitrosalicylic acid and 4-aminosalicylic acid have been described, including derivatives of 4-nitro-2-benzyloxybenzoic acid. By catalytic hydrogenation the benzyl group could easily be split of from the free acid, from the amide and the anilide, but not from the heterocyclic derivatives.

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Tuberculostatic Derivatives of 4-Aminobenzoic Acid

IV. Heterocyclic Derivatives of 4-Aminosalicylic Acid (PAS)

KAI ARNE JENSEN and SVEND Å. K. CHRISTENSEN

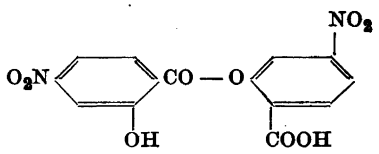
Chemical Laboratory of the University of Copenhagen, Copenhagen, Denmark

As mentioned in the preceding paper [1] it was attempted to prepare 4-aminosalicylamides with heterocyclic substituents in the amide group by reduction of 4-nitro-2-benzyloxybenzamides. We did not, however, succeed in splitting off the benzyl group when the amides contained heterocyclic substituents. While heterocyclic substituted amides of salicylic acid could easily be prepared by reaction of salicyloyl chloride or acetylsalicyloyl chloride with heterocyclic amines in pyridine solution (Jensen and Linholt [2]), great difficulties were encountered in obtaining well defined compounds by application of this process to the corresponding 4-nitroderivatives. Numerous experiments were performed with the condensation of 2-aminopyridine with 4-nitrosalicyloyl chloride or with 2-acetoxy-4-nitrobenzoyl chloride, with or without excess of 2-aminopyridine, with or without pyridine as condensing agent, at 0° or in boiling benzene or carbon tetrachloride, or in aqueous solution by the Schotten-Baumann procedure. Products were obtained which were obviously inhomogenous, their melting points ranging from 100° to 270°, their nitrogen content from 8 to 14 %, and their colour from almost white to an intense yellow, without any apparent connection between melting point or colour and nitrogen content. On boiling the products with ethanol a certain fractionation could be obtained, but it was impossible to isolate a pure compound, because the products were almost insoluble in the common solvents. In sodium hydroxide they dissolved with an intense reddish-brown colour. When the solution was boiled for a short time and acidified 4-nitrosalicylic acid was obtained.

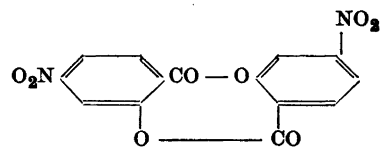
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* After completion of this work condensation products of 2-acetoxy-4-nitrobenzoyl chloride with aminoheterocycles have been described by Doub *et al.*[3], but their attempts to reduce these compounds were unsuccessful.

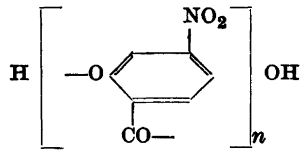
It seems that salicyloyl chloride and acetylsalicyloyl chloride by the action of pyridine or 2-aminopyridine are in part transformed in di- and polysalicylides:



Disalicylide 8.05 % N

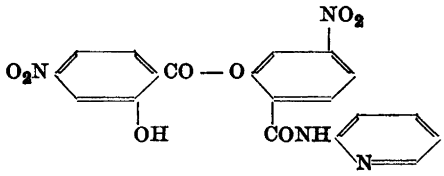


"Diplosal" 8.5 %

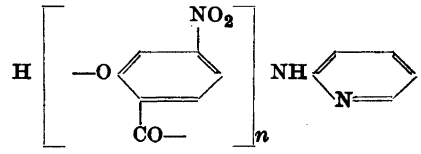


Polysalicylides 8.17 – 8.50 % N

and in part in 2-pyridylamides of these acids:



13.5 % N



11.9 – 8.5 % N

It was observed, however, that a compound having the desired composition (16.2 % N) could be obtained when a solution of 4-nitrosalicyloyl chloride and 2-aminopyridine in pyridine was heated at 120° for some time. Under these circumstances the polymeric anhydrides of 4-nitrosalicylic acid apparently are split by the action of 2-aminopyridine.

Amides of 4-nitrosalicylic acid containing a thiazole, thiadiazole or triazole group were obtained more easily in pure form. In these cases, too, inhomogenous products with low nitrogen content were obtained when the condensation was carried out at lower temperature, but in boiling pyridine products with the calculated composition were formed. No well defined compounds could be obtained from condensations of 4-nitrosalicyloyl chloride with 2-amino-4,6-dimethyl-pyrimidine, 2-amino-4-methyl-pyrimidine, imidazole, indole or 2-methyl-indole. In all these cases the reaction mixture became very dark brown.

The reduction of the nitro derivatives were beset by great difficulties. Catalytic hydrogenation of the nitro compounds were first attempted, using PtO_2 as a catalyst. The pyridine compound could be hydrogenated in hot acetic acid, where it is fairly soluble, but no well defined product could be isolated. The products prepared from 2-aminothiazole and 2-amino-5-methylthiadiazole on the other hand are almost insoluble in all common solvents and although hydrogen was slowly absorbed by suspensions of the compounds in ethanol no amino compounds were formed: the yellow compounds turned white without going in solution and the reaction products were amorphous, insoluble in all solvents except concentrated acids and bases, gave no reaction for an aromatic amino group and could not be purified to give a well defined product. Only in the case of the nitrosalicyloyl derivative of 2-amino-4,5-dimethylthiazole could an amino compound of the desired composition be isolated from the hydrogenation mixture. In this case the nitro compound is fairly soluble in ethanol.

Several other means of reduction had been tried with no success when it was found that reduction proceeded smoothly with tin and hydrochloric acid in acetic acid solution. Under these conditions also the difficultly soluble nitrosalicyloyl derivatives of 2-aminothiazole and 2-amino-5-methylthiadiazole went into solution in few minutes. The amino compounds could be isolated after precipitation of tin with hydrogen sulfide. The amino compounds form colourless crystals and are soluble even in dilute ethanol. They dissolve easily in alkali and in dilute hydrochloric acid but form slightly soluble hydrochlorides with concentrated hydrochloric acid. With ferric chloride they give a violet colour.

The compounds have been found to possess tuberculostatic activity but less than the parent compound, PAS (experiments by J. Lehmann, to be published later).

EXPERIMENTAL

Microanalyses by Mr. A. Grossmann

2-Acetoxy-4-nitrobenzoic acid. A mixture of 183 g of 4-nitro-salicylic acid, 110 g of acetic anhydride and 500 ml of benzene was refluxed for 4 hours. The solution was concentrated to half the volume *in vacuo*. On cooling light yellow crystals deposited, which were filtered and recrystallized from dry benzene. Yield 198 g (88 %). M. p. 156°.

$C_9H_7O_6N$ (225.1) Calc. N 6.22 Found N 6.27

On boiling with water the compound is easily hydrolyzed to 4-nitrosalicylic acid.

2-Acetoxy-4-nitrobenzoyl chloride. A mixture of the acid (11.2 g) and thionyl chloride (10 ml) was heated on a water bath for 15 minutes. The excess of thionyl chloride was

removed *in vacuo* and the residue recrystallized from hexane. Yield 8 g (66 %). Light yellow crystals; m. p. 57.5°.

$C_9H_6O_5NCl$ (243.6) Calc. N 5.75 Found N 6.14

2-Hydroxy-4-nitrobenzoyl chloride (4-nitrosalicyloyl chloride). A mixture of 5 g of 4-nitrosalicylic acid, 20 ml of benzene and 4 ml of thionyl chloride was heated to 90° for 1—2 hours. Generally the acid dissolved in the course of ½ hour (depending on the fineness of the powder). After removing of benzene and excess of thionyl chloride in vacuo the acid chloride crystallized in yellow rosettes which were washed with dry petroleum ether and dried *in vacuo* over paraffin and phosphorus pentoxide. Humidity should be excluded very cautiously during the preparation. Yield quantitative. M. p. 59—60°.

$C_7H_4O_4NCl$ (201.5) Calc. N 6.95 Found N 6.97

4-Nitrosalicylamides. Condensation of heterocyclic amines with 4-nitrosalicyloyl chloride or 2-acetoxy-4-nitrobenzoyl chloride at lower temperature gave products with low nitrogen content. Products with the desired nitrogen content were obtained in the following way: 0.01 mole of 4-nitrosalicyloyl chloride was melted and a solution of 0.01 mole of the amino compound in 15 ml of dry pyridine was added at one time. A vigourous reaction took place. The solution was heated to 120° for ½ hour, cooled and poured into water. The precipitation was completed by addition of acetic acid, and after filtration the precipitate was washed thoroughly with water and digested with 20 ml of hot ethanol. The crude products (yields 50—70 %) were used directly for reduction. They are far from pure, but with exception of compounds nos. II and III their solubility was so extremely slight that it was inconvenient to recrystallize more than small portions for analysis. At least two recrystallizations were necessary to obtain pure products. With exception of compound no III they have no sharp melting points but decompose before melting, the melting point therefore being very dependent on the heating rate.

I. 2-(p-Nitrosalicyloyl)-aminothiazole. Light yellow crystals from acetic acid. M. p. ca. 300° (dec.). Almost insoluble in ethanol.

$C_{10}H_7O_4N_3S$ (265.3) Calc. C 44.60 H 2.62 N 15.84
Found » 44.20 » 3.12 » 15.96—16.04—15.87 *

II. 2-(p-Nitrosalicyloyl)-4,5-dimethylthiazole. Yellow crystals from ethanol (fairly soluble). M. p. ca. 300° (dec.).

$C_{12}H_{11}O_4N_3S$ (293.3) Calc. N 14.33 Found N 14.20—13.94—13.97 *

III. 2-(p-Nitrosalicyloyl)-amino-4-methyl-5-carbethoxy-thiazole. Light yellow crystals from ethanol or acetic acid. M. p. 237—38°.

$C_{14}H_{13}O_6N_3S$ (351.3) Calc. N 11.96 Found N 11.53

* Different preparations.

IV. 2-(p-Nitrosalicyloyl)-aminopyridine. Intensely brownish yellow crystals from propanol; almost insoluble in ethanol. M. p. ca. 240° (dec.).

$C_{12}H_9O_4N_3$ (259.2) Calc. N 16.21 Found N 16.05—16.39—16.49 *

V. 3-(p-Nitrosalicyloyl)-amino-1,2,4-triazole. Light yellow crystals from ethanol. M. p. ca. 315 (dec.).

$C_9H_7O_4N_5$ (249.2) Calc. N 28.10 Found N 27.81

VI. 2-(p-Nitrosalicyloyl)-amino-5-methyl-1,3,4-thiadiazole. Light yellow (almost white) crystals from acetic acid (very slightly soluble). Insoluble in most solvents. M. p. ca. 300° (dec.).

$C_{10}H_8O_4N_4S$ (280.3) Calc. C 42.87 H 3.62 N 19.99
Found » 43.02 » 3.28 » 19.77—19.72—20.16—20.26 *

4-Aminosalicylamides. Reduction of the nitro compounds was performed in the following way: The nitro compound (1 g) was mixed well with 100 g of granulated tin and 20 ml of acetic acid and 5 ml of conc. hydrochloric acid were added. The mixture was heated to 100° for 10 minutes, the compounds usually dissolving in 1 or 2 minutes. The solution was decanted from the tin (which was used for the next preparation), diluted with 150 ml of water and precipitated with hydrogen sulfide. After filtration the solution was brought to pH = 5, the precipitates filtered and washed with water. They were recrystallized from 50 % ethanol and dried at 100°. Yields 0.60—0.70 g. The products form colourless crystals.

2-(p-Aminosalicyloyl)-aminothiazole. M. p. 252° (dec.), 266° on the Kofler-stage.

$C_{10}H_9O_2N_3S$ (235.3) Calc. C 51.09 H 3.85 N 17.85
Found » 50.86 » 4.04 » 17.60

2-(p-Aminosalicyloyl)-amino-4,5-dimethylthiazole. M. p. 253—54°, 268° on the Kofler stage.

$C_{12}H_{13}O_2N_3S$ (263.3) Calc. N 15.95 Found N 16.33

2-(p-Aminosalicyloyl)-aminopyridine. M. p. 185—86°, 190° on the Kofler stage.

$C_{12}H_{11}O_2N_3$ (229.3) Calc. N 18.33 Found N 17.98

2-(p-Aminosalicyloyl)-amino-5-methyl-1,3,4-thiadiazole. M. p. ca. 300° (dec.).

$C_{10}H_{10}O_2N_4S$ (250.2) Calc. N 22.40 Found N 21.94

* Different preparations.

SUMMARY

Condensation of *p*-nitrosalicyloyl chloride with heterocyclic amines in pyridine solution at 120° gave the corresponding heterocyclic substituted *p*-nitrosalicylamides. At lower temperature more complicated products were formed.

By reduction of the nitro compounds with tin and hydrochloric acid in acetic acid solution the corresponding heterocyclic substituted *p*-aminosalicylamides were obtained.

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ACTA CHEMICA SCANDINAVICA 6 (1952) 172–175

Tuberculostatic Derivatives of *p*-Aminobenzoic Acid

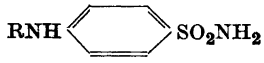
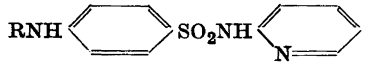
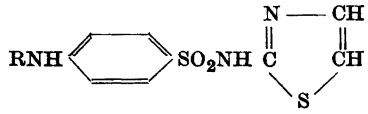
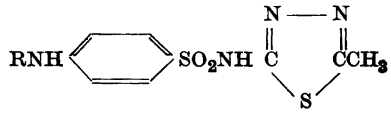
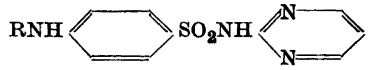
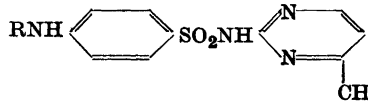
V. 4-Aminosalicyloyl Derivatives of Sulfanilamide and Related Compounds

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With the purpose of investigating their tuberculostatic activity some compounds which at the same time are derivatives of p-aminosalicylic acid (PAS) and sulfonamides or sulfones were prepared.

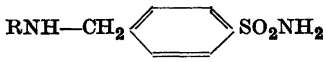
From the reaction of *p*-nitrosalicyloyl chloride with aniline a well defined product could more easily be obtained than from the reaction of this acid chloride with heterocyclic amines (*cf.* the preceding paper [1]). Analogously *p*-nitrosalicyloyl derivatives of sulfanilamide, sulfathiazole *etc.* were obtained without difficulty and generally in good yields. The nitro compounds form intensely yellow powders which are very slightly soluble in most solvents. The following compounds were prepared (R = O_2N–C$_6$H$_3$(OH)–CO–):

		PNS-Derivative of:
I		Sulfanilamide
II		Sulfapyridine
III		Sulfathiazole
IV		Sulfamethylizole
V		Sulfadiazine
VI		Sulfamerazine

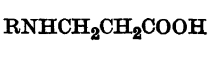
In addition to the compounds of this type we also prepared *p*-nitrosalicyloyl derivatives of 4,4'-diamino-diphenylsulfone (VII), *p*-aminomethylbenzenesulfonamide (Marfanil, Sulfamylon. VIII) and β-alanine (IX):



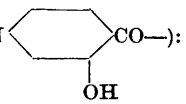
VII

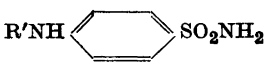
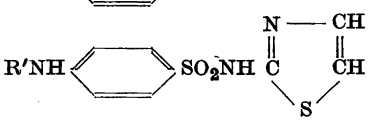
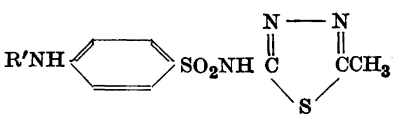
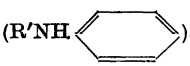
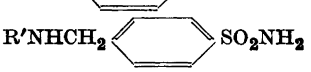


VIII



IX

The nitro compounds were reduced to the corresponding amino compounds with tin and hydrochloric acid in acetic acid solution. The following compounds were isolated and characterized (R'= ):

		PAS-Derivative of:
X		Sulfanilamide
XI		Sulfathiazole
XII		Sulfamethylizole
XIII		4,4'-Diamino-diphenylsulfone
XIV		Marfanil

EXPERIMENTAL

p-Nitrosalicylanilide (2-Hydroxy-4-nitrobenzanilide). *p*-Nitrosalicyloyl chloride (2 g) was added with cooling to aniline (5 ml) and the reaction product recrystallized from benzene. Yield 2.1 g (80 %). M. p. 238°.

$C_{13}H_{10}O_4N_2$ (258.2) Calc. N 10.85 Found N 10.65

By catalytic hydrogenation at room temperature and atmospheric pressure (*cf.* Jensen, Rosdahl and Ingvorsen [2]) this compound was transformed into *p*-aminosalicylanilide (m. p. 143°) identical with the product prepared by Jensen and Ingvorsen [3].

N^4-(p-Nitrosalicyloyl)-sulfanilamide (I). Sulfanilamide (0.005 mole; 0.85 g) dissolved in 10 ml of pyridine was added to *p*-nitrosalicyloyl chloride (1 g) and the solution heated for ½ hour at 120°. The mixture was diluted with water and the precipitate filtered and washed thoroughly with water. Yield of crude product 1.55 g (89 %). Recrystal-

lized from ethanol it formed orange-yellow crystals which turned almost white at 200° and melted at 249–50°.

$C_{13}H_{11}O_6N_3S$ (337.3) Calc. N 12.46 Found N 12.54

In a similar way the other nitro compounds (Table I) were prepared. In preparing the comp. no. VIII the hydrochloride of Marfanil instead of the free amine was used. Yields in most cases were 70–90 %. The crude products were recrystallized from ethanol or acetic acid. The derivatives of the heterocyclic sulfonamides and comp. no. VII are very slightly soluble in the common solvents, whereas the other compounds were rather soluble in hot ethanol. The compounds form light yellow crystals.

By reaction of *p*-nitrosalicyloyl chloride with 4,4′-diaminodiphenylsulfone a bis-*p*-nitrosalicyloyl derivative was obtained. With 4-aminophenyl-5′-amino-2′-thiazolyl-sulfone (Promizole) on the other hand a product was obtained which apparently is a mono-*p*-nitrosalicyloyl derivative (calc. 13.34 % N, found 13.10 %). Other preparations, however, gave lower nitrogen values and the compound was not examined further.

The derivative of β-alanine was prepared by using a Schotten-Bauman procedure in the same way as salicyloyl-β-alanine (Moss *et al.*[4]).

Reduction was performed with tin and hydrochloric acid in acetic acid solution in the same way as described in the preceding paper.

N^4-(p-Aminosalicyloyl)-sulfanilamide. On recrystallizing from dilute ethanol (70 %) the compound separated as white shiny plates. Slightly soluble in hot ethanol, easily soluble in acetone. M. p.: decomposes gradually at 270–300°.

$C_{13}H_{13}O_4N_3S$	(307.3)	Calc.	N 13.74	S 10.64
		Found	» 13.70	» 10.64

In a similar way the other amino compounds (Table 2) were prepared.

Table 1. p-Nitrosalicylic acid derivatives of sulfanilamide etc.

No.	Empirical formula	M. p.	% N	
			calc.	found
I	$C_{13}H_{11}O_6N_3S$	250°	12.46	12.50
II	$C_{18}H_{14}O_6N_4S$	205° (dec.)	13.52	13.45
III	$C_{16}H_{12}O_6N_4S_2$	290° (dec.)	13.36	13.80
IV	$C_{16}H_{13}O_6N_5S_2$	296° (dec.)	16.08	16.60
V	$C_{17}H_{13}O_6N_5S$	295° (dec.)	16.86	16.63
VI	$C_{18}H_{15}O_6N_5S$	270° (dec.)	16.31	16.45
VII	$C_{26}H_{18}O_{10}N_4S$	245° (dec.)	9.68	9.97
VIII	$C_{14}H_{13}O_6N_3S$	254°	11.94	11.96
IX	$C_{10}H_{10}O_6N_2S$	212–13°	11.03	11.15

Nos. III and IV were recrystallized from acetic acid, the others from ethanol. No. VI melts first at ca. 160° but solidifies again.

Table 2. p-Aminosalicyloyl derivatives of sulfanilamide etc.

No.	Empirical formula	M. p.	% N	
			calc.	found
X	$C_{13}H_{13}O_4N_3S$	dec.	13.74	13.70
XI	$C_{16}H_{14}O_4N_4S_2$	270° (dec.)	14.35	13.82
XII	$C_{16}H_{15}O_4N_5S_2$	280° (dec.)	17.50	18.07
XIII	$C_{26}H_{20}O_8N_4S$	264°	10.81	10.68
XIV	$C_{14}H_{15}O_4N_3S$	219°	13.08	12.98

SUMMARY

p-Nitrosalicylic acid derivatives of sulfanilamide and substituted sulfanilamides, of diaminodiphenylsulfone and *p*-aminomethylbenzenesulfonamide (Marfanil, Sulfamylon) were prepared by the reaction of *p*-nitrosalicyloyl chloride with the appropriate amino compound in pyridine solution. The corresponding *p*-amino derivatives were obtained by reduction of the nitro compounds with tin and hydrochloric acid in acetic acid solution.

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Tuberculostatic Derivatives of *p*-Aminobenzoic Acid

VI. Preparation of Amides of p-Aminosalicylic Acid (PAS) by Means of 2-Acetoxy-4-acetamido-benzoyl Chloride

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The difficulties encountered in preparing heterocyclic derivatives of p-aminosalicylamide (Jensen and Ingvorsen [1], Jensen and Christensen [2]) via 4-nitrosalicylic acid caused us to investigate the possibility of preparing compounds of this type more directly from 4-aminosalicylic acid (PAS). To transform this acid into an acid chloride the amino group, and probably also the hydroxy group, should be protected, for instance by introduction of a carbobenzyloxy group or an acetyl group. The first possibility would offer the advantage that the carbobenzyloxy group might be removed by hydrogenolysis, whereas the acetyl group has to be removed by hydrolysis which will also involve the other amide group. After some preliminary experiments with 4-carbobenzyloxyamino-2-hydroxybenzoic acid we, however, concentrated our efforts on the reactions of 2-acetoxy-4-acetamidobenzoyl chloride, which turned out to be rather easily accessible. This acid chloride couple easily with amines to give 4-acetamidosalicylamides. By hydrolysis these give the corresponding amides of p-aminosalicylic acid, although not in good yields, because the hydrolysis of course attacks both the acetamido and the benzamido group. Because of the easy accessibility of the acid chloride this method of preparing amides of *p*-aminosalicylic acid nevertheless seems to be preferable to their preparation via 4-nitrobenzoyl chloride.

EXPERIMENTAL

Microanalyses by Mr. A. Grossmann.

2-Hydroxy-4-acetamidobenzoic acid *. 2-Hydroxy-4-aminobenzoic acid (7.7 g) was dissolved in 2 *N* NaOH (25 ml) and acetic anhydride (10.2 g) was added successively with stirring, sodium hydroxide being added to maintain the pH at 7. When the acetic anhydride had dissolved the solution was acidified strongly with hydrochloric acid (19 ml). The solid was filtered off and recrystallized from 50 % ethanol. Yield 7.3 g (74 %). M. p. 231° (dec.).

$C_9H_9O_4N$ (195.2) Calc. N 7.18 Found N 7.36

2-Acetoxy-4-acetamidobenzoic acid *. 2-Hydroxy-4-aminobenzoic acid (10 g) was suspended in a mixture of dry benzene (30 ml) and acetic anhydride (25 ml) and the mixture refluxed for 2 hours. After cooling the solid was filtered off (yield 15.5 g or 100 %) and recrystallized from acetic acid containing 20 % acetic anhydride. Yield of recrystallized product: 12 g (78 %). M. p. 195°.

$C_{11}H_{11}O_5N$ (237.2) Calc. N 5.91 Found N 5.93

2-Hydroxy-4-carbobenzyloxyaminobenzoic acid. To an icecold solution of 2-hydroxy-4-aminobenzoic acid (1 g) in 1 *N* sodium hydroxide (6.5 ml) was added alternately and with thorough stirring 6.5 ml of 1 *N* sodium hydroxide and a solution of benzyl chloroformate (2 g) in toluene. After standing for one hour in an ice bath the solution was extracted with ether and to the aqueous solution was added concentrated hydrochloric acid until pH = 2. The precipitate was recrystallized from dilute ethanol (1 : 1). Yield 1.8 g (69 %). M. p. 187—88°.

$C_{15}H_{13}O_5N$ (287.3)	Calc.	C 62.69	H 4.62	N 4.88
	Found	» 62.72	» 4.53	» 4.71

Even by application of a large excess of benzyl chloroformate only the mono-derivative was formed.

2-Acetoxy-4-acetamidobenzoyl chloride. Finely powdered 2-acetoxy-4-acetamidobenzoic acid (1 g) was mixed with thionyl chloride (2 ml). After standing for about one hour at room temperature a reaction sat in: hydrogen chloride was evolved and the solid went in solution; separation of another solid began as a rule before all had dissolved. After two hours a homogenous, almost white precipitate of the acid chloride had formed. The process can be considerably accelerated by addition of a trace of pyridine, but the product then becomes yellow. After removing of excess thionyl chloride *in vacuo*, addition of dry benzene and evaporating again, the product could be directly used for preparation of the amides. When working with larger amounts 1 ml of thionyl chloride for each g of the acid is sufficient.

For analysis the acid chloride was prepared in the following way: 1 g of the acid was suspended in 5 ml of chloroform and 1 ml of thionyl chloride was added. After 2—3

* While this work was in progress these two acids were also reported by Drain *et al.*[4].

hours all had dissolved with evolution of hydrogen chloride. After standing for 24 hours colourless prismatic crystals had separated, which were filtered off and washed with chloroform. M. p. 144°.

$C_{11}H_{10}O_4NCl$ (255.7) Calc. Cl 13.87 Found Cl 13.84

Amides of 2-hydroxy-4-aminobenzoic acid. To the acid chloride prepared from 1 g of the acid was added 10 ml of dry benzene and either 2.5 mole of the amine or 1 mole of the amine and 1.5 mole of pyridine. The mixture was cooled and stirred well. After ½ hour 10—20 ml of warm (40°) 2-N sodium hydroxide was added and the mixture shaken in separatory funnel. The aqueous layer was separated and acidified. The acetamido compound was filtered off and either recrystallized or hydrolyzed directly. Hydrolysis of the acetoxy group was attained by boiling with 25 ml of 6-N sulfuric acid for 10—15 minutes (or until the solid had dissolved); when the cooled solution was brought to pH 5—6 the amino compound separated. Yields of the crude acetamido compounds were 80—100 %, yields of the hydrolysed products 40—60 %. Identity of the amino compounds with those prepared via the nitro compounds was shown by mixed melting points.

In this way the following amides were prepared:

2-Hydroxy-4-acetamidobenzoic isopropylamide. Recrystallized from 50 % ethanol. Melts at 185°, solidifies and melts again at 210°.

$C_{12}H_{16}O_3N_2$ (236.7) Calc. N 11.84 Found N 11.94

By hydrolysis 2-hydroxy-4-aminobenzoic isopropylamide was obtained. M. p. 161°, with no depression on mixing with a product prepared via the nitro compound [3].

2-Hydroxy-4-acetamidobenzoic diethylamide. Recrystallized from water.

$C_{13}H_{18}O_3N_2$ (250.3) Calc. N 11.20 Found N 11.09

Melts at 214°, solidifies and melts again at 230°; no depression on mixing with a product prepared by acetylation of the amino compound obtained via the corresponding nitro compound [3].

2-Hydroxy-4-aminobenzanilide. Recrystallized from ethanol. M. p. 143°.

N^2-(2-Hydroxy-4-aminobenzoyl)-sulfanilamide. Recrystallized from ethanol. Decomposes at about 300°. N 13.36 %, calc. 13.70 %.

2-(2'-Hydroxy-4'-aminobenzoyl)-aminopyridine. Recrystallized from 50 % ethanol. M. p. 186°. N 17.98 %, calc. 18.30 %.

2-(2'-Hydroxy-4-aminobenzoyl)-amino-5-methyl-1,3,4-thiadiazole. Recrystallized from 50 % ethanol. M. p. 300° (dec.). N 21.94 %, calc. 22.40 %.

2-(2'-Hydroxy-4'-aminobenzoyl)-aminothiazole. To the acid chloride prepared from 2 g of 2-acetoxy-4-acetamidobenzoic acid was added a solution of 1 g of 2-aminothiazole in 5 ml of pyridine with good mixing. After 2 hours 100 ml of water was added and the precipitate was filtered. After recrystallization from acetic acid containing 20 % acetic anhydride 0.75 g of 2-(2'-acetoxy-4'-acetamidobenzoyl)-aminothiazole was obtained. M. p. 294—95°.

$C_{14}H_{13}O_4N_3S$ (319.3) Calc. N 13.13 Found N 13.23

On hydrolysis 0.35 g of the deacetylated compound was obtained. This compound is slightly soluble in boiling water; on cooling colourless needles were obtained. When dried at room temperature over $CaCl_2$ the composition corresponds to a hemihydrate:

$C_{10}H_9O_2N_3S \cdot \frac{1}{2}H_2O$ (244.3)	Calc.	C 49.18	H 4.22	N 17.10	S 13.11
	Found	» 49.18	» 4.10	» 17.21	» 13.05

After drying at 100° the composition corresponds to an anhydrous compound:

$C_{10}H_9O_2N_3S$ (235.3)	Calc.	N 17.84	Found	N 17.50

M. p. 266° (dec.) in accordance with the value found for the product prepared via the nitro compound [2].

SUMMARY

2-Acetoxy-4-acetamidobenzoic acid, prepared by acetylation of *p*-aminosalicylic acid (PAS), has been transformed into the acid chloride. By condensation of this with different amino compounds and subsequent hydrolysis of the acetoxy group the corresponding substituted *p*-aminosalicylamides have been obtained.

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On *cis*-β-Naphthylacrylic Acids

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In connection with investigations on 1-naphthylacetaldehyde (Jensen and Christensen [1]) experiments were undertaken to prepare *cis*-β-(1-naphthyl)-acrylic acid (V) and related compounds.

In analogy with *allo*-cinnamic acid, *cis*-β-(1-naphthyl)-acrylic acid could be obtained through the following series of reactions:

$$\underset{\text{I } \textit{trans}}{1\text{-}C_{10}H_7\text{-}CH=CH\text{-}COOH} \longrightarrow \underset{\text{II}}{1\text{-}C_{10}H_7\text{-}CH=CH\text{-}COOEt} \longrightarrow$$

$$1\text{-}C_{10}H_7\text{-}CHBr\text{-}CHBr\text{-}COOEt \longrightarrow \underset{\text{III}}{\phantom{1\text{-}C_{10}H_7\text{-}CHBr\text{-}CHBr\text{-}COOEt}} \quad 1\text{-}C_{10}H_7\text{-}C\equiv C\text{-}COOH \xrightarrow[Pd/BaSO_4]{H_2} \underset{\text{IV}}{\phantom{1\text{-}C_{10}H_7\text{-}C\equiv C\text{-}COOH}}$$

$$\underset{\text{V } \textit{cis}}{1\text{-}C_{10}H_7\text{-}CH=CH\text{-}COOH}$$

1-Naphthaldehyde on treatment with malonic acid in pyridine afforded the *trans*-acid (I), melting at 210-212° in agreement with previous statements [2,3]. Bromination of (I), as described by Wojack *et al.*[4], did not proceed very satisfactorily in our hands, nor did the subsequent alkali-induced dehydrobromination. Therefore attention was turned to the procedure used by West [3] according to which the ester (III) was treated with potassium hydroxide but with no better success. After some trials it became obvious that the employment of rigorously purified ester (III) and high alkali concentration were factors of prime import, increasing the yield of the propiolic acid (IV) to 80-90 %. When submitted to hydrogenation with a palladium catalyst precipitated on barium sulfate, the propiolic acid readily took up one mole of hydrogen and the desired β-(1-naphthyl)-acrylic acid (V) was isolated as colourless plates,

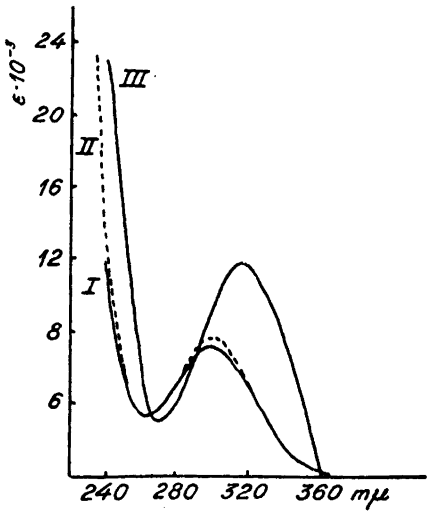


Fig. 1. Ultraviolet absorption spectra of: cis-β-(1-naphthyl)-acrylic acid prepared by hydrogenation of the corresponding propiolic acid I; cis-β-(1-naphthyl)-acrylic acid prepared by rearrangement of the trans-acid II; trans-β-(1-naphthyl)-acrylic acid III.

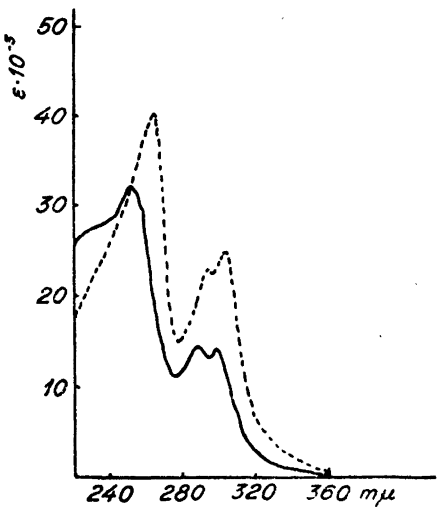


Fig. 2. Ultraviolet absorption spectra of: cis-β-(2-naphthyl)-acrylic acid ————; trans-β-(2-naphthyl)-acrylic acid – – – –.

melting at 156°. This acid is considered as a *cis*-form on account of the mode of formation, melting point, solubility and UV-spectrum (Fig. 1).

At a late stage of the present investigation Havinga and Nivard [5] reported on the preparation of *cis*-β-(1-naphthyl)-acrylic acid, m.p. 141—143°, by ultraviolet irradiation of the *trans*-acid, and presented UV-data for the two geometrically isomeric acids, which although quite similar to our spectroscopical findings differed significantly from these in having the peak for the *cis*-acid displaced about 10 mμ towards the long wave region.

Shortly afterwards the communication by Lock and Gergely [6] appeared in which the *cis*-configuration of a photorearranged *trans*-acid (m.p. 143-145°) seemed to be convincingly established.

These independent findings with their mutual accord in melting points, impelled us to re-examine our *cis*-acid as to homogeneity and identity. Several attempts to repeat the photoinduced rearrangement as described by Lock and Gergely *(l. c.)* invariably yielded an acid (m.p. 156°) which by means of mixed melting point, ultraviolet (Fig. 1) and infrared (Fig. 5) spectra proved to be identical with the specimen prepared *via* the propiolic acid.

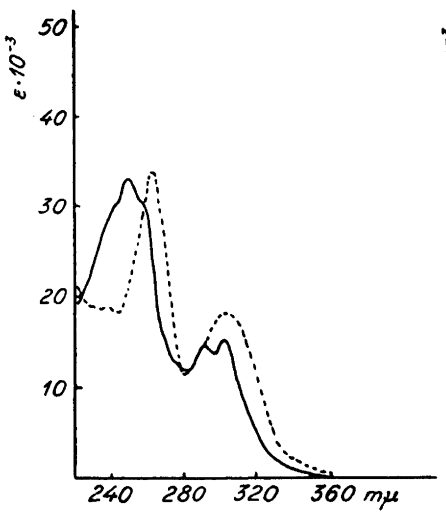


Fig. 3. Ultraviolet absorption spectra of: cis-α-bromo-β-(2-naphthyl)-acrylic acid ————; trans-α-bromo-β-(2-naphthyl)-acrylic acid – – – – – –.

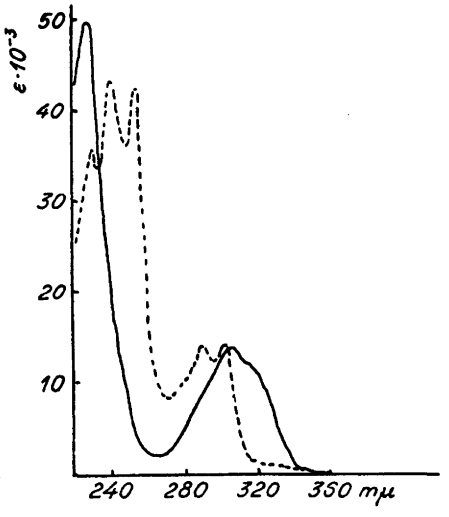


Fig. 4. Ultraviolet absorption spectra of: β-(1-naphthyl)-propiolic acid ————; β-(2-naphthyl)-propiolic acid – – – – – –.

In view of the experimental evidence it appears safe to conclude that our acid (m. p. 156°) possesses *cis*-configuration and that the reported lower-melting acids merit this designation as well. The question remains whether the lower melting preparation represents a dimorphous form. The occurrence of polymorphism in this series would not be surprising, recalling the existence of no less than four different forms of *allo*-cinnamic acid. The discrepancy in melting points and UV-spectra between Havinga and Nivard's (*l. c.*) and our *cis*-acid may, however, be accounted for by the presence of a slight amount of *trans*-acid in their preparation.

An identical sequence of reactions starting with 2-naphthaldehyde yielded *cis*-β-(2-naphthyl)-acrylic acid (VI), for which ultraviolet data are presented in Fig. 2.

$2\text{-}C_{10}H_7\text{-}CH=CH\text{-}COOH$ VI

$2\text{-}C_{10}H_7\text{-}CHBr\text{-}CHBr\text{-}COOEt$ VII

$2\text{-}C_{10}H_7\text{-}CH=CBr\text{-}COOH$ VIII

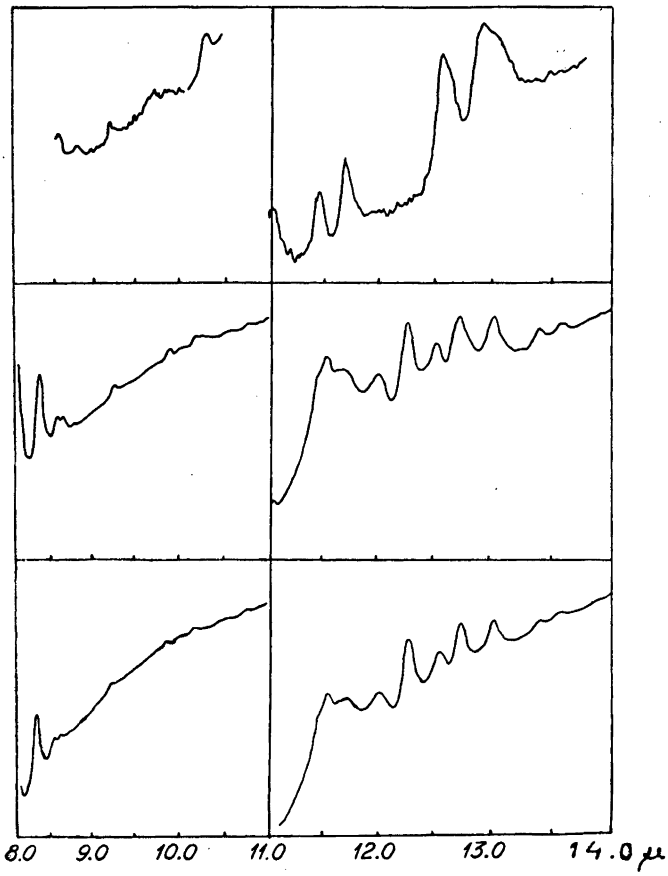


Fig. 5. Infrared absorption spectra of: trans-β-(1-naphthyl)-acrylic acid (upper curve); cis-β-(1-naphthyl)-acrylic acid prepared by rearrangement (middle curve); cis-β-(1-naphthyl)-acrylic acid prepared by hydrogenation of the corresponding propiolic acid (lower curve).

In contradistinction to what was found in the first series, bromination of the free *trans*-β-(2-naphthyl)-acrylic acid proceeded smoothly in high yield. Upon short heating of ethyl α,β-dibromo-β-(2-naphthyl)-propionate (VII) with ethanolic potassium hydroxide two isomeric monobromonaphthylacrylic acids were obtained (m.p. 200-202° and 185-186°), which are tentatively designated as *trans*- and *cis*-α-bromo-β-(2-naphthyl)-acrylic acids (VIII) respectively, the allocation of structure being made merely on basis of melting points, solubilities and UV-spectra (Fig. 3) of the two isomerides. Again, in the phenyl series the analogous transformation of β-phenyl-α,β-dibromopropionic acid into two isomeric α-bromo-cinnamic acids is well known from in the literature [7,8].

On further hydrogenation the *cis*-β-naphthylacrylic acids were transformed into β-(1-naphthyl)- and β-(2-naphthyl)-propionic acids respectively, having melting points identical with those of the corresponding acrylic acids, although a depression of about 20° was noted on admixture with these. A preliminary suspicion that the reason for the accord in melting points should be sought in nearly identical crystal lattices for the acrylic- and propionic acids were not borne out by the Debye-diagrams which appeared to be quite different.

In Fig. 4 the UV-spectra of the two isomeric β-naphthylpropiolic acids are presented.

EXPERIMENTAL

1- and 2-Naphthaldehyde. It was found that these aldehydes could very conveniently be prepared by oxidation of 1- and 2-methylnaphthalene with selenium dioxide (*cf.* ref. 9).

In a three-necked flask provided with a stirrer, thermometer and an air-cooled condenser set for distillation, 2-methylnaphthalene (175 g) was melted and freshly sublimed selenium dioxide (65 g) added with stirring. The well agitated mixture was heated cautiously to 200°, whereupon a vigorous reaction started with water, methylnaphthalene and some aldehyde distilling over. When the reaction subsided, heating was continued for about 10 minutes. The distillate (60 ml) diluted with 150 ml of benzene was added to the cooled reaction mixture and the selenium filtered off. After removal of benzene at atmospheric pressure the residue was fractionated *in vacuo*. The excess of 2-methylnaphthalene distilled at 110°/10 mm followed by the aldehyde at 150°/12 mm. After recrystallization from ethanol the aldehyde melted at 57°. Yield 55 g or 70 % (calculated on SeO_2). A second recrystallization from ethanol raised the melting point to 59° in accordance with the values in the literature (*cf.* ref. 10).

The same procedure was employed to prepare 1-naphthaldehyde in 50 % yield. The yields reported here may perhaps be increased by further modification of the procedure.

1-Naphthyl-derivatives

trans-β-(1-Naphthyl)-acrylic acid (I). Prepared by condensation of 1-naphthaldehyde with malonic acid in pyridine according to Fulton and Robinson [2]. Yield 60 %. M.p. 210—212°, in accordance with the value given by most authors. Willstaedt and Scheiber [11] report the melting point 129-130°, probably attributable to a misprint.

$C_{13}H_{10}O_2$ (198.2)	Calc.	C	78.76	H	5.08
	Found	»	78.96	»	5.20

Micro-hydrogenation: 9.935 mg ~ 1.130 ml H_2 (0°, 760 mm) Calc. 1.124 ml

Ethyl trans-β-(1-naphthyl)-acrylate (II). Prepared by esterification of the acid, according to known procedures [3,12].

B.p. 250° at 13 mm. Yield 90 %.

$C_{15}H_{14}O_2$ (226.3)	Calc.	C	79.43	H	6.24
	Found	»	79.35	»	6.18

Ethyl β-(1-naphthyl)-α,β-dibromopropionate (III). By bromination of the ester (II) according to West [3]. Yield 92 %. M.p. 77-78°.

$C_{15}H_{14}O_2Br_2$ (386.1)	Calc.	Br	41.45
	Found	»	41.31

β-(1-Naphthyl)-propiolic acid (IV). This acid has been decribed by Wojack *et al.*[4], and by West [3] who obtained it from the dibromopropionic acid and the ethyl dibromopropionate respectively. The ester method seemed to be preferable as the yield by bromination of the free acid is much lower than for the corresponding ester, and since the elimination of hydrogen bromide from the dibromo acid gave only a 40 % yield of the propiolic acid. Following the directions of West [3], however, we obtained no propiolic acid at all. After some experiments it became clear that it is essential to use a pure dibromo ester (West used the crude ester) and a very high concentration of potassium hydroxide. In the following way a very satisfactory yield was obtained:

A solution of 0.01 mole (3.86 g) of ethyl β-(1-naphthyl)-α,β-dibromopropionate (III) and 0.12 mole (6.7 g) of potassium hydroxide in 75 ml of 95 % ethanol was refluxed for 4 hours. The solution was cooled, filtered and the ethanol removed *in vacuo*. After addition of 25 ml of water the solution was extracted twice with ether. The aqueous solution was heated to expel dissolved ether, cooled in ice and the acid precipitated by addition of dilute sulfuric acid. The crude product was recrystallized from carbon tetrachloride. M.p. 138°. Yield 1.60 g raised by an additional crop from the mother liquor to 1.80 g (92 %).

$C_{13}H_8O_2$ (196.2)	Microhydrogenation:		
	Found	4.074 mg ~ 0.922 ml H_2	(0°, 760 mm)
	Calc.	0.930 » »	

cis-β-(1-Naphthyl)-acrylic acid. The propiolic acid (2.25 g) was dissolved in 50 ml of ethanol; 1.5 g of $Pd/BaSO_4$ (5%) was added and the mixture hydrogenated at room temperature and atmospheric pressure. After *ca.* two hours, 95 % of the theoretical amount of hydrogen (260 ml) had been absorbed. The catalyst was filtered off, the filtrate concentrated to 25 ml and after cooling in the ice-box, 1.5 g of crystals with m.p. 140° were isolated. Three recrystallizations from benzene followed by one from a mixture of carbon tetrachloride and chloroform (1 : 1) raised the melting point to 154-156° (yield 1.06 g).

$C_{13}H_{10}O_2$ (198.2)	Calc.	C	78.76	H	5.08	Eq. w. 198.2
	Found	»	79.03	»	4.96	» » 198.4

Microhydrogenation:

Found	10.00 mg ~	1.138 ml H_2	(0°, 760 mm)
Calc.		1.132 » »	

The acid crystallizes from ethanol, benzene, carbon tetrachloride or mixtures of carbon tetrachloride and chloroform in nacreous plates, easily soluble in methanol and acetone. The sodium salt is soluble in methanol but insoluble in acetone. The acid sublimates at 140°/0.1 mm without decomposition and with unchanged melting point.

The *cis*-acid was also prepared by ultraviolet irradiation of the *trans*-acid, mainly following the directions of Lock and Gergely [6]. The purification of the crude product was

performed in the following way. After evaporation of the ether, the residue was suspended in methanol and unchanged *trans*-acid filtered off, methanol was removed *in vacuo* and the residue recrystallized several times from benzene. Starting with 2 g of *trans*-acid, we obtained 200 mg (m.p. 154-156°), 100 mg (m.p. 151-153°) and 270 mg (m.p. 146-148°), *i.e.* a total yield of 27 % (Lock and Gergely: 29 %). No depression was observed on admixture with the acid prepared *via* the propiolic acid. Analysis of the product melting at 154-156° gave the following values:

$C_{13}H_{10}O_2$ (198.2)	Calc.	C	78.76	H	5.08
	Found	»	78.94	»	5.15

2-Naphthyl-derivatives

The 2-naphthyl compounds were in most cases prepared according to the directions given for the 1-naphthyl derivatives:

trans β-(2-Naphthyl)-acrylic acid. M.p. 210°.

$C_{13}H_{10}O_2$ (198.2)	Calc.	C	78.76	H	5.08
	Found	»	78.47	»	5.06

Microhydrogenation: 15.308 mg ~ 1.720 ml H_2 (0°, 760 mm)
Calc. 1.731 » »

Ethyl trans-β-(2-naphthyl)-acrylate. Yield 94 %. M.p. 68-69° (recrystallized from ethanol).

$C_{15}H_{14}O_2$ (226.3)	Calc.	C	79.43	H	6.24
	Found	»	79.66	»	6.17

Ethyl β-(2-naphthyl)-a,β-dibromopropionate. Yield 92 %. M.p. 82-83° (recrystallized from abs. ethanol).

$C_{15}H_{14}O_2Br_2$ (386.1)	Calc.	C	46.63	H	3.63	Br.	41.45
	Found	»	46.54	»	3.79	»	41.68

β-(2-Naphthyl)-propiolic acid. In contrast to the corresponding 1-naphthyl compound this acid forms a slightly soluble potassium salt and the directions were altered accordingly.

A solution of 0.04 mole (15.4 g) of the dibromo ester (VII) and 0.48 mole (26.8 g) of potassium hydroxide in ethanol (300 ml) was refluxed on a steam bath for 4 hours. After standing in the ice-box overnight, the precipitate was filtered off, washed with a little cold ethanol and dissolved in 400 ml of water at 50—60°. After filtering the solution was cooled to 30°, and the acid precipitated by slow addition of 30 ml of 4-N sulfuric acid. After drying, the crude product was crystallized from carbon tetrachloride. Yield 5.00 g, increased by 0.60 g from the mother liquor. Total yield 71 %. M.p. 145-147°. The acid

separates from carbon tetrachloride in long colourless needles, easily soluble in the common solvents.

$C_{13}H_8O_2$ (196.2)	Calc.	C	79.56	H	4.11	
	Found	»	79.41	»	4.27	
Microhydrogenation:	9.614 mg	~	2.197 ml	H_2	(0°, 760 mm)	
	Calc.		2.190	»	»	

cis-β-(2-Naphthyl)-acrylic acid. Prepared in the same way as the 1-naphthyl compound. The ethanolic solution was concentrated to 15 ml and the crystals deposited (1.35 g, m.p. 130°) recrystallized from carbon tetrachloride; yield 1.20 g, m.p. 134°.

$C_{13}H_{10}O_2$ (198.2)	Calc.	C	78.76	H	5.08	Eq. w.	198.2
	Found	»	78.84	»	5.11	» »	198.5
Microhydrogenation:	Found	12.738 mg	~	1.432 ml	H_2	(0°, 760 mm)	
	Calc.			1.441	»	»	

The acid resembles very much the isomeride, but differs in being more soluble in ethanol and benzene.

β-(2-Naphthyl)-α,β-dibromopropionic acid. trans-β(2-Naphthyl)-acrylic acid (1 g) was suspended in carbon disulfide (20 ml) and a solution of bromine (0.8 g) in carbon tetrachloride added with stirring. During the addition the reaction mixture was exposed to sun light. After removal of excess of bromine by shaking with sulfurous acid, carbon disulfide was removed and the residue recrystallized from ethanol. Yield practically quantitative (1.75 g). M.p. 177-180°.

$C_{13}H_{10}O_2Br_2$ (358.0)	Calc.	Br	44.70
	Found	»	44.65

cis- and trans-β-(2-Naphthyl)-α-bromo-acrylic acid. A solution of ethyl β-(2-naphthyl)-α,β-dibromopropionate (3.86 g) and potassium hydroxide (6.7 g) in ethanol (50 ml) was heated to a short boil. After standing overnight a precipitate had formed which contained the slightly soluble potassium salt of the *trans*-acid and potassium bromide. It was filtered off and dissolved in hot water (175 ml). After filtering, 10 ml of 1-N hydrochloric acid was added to the hot solution. A precipitate of the acid separated and was recrystallized from 50 % (v/v) ethanol. Yield 0.75 g (27 %). M.p. 200-202°.

$C_{13}H_9O_2Br$ (277.1)	Calc.	C	56.35	H	3.27	Br 28.83	Eq. w. 277.1
	Found	»	56.53	»	3.26	» 28.98	» » 276.0

The filtrate from the potassium salt of the *trans*-acid was evaporated to dryness *in vacuo*, the residue dissolved in 20 ml of water and the *cis*-acid precipitated by addition of 30 ml of 1-N hydrochloric acid. Recrystallized from dilute ethanol it melted at 185-187°. Yield 0.85 g (31 %).

$C_{13}H_9O_2Br$ (277.1)	Calc.	C	56.35	H	3.27	Br 28.83	Eq. w. 277.1
	Found	»	56.60	»	3.25	» 28.98	» » 276.1

The two acids form pale yellow crystals, slightly soluble in water and carbon tetrachloride, easily soluble in ether. In ethanol the *trans*-acid is moderately, the *cis*-acid easily soluble.

Microanalyses were performed in this laboratory by Mr. A. Grossmann. Microhydrogenations were run in the apparatus described by Clauson-Kaas and Limborg [13]. The ultraviolet absorption spectra were determined in methanol using the Beckman model DU spectrophotometer. The infrared data were obtained in Nujol mulls on the recording Beckman IR-2 equipment.

We thank Mr. Chr. Pedersen for valuable assistance in the spectroscopical work.

SUMMARY

The following new substances are described: β-(2-naphthyl)-propiolic acid, *cis*-β-(2-naphthyl)-acrylic acid, β-(2-naphthyl)-α,β-dibromopropionic acid and its ethyl ester and *trans*-β-(2-naphthyl)-α-bromoacrylic acid.

Improved procedures for preparing 2-naphthaldehyde, β-(1-naphthyl)-propiolic acid and *cis*-β-(1-naphthyl)-acrylic acid are given.

Discrepancies in the physical data of *cis*-β-(1-naphthyl)-acrylic acid are discussed.

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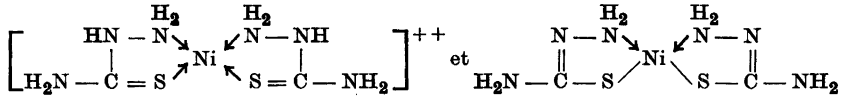
ACTA CHEMICA SCANDINAVICA 6 (1952) 189–194

Complexes de nickel avec la thiobenzhydrazide et avec des composés analogues

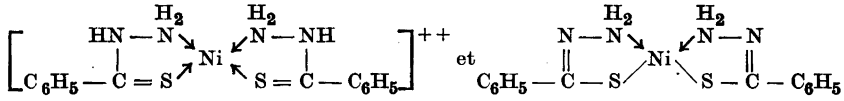
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Laboratoire de chimie de l'Université de Copenhague, Copenhague, Danemark

En 1934 Jensen [1] a montré que le nickel forme avec la thiosemicarbazide des complexes du type:

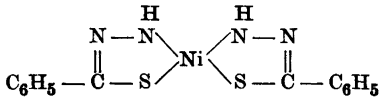


Ce présent travail montre que la thiobenzhydrazide, qui peut être dérivée de la thiosemicarbazide en substituant un des groupes amino par un radical phényl, peut donner avec les sels de nickel des complexes qui ressemblent fortement aux complexes correspondant de la thiosemicarbazide, et qui possèdent sans doute une constitution analogue:

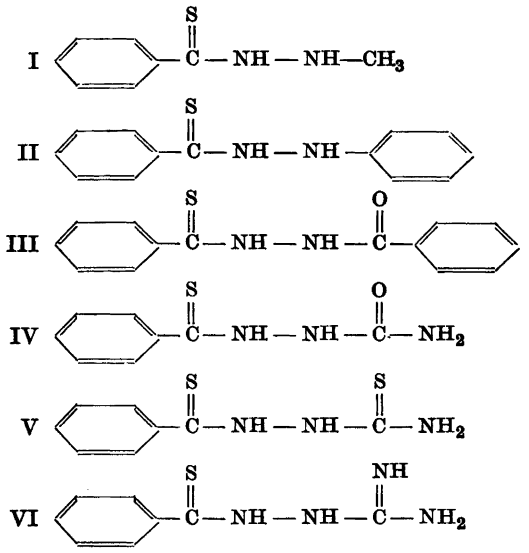


Les sels complexes sont bruns ou bruns verdâtres, le complexe interne est beige. Les sels complexes sont stables à l'air, alors que le complexe interne, dès sa formation, se colore en bleu sous l'action de l'air, et plus rapidement sous l'action de l'eau oxygénée. Poursuivant nos recherches sur la formation des complexes de nickel trivalent [2], ce composé bleu a eté étudié en vue de déterminer la présence d'atomes de nickel trivalent, mais l'étude de ses propriétés magnétiques a montré que le complexe ne possède point le paramagnétisme calculé pour un composé de nickel trivalent. Au contraire nous avons trouvé que le composé est pratiquement diamagnetique.

L'analyse du composé bleu correspond a celle du composé non oxydé moins deux atomes d'hydrogene. On peut donc représenter le complexe par une formule avec le nickel tetravalent:



Dans le même sens, nous avons également étudié la formation des complexes des dérivés de la thiobenzhydrazide, obtenus en substituant un des atomes du groupe NH_2, ces composés n'étant point encore décrit dans la litterature:



I — N^2-Methyl-thiobenzhydrazide

II — N^2-Phenyl-thiobenzhydrazide

III — N^2-Benzoyl-thiobenzhydrazide

IV — 1-Thiobenzoyl-semicarbazide

V — 1-Thiobenzoyl-thiosemicarbazide

VI — 1-Thiobenzoyl-aminoguanidine

Les substituants ne sont point liés au même atome d'azote, car les composés ne donnent point de condensation avec la benzaldehyde. Leur préparation résulte de l'action de l'acide thiobenzoylthioglycolique sur l'hydrazine substituée en milieu alcalin (pH = 9). Cette réaction ayant lieu à 0° et en présence d'un excès d'hydrazine pour éviter toute décomposition. Après plusieurs heures l'acidification donne un précipité qui, soit lavé à l'éther ou au benzène, soit recristallisé, lorsque la stabilité l'autorise, conduit à des cristaux jaunes, bien solubles en milieu ammoniacal, peu solubles dans l'eau à froid. En milieu ammoniacal les composés peuvent être chauffés sans décomposition. En milieu acide ou isolés ils se transforment, mais nous n'avons pas encore réussis à identifier les composés formés.

Par l'addition d'un sel de nickel à une solution alcoolique de ces composés la solution se colore en brun. Avec l'exclusion du composé III, qui se décompose, les composés forment des complexes internes, insolubles, analogues à ceux obtenus avec la thiobenzhydrazide. Les composés II, IV et V forment seulement des complexes internes pareillement aux thiosemicarbazides substituées avec des radicaux aromatiques en position 1 [1]. Les composés I et VI au contraire forment aussi des sels complexes.

PARTIE EXPÉRIMENTALE

La *thiobenzhydrazide* fut préparée selon les indications de Holmberg [3].

N^2-Méthyl-thiobenzhydrazide (I). 2,12 g de carboxyméthyl dithiobenzoate [4] (acide thiobenzoyl thioglycolique) sont dissous dans 10 cm³ de NaOH, 1-N, refroidit à 0° et additionnés d'une solution de 1,4 g de sulfate de méthylhydrazine dans 20 cm³ de NaOH, 1-N, également refroidis. Une huile se sépare qui cristallise par refroidissement. Recristallisé dans un mélange d'éther et d'éther de pétrole (1 : 1), le composé donne des cristaux jaunâtres, fondant à 88°, bien solubles dans l'éther et dans l'alcool, moins solubles dans le benzène, peu solubles dans l'éther de pétrole.

$C_8H_{10}N_2S$ (166,2)	calc.	C 57,83	H 6,06	N 16,86
	trouvé	» 56,98	» 6,46	» 17,04

N^2-Phenyl-thiobenzhydrazide (II). 2,12 g de l'acide thiobenzoylthioglycolique sont dissous dans 10 cm³ NaOH 1-N et additionnés de 1,25 g de phénylhydrazine. Une huile se sépare qui, après deux jours à 0° cristallise. Par recristallisation dans un mélange d'éther et d'éther de pétrole (1 : 1) on obtient l'hydrazide sous forme de microcristaux jaune paille, fondant à 87°. Rendement 2,1 g (92 %). Le composé est bien soluble dans l'éther et dans l'alcool, peu soluble dans l'éther de pétrole.

$C_{13}H_{12}N_2S$ (228,3)	calc.	C 68,40	H 5,29	N 12,28
	trouvé	» 68,50	» 5,18	» 12,39

N^2-Benzoyl-thiobenzhydrazide (III). On mélange une solution de 2,12 g de l'acide thiobenzoylthioglycolique dans 15 cm³ de NaOH 1-N avec une solution de 2,00 g de benzhydrazide dans 35 cm³ de NaOH 1-N et laisse le mélange dans la glacière pendant 14 heures. Le précipité est essoré, lavé à l'éther et séché dans le vide. Le composé se présente sous forme d'une poudre microcristalline jaune, légèrement soluble dan l'éther, bien soluble dans le benzène. Par chauffage progressif le composé jaune perd sa coloration aux environs de 100° et commence de suinter vers 135° sans point de fusion définitif. De même à la température ordinaire, il y a une décomposition progressive.

$C_{14}H_{12}ON_2S$ (256,3)	calc.	N 10,93	S 12,52	
	trouvé	» 10,64	» 12,84	

1-Thiobenzoyl-semicarbazide (IV). 2,12 g de l'acide thiobenzoylthioglycolique sont dissous dans 15 cm³ de NaOH 1-N, puis refroidis à 0°. On ajoute en une seule fois une

solution de 2,24 g de chlorhydrate de semicarbazide dans 20 cm³ de NaOH 1-N, refroidit à 0°, et abandonne 12h à 0°. La coloration primitivement rouge est devenue jaune, et la solution doit être encore limpide. On ajoute SO_4H_2 2-N, et il précipite un composé jaune pâle, qui est essoré et trituré avec du benzène à 40°. Rendement 1,75 g (90 %). Par recristallisation d'un mélange d'alcool et d'eau (1 : 1) on obtient le composé sous forme de belles aiguilles d'un jaune vif, fondant à 155° en suintant quelques degrés auparavant.

$C_8H_9ON_3S$ (195,2)	calc.	C	49,23	H	4,65	N	21,53	S	16,43
	trouvé	»	50,51	»	4,59	»	21,63	»	16,52

1-Thiobenzoyl-thiosemicarbazide (V). Preparé de la même manière que la thiobenzoyl-semicarbazide (IV) en employant une solution de 1,4 g de thiosemicarbazide dans 30 cm³ de NaOH N/3. En acidifiant la solution, un composé jaune citron ou jaune orange précipite. On essore, met en suspension dans le benzène, chauffe environ jusqu'à 40°, et essore une poudre microcristalline jaune citron. Le composé est très soluble dans le benzène, un peu plus soluble dans l'éther, et bien soluble dans l'alcool et l'acetone; il se décompose à chaud dans l'eau. Par chauffage progressif il commence à marquer une transformation aux environs de 120—23°, la couleur jaune citron des cristaux disparaissant, pendant que ceux-ci devenus presque liquides se transforment lentement en plaquettes incolores qui commencent à fondre vers 219°.

$C_8H_9N_3S_2$ (211,3)	calc.	N	19,90	S	30,36
	trouvé	»	20,00	»	30,11

Thiobenzoylaminoguanidine (VI). Préparé de la même manière que les composés précédents, en employant une solution de 1,5 g de sulfate d'aminoguanidine dans 20 cm³ NaOH 1-N. Rendement 1,6 g d'un composé jaune pâle (82 %). Par recristallisation dans 30 cm³ d'eau, on obtient de belles aiguilles jaunâtres. Le composé est tres soluble dans l'alcool, insoluble dans le benzène, l'éther, l'éther de pétrole, le tétrachlorure de carbone etc., et soluble dans l'eau à chaud. P. f. 146—47°.

$C_8H_{10}N_4S$ (194,2)	calc.	C	49,48	H	5,15	N	28,81
	trouvé	»	49,57	»	5,00	»	28,85

Complexes de nickel. En mélangeant une solution de 0,320 g de thiobenzhydrazide dans 5 cm³ d'alcool, avec une solution de 0,290 g de nitrate de nickel (hexahydrate) dans 5 cm³ d'alcool acidifié par quelques gouttes d'acide nitrique 4-N, il se dépose au cours de quelques heures des cristaux brun clair. Le chlorure est préparé de la même manière, le sulfate en employant une solution aqueuse de sulfate de nickel acidifié par l'acide sulfurique. Rendements presque quantitatifs. Le chlorure et le nitrate sont solubles dans l'eau, mais très peu solubles dans l'alcool dilué; le sulfate est presque insoluble aussi dans l'eau.

	N %		couleur
	calc.	trouvé	
$[Ni(C_7H_8N_2S)_2]\ (NO_3)_2$	17,26	17,16	brun clair
$[Ni(C_7H_8N_2S)_2]Cl_2$	12,93	13,03	vert moutarde
$[Ni(C_7H_8N_2S)_2]SO_4$	12,21	12,14	brun clair

Quand on mélange une solution alcoolique de thiobenzhydrazide avec une solution alcoolique de la quantité théorique de chlorure de nickel et fait alcalin avec l'ammoniaque, on obtient immédiatement un précipité beige clair qui s'oxyde rapidement en devenant verdâtre. On essore rapidement dans un atmosphêre d'azote.

$Ni(C_7H_7N_2S)_2$	calc.	N 15,50
	trouvé	» 15,64

Si, avant d'essorer, on ajoute de l'eau oxygénée le composé devient bleu foncé. On essore, lave avec l'eau et sèche sous P_2O_5. Le composé semble être dérivé du composé précédent par départ de deux atomes d'hydrogène.

$Ni(C_7H_6N_2S)_2$	calc.	C 46,53	H 3,33	N 15,52
	trouvé	» 47,63	» 3,26	» 15,57

Avec les dérivés de la thiobenzhydrazide il se forme des composés analogues:

composé organique	formule du complexe	couleur	N % calc.	trouvé
$C_6H_5CSNHNHCH_3$	$Ni(C_8H_9N_2S)_2$	ocre	14,40	14,28
$C_6H_5CSNHNHC_6H_5$	$Ni(C_{13}H_{11}N_2S)_2$	brun foncé	10,93	10,92
$C_6H_5CSNHNCSNH_2$	$Ni(C_8H_7N_3S_2)$	brun verdâtre	15,67	15,69
$C_6H_5CSNHNHC(NH)NH_2$	$Ni(C_8H_{10}N_4S)_2SO_4$	brun clair	20,66	19,66
	$Ni(C_8H_9N_4S)_2$	brun clair	25,11	25,19

Le phénylhydrazide (II) et le thiosemicarbazide (V) donnent seulement des complexes internes, qui se forment même en milieu acide. Le methylhydrazide (I) et le semicarbazide (IV) ne donnent pas une coloration en milieu acide; en milieu ammoniacal le semicarbazide donne un composé beige clair. En milieu ammoniacal le composé de la phenylhydrazide se colore presque en noir en s'oxydant. Le thiobenzoyl-benzhydrazide (III) donne avec le nitrate de nickel une coloration brun rouge intense, mais les analyses indiquent que la formation des composés de nickel s'accompagne d'une décomposition de la partie organique.

RÉSUMÉ

La thiobenzhydrazide forme des complexes avec le nickel par l'addition de deux molecules des composés organiques à une molecule d'un sel de nickel. En milieu alcalin, l'acide est éliminé avec formation d'un complexe interne. Celui ci s'oxyde a l'air en devenant bleu foncé et se transforme dans un composé qui probablement contient le nickel tetravalent.

Des derivés nouveaux de la thiobenzhydrazide de type $C_6H_5CSNHNHR$, avec R = CH_3, C_6H_5, COC_6H_5, $CONH_2$, $CSNH_2$ et $C(NH)NH_2$ ont été préparés et leur possibilité de donner des complexes avec le nickel a été etudié.

Les microanalyses ont été effectués dans ce laboratoire par M[r] A. Grossmann. Nous remercions M[r] E. Wismer Petersen, qui nous a assisté dans quelques unes des préparations.

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Derivatives and Reactions of Sulfanilhydrazide *

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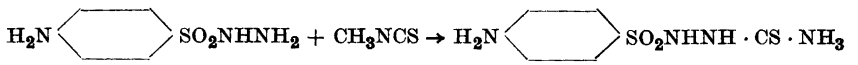
Sulfanilhydrazide was first described, in 1926, by Curtius and Stoll [1], who prepared it by the action of concentrated hydrochloric acid for only a few minutes on 4-acetamido-benzenesulfonyl-hydrazide (acetylsulfanilhydrazide). Unaware of this publication we tried to hydrolyze acetylsulfanilhydrazide by boiling with dilute hydrochloric acid, but in this way the hydrazide was decomposed. We therefore sought for another method of preparing sulfanilhydrazide and found that it could very conveniently be prepared from sulfanilyl fluoride, which we had previously found useful in the preparation of esters of sulfanilic acid [2], where deacetylation would involve also hydrolysis of the ester group. The sulfanilhydrazide prepared from sulfanilylfluoride had, of course, the same properties as that prepared by the method of Curtius and Stoll, but the fluoride method is more convenient and gives a better yield.

Sulfanilhydrazide may be recrystallized from water or dilute ethanol and is a beatifully crystalline substance with m.p. 133°. It is difficultly soluble in cold water, but dissolves as well in acids as in alkalis. It reduces Fehling's solution, Tollen's reagent, an iodine solution by warming (or in the cold by addition of NaOH), and it combines with different carbonyl compounds: a solution of the hydrazide in dilute hydrochloric acid gives white crystalline precipitates with benzaldehyde, acetone, pyruvic acid *etc.* With glucose and glyoxal it forms water soluble condensation products and with *p*-dimethylamino-benzaldehyde it gives a yellow colour. With isatin slightly soluble, yellow isatin-sulfanilylhydrozone is formed. This compound was prepared with a view to using it at an intestinal antiseptic. It was found, however, that both this compound and the other derivatives of sulfanilhydrazide had

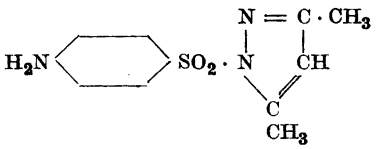
* This investigation was completed in 1943–45. Since then a few of the compounds here described have been reported by other workers (Seppi [3], Roth and Degering [4]).

only a slight bacteriostatic eefect ($^1/_4$—1/10 of the effect of sulfanilamide against pneumococci *).

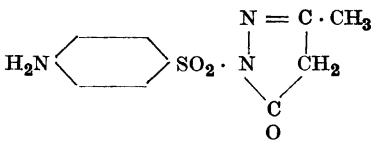
As sulfonylhydrazines have not been very thoroughly investigated, we have studied in more detail the reactions of sulfanilhydrazide. With methylisothiocyanate it reacts slowly with formation of a thiosemicarbazide:



With potassium cyanate and acetic acid a semicarbazide is formed. With acetylacetone and with ethyl acetoacetate the following pyrazole derivatives are formed:

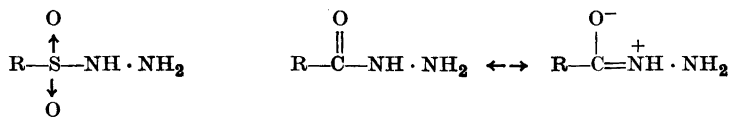


1-Sulfanilyl-3,5-dimethylpyrazole



1-Sulfanilyl-3-methyl-pyrazolone(5)

It is evident that sulfanilhydrazide more resembles a monosubstituted alkylhydrazine than an acylated hydrazine, and this is without doubt due to the fact that the influence of the sulfonyl group on the hydrazine residue is purely inductive, whereas in a carboxylic acid hydrazide resonance is possible:



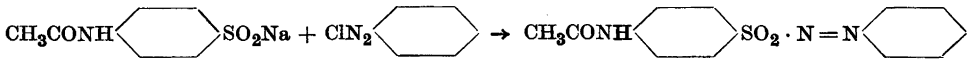
The inductive effect of the sulfonyl group, however, makes sulfanilhydrazide less reactive than an alkylhydrazine. No reaction took place with carbon disulfide and potassium hydroxide, with methylthiuronium sulfate, or with cyanamide. No thiadiazole could be prepared from the thiosemicarbazide.

Sulfanilyl fluoride reacts only slowly with phenylhydrazine, and no pure phenylhydrazide could be prepared in this way. Acetylsulfanilyl chloride on the other hand reacts easily with phenylhydrazine to give the corresponding phenylhydrazide. This could be oxidized with mercuric oxide to a yellow azo

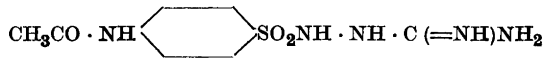
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* We thank Dr. K. Schmith of the State Serum Institute, Copenhagen, for making these measurements.

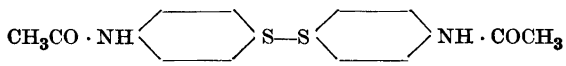
compound. The same compound could also be prepared (but in less pure state) by the reaction of sodium-4-acetylamino-benzenesulfinate and benzenediazonium chloride:



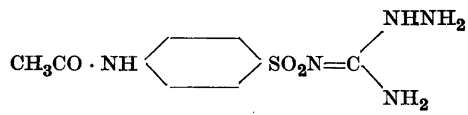
By reaction of acetylsulfanilhydrazide with cyanamide we hoped to prepare an acetylsulfanilylaminoguanidine with the formula:



The only pure product which could be isolated from the reaction mixture was, however, di-*p*-acetylaminodiphenyl-disulfide:



By reaction of acetylsulfanilylcyanamide with hydrazine we prepared an aminoguanidine with the formula:



The compound is only slowly in sodium hydroxide and therefore has no hydrogen at the nitrogen atom bound to the SO_2-group. By reaction of this aminoguanidine with nitrous acid a tetrazole is formed, which was shown to be identical with the compound prepared from aminotetrazole and acetylsulfanilyl chloride (Jensen [5]). By treatment with dilute sodium hydroxide this tetrazole is transformed into the isomeric compound of Veldstra and Wiardi [6]. The constitution of these acetylsulfanilylaminotetrazoles have been subjected to a closer investigation which will be published in another paper.

EXPERIMENTAL

Sulfanilhydrazide. Sulfanilylfluoride (8.75 g) is suspended in ethanol (15 ml) and hydrazine hydrate (5 ml) is added. By warming a reaction sets in and the solution begins to boil. After the reaction has finished the solution is cooled and water (10—20 ml) is added. The crystals which separate are filtered, washed with water, and recrystallized from water or dilute (30 %) ethanol. Yield 7 g = 75 %. M. p. 133—135° with destruction

(colour change to yellow, formation of nitrogen, sulfur dioxide and aniline). The hydrazide forms long, colourless needles.

$C_6H_9O_2N_3S$ (187.2) Calc. N 22.45 Found N 22.52

Acetylsulfanilhydrazide. Acetylsulfanilyl chloride (11.5 g) is dissolved in a mixture of abs. ethanol (100 ml) and hydrazine hydrate (5 ml). White crystals separate, which are filtered and washed with ethanol. Yield 11.0 g = 96 %. The compound can be recrystallized from water, but as it is gradually decomposed by heating with water, it should be dissolved in boiling water, the solution filtered and cooled rapidly. The melting point is 176—178° (dec.) by slow heating, but is 191—192° on a preheated block.

$C_8H_{11}O_3N_3S$ (229.2) Calc. N 18.33 Found N 18.54

Benzaldehyde sulfanilhydrazone. Sulfanilhydrazide (2.0 g) is suspended in ethanol (20 ml), benzaldehyde (1.0 g) is added. The mixture is heated to boiling and on diluting with water the hydrazone is precipitated. It is recrystallized from 50 % ethanol. Yield 2.4 g = 87 %. M. p. 172—73°.

$C_{13}H_{13}O_2N_3S$ (275.3) Calc. N 15.26 Found N 15.20

Isatin β-sulfanilhydrazone. Sulfanilhydrazide (0.94 g) and isatin (0.73 g) are dissolved together in hot ethanol (25 ml) and the solution heated to boiling for a few minutes. On cooling yellow crystals form. Yield 1.45 g = 92 %. The compound was recrystallized from dilute ethanol (50 %). It is very slightly soluble in abs. ethanol, insoluble in acids and bases.

$C_{14}H_{12}O_3N_4S$ (316.3) Calc. N 17.71 Found N 17.84, 17.66

Acetylsulfanil-phenylhydrazide. A mixture of acetylsulfanilyl chloride (12 g), ethanol (100 ml) and phenylhydrazine (10.8 g) is heated to boiling. On cooling a precipitate of phenylhydrazine hydrochloride separates and is filtered off. By dilution with water (200 ml) the filtrate gives a precipitate of the phenylhydrazide. Yield 14 g = 90 %. Recrystallized from dilute ethanol. White crystals with m. p. 160—61° (dec.). Insoluble in water, very soluble in ethanol. The compound has also been described by Seppi [3].

$C_{14}H_{15}O_3N_3S$ (305.3) Calc. N 13.77 Found N 13.72, 13.83

We tried to prepare sulfanil phenylhydrazide by reaction of sulfanilyl fluoride with phenylhydrazine (either in alcoholic solution or by direct heating of the fluoride with phenylhydrazine to 180°), but we did not succeed in isolating a pure compound. The melting point is practically constant (67—69°) after a few recrystallizations from benzene + ligroin (the product being very soluble in benzene or in ethanol). The nitrogen was much too low, however, and the product gave positive reaction for fluorine.

Acetylsulfanilyl-azo-benzene. Acetylsulfanil-phenylhydrazide (0.5 g) is dissolved in boiling ethanol (10 ml), benzene (20 ml) is added. The solution is heated to boiling, shaken vigourously with mercuric oxide (5 g) and filtered. The solvent is removed *in vacuo* at room temperature and the residue is dissolved in 10 ml hot ethanol. The solu-

tion is filtered and cooled in ice and on rubbing orange-yellow crystals separate. Yield 0.20 g = 40 %. Decompose by heating at 100—110°.

$C_{14}H_{13}O_3N_3S$ (303.3) Calc. N 13.85 Found N 13.90

The same compound was prepared by mixing a solution of sodium acetamidobenzenesulfinate with a solution of benzenediazoniumchloride.

1-Acetylsulfanilyl-4-methyl-thiosemicarbazide. A mixture of acetylsulfanilhydrazide (7.6 g), methyl isothiocyanate (2.5 g) and ethanol (50 ml) is boiled for 1 hour. Without going into solution the precipitate is altered. Yield 5.5 g = 55 %. M. p. ca. 230°. Very slightly soluble in most solvents. A small portion was recrystallized from ethanol (80 %) in which it is also slightly soluble. M. p. 248° (dec.). The compound is soluble in dilute sodium hydroxide.

$C_{10}H_{14}O_3N_4S_2$ (302.4) Calc. N 18.53 Found N 18.51

1-Sulfanilyl-4-methyl-thiosemicarbazide. A mixture of sulfanilhydrazide (1.9 g). methyl isothiocyanate (0.75 g) and ethanol (20 ml) is boiled for 2 hours. The solution is cooled and diluted with 20 ml of water. White crystals separate and are recrystallized from dilute ethanol (30—40 %). Colourless needles. M. p. 195—96°. Yield 75 %. The compound is soluble both in acids and bases and contains a free aminogroup (diazotates and couples).

$C_8H_{12}O_2N_4S_2$ (260.3) Calc. N 21.53 Found N 21.56

1-Sulfanilyl-semicarbazide. Sulfanilhydrazide (1.9 g) is dissolved in acetic acid (20 %; 5 ml) by warming. The solution is then cooled and a solution of 1 g of potassium cyanate in 2 ml of water is added with stirring. After half an hour the mixture is filtered, and the precipitate is recrystallized from water (easily soluble in hot water). Yield 1.3 g = 56 %. The compound decomposes with evolution of gas above 200°, without a sharp melting point.

$C_7H_{10}O_3N_4S$ (230.2) Calc. N 24.34 Found N 25.12

1-Sulfanilyl-3,5-dimethyl-pyrazole. A mixture of sulfanilhydrazide (4.4 g), acetylacetone (3 g) and ethanol (15 ml) is heated on steam bath for 2 hours. The ethanol is evaporated, whereupon the reaction product crystallizes in leaves. These are filtered off, washed with ethanol and ether, and recrystallized from ethanol. Yield 3.1 g = 53 %. M. p. 182°. Diazotation shows the presence of free amino group.

$C_{11}H_{13}O_2N_3S$ (251.3) Calc. N 16.72 Found N 16.78

1-Sulfanilyl-3-methyl-pyrazolone (5). A mixture of sulfanilhydrazide (1.9 g), ethyl acetoacetate (1.3 g) and ethanol (10 ml) is refluxed for one hour. When cool the solution was diluted with water, and the precipitate was recrystallized from water with the addition of activated carbon. Yield 1.7 g = 67 %. M. p. 148°. White needles, easily soluble in ethanol and in hot water.

$C_{10}H_{11}O_3N_3S$ (253.3) Calc. N 16.59 Found N 16.60

4-Acetylsulfanilylaminoguanidine. Ten grams of the calcium salt of acetylsulfanilylcyanamide, 2.5 g of hydrazine sulfate and 0.7 g of hydrazine hydrate were thoroughly mixed together and the mixture heated in an oil bath at 160—70° for one hour. The reaction mixture was extracted with boiling water; after filtering and cooling a finely crystalline white precipitate separated. It was recrystallized from boiling water with addition of activated carbon. M. p. 256°.

$C_9H_{13}O_3N_5S$ (271.3) Calc. N 25.90 Found N 25.56

4-Sulfanilylaminoguanidine. The acetyl compound (2 g) was dissolved in hot 2N NaOH (50 ml) and the solution boiled for 15 min. The hot solution was neutralized to pH 6, carbon was added and the solution filtered. On cooling colourless crystals separated. Recrystallized from water. M. p. 202°. Yield 0.9 g or 53 %.

$C_7H_{11}O_2N_5S$ (229.3) Calc. N 30.54 Found N 30.07

Acetylsulfanilylaminotetrazole. 4-Acetylsulfanilylaminoguanidine (0.7 g) was dissolved in 15 ml 4N H_2SO_4 and a solution of sodium nitrite (0.3 g) in 2 ml of water was slowly added. Yellowish crystals separated. On recrystallization from hot water under addition of activated carbon they became almost colourless. M. p. 172—73° (dec.). Yield 0.5 g.

$C_9H_{10}O_3N_6S$ (282.3) Calc. N 29.77 Found N 29.46

According to the melting point this compound is identical with the acetylsulfanilylaminotetrazole previously described. It also gave azoimide by hydrolysis with hot sodium hydroxide and on treatment with cold 0.1 N sodium hydroxide was transformed into an isomer with m. p. 202°.

SUMMARY

Sulfanilhydrazide has been prepared by reaction of sulfanilylfluoride with hydrazine and some of its reactions have been studied.

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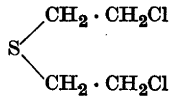
ACTA CHEMICA SCANDINAVICA 6 (1952) 201–204

Attempts at Synthesizing Compounds Isosteric with Methyl-bis-(β-chloroethyl)amine ("Nitrogen Mustard")

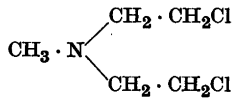
KAI ARNE JENSEN and BØRGE ALHEDE

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Methyl-bis-(β-chloroethyl)amine was first synthesized by Prelog [1] and was later used for the synthesis of the analgesic substance dolantin [2] (demerol, pethidin). That this compound is a vesicant with an effect on skin and mucous membranes similar to that exerted by mustard gas was in 1941 observed by Jensen and Lundquist [3]. This property was explained by the fact that methyl-bis-(β-chloroethyl)amine is isoelectronic (or isosteric in a broader sense) with mustard gas, bis-(β-chloroethyl)sulfide:



bis-(β-chloroethyl)sulfide
(mustard gas)

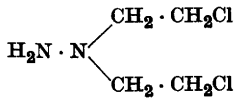


methyl-bis-(β-chloroethyl)amine
("nitrogen mustard")

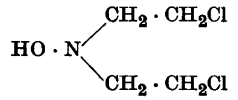
Considerable work was done during world war II on the toxicology and pharmacology of nitrogen mustard with a view to a possible use of this compound as a chemical warfare agent. During this work it was observed that the biological effects both of sulfur mustard and nitrogen mustard show a very striking similarity to those of ionizing radiations [4]. The toxic effect of the mustards is especially pronounced toward bone marrow and lymphoid tissued, and because of this effect intravenous injection of the hydrochloride of „nitrogen mustard" has been used for the treatment of leucaemia and other neoplastic diseases of the hematopoietic system (the very extensive literature on the clinical use has been reviewed by Gellhorn and Jones and Reinhard *et al.*[5]). From a theoretical point of view, however, the most interesting effect of "nitrogen mustard" is that it is mutagenic. Mutations have been caused in

Drosophila (Auerbach and Robson [6] as well as in Neurospore (Horowitz *et al.*[7], Tatum [8]). Several other compounds have been claimed to be mutagenic, but a critical analysis of the results show that a true reversible mutagenic action, similar to that exerted by ultraviolet rays, had until quite recently only been demonstrated for sulfur mustard, nitrogen mustard and a few closely related compounds (cf. Auerbach [9] and Jensen, Kølmark and Westergaard [10]).

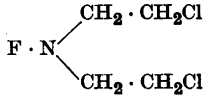
In connection with a more extensive investigation on the mutagenic effect of chemical substances we wished to try the effect of some compounds isosteric with "nitrogen mustard", *viz.* the following compounds:



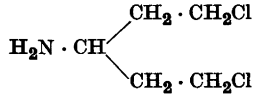
III. N,N-bis-(β-chloroethyl)hydrazine



IV. bis-(β-chloroethyl) hydroxylamine



V. N-fluoro-bis-(β-chloroethyl)amine



VI. 1,5-dichloro-3-amino-pentan

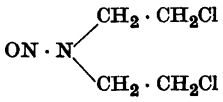
Of these the last compound (no. VI) had been synthesized previously by Hahn, Prelog and Cerkovnikov [11] and was prepared according to their directions. It was found to be devoid of mutagenic effect (Jensen, Kirk and Westergaard [12]). In spite of numerous attempts, we did not succeed in synthesizing the compounds III—V. The hydroxy compounds corresponding to compounds III and IV are known (formed from ethylene oxide and hydrazine and hydroxylamine, respectively [13]), but treatment of the hydrochlorides of these hydroxyamines with concentrated hydrochloric acid resulted in complete decomposition, ammonium chloride being formed. By treatment with thionyl chloride the hydrochloride of N,N-bis-(β-hydroxyethyl)hydrazine gave a dark, viscous, seemingly highpolymeric substance.

From bis-(β-chloroethyl)amine the nitrosamine (VII) could be prepared. This could be reduced catalytically in acetic acid solution, but although the hydrazine was presumably formed (the solution reduced cupric hydroxide in the cold), we did not succeed in isolating it in form of a salt or a derivative with a carbonyl compound.

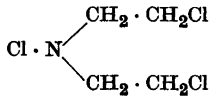
The formation of the hydroxylamine (IV) was also attempted by oxydation of bis-(β-chloroethyl)amine with hydrogen peroxide, but no hydroxylamine was formed in acid or neutral solution, and in alkaline solution the chlorine atoms are split off.

N-Fluoro compounds are only known in the form of perfluoro derivatives. Attempts at preparing simple N-fluoro compounds by reaction of N-chloro- or N-bromo-amines with silver or mercuric fluoride were not successful.

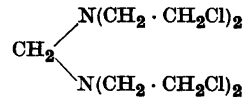
In connection with these experiments we prepared N-chloro-bis-(β-chloroethyl)amine and tetra-β-chloroethyl-methylenediamine (VIII and IX).



VII. N-nitroso-bis-(β-chloroethyl)amine



VIII. N-chloro-bis-(β-chloroethyl)amine



IX. tetra-β-chloroethyl-methylenediamine

EXPERIMENTAL

Microanalyses by Albert Grossmann

N-Nitroso-bis-(β-chloroethyl)amine (VII). To an ice-cold solution of 3.6 g (0.02 mole) of bis-(β-chloroethyl)amine hydrochloride in 5 ml of water was added 1 drop of conc. hydrochloric acid and a solution of 0.7 g of sodium nitrite in 2 ml of water. After standing in an ice-bath for half an hour the nitrosamine was extracted with ether. The ethereal solution was washed with water and dried with sodium sulfate. After removing the ether *in vacuo* the nitrosamine was left as a yellow oil which could not be distilled. Yield 2.5 g = 73 %.

$C_4H_8ON_2Cl_2$ (171.1)	Calc.	C	28.08	H	4.71	N	16.38	Cl 41.45
	Found	»	27.84	»	4.64	»	16.44	» 41.49

N-Chloro-bis-(β-chloroethyl)amine (VIII). To an icecold solution of sodium hypochlorite (prepared from 30 g of NaOH in 250 ml of water + 100 g of ice) a solution of 18 g of bis-(β-chloroethyl)amine hydrochloride in 50 ml of water was added. An oil separated. After standing in an ice-bath for a few hours the solution was extracted with ether. The ethereal layer was dried with sodium sulfate, and the ether removed *in vacuo*. The liquid residue was filtered into a Claisen-flask and distilled *in vacuo*. B.p. 110° at 18 mm. Yield 10 g = 55 %.

$C_4H_8NCl_3$ (176.5)	Calc.	N	7.94	Cl	60.30
	Found	»	8.10	»	60.46

On standing the compound is rapidly decomposed with deposition of crystals.

N,N-Tetra-β-chloroethyl methylenediamine (IX). A solution of 3.6 g of bis-(β-chloroethyl)amine hydrochloride in 50 ml of formaldehyde (40 %) was added slowly with stirring to a solution of 1.25 g of KOH in 5 ml of water. The solution was warmed a few minutes

on a steam bath, cooled and extracted with ether. The ether solution was dried with K_2CO_3 and when the ether was removed *in vacuo* the amine was left as an oil which could not be distilled without decomposition. On passing dry hydrogen chloride through the ethereal solution the hydrochloride separated as an oil. The picrate was obtained in crystalline form by addition of an aqueous or ethanolic solution of picric acid to the amine and could be recrystallized from water. M.p. 175°.

$C_{21}H_{24}O_{14}N_8Cl_4$ (754.3)	Calc.	N	14.85
	Found	»	15.01

The melting point of the picrate of bis-(β-chloroethyl)amine is 112—113°.

The maleate was prepared in the following way: To a dry ethanolic solution of the amine a solution of 2.3 g of maleic acid in 150 ml of dry ether was added. Colourless crystals separated. After some hours they were filtered and dried. Yield 1.3 g (25 %). M.p. 129—130°. No pure substance could be isolated from the mother liquor.

$C_{17}H_{26}O_8N_2Cl_4$ (528.2)	Calc.	N	5.31	Cl	26.84
	Found	»	5.47	»	27.77

SUMMARY

The following compounds have been synthesized for the first time: N-nitroso-bis-(β-chloroethyl)amine, N-chloro-bis-(β-chloroethyl)amine and N,N-tetra-β-chloroethyl-methylenediamine. Attempts were made to synthesize N,N-bis-(β-chloroethyl)hydrazine, bis-(β-chloroethyl)hydroxylamine and N-fluoro-bis-(β-chloroethyl)amine, but were unsuccessful.

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Deviation from Coplanarity in the 1,3,5-Triphenylbenzene Molecule

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All biphenyl derivatives studied by electron diffraction so far show a deviation from the planar configuration in the gaseous state [1,2]. This is not only the case for the orthosubstituted but also for the meta- and para-substituted derivatives which X-ray crystallographic work has shown to consist of planar molecules. This apparent discrepancy is accounted for by taking into consideration the inter-molecular forces which are of much greater significance in the crystalline than in the gaseous phase. It is easily understood that a planar molecule might be preferred as a unit for the construction of a stable lattice, even if the isolated molecule itself is generally found in a non-planar configuration. The coplanarity of non-orthosubstituted biphenyls is accordingly not entirely caused by resonance phenomena as is generally believed. If this argument is correct it is not necessarily true that all non-orthosubstituted biphenyl molecules are planar in the crystalline state. In certain cases sufficient stability might be obtained to maintain the non-planarity characteristic of the isolated molecule even in the lattice.

In this connection the result obtained by Lonsdale [3] concerning sym. triphenylbenzene is of great interest. She finds that the molecule is probably non-planar in the crystalline state. The planes of the phenyl groups are rotated through 25° out of the plane of the central nucleus. In the opinion of the present author this deviation from coplanarity is not caused by the interaction between the meta-substituted phenyl groups, but by the interactions between the ortho-hydrogens in neighbouring rings. The shortest hydrogen-hydrogen distance between two of the outer phenyl groups in a planar molecule is about 3.3 Å, which is definitely so great as to exclude any possible van der Waals repulsion. The effect must be the same as that present in biphenyl and its meta- and para-substituted derivatives. The angle between the planes of

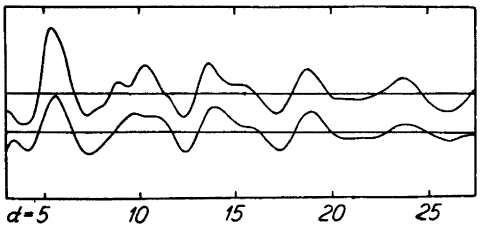


Fig. 1. (I_E/B_E-1) function for 1,3,5-triphenylbenzene (upper curve) and benzene (lower curve).

two adjacent phenyl rings should accordingly be approximately 50° in gaseous 1,3,5-triphenylbenzene, as found for other non-orthosubstituted biphenyls [2]. The fact that the angle seems to be smaller in the crystal is easily explained.

EXPERIMENTS AND CALCULATIONS

The 1,3,5-triphenylbenzene used in this work was prepared by heating acetophenone with aniline and iodine. The compound was recrystallized from glacial acetic acid and melted at 172° C.

Electron diffraction photographs were taken using an s^3 sector. At the same time new photographs of benzene were taken for comparison and subtraction purposes. In Fig. 1 the intensity curves of triphenylbenzene (upper curve) and benzene (lower curve) are given. The background is substracted in both cases. (N. B. the abscissa here is not the usual $s = \frac{4\pi}{\lambda} \sin \Theta$ but $d = \frac{2\pi}{\lambda} \tan 2\Theta$). In Fig. 2 the $\frac{\sigma(r)}{r}$ curves for the two compounds are given. They are calculated from the following relation:

$$\frac{\sigma(r)}{r} = \int_{s=3}^{s=26} \left(\frac{I_E}{B_E} - 1 \right) s \, e^{-k s^2} \, B_T \sin rs \, ds$$

where I_E and B_E are the corrected experimental intensity and background respectively. B_T is $\Sigma_i[Z_i - f_i)^2 + S_i]$ summed over all atoms in the molecules, and k is here 0.007. — The corresponding normal curve [4] for the carbon-carbon distance was calculated using the relation:

$$N(\varrho) = \int_{s=3}^{s=26} \left(1 - \frac{f_c}{Z_c} \right)^2 e^{-k s^2} \cos \varrho \, s \, ds$$

The Fourier inversion was carried out using I.B.M. calculating techniques [5].

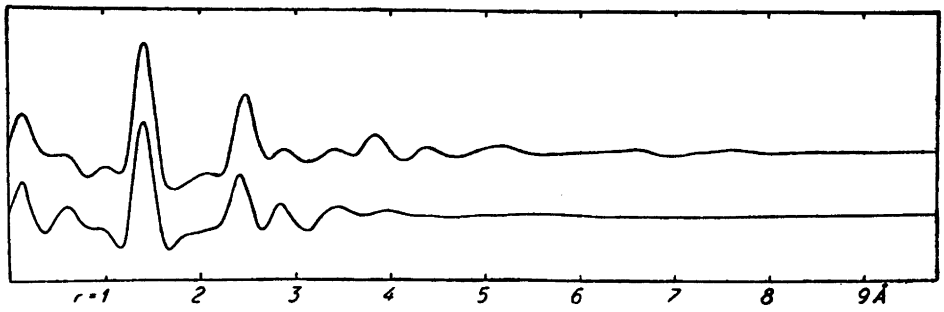


Fig. 2. $\frac{\sigma(r)}{r}$ *curve for 1.3.5-triphenylbenzene (upper curve) ond benzene (lower curve).*

The $\frac{\sigma(r)}{r}$ curve of benzene (Fig. 2) was multiplied by a factor so that the ratio of the heights of the first peaks in the two curves should have the correct theoretical value. Theoretically the normalization factor should be 4, but a deviation from this value might be expected because of the uncertainty in the drawing in of the background. As a matter of fact the factor was found to be 4.4.

If we now assume that the rings in triphenyl-benzene have the same dimensions as in benzene, the distances within the rings of triphenylbenzene can be subtracted. This assumption is not of course strictly correct, but for the purpose of determining the angle between the ring planes of triphenylbenzene it may be regarded as a valid approximation. The upper curve of Fig. 3 shows the difference between the two curves of Fig. 2. It is thus, to the approximation mentioned, and disregarding some unimportant carbon-hydrogen contributions, the $\frac{\sigma(r)}{r}$ curve for triphenylbenzene containing only distances between different rings. (The ordinate scale in Fig. 3 is twice that of Fig. 2.)

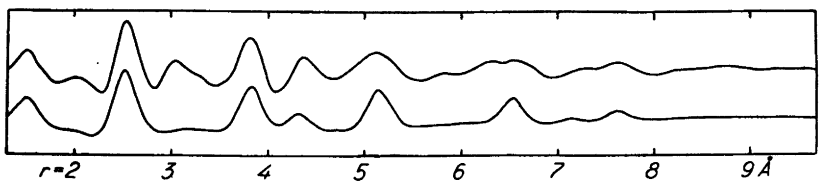


Fig. 3. The upper curve is the difference between the two curves of Fig. 2. The lower curve is the $\frac{\sigma(r)}{r}$ *curve calculated for the unvariable distances between different rings in 1,3,5-triphenylbenzene.*

Table 1. C—C distances occuring in the sym. triphenylbenzene. n and m refer to the numbers given in Fig. 4. N is the number of the times the particular type of distance occurs in the molecule. r_{nm} is the distance in Å.

Part I

C_n-C_m	N_{nm}	r_{nm}
* 1—2	24	1.40
1—3	24	2.42
1—4	12	2.80
1—7	3	1.52
1—8	12	2.53
1—9	12	3.82
1—10	6	4.32
10—2	12	5.16
10—3	12	6.53
10—14	3	7.12
7—13	3	5.06
7—16	6	7.61
10—16	3	9.91

Part II

C_n-C_m	N_{nm}	* r_{nm}	** r_{nm}
2—12	6	2.92	3.07
2—8	6	3.80	3.67
2—11	12	4.32	4.43
2—9	12	4.95	4.86
3—11	6	5.72	5.80
3—9	6	6.21	6.14
7—14	6	5.25	5.43
7—15	6	6.61	6.75
7—18	6	6.31	6.16
7—17	6	7.48	7.35
10—14	6	7.48	7.73
10—15	6	8.72	8.93
10—18	6	8.95	8.73
10—17	6	10.00	9.82

Part III

C_n-C_m	N_{nm}	* r_{nm}	** r_{nm}	** r_{nm}
12—14	3	5.06	5.71	5.41
12—15	6	6.31	6.91	6.68
12—18	6	6.61	6.44	6.68
12—17	6	7.61	7.52	7.72
11—18	6	7.99	7.78	7.98
11—17	6	8.95	8.81	8.99
11—15	3	7.48	8.05	7.86
9—18	6	8.72	8.53	8.35
9—17	3	9.91	9.69	9.53
8—18	3	7.48	7.32	7.11

* $\varphi = 0$
** $\varphi = 46.4°$

These distances can be devided into two kinds: 1) those which are constant during a rotation about the carbon-carbon bridges linking the rings together, and 2) those which vary with such a rotation. Using the normal curve mentioned above the contribution of the invariable distances has been calculated. The result is given as the lower curve in Fig. 3. The carbon-carbon bond distances in the ring are determined by the curves in Fig. 2 to be 1.40 Å, and

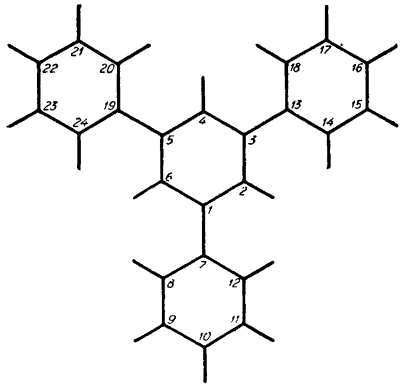


Fig. 4. A planar model for 1,3,5-triphenylbenzene.

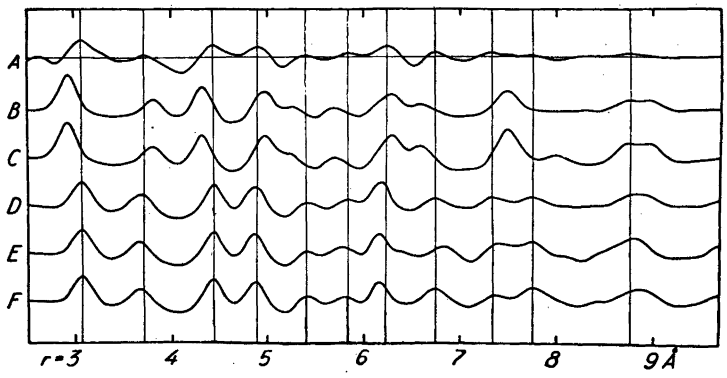


Fig. 5. Curve A is the $\frac{\sigma(r)}{r}$ *curve of 1,3,5-triphenylbenzene for the variable distances. Curves B—F are theoretical curves for various structures.*

the bond distance between neighbouring carbon atoms from adjacent rings is determined from the upper curve in Fig. 3 to be 1.52 Å. (The corresponding distance for biphenyl, 3,3'-dibromobiphenyl, 3,3', 5,5'-tetrabromobiphenyl, and ortho-dibromobiphenyl is found to be 1.48, 1.49, 1.50, and 1.50 Å respectively [1,2], and all these measurements agree within the limits of their respective errors.) All carbon-carbon distances in triphenylbenzene used in the calculation of theoretical curves are given in Table 1. The numbering of the atoms in the table refers to the planar model of Fig. 4.

As might have been expected, the two curves in Fig. 3 show many similarities. The difference between them must contain contributions from all the carbon-carbon distances which vary when the angle φ between the planes of two adjacent rings is varied. This difference is given as the upper curve (A) of Fig. 5 and should be well suited for the determination of φ, since all the disturbing distances are removed. Curve A of Fig. 5 is now compared with corresponding theoretical curves for planar models, B and C. In B we have only taken into account the distances given in Part II of Table 1, while in C

Table 2. Angle φ *calculated from four pronounced peaks of curve A, Fig. 5.*

C_n-C_m	Distances for plane model	Observed values	Calculated φ-values	
2—12	2.92 Å	3.065 Å	45.2°	
2—11	4.32 »	4.425 »	46.6°	
2—9	4.95 »	4.88 »	41.0°	Mean value 46.4°
3—11	5.72 »	5.82 »	52.6°	

the rest of the variable distances have been included. The general appearance of the theoretical curves is very much the same as that of curve A as most of the peaks in A have corresponding peaks in B and C. The positions of the peaks of the theoretical curves, however, by no means coincide with those of curve A. We must therefore abandon the planar model.

Most of the peaks in the difference curve are composed of contributions from two or more distances. To calculate the angle φ from direct measurements of the positions of the peaks in curve A is therefore not straight-forward. To find φ we have chosen the four peaks whose positions are given in Table 2. Each of these peaks is virtually caused by only one distance. We thus get four independent measurements of φ giving an average value $\varphi = 46.4°$. Of course the positions of the four peaks chosen are not entirely uninfluenced by neighbouring distances.

It can easily be shown, for instance, that the maximum at 5.82 Å is markedly influenced by other distances than 3—11. This explains the high value of φ calculated from this particular peak.

If we now use the value $\varphi = 46.4°$ to calculate the carbon-carbon distances and theoretical difference curves of the type under discussion we get curves D, E, and F. Curve D contains only contributions from the distances given in Part II of Table 1, while E and F include the rest of the variable distances. E is calculated for a propeller-shaped model and F for a model where the metaphenyl groups are statistically arranged in relation to each other but in such a way that the angle between each of them and the central nucleus is 46.4°. Taking into consideration the fact that we are dealing with difference curves,

Table 3. Deviation between experimental and theoretical positions of the maxima for a planar and a non-planar ($\varphi = 46.4°$) structure.

Exp. positions of maxima	Calculated positions $\varphi = 0°$	$\lvert\Delta\rvert$ $\varphi = 0°$	Calculated positions $\varphi = 46.4°$	$\lvert\Delta\rvert$ $\varphi = 46.4°$
3.06 Å	2.93 Å	0.13 Å	3.06 Å	0.00 Å
3.71 »	3.81 »	0.10 »	3.67 »	0.04 »
4.43 »	4.32 »	0.11 »	4.43 »	0.00 »
4.88 »	4.98 »	0.10 »	4.87 »	0.01 »
5.40 »	5.25 »	0.15 »	5.43 »	0.03 »
5.82 »	5.70 »	0.12 »	5.82 »	0.00 »
6.23 »	6.28 »	0.05 »	6.17 »	0.06 »
6.74 »	6.58 »	0.16 »	6.75 »	0.01 »
7.34 »	7.48 »	0.14 »	7.34 »	0.00 »
		$\overline{\lvert\Delta\rvert} = 0.12$ Å		$\overline{\lvert\Delta\rvert} = 0.017$ Å

where essential and easily reproducible information is subtracted leaving only a fraction of the original information contained in the experimental intensity curve, we think that the correspondence between curve A and the curves D—F is remarkably good. The degree of correspondence of the positions of the maxima is shown in Table 3 in which the Δs represent the differences in Å between the positions of the peaks for the pairs of curves A & B and A & D. The average $|\Delta|$ value is found to be 0.12 Å between curves A and B and only 0.017 Å between curves A and D.

DISCUSSION OF THE RESULTS

All the theoretical difference curves are calculated on the assumption of rigid molecules. One might expect this assumption to be a rather unsatisfactory working hypothesis, particularly when a well pronounced restricted rotation about the equilibrium position would be expected. It is, of course, difficult to obtain a complete picture of the amount of restrited rotation in a molecule of such high complexity from electron diffraction data. This much can, however, be said: by comparing the two curves A and D we see that the heights of corresponding peaks are not very different, though we can as a general rule say that the peaks of curve D are more pronounced and higher than those of curve A. This is to be expected if restricted rotation occurs. Furthermore we see by inspecting the curves D, E, and F that D agrees best with curve A although E or F would be expected to be better on theoretical ground, assuming a rigid molecule. E and F include all carbon-carbon distances that are included in the semi-experimental curve A. Internal rotation should, however, reduce the contribution of the various types of distances to a different degree. In calculating curve D we have only considered carbon-carbon distances varying with rotation about one single carbon-carbon bond. In E and F the rest of the carbon-carbon distances are included, and these distances would be expected to be influenced more by restricted rotation as they vary with rotation about two single bonds. For instance if we rotated through an angle 3.5° from the value of 46.4° which we have used in our calculation, we find that a distance of the type 2—12 will vary by about 0.03 Å, while a distance of the type 12—14 will vary by about 0.08 Å.

The above arguments indicate that some restricted rotation certainly occurs, but that it is of limited amplitude. Hence the rigid model can be used as a reasonable approximation.

Comparing the curves E and F we see that curve F gives the better agreement with curve A. Although the difference between curves E and F might be of the order of magnitude of the errors in curve A, we should like to point

out that the model corresponding to F is more likely from a chemical point of view than that corresponding to curve E. As has been pointed out earlier, two meta phenyl groups would not be expected to influence each other to any appreciable extent. A statistical arrangement should accordingly be more probable than the symmetrical propeller-like model with a three-fold axis of symmetry. Even if this is true in the gaseous state, however, there is no reason to suppose that it should be the case in the solid state. The symmetrical form of the molecule might conceivably pack better into a crystal than the disordered form.

Finally we should say something about the accuracy of the determination of the angle φ. The value 46.4° is not, of course, particularly accurate: it is only the average of the values of Table 2, of which at least the result for the distance 3—11 is unreliable. To estimate the error we could have calculated curves corresponding to curve D for a whole series of φ-values. This is, however, a very long and tedious method, and the same result can be obtained more simply by studying the influence of the variation in φ on the most pronounced peaks. By doing so we find that the error in the determination is less than 5°. We therefore feel that we can safely state that the angle φ is $46° \pm 5°$.

This value is, within the limits of error, the same as has been found for biphenyl and its meta- and para-derivatives by electron diffraction [1, 2]. There is, however, some reason to believe that small differences do exist. For instance the limits of the errors for the φ-value obtained for 3,3'-dibromobiphenyl ($54° \pm 5°$) do certainly overlap with those found for sym. triphenylbenzene, but the average values are rather different. We believe that the difference is a real one, though its precise interpretation does not seem to be straightforward.

SUMMARY

The structure of 1,3,5-triphenylbenzene has been studied by the electron diffraction sector method. By using the subtraction procedure it has been shown that the molecule is non-planar. The angle between two adjacent ring planes is found to be $46° \pm 5°$.

The Author wishes to express his gratitude to *Norges Almenvitenskapelige Forskningsråd* for financial aid without which the present work could not have been accomplished. He must also acknowledge his indebtedness to the I. B. M. office in Oslo which has kindly placed their equipment at the disposal of the Chemistry Department of the University in Oslo.

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ACTA CHEMICA SCANDINAVICA 6 (1952) 214–218

Molecular Structures of Dodecafluoro-*n*-Pentane, Hexadecafluoro-*n*-Heptane and Dodecafluoro-Isopentane

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During the last years many chemists have focused their attention on the fluorocarbons, partly because of their industrial importance, but also because of their interesting similarities to the hydrocarbons.

The low scattering power of the hydrogen atom makes electron diffraction studies of the hydrocarbons difficult, and in many cases important details of the structures are lost. This difficulty does not occur with fluorocarbons, and we have therefore found it worth while to study some of these compounds. Results of earlier investigations of some ring fluorocarbons have already been published[1]. This paper deals with three open-chain aliphatic perfluorocarbons.

Dodecafluoro-*n*-pentane

The $\frac{\sigma(r)}{r}$ curve obtained for this molecule using the electron diffraction method with a rotating sector is shown in Fig. 1. The peaks which are important for determining the structure are numbered with Roman numerals. Peak I, occuring at 1.38 Å, is mainly caused by the C-F bond distance; this peak is very little influenced by the C-C bond distance.

Peak II is composed of contributions from distances of the type $F_1—F_1'$ and $C_1—F_2$. The position of the maximum is in agreement with the assumption of tetrahedral symmetry around the carbon atoms. Peak III and IV are caused by distances of the type $F_1—F_2$ and $C_1—F_3$. The heights of the peaks and their positions indicate that no free rotation occurs about the C—C bond. Fluoro atoms linked to neighbouring carbon atoms must be either in the trans-position or in the so-called "gauche" position. However, it is clear that we are by no means dealing with a rigid molecule for which peak III would be about twice as high as peak IV. Sterically hindred rotation about the equili-

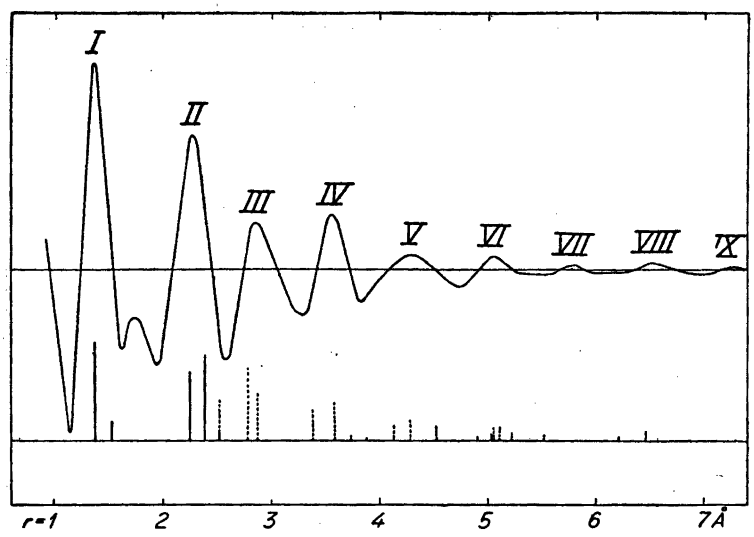


Fig. 1. $\frac{\sigma(r)}{r}$ *-curve of dodecafluoro-n-pentane.*

brium position would be expected to diminish the heights of both peaks, but would have more influence on the peak due to "gauche" F—F distances. A detailed study of the relative heights and shapes of these two peaks might have yielded quantitative information about the amplitude of the restricted rotation about the C—C bonds. The reason, why we did not undertake such a study is because we were afraid of overestimating the method in its present state. We do feel, however, that discussions of the type indicated might be satisfactorily carried out in less complex cases.

Peaks V—IX are of significance for the shape of the carbon skeleton as a whole. The line diagram under the $\frac{\sigma(r)}{r}$ curve corresponds to a rigid molecule with a planar carbon skeleton. This model seems to explain the experimental curve satisfactorily, particularly when allowance is made for vibrations. The following additional models giving a satisfactory fit with the four inner peaks of the $\frac{\sigma(r)}{r}$ curve, have been studied:

1) Carbon atoms C_1, C_2, C_3 and C_4 lie in one plane, and the plane containing C_3, C_4 and C_5 is rotated 60° out of this plane.
2) The carbon atoms C_2, C_3 and C_4 lie in one plane, and the C_1 and C_5 atoms are rotated out of the plane in *opposite* directions.

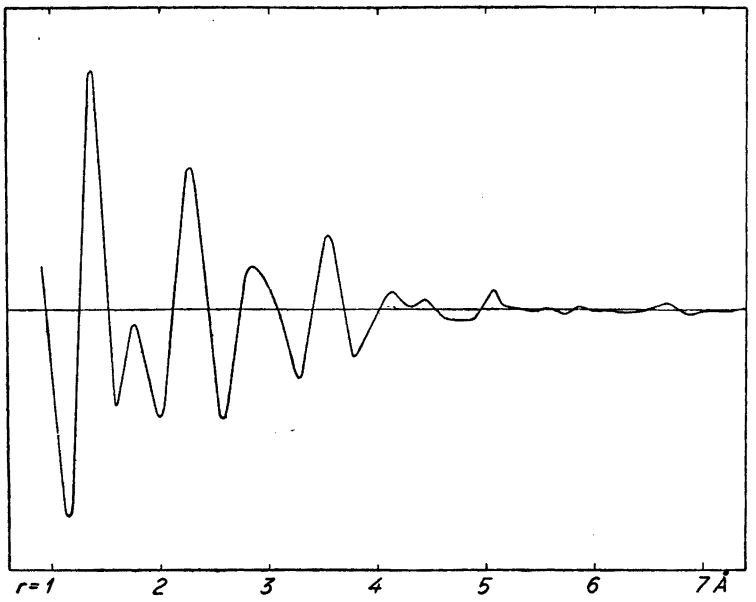


Fig. 2. $\frac{\sigma(r)}{r}$ *-curve of hexadecafluoro-n-heptane.*

3) Like 2), but the atoms C_1 and C_5 are rotated out of the plane in the *same* direction.

None of these three models gave very good agreement with the outer part of the experimental $\frac{\sigma(r)}{r}$ curve. We therefore concluded that a planar model is most probable. The possibility of an equilibrium between the planar model and a certain proportion of model 1) and/or 2) cannot, however, be excluded, and is indeed probable from other evidences [2]. (Model 3 is excluded of steric reasons.)

Hexadecafluoro-*n*-heptane

Fig. 2 shows the $\frac{\sigma(r)}{r}$ curve obtained for hexadecafluoro-*n*-heptane. The form of the curve is very similar to that obtained for perfluoro-*n*-pentane, and C—F bond distance is found to be the same as in that compound, *viz.* 1.38 Å. No deviation from the tetrahedral angle is found. The striking similarity between the two $\frac{\sigma(r)}{r}$ curves of Fig. 1 and Fig. 2 suggested that there must be similarities in the two structures. A detailed study shows that here,

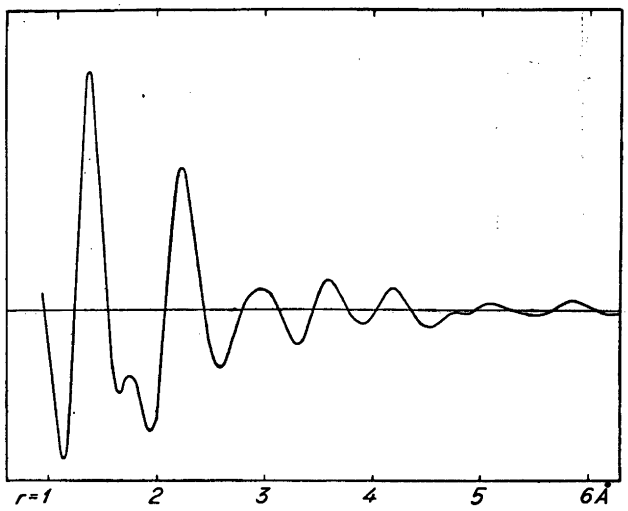


Fig. 3. $\frac{\sigma(r)}{r}$*-curve of dodecafluoro-isopentane.*

as before, the model containing a planar zigzag carbon skeleton is in very good agreement with the $\frac{\sigma(r)}{r}$ curve. Other models, analogous to those mentioned for the dodecafluoro-*n*-pentane, have been studied, but none of them agree satisfactorily with the $\frac{\sigma(r)}{r}$ curve. Once again the possibility of a mixture, consisting mainly of the structure with the planar zigzag carbon skeleton together with contributions from non planar structures, cannot be excluded.

Dodecafluoro-isopentane

As might be expected the $\frac{\sigma(r)}{r}$ curve of dodecafluoro-isopentane (Fig. 3) differs in several details from those of the two compounds already discussed. However, the similarities are still very striking. The C—F bond distance of 1.38 Å, the presence of tetrahedral angles, and the trans configuration of the fluorines are easily verified. Two models were considered:
1) The carbon atoms C_1, C_3, C_4 and C_5 lie in one plane. (Fig. 4), 2) The carbon atoms C_1, C_3 and C_4 lie in one plane, C_2 and C_5 lie in a parallel plane.

It was found that the first model gave the better agreement with the experimental $\frac{\sigma(r)}{r}$ curve.

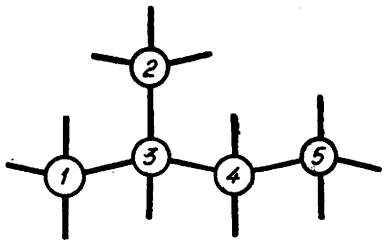


Fig. 4. Dodecafluoro-isopentane.

We have tried to estimate, in each case, the stability of the various alternative models by consideration of the van der Waals forces between non-bonded atoms. Our conclusions support the results of the electron diffraction investigation.

SUMMARY

The structures of dodecafluoro-*n*-pentane, hexadecafluoro-*n*-heptane and dodecafluoro-isopentane have been studied by the electron diffraction sector method. In all the three cases the C—F bond distance is found to be 1.38 Å. No attempt of precision assignment of the bond lengths have been made. The most stable forms of the molecules are found to be those with zigsag carbon skeletons. There is restricted rotation about the C—C bonds.

Samples of the three compounds in a high state of purity were placed at the disposal of our laboratory by Professor G. H. Cady, of the University of Washington, Seattle, and we wish to express our gratitude to him. We also want to thank the Head of the Department Professor O. Hassel for his encouraging interest in our work.

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ACTA CHEMICA SCANDINAVICA 6 (1952) 219–222

Detection, Separation, and Identification of Aliphatic Amines by Paper Chromatography

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It has previously been difficult to detect, separate, and identify volatile aliphatic amines in quantities of a few gammas. The microdiffusion method of Conway, as applied by Richter [1] probably has been the best approach to this problem. For the purpose of studying the uptake and metabolism of lower aliphatic amines by plants, we have developed a paperchromatographic method, which allows the detection, separation, and identification of as little as 1γ of aliphatic amines in biological materials. The method is very simple and quick to carry out, the results being ready in about two hours. A minimum of preparatory operations are required, as very few naturally occurring substances interfere.

The chromatogram is run with butanol-acetic acid and the amine spots are made visible by spraying with a suitable indicator solution (bromphenol-blue). Blue spots appear immediately on a yellow background, varying in the intensity of colour according to the quantity of amine present. Basic amino acids, of course, give similar spots.

EXPERIMENTAL

Procedure

According to our experience the fast running paper Munktell OB (J. H. Munktells Pappersfabriksaktiebolag, *Grycksbo*, Sweden) is very well suited for our purpose. It is somewhat more acid than the Whatman No. 1, thereby offering a better and more evenly yellow background when sprayed with indicator solution. With Whatman No. 1 it is possible, though, to detect somewhat smaller amounts of amines (about 1 γ against 2 γ with Munktell).



a b

Fig. 1. a) Chromatogram of 5, 10, 15, and 20 γ (from left to right) of dimethylamine-HCl, ethylamine-HCl, n-*propylamine-HCl, and* i-*propylamine-HCl. b) Separation of ethylamine-HCl,* n-*propylamine-HCl,* i-*propylamine-HCl, and trimethylamine-HCl from ammonia (2, 5, 10 resp. 30 γ of amine).*

The ascending chromatogram method has been used, placing the strips or cylinders of paper in small receptacles and running the chromatogram for 1 hour with Munktell-paper (solvent ascends 13—14 cm) or 3 hours with Whatman, thus keeping to a minimum the losses of amines due to volatilization.

The amine solution is transferred to the paper in a current of warm air, evaporating the solvent during application. Care is taken to keep the diameter of the initial spots below 6 to 8 mm. Immediately after transfer and drying of the spots, the paper is placed into the chamber containing the solvent, and the chromatogram is started.

Care must be taken in preparing the amine solution, as strong acids (mineral acids, trichloroacetic acid) interfere with the chromatogram. Solutions containing such acids must, previous to application, be neutralized to a pH value of 3—6 by sodium acetate or by other means. It is also possible to neutralize acid solutions on the paper by ammonia vapour, the amine spots then appearing above a diffuse blue band of ammonium salts. The R_F values are in this case somewhat enhanced due to a replacing effect of the ammonium ions. Strong acids appear as bright yellow spots below the amines and the ammonia. The best solvent for preparing extracts of amines from biological material is absolute alcohol containing 0.1 to 2 per cent of glacial acetic acid.

The chromatograms are developed with *n*-butanol saturated with 25 % acetic acid. Before use, the solvent must be left at room temperature for about three days to allow esterification to proceed to equilibrium. Phenol-water may also be employed, although the high R_F values and long drying-time are objections to its use.

After running, the paper is dried at room temperature for one half to three quarters of an hour. A slow current of acid- and base-free air is very helpful. Spraying the chromatogram prepared in this manner immediately after drying with 0.2 % bromphenolblue in absolute alcohol develops the spots. As the

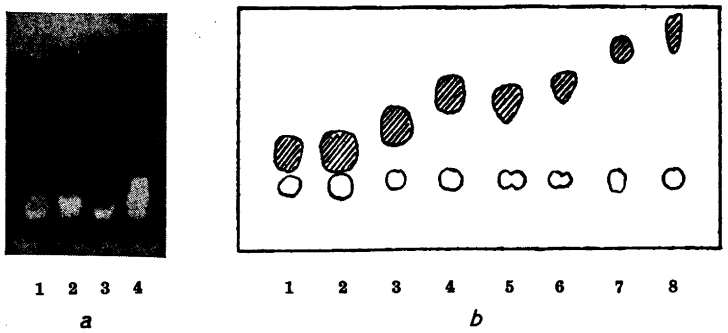


Fig. 2. a) Separation of mixtures: (1) dimethylamine-trimethylamine, (2) ethylamine-n-propylamine, (3) dimethylamine-ethylamine, (4) n-propylamine-i-propylamine. b) Chromatogram of some aliphatic and aromatic amines and piperidine.

1 = Dimethylamine-HCl	*5 γ*
2 = Trimethylamine-HCl	*10 γ*
3 = Ethylamine-HCl	*5 γ*
4 = n-Propylamine-HCl	*5 γ*
5 = i-Propylamine-HCl	*5 γ*
6 = Piperidine-HCl	*5 γ*
7 = Benzylamine-HCl	*5 γ*
8 = Phenylethylamine-HCl	*5 γ*

colour tends to fade after a few days, due to evaporation of amine (trimethylamine spots will fade especially rapidly), we have usually made photographic prints on document contact paper immediately after spraying.

Experiments

Amine	R_F	Minimum amount		Amine	R_F	Minimum amount	
Methylamine	0.28	3 γ	HCl-salt	Benzylamine	0.59	2 γ	HCl-salt
Dimethylamine	0.30	3 γ	»	Phenylethylamine	0.64	2 γ	»
Trimethylamine	0.31	4 γ	»	Cadaverine	0.12	2 γ	»
Ethylamine	0.35	3 γ	»	Piperidine	0.49	2 γ	»
n-Propylamine	0.46	2 γ	»	Arginine	0.13	1 γ	free base
i-Propylamine	0.45	2 γ	»				

Fig. 1a and 2b illustrate the chromatograms of these amines and of piperidine.

To test the efficacy of separation we have tried separation of the following pairs of substances (Figs. 1b and 2a):

NH_3 — all amines	neat separation.
Dimethylamine-trimethylamine	trimethylamine is somewhat ahead and evaporates completely in 12 hours.
Trimethylamine-ethylamine	trimethylamine is somewhat behind and evaporates, leaving ethylamine.
Dimethylamine-ethylamine	partial separation with constriction of area between the spots.
Ethylamine-*n*-propylamine	neat separation.
n-Propylamine — *i*-propylamine	partial separation with constriction.

Pairs of substances with greater differences in R_F values may, of course, easily be separated.

By applying different volumes of an unknown solution side by side with a known solution, it has been possible to determine the amount of amine present in the initial spot to about $\pm$ 1 γ. Best results are obtained by applying dilution series and by observing the minimum quantity giving a just visible spot after chromatography.

For identifying the more volatile amines we have also used the microdiffusion methods of Richter [1] and of Opfer-Schaum [2]. After spraying with indicator we have cut out the areas containing about 5 γ of amine, placed them in small crucibles of about 1 ml volume, and covered the containers with airtight lids which carried micro drops of picric acid solution (less than the required amount). After moistening the paper disc with saturated K_2CO_3 solution, crystals of the picrates appeared on standing. After draining the mother liquor, they were dried, and identified by their melting-point.

SUMMARY

A simple and rapid method of detecting, separating and identifying volatile aliphatic amines by paper chromatography is presented. By comparing the spots with those derived from solutions of known amine content, semiquantitative estimations of the amines may be made. Combination of the method with microdiffusion technique offers a possibility of separating amine mixtures and preparing derivatives of the components.

I wish to thank Prof. P. Karrer for a leave of absence from his institute, the *Erziehungsdirektion des Kantons Zürich* for a grant from the *Stiftung zur Förderung des akademischen Nachwuchses*, and Prof. A. I. Virtanen for suggesting this work and for his hospitality.

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On the Influence of Colloids on the Stability of Supersaturated Solutions of Carbon Dioxide. With Special Reference to Wild Beer

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Moderately supersaturated, homogeneous systems are apparently stable, metastable.

In supersaturated solutions of carbon dioxide in water small quantities of colloid ferric oxide or aluminium hydroxide can cause spontaneous release of the carbon dioxide. The colloid particles participate in the formation of the nuclei on which the supersaturation is released. Knowledge of the processes leading to nuclei formation may be of importance to the understanding of the causes of the phenomena of over-foaming sometimes occuring in beer and other carbonated beverages.

This paper presents the results of some experiments which show that the boundary surface between the liquid and gaseous phases seems to play a rôle in the processes leading to the formation of nuclei.

In experiments with ultrafiltration and diffusion through a collodion membrane it is demonstrated that the bicarbonate ions displace the Gegen-ions of the colloid particles. The consequences which the latter process may have for the stability of the colloid system and the possibilities of nuclei formation hereby arising are finally discussed.

In 1897 Ostwald [1] introduced the expression metastability as a designation for the apparent stability, which may exist in homogeneous systems which are not too strongly supersaturated. The supersaturation may be released by seeding, but Ostwald showed that this requires a certain amount of seeding material; smaller amounts have no effect.

The theoretical explanation for these facts has been given by Gibbs [2] and Volmer [3].

For solutions of gases in liquids, an equilibrium bubble of definite radius corresponds to every degree of supersaturation. The stronger the supersatura-

tion, the smaller the equilibrium bubble. Supersaturation can only be released when, for some reason, a bubble is formed, the radius of which is equal to, or larger than, the radius of the equilibrium bubble. All bubbles of smaller radius will be forced into solution again.

This is true for homogeneous systems without phase boundaries. Such systems do not in fact occur, since there will always be a phase boundary. Gibbs, however, has also investigated the conditions for stability in such cases. It is possible to conclude from his results that supersaturation will be released earlier at the phase boundary than in the liquid, if the surface tension of the foreign phase against the dissolved gas is equal to or less than the surface tension of the liquid.

In a paper, *On the stability of supersaturated carbon dioxide solutions — with special reference to beer*, Krause [4] has given a more detailed account of the conditions described above, and on the basis of this has put forward a hypothesis on the causes of the phenomenon of over-foaming in beer.

Knowledge of the factors which influence the stability of supersaturated carbon dioxide solutions is of great importance to all industries which prepare carbonated beverages.

In the brewing industry, the phenomenon of over-foaming in bottled beer is well known. Over-foaming appears as a spontaneous release of part of the carbon dioxide dissolved in the beer immediately the bottle is opened. This results in such strong foaming that some of the beer is spilled from the bottle.

Over-foaming does not usually occur or appears only to a very slight extent just after bottling. Only when the beer has been subjected to shaking, *e. g.* during transport to the consumers, does over-foaming appear.

The tendency of the beer to over-foaming is usually investigated in the brewery in the following way. A suitable number of bottles, 10—20, is placed in an apparatus in which they are subjected to shaking, hopping or rocking movements in order to give an exaggerated imitation of the movements during transport, to which the beer is subjected on the way to the consumers. When the bottles have remained here for a certain number of days, they are taken out and placed at rest for temperature adjustment for a definite number of hours. They are then opened carefully and the amount of beer which runs out is determined. Over-foaming often occurs in periods, and the cause or causes of the phenomenon are not known. Many experiments have been carried out and many hypotheses put forward, but a satisfactory explanation, based on experimental facts, has not so far been obtained.

Krause's hypothesis, however, is one of the most important contributions which have been given in recent years to the understanding of the causes for this phenomenon. It is based on generally valid considerations, but has no

experimental support. As so many others, Krause imagines that over-foaming is due to nuclei which are formed in the beer during shaking, and he explains the formation of the nuclei thus:

"Their formation is best explained by assuming that the beer during bottling or during transport has foamed and splashed, causing large or small bubbles to be whipped down into the beer. In this case the large bubbles will rise rapidly, small bubbles, rather slowly and very small bubbles, very slowly. In this connection it must be remembered that no supersaturation is found in the closed bottle, hence there is no possibility for the formation of a bubble which is in equilibrium with the surrounding liquid — the equilibrium bubble would have radius ∞. This means that all bubbles which are shaken down into the solution will decrease in size so long as they remain there, and in the case of the smallest and most slowly rising bubbles, this means that they are forced into solution before they reach the surface.

The substances adsorbed onto the surface of the bubbles will be "compressed" during the shrinkage of the bubble surface and the least surface-active will be forced away from the surface by the more surface-active.

As a result of the decrease in size of the bubble, the compression of the adsorbed substances causes, in some cases, a change in the nature of the surface from gaseous-liquid to gaseous-solid. Due to this change, the pressure in the bubble must become less and less as the motility of the surface gradually decreases, the carbon dioxide coming into equilibrium with the carbon dioxide concentration in the beer by diffusion through the adsorbed layer. This will normally be drawn by capillary action into the small cavity in the interior of the aggregate, so that the final result will be the same as before, but if the adsorbed layer has a smaller surface tension to carbon dioxide than to beer, capillary action will not occur and in this case we have a bubble in equilibrium with the beer. Since this bubble will always be very small, its bouyancy will be reduced or completely counteracted by the weight of the adsorbed substances; when the bottle is opened, the fall in pressure will cause expansion, hence a bubble of above equilibrium size may be formed.

If the explanation given here is feasible, then the beer must contain certain colloid particles with surface activities higher than those of the other components of the beer, and in addition, their surface tension to beer — in any case when they occur in compact form — must be relatively high."

Quite apart from whether this hypothesis is correct, it is worth emphasizing the importance for nuclei formation which Krause ascribes to the surface between the liquid and gaseous phase. In the absence of this boundary, as in completely filled bottles, there is no possibility for nuclei formation.

In 1942 the author of this paper began some experiments in order to investigate which substances reduce the stability of supersaturated carbon dioxide solutions. The first experiments were quite simple, but since their results were decisive for the direction in which the later experiments proceeded, they will be discussed briefly here. Similar experiments will be described later in greater detail.

0.1 Molar acetate buffer with pH 4.4, *i. e.* with the same pH as normal beer, was saturated with carbon dioxide at 0° and a counter-pressure of about 200 mm Hg. Under these conditions the concentration of saturation is about 0.42 per cent. In normal bottled beer the concentration of carbon dioxide is of the same magnitude. The carbonated buffer solution was then filled into ordinary beer bottles and examined for "over-foaming" in the usual way. Contrary to expectation considerable "over-foaming" occurred. As soon as a bottle was opened, so much carbon dioxide was released spontaneously that the solution shot out of the bottle in a 10—20 cm high spray. The "over-foaming" did not occur when water, redistilled in a glass apparatus, was used for the preparation of the buffer solution. A further investigation of the cause for this phenomenon showed that the distilled water used contained very small amounts of colloidal aluminium hydroxide. "Over-foaming" could also be produced by the use of redistilled water to which 1—2 mg of colloidal aluminium hydroxide per 1 were added.

At the pH in question this colloid is positively charged. Colloidal ferric oxide, which also has positively charged particles, was found to behave in exactly the same way. Colloidal silicic acid, which has negatively charged particles, had no effect as will be shown later.

Findlay and co-workers [5,6] have published comprehensive and accurate investigations on the influence of various compounds on the solubility of carbon dioxide in water and on the stability of supersaturated solutions. The stability of the supersaturated solutions was determined by measuring the velocity with which the carbon dioxide escaped when the pressure above the solution was reduced to atmospheric pressure. Findlay showed that water which contains colloidal ferric oxide dissolves larger amounts of carbon dioxide than pure water, and that the solubility does not follow Henry's law. Findlay considers that this is due to the adsorption of the carbon dioxide on the colloid.

The observations made by Findlay on the effect of this compound on the stability of supersaturated solutions are, however, especially interesting in this connection. Immediately the pressure was reduced to atmospheric pressure, the solutions spontaneously released part of the carbon dioxide. The velocity with which this occurred was so high that it was not possible to

measure it with the apparatus used. Findlay has not investigated this phenomenon further. He concludes thus: "It would therefore appear that in the case of solutions of ferric hydroxide there exists no essential difference as compared with other colloids, but that the metastable region with regard to gaseous supersaturation is much less."

This spontaneous release of carbon dioxide, however, can only be due to the presence of a large number of particles, which act as nuclei for bubble formation.

As previously mentioned, Findlay is of the opinion that the carbon dioxide is adsorbed on the colloid. Gatterer [7] thinks that the dissolved carbon dioxide reacts chemically with the colloid, and Luther and Krsnjavi [8], that it is a question of formation of complexes, but this alone is not sufficient to explain the formation of nuclei. As will appear from some experiments to be described later, the formation of the nuclei, which cause the spontaneous release of carbon dioxide, also seems to be conditioned in this case by the presence of a boundary between the liquid and gaseous phases.

EXPERIMENTAL

The following preparations were used in the experiments:

I. Hydrosol of aluminium hydroxide, prepared according to Crum [9], concentration 0.46 per cent, calculated as $Al(OH)_3$.

II. Hydrosol of hydrated ferric oxide, commercial preparation Merck, "Liquor ferri oxydati dialysati", concentration 10.1 per cent, calculated as Fe_2O_3.

The effect exerted by these colloids on the stability of supersaturated carbon dioxide solutions can be demonstrated very simply. The method used and the results of a few typical experiments will be described in detail here.

The bottles which were used were Danish beer bottles of the old standard type. When normally filled, *i. e.* half way up the neck, they contain about 335 ml, with an air space of about 20 ml. The crown corks used were of the normal type with plastic discs.

The carbon dioxide solution was prepared by leading carbon dioxide through distilled water at 0° C and at a counter-pressure of about 200 mm Hg. When the water is saturated with carbon dioxide it contains about 0.42 per cent CO_2. At 20° C and atmospheric pressure, the supersaturation is hence about 150 per cent. The filling of the bottles was also carried out at 0° C and without counter-pressure. When the bottles were closed immediately after filling, there was no appreciable loss of carbon dioxide.

The apparatus used for shaking was constructed by Krause in 1930; it was built as a cupboard into which the bottles can be placed horizontally, separated from each other and fastened by rubber rollers. The apparatus performs rapid ascending and descending movements, about 180 times a minute. The designation "hopping-cupboard" is usually used for apparatus of this type, and the process to which the bottles are subjected is by analogy called "hopping". These designations will be used in the present paper. The hopping-cupboard is placed in a room with a constant temperature of 10° C.

[The effect of colloidal aluminium hydroxide and ferric oxide

The aluminium hydroxide sol was diluted 1 : 10. Hence 5.0 ml of the diluted sol contained 2.3 mg $Al(OH)_3$.

30 bottles were used for the experiment. 5.0 ml of the diluted sol was measured into each bottle, the bottles were then filled up with carbon dioxide solution and closed.

10 bottles were kept for 4 days in the hopping-cupboard.

10 » » » » 4 » » » » » and

then for 4 days at rest at room temperature.

10 bottles were kept for 8 days at rest at room temperature.

Before the bottles were opened their temperature was adjusted by standing for 2 hours in a thermostat at 20° C.

The experiments with colloidal ferric oxide were carried out in the same way. A sol, containing 3.3 mg Fe_2O_3 per 5.0 ml was prepared by dilution of the ferric oxide sol.

The results are given in Table 1. The individual figures in the table show the number of g of the solution, which ran out of the bottle in question on opening.

In the bottles which were kept in the hopping-cupboard for 4 days, some of the dissolved carbon dioxide was released spontaneously immediately the bottles were opened, causing about 1/5 of the solution to be spilled.

The same result was obtained even when the bottles were kept at rest at room temperature for 4 days after shaking in the hopping-cupboard. The added colloids had no effect in the control bottles which stood at rest at room temperature.

Table 1. The effect of colloidal aluminium hydroxide and ferric oxide.

Bottle no.	4 days in hopping-cupboard		4 days in hopping-cupboard + 4 days at rest at room temperature		8 days at rest at room temperature	
	$Al(OH)_3$	Fe_2O_3	$Al(OH)_3$	Fe_2O_3	$Al(OH)_3$	Fe_2O_3
1	83	72	94	72	0	0
2	71	67	72	66	0	0
3	72	72	69	74	0	0
4	74	62	84	61	0	0
5	70	63	69	76	0	0
6	79	69	74	71	0	0
7	36	71	82	63	0	0
8	81	63	65	65	0	0
9	64	69	60	68	0	0
10	32	57	61	65	0	0
Mean	66	67	73	68	0	0

When small amounts of these colloids are present in the supersaturated carbon dioxide solution, shaking in the hopping-cupboard causes changes in the system which influence the stability, and which can be recognized clearly by the spontaneous evolution of carbon dioxide. These changes are apparently permanent. In this respect the system behaves in a similar way to beer which has a tendency to over-foaming.

The effect of colloidal silicic acid

As mentioned in the introduction, colloidal silicic acid is without effect. A sol, containing 3.0 mg per ml calculated as SiO_2, was prepared according to a method given by Schwarz [10]. The results of an experiment with this sol are given in Table 2. Each bottle contained about 4.0 mg colloidal silicic acid, calculated as SiO_2. The bottles were kept in the hopping-cupboard for 7 days. When the bottles were opened only slight foaming was observed, in some cases this caused a few grams to spill from the bottle.

The influence of the surface

Some experiments performed similarily to those just described were carried out in order to determine whether the liquid surface plays any part in the processes which lead to the formation of nuclei.

As previously mentioned, the existence of a boundary between the liquid and gaseous phases is, according to Krause's hypothesis, an essential condition for the formation of the nuclei, which cause over-foaming in beer.

Since ordinary beer bottles which are closed with crown corks were unsuited to the experiments, they were replaced by 1/4 l mineral water bottles with patent closures. These bottles could be filled practically completely.

In normally filled bottles the bubbles will be continually whipped down into the liquid during hopping and hence will cause circulation. If, however, the bottles are completely filled, the circulation in the liquid will be considerably reduced. For this reason, a rotation apparatus was used for these experiments in place of the hopping-

Table 2. The effect of colloidal silicic acid.

	g "over-foaming"
Bottle no.	7 days in hopping-cupboard
1	4
2	0
3	2
4	0
5	4
6	3
7	0
8	2
	Mean 2

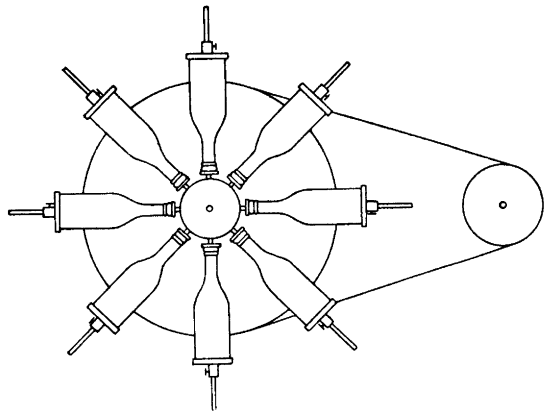


Fig. 1. Rotation apparatus.

cupboard; this is illustrated in Fig. 1. The bottles are placed radially around the axis of rotation, with their mouths pointing towards it. The apparatus, which is placed in a room at constant temperature, 10° C, rotates at 56 revolutions per minute and can contain 8 bottles. 10 5 mm glass balls were placed in each bottle. Satisfactory liquid circulation was thus obtained in the completely filled bottles during rotation.

The aluminium hydroxide sol was diluted 1 : 10. 3.0 ml of the diluted sol thus contained 1.4 mg $Al(OH)_3$. 8 bottles were used for the experiment with this sol. 3.0 ml of the diluted sol were measured into each bottle, and the bottle was then filled with carbon dioxide solution. 4 bottles were normally filled and 4 bottles were completely filled. The bottles were then placed in the rotation apparatus and rotated for 4 days. Before opening their temperature was adjusted for 2 hours at 20° C.

The experiment with colloidal ferric oxide was performed in exactly the same way. A sol, containing 1.5 mg Fe_2O_3 per 3.0 ml, was prepared by dilution of the ferric oxide sol. The results are presented in Table 3.

Table 3. Influence of the surface

Bottle no.	g "over-foaming"			
	Normally filled		Completely filled	
	$Al(OH)_3$	Fe_2O_3	$Al(OH)_3$	Fe_2O_3
1	55	63	0	0
2	57	57	0	0
3	49	60	0	0
4	58	56	0	0
Mean	55	59	0	0

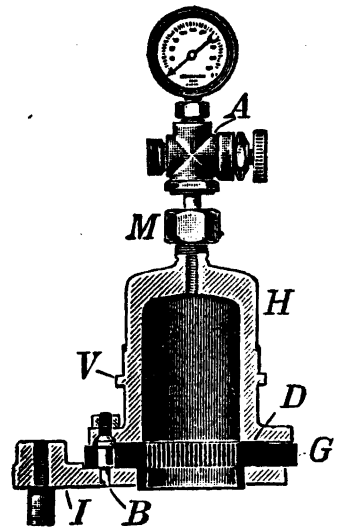


Fig. 2. Ultrafiltration apparatus. For explanation of letters see text.

In the bottles which were filled normally, the release of carbon dioxide on opening was so violent that the liquid shot out of the bottle in a 30—40 cm high spray; about 1/5 of the content was thus spilled. In the bottles which were completely filled, on the other hand, it was not possible to demonstrate any effect of the added colloid.

Hence the experiment appears to confirm the assumption that the liquid surface plays a part in the processes which lead to the formation of nuclei.

The addition of these colloids to a supersaturated solution of carbon dioxide thus gives a system which behaves essentially in the same way as beer which has a tendency to over-foaming.

The adsorption on colloidal ferric oxide

If the carbon dioxide is bound to the colloid, it must be assumed that complex compounds are formed in the surface of the particles by reaction between the bicarbonate ions and the positive ions in the surface of the particles. Hence this reaction is presumably the first step in the processes which lead to the formation of nuclei.

The experiments described here are designed for the purpose of confirming the assumption that the particles bind carbon dioxide.

The experiments were performed with the same ferric oxide sol used in the experiments described previously.

When the particles bind negative ions, in this case bicarbonate ions, some of the Gegen-ions already present will be displaced. An analysis of the ferric oxide sol showed that it contained 0.90 per cent Cl, which is present as

1. Chlorine ions, bound *in* the particles.
2. Chlorine ions, bound *to* the particles as associated ions (Gegen-ions).
3. Intermicellary ions, *i. e.* free chlorine ions.

The concentration of the chlorine ions, which are present as intermicellary ions, can be determined by ultrafiltration. Since the ions are not bound to the particles, they pass

Table 4. Filtration under oxygen pressure.

Fraction no.	ml	c_{Cl^-} $n \times 10^3$	c_{Fe} $m \times 10^3$
I	23	3.1	0.05
II	24	3.7	0.08
III	30	3.7	0.16
IV	25	3.7	0.15
V	25	3.9	0.16

freely through the filter and the concentration can be determined by analysis of the ultrafiltrate.

When bicarbonate ions are bound to the particles, some of the Gegen-ions will be released, resulting in a rise in the concentration of intermicellary chlorine ions. In order to demonstrate this, an ultrafiltration apparatus was used; this is illustrated in Fig. 2.

The liquid to be filtered is poured into the bell-jar H. The filter is placed between the flange D of the bell-jar and the perforated plate G. The liquid is forced through the filter by means of compressed gas which is led into the bell-jar at M. The apparatus is designed for pressures of up to 125 atmospheres and the bell-jar has a volume of about 250 ml.

Zsigmondy's ultrafilters from the firm Membranfilter Gesellschaft, Göttingen, were used for the experiments, and the procedure was as follows:

The ferric oxide sol was diluted 1 : 20. Hence the diluted sol contained:

Fe_2O_3: 0.505 per cent
Cl, (total): 0.045 per cent $\sim$ 12.7 millinormal.

1. Filtration under oxygen pressure

200 ml of the diluted sol were forced through the filter by means of compressed oxygen at about 50 atmospheres. As the filtration gradually proceeded, the ultrafiltrate was removed in fractions of 25—30 ml. The chlorine ion concentration in the first 5 fractions was determined by titration according to Mohr's method. The filtration lasted about two hours.

2. Filtration under carbon dioxide pressure

The filtration experiment was repeated in exactly the same way, except that carbon dioxide at about 50 atmospheres replaced the oxygen.

The results of the filtration experiments are presented in Tables 4 and 5.

Table 4 shows that the concentration of chlorine ions remains rather constant during the whole filtration. Assuming that the oxygen which dissolves in the sol during filtra-

Table 5. Filtration under carbon dioxide pressure.

Fraction no.	ml	c_{Cl^-} $n \times 10^3$	c_{Fe} $m \times 10^3$
I	23	3.9	0.02
II	24	5.4	0.04
III	28	6.2	0.11
IV	31	7.1	0.20
V	16	6.8	0.21

tion does not cause any change in the sphere of ions of the particles, the concentration of the intermicellary chlorine ions is thus determined. In the diluted sol the total chlorine content is 0.045 per cent, corresponding to 12.7 millinormal, of which about 29 per cent is present as intermicellary ions. On the other hand, when carbon dioxide is used for the filtration, it is dissolved in the sol during filtration and the bicarbonate ions thus formed will displace some of the associated ions. Hence the concentration of chlorine ions in the ultrafiltrate rises during filtration and gradually reaches about 56 per cent of the total chlorine content.

The concentration of ferric ions was also determined in the different fractions with *o*-phenanthroline. The figures are given in the last column of the tables. It can be seen that the concentration in the last fractions is slightly higher when carbon dioxide is used for filtration instead of oxygen, but the difference is relatively small and without significance for the conclusions which can be drawn from the experiment.

It can thus be shown in this way that the particles bind carbon dioxide. It must, however, be pointed out that shortly after the beginning of filtration complete coagulation occurs in the sol, and that a continuously increasing layer of precipitated ferric oxide deposits on the filter as the filtration proceeds. The possibility of further investigation of the process by means of ultrafiltration is thus very limited.

If it is possible to determine the concentration of the intermicellary chlorine ions in the sol itself by electrometric methods, *e. g.* by means of a silver chloride electrode, it should be possible to follow the reaction without simultaneously causing changes in the system. This is only possible if the electrode potential is determined by the intermicellary ions alone.

Lamb and Jacques [11] have used the silver chloride electrode in some investigations on the slow hydrolysis which occurs in diluted solutions of ferric chloride. The electrode potential was measured at suitable intervals during the hydrolysis, and a silver chloride electrode dipping into a hydrogen chloride solution of known concentration was used as a reference electrode. The two half cells were connected by a salt bridge of saturated ammonium nitrate. It appeared that it was not possible to determine changes in the activity of the chlorine ion during hydrolysis in a 0.001 *N* solution, and measurements in a 0.004 *N* solution showed only a very slight, but nevertheless definite decrease in activity.

Thus by this method Lamb and Jacques did not obtain results which could serve as a basis for calculations on the course of hydrolysis. The author, using the same method, has tried to determine the concentration of free chlorine ions in the ferric oxide sol. The results in this case also indicate that it is not possible to be certain that the electrode potential is determined by the free chlorine ions alone. The use of a salt bridge must also be considered as rather unfortunate, especially when the potential measurements are repeated over a long period in the same sol. It will not be possible to avoid gradual leakage of the ammonium nitrate into the sol, where it may be able to influence the chlorine ion activity or even produce coagulation in the sol.

As a result of these experiments and considerations, an apparatus, shown in Fig. 3, was used for the further experiments.

The triple-necked flask A contains about 1 l. The collodion sack B is prepared according to the method given by Sørensen [12] and mounted on the glass tube C. A hydrogen electrode D and a silver chloride electrode E are placed inside the sack. The two glass tubes F and G serve as delivery tubes for carbon dioxide and hydrogen respectively. The silver chloride electrode was prepared according to a method given by Brown [13]. These electrodes are very simple to prepare and many measurements in dilute hydrogen chloride

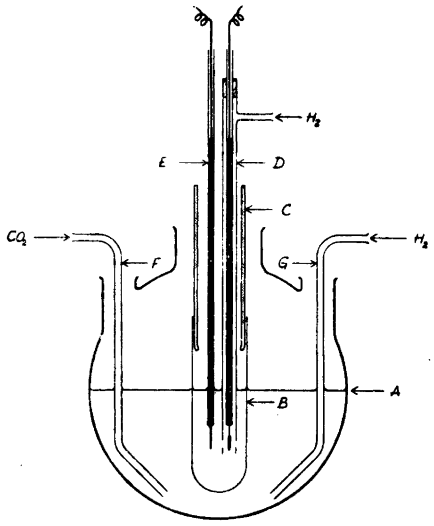


Fig. 3. Apparatus for measuring the activity of intermicellary ions. A triple-necked flask. B collodion sack. C glass tube. D hydrogen electrode. E silver chloride electrode. F and G delivery tubes for carbon dioxide and hydrogen respectively.

solutions have shown that the setting of potential occurs rapidly and that the accuracy is adequate for the present purpose.

The flask was placed in a water thermostat at 25.0° C. The separate parts of the apparatus were supported by means of clamp stands.

The carbon dioxide used for the experiments was taken from a steel cylinder and washed by passing through a solution of sodium bicarbonate and then through water. The hydrogen, which was also used to supply the hydrogen electrode, was similarily taken from a steel cylinder and cleaned by passing through five spiral wash bottles which contained, 1. alkaline pyrogallol solution, 2. ditto, 3. saturated potassium permanganate, 4. 0.1 *N* silver nitrate solution, 5. water.

500 ml of the solution to be examined were measured into the flask and 25 ml of water were measured into the sack. Hence the intermicellary ions will be free to wander through the collodion membrane and the concentration of these ions within the sack will thus rise until, at equilibrium, the same concentration is present on both sides of the membrane.

The use of a cell consisting of a hydrogen electrode and a silver chloride electrode has the great advantage that the salt bridge can be omitted. The potential is determined by the activity of the hydrogen ions and of the chlorine ions.

In pure hydrochloric acid solutions the potential E of the cell is determined by the expression:

$$E = E_0 - 2 \cdot 2.303 \frac{RT}{NF} \log a_{HCl}$$

where a_{HCl} is the activity of the hydrochloric acid and E_0 the normal potential of the silver chloride electrode. At 25.0° C $E_0 = 0.2224$ Volt (Harned and Ehlers [14]). If the potential of the cell at this temperature is E_{25}, we obtain:

$$\log a_{HCl} = \frac{0.2224 - E_{25}}{0.1182}$$

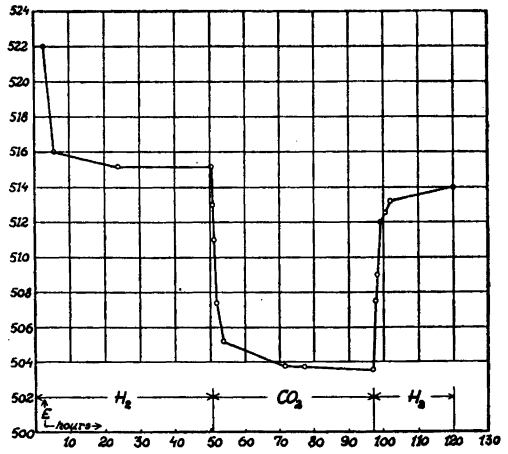


Fig. 4. Results of an experiment with the ferric oxide sol. Ordinate: Potentials in millivolt. Abscissa: Time in hours.

The procedure in experiments with this apparatus is as follows:

During the first period a weak current of hydrogen is led through the sol in order to obtain circulation in it, and hence promote diffusion of ions through the collodion membrane. When the measurements show that the potential has become constant, equilibrium has been reached and the chlorine ion concentration is the same on both sides of the membrane. The stream of hydrogen is then stopped, and a stream of carbon dioxide led through the sol. The concentration of chlorine ions rises again and after an interval the potential is again constant. If the dissolved carbon dioxide is now driven out by leading hydrogen through the sol, the process is reversed until the state present in the system before the carbon dioxide was introduced, is reached. On the basis of the potential measured after the introduction of hydrogen, the concentration of free chlorine ions in the sol can be calculated.

The first experiments were carried out with the ferric oxide sol. These showed that the measurements could not be reproduced with the necessary accuracy. Further investigation showed that the difficulties arose from the fact that the electrodes were placed side by side inside the sack. Measurements in hydrochloric acid solutions of known concentrations thus gave values that were too low, when the electrodes were placed side by side in the electrode vessel. On the other hand, good agreement between the measured and the theoretically calculated potentials was obtained when a U-tube was used, in which the electrodes were placed in the two arms. It must therefore be assumed that when the electrodes are placed side by side, the hydrogen affects the potential of the silver chloride electrode. For this reason all measurements in the following experiments were carried out in a U-tube. The solution in the sack was transferred to the U-tube and poured back into the sack again after completion of each measurement.

The electrodes were controlled during each experiment at suitable intervals by measuring in two hydrochloric acid solutions with c_{HCl}, 0.1006 *N* and 0.0101 *N* respectively. Hydrochloric acid "pro analysi" was used for the preparation of these solutions; it was first evaporated to about half the volume, small amounts of potassium permanganate being added, and then distilled twice in a quartz distillation apparatus. A U-tube was used as electrode vessel and the electrodes were placed in the two arms of the U-tube.

The potentials of the two elements:

I. H_2 (1 atm.) / HCl 0.1006 N / AgCl / Ag

II. H_2 (1 atm.) / HCl 0.0101 N / AgCl / Ag

at 25.0° C are 0.3519 Volt and 0.4639 Volt respectively.

The control measurements showed that in no case there were deviations, which exceeded the accuracy of measurement, $\pm$ 0.2—0.3 mV.

The results of an experiment with the ferric oxide sol are presented graphically in Fig. 4. The ferric oxide sol was used in the same dilution as in the ultrafiltration experiment. Fig. 4 shows that constant potential was reached after leading hydrogen through for 24 hours, as the measured value of 0.5152 Volt did not change demonstrably after passing hydrogen through for further 26 hours.

0.5152 Volt corresponds to $a_{HCl} = 0.00335\ N$. c_{HCl} can be calculated from the equations:

$$c_{HCl}\,\gamma = 0.00335$$

$$\log \gamma = -0.5\ \sqrt{c}_{HCl}$$

and hence $c_{HCl} = c_{Cl^-} = 0.0036\ N$. After correction for the 25 ml of water in the sack, $c_{Cl^-} = 0.0038\ N$, is obtained for the diluted sol, which is in good agreement with the value found in the ultrafiltration experiment, $c_{Cl^-} = 0.0037\ N$ for the concentration of free chlorine ions in the sol.

The effect of the stream of carbon dioxide soon shows itself by a sudden fall in the potential; after about 21 hours it is 0.5038 Volt and it does not change in the next 24 hours.

0.5038 Volt corresponds to $a_{HCl} = 0.0042\ N$ and $c_{Cl^-} = 0.0045\ N$. About 0.9 milliequivalents of chlorine ion per l. of the diluted sol is thus displaced by the bicarbonate ions.

When the carbon dioxide is finally driven out of the sol by the hydrogen, the potential rises rapidly indicating that the process is now reversed.

In order to calculate $c_{HCO_3^-}$ in the intermicellary liquid, it must be presupposed that the solubility and dissociation of the carbon dioxide in this liquid is the same as it is in water, and that the hydrogen ion activity is the same on both sides of the collodion membrane.

Inside the sack $a_{H^+} = a_{HCl} = 4.2 \cdot 10^{-3}\ N$. In a saturated solution of carbon dioxide in water, $a_{HCO_3^-}$ can be calculated from the equilibrium expression:

$$a_{HCO_3^-} = K \frac{a_{CO_2}}{a_{H^+}}$$

At 25° C K is about $3.6 \cdot 10^{-7}$ (Faurholt [15]). If the solubility of carbon dioxide in water at 25° C and atmospheric pressure, which is about $3.3 \cdot 10^{-2}$, is substituted for a_{CO_2}, then

$$a_{HCO_3^-} = 2.8 \cdot 10^{-6}\ N$$

If the concentration of the bicarbonate ions in the intermicellary liquid is of this order of magnitude, it can be seen from the figures that these ions are rather strongly bound by the particles.

DISCUSSION

Krause's hypothesis presupposes, besides the existence of a surface between liquid and gaseous phase, that the substances which form the nuclei are present in higher concentration in the surface than in the liquid. When used as an explanation for the observations made in the previously described experiments, it indicates that the colloid particles must occur in the highest concentration in the surface and that an association (coagulation) of the particles occurs here during shaking. Among earlier investigations on coagulation in surfaces, a paper by Freundlich and Kroch [16] must be mentioned in this connection. These authors investigated ferric oxide sols, prepared by hydrolysis of ferric carbonyl and found that coagulation occurs in the surface when the sol is subjected to mechanical stirring, while this is not the case in the more stable sols prepared by hydrolysis of ferric chloride.

Hence from these results it can not be expected that the ferric oxide sol used for the experiments described here has a tendency to coagulate directly in the surface. The fact that the particles bind bicarbonate ions, possibly during formation of complex compounds, however, causes a decrease in the net charge of the particles. Decreasing charge of the particles in a sol usually indicates an increased tendency to coagulation. Hence the possibility can not be excluded that the reaction with the bicarbonate ions causes a change in the properties of the sol, and that the tendency on the part of the particles to adsorption and coagulation in the surface is thus increased.

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Molecular Configuration of 1,2-Dichloro-4,5-dibromocyclohexane ($\varepsilon, \varepsilon-\varkappa, \varkappa \rightleftharpoons \varkappa, \varkappa-\varepsilon, \varepsilon$) in the Crystal

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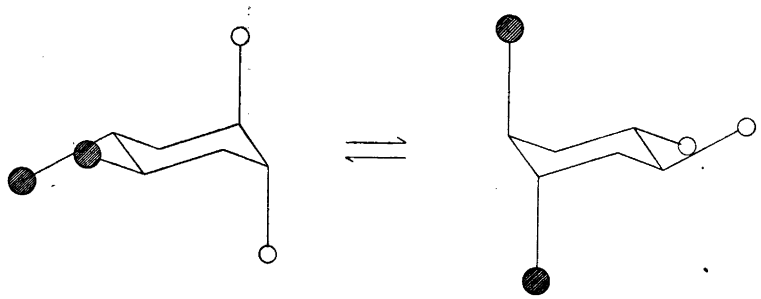
Recently Hassel and Lunde [1] prepared a new member of the series of 1,2,4,5-tetrahalogeno-cyclohexanes (1,2-dichloro-4,5-dibromocyclohexane, m.p. 171° C) from 1,2-dichlorocyclohexene and bromine. They expected to obtain a substance corresponding to the tetrachloro- and tetrabromocyclohexanes melting at 176° C and 187° C respectively, the structure of which has been determined [2,3] and corresponds to the configuration 1ε, 2ε, $4\varkappa$, $5\varkappa$.

We actually found the crystals of the new substance to be isomorphous with those of the two tetrahalogeno-compounds just mentioned. In Table 1 the lattice constants are listed. The number of molecules in the unit cells is 2, the space group is $P2_1\,2_1\,2$ and the molecules therefore have a two-fold axis of symmetry.

Table 1. Lattice constants of the three tetrahalogeno-compounds in Å.

	a	*b*	*c*
$C_6H_8Cl_4$	7.60	7.54	7.72
$C_6H_8Br_4$	7.90	7.98	7.89
$C_6H_8Cl_2Br_2$	7.69	7.67	7.83

A more detailed analysis of the structure was subsequently carried out in order to decide whether the structure is of a disordered type, the chlorine and bromine atoms occupying ε and $\varkappa$ positions in a statistical way, or if all the molecules have the same configuration. In the latter case it seemed of interest to determine which of the two interconvertible forms is present in the crystal, the form (Fig. 1a) having the chlorine atoms in ε positions or the form (Fig. 1b) in which the chlorine atoms are in $\varkappa$ positions. A priori it would seem most probable that the chlorine atoms are in ε position because the repulsion



Figs. 1 a and b. Possible configurations of the 1,2-dichloro-4,5-dibromocyclohexane (ε, $\varepsilon-\varkappa$, $\varkappa$).

between a halogen atom in ε position and the two nearest hydrogen atoms, also in ε positions, would be expected to raise the energy of the molecule more if it is a bromine atom than if it is a chlorine atom. The repulsion effect of two halogen atoms in neighbouring $\varkappa$ positions would probably be less important to the energy of the molecule.

It was a great advantage to our investigation that the structure of the tetrachloro-compound was already known in some detail. In the case of the tetrabromo-compound the parameters of the bromine atoms had also been determined, but the positions of the carbon atoms were rather uncertain. K. Taugböl of our laboratory therefore worked out a Fourier projection of the bromine compound which settled with certainty that the parameters of all corresponding atoms have nearly identical values in the tetrachloro- and the tetrabromo-compounds.

Rotation and Weissenberg photographs obtained from the new compound were so similar to those of the compounds containing only chlorine or bromine that it must be assumed that the halogen atoms have nearly the same parameters in the three cases. In order to work out a Fourier projection along the c axis in which the halogen atoms would be well separated, intensity values of ($hk0$) reflexions were determined from Weissenberg photographs with rotation about [001]. The exposure times of the double films were 1, 5 and 20 hours respectively. The intensities of both CuK_α and K_β reflexions were considered. A standard scale was prepared using a strong reflexion from the crystal itself as a source of radiation.

As the first structure alternative, a statistical distribution of halogen atoms over ε and $\varkappa$ positions did not give a satisfactory agreement between observed and calculated intensities of ($hk0$) reflexions, we calculated the inten-

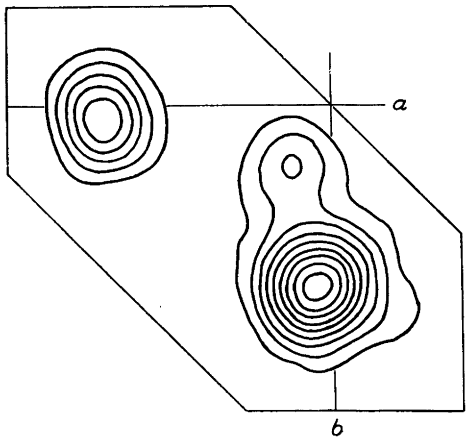


Fig. 2 a. Projection of the electron density of dichloro-dibromocyclohexane along the c axis.

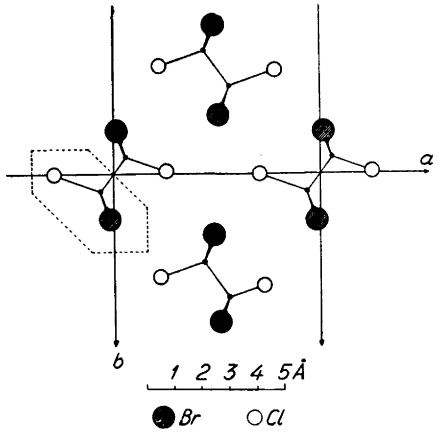


Fig. 2 b. The structure of dichloro-dibromocyclohexane projected along the c axis.

sities assuming the chlorine atoms to be in ε positions, the bromine atoms in $\varkappa$ positions (Fig. 1a) and obtained a very satisfactory agreement with observed intensities. The resulting electron-density map showed a marked difference in the heights of the ε and $\varkappa$ halogen peaks. The x and y parameters thus obtained differed very little from those first assumed, and a single amplitude only changed its sign.

The refined Fourier map is reproduced in Fig. 2a. Neighbouring contour lines correspond to a drop in electron density equal to 5 electrons per square Ångström. Comparing Fig. 2a with Fig. 2b which gives a full projection of the structure along the c axis (the area whithin the dotted hexagon corresponds to the Fourier map of Fig. 2a) it is easily seen that the chlorine atoms are situated in ε positions, the bromine atoms in $\varkappa$ positions.

The presence of two of the carbon atoms is indicated by a smaller peak extending from the peak due to the ($\varkappa$) bromine atom. In the case of an undeformed carbon ring (with strictly tetrahedral valency angles) the projections of these two carbon atoms would coincide. The projection of the third, crystallographically independent, carbon atom nearly coincides with that of the bromine atom and thus contributes to the height of the bromine peak. Taking this contribution into account, the relative heights of the two halogen peaks are in good agreement with the ratio to be expected.

The aim of the crystal structure investigation was to determine the configuration of the molecules and not the exact parameters of the atoms. It is possible, however, from the Fourier projection along [001] to obtain fairly accurate x and y values for the halogen atoms (compare Table 2).

Table 2. x and y parameters of the dichloro-dibromo-compound.

	x	y
Cl	− 0.282	0.020
Br	− 0.019	0.222
C_1,C_5	− 0.062	0.081
C_6	− 0.028	0.194

Table 3. Observed and calculated $|F|$ values of (hkO) reflexions.

*hk*0	F_{obs}	F_{calc}	*hk*0	F_{obs}	F_{calc}
200	22.3	23.9	440	10.5	9.4
400	23.5	21.0	540	0	1.4
600	2.8	4.4	640	2.3	2.7
800	0	1.5	740	0	2.2
110	11.5	11.7	840	0	1.4
210	7.4	9.1	150	5.0	7.9
310	13.8	10.9	250	1.6	1.4
410	9.9	10.6	350	8.4	9.0
510	5.0	2.9	450	4.4	4.2
610	6.0	6.4	550	0	2.9
710	3.2	2.3	650	1.8	1.1
810	3.9	3.7	750	3.9	3.3
910	2.9	1.3	850	2.2	1.5
020	9.1	12.3	060	2.5	2.5
120	4.9	6.4	160	3.1	3.5
220	35.4	30.0	260	8.5	6.6
320	3.5	4.7	360	0	1.0
420	5.5	9.0	460	0	0.8
520	4.4	5.0	560	3.1	3.3
620	6.8	7.5	660	0	1.0
720	2.3	2.0	760	0	1.7
820	2.4	2.4	170	6.5	7.4
920	0	1.0	270	0	1.2
130	13.9	14.2	370	2.9	4.0
230	2.1	2.5	470	0	0.3
330	3.2	5.3	570	3.5	3.2
430	0	1.5	670	1.6	1.1
530	8.0	6.7	080	0	0.5
630	3.8	4.1	180	0	0.6
730	2.6	0.1	280	0	1.1
830	1.6	1.9	380	2.5	2.5
930	1.4	1.3	480	0	0.6
040	21.1	17.6	580	0	1.1
140	2.8	2.6	190	0	2.2
240	2.5	5.5	290	0	0.6
340	2.9	3.4	390	1.4	2.1

As regards the carbon atoms, parameter values obtained from the structure analysis of the tetrachloro-compound were used, when calculating F values (compare Table 2). A temperature factor $e^{-5,15\left(\frac{\sin\vartheta}{\lambda}\right)^2}$ was introduced.

It is worth mentioning that the good agreement obtained between calculated and observed $|F|$ values (compare Table 3) would not have been reached if the influence of the carbon atoms had been disregarded. The low value of the reliability factor (0.20) seems indeed to justify the choice of the carbon parameters mentioned above.

The final result of our investigation thus confirms the anticipation concerning the relative stability of the two possible configurations of the molecule. It should be borne in mind, however, that the molecules are not "free" and that the effect of intermolecular forces has not been considered. Although we think it very probable that the configuration found in the crystal will be that which predominates in the vapour, it would indeed seem worth while to determine the configuration in the vapour phase also, using electron diffraction technique. Such investigations have already been started in our laboratory, but experimental difficulties due to thermal instability of the dichloro-dibromo-compound have prevented us from giving final results in the present communication.

SUMMARY

A new substance, 1,2-dichloro-4,5-dibromo-cyclohexane [1], isomorphous with the tetrachloro- and tetrabromocyclohexanes in which the halogen atoms occupy 1ε, 2ε, $4\varkappa$, $5\varkappa$ positions, has been studied in the solid state. It was found that the chlorine atoms are in ε positions, the bromine atoms in $\varkappa$ positions. It seems probable that this configuration corresponds to a lower energy of the molecule than the configuration in which the chlorine atoms occupy $\varkappa$ positions, the bromine atoms ε positions even in the gasous state.

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The Dissociation Constants of Pyruvic and Oxaloacetic Acid

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The two important acids, pyruvic and oxaloacetic acid, are both fairly strong acids, but only very scant information of their dissociation constants is found in the earlier literature [1–3]. Oxaloacetic acid is unstable in aqueous solution. It decomposes into pyruvic acid and carbon dioxide. In order to give a satisfactory interpretation of the results of a kinetic study of this reaction (preliminary communication [4]), it was desirable to have better data for the dissociation constants of the two acids. They were therefore determined by means of the glass electrode in solutions containing sodium or, in some cases, potassium chloride, at the temperatures 25 and 37° C.

MATERIALS

In the preparation and purification of oxaloacetic acid, the method of Wohl and Oesterlin [5] was followed with some modifications. First, *diacetyltartaric anhydride* was prepared as described by these authors. Next, the *pyridinium salt of oxymaleic anhydride* was prepared by the following procedure worked out and kindly communicated to me by Dr. A. Klit [6]:

In a 300-ml beaker is placed 80 ml of dry pyridine. At room temperature, and with good stirring by means of a thermometer, 60 g of finely powdered diacetyltartaric anhydride is added. After 15–30 seconds, when practically all has gone into solution with a yellow to green colour, 24 ml of glacial acetic acid is run into the beaker. The turbidity disappears, and the temperature rises to about 40°. The mixture is quickly cooled in a good ice-salt bath while it is stirred vigorously by means of the thermometer. Very soon precipitation of the pyridine compound sets in. Owing to the heat generated by the reaction, the temperature has at first a tendency to rise in spite of good cooling. When it falls, it is best to keep it for about two minutes between 30 and 25° since the reaction stops at about 20°. The mixture is cooled to 0° and 100 ml of dry ether is added. The ether promotes the precipitation of the pyridinium salt. The mixture is then filtered by suction on a glass filter. The salt is washed twice with absolute alcohol and two or three times with dry ether, both liquids being strongly cooled. The ether is removed by sucking air through the preparation. In order to prevent condensation of water from the air, it is

necessary to heat the filter with the crystals. It is therefore placed in a steam-heated drying box for one or two minutes, after which air is again sucked through the crystals. This is repeated until the preparation does not smell of ether. The preparation is only faintly green. It melts at 108 – 110° C. Yield 42 g or 78 per cent of the theoretical amount based on diacetyltartaric anhydride.

Finally, *oxaloacetic acid* was prepared by the following procedure. 36 g of the pyridinium salt of oxymaleic anhydride was, in small portions, dissolved in 120 ml of 38 per cent hydrochloric acid. During the addition, the solution heated itself a little. When all was dissolved, oxaloacetic acid began to deposit. At the same time, the colour of the solution changed from greenish blue to reddish. The mixture was placed in a good ice-salt bath where it stayed for several hours. It was then filtered by suction, and the crystals were washed twice with altogether 50 ml of concentrated hydrochloric acid, which had first been cooled in an ice-salt bath. They were dried in vacuum over concentrated sulphuric acid and solid sodium hydroxide. The dry preparation had a faint greenish-gray colour. The yield of crude acid was 90 per cent of the theoretical amount.

Recrystallisation of oxaloacetic acid. 25 g of the crude acid was dissolved in 205 ml of hot acetone. 325 ml of 55° warm benzene was added to the nearly boiling solution. On cooling in an ice-salt bath, oxaloacetic acid crystallised. After filtration, it was washed with benzene and dried in vacuum over concentrated sulphuric acid and paraffin-wax shavings. Yield 18 g. More, but less pure, acid could be obtained by partial evaporation of the mother liquor. The recrystallised acid was nearly white with a faint brown tint, which remained unchanged after renewed recrystallisation. It was not hygroscopic and could be stored in an ordinary glass-stoppered vessel without decomposing. The oxaloacetic acid used for the measurements had been recrystallised twice. The molecular weight found by dissolving a sample in a small excess of sodium hydroxide and titrating back with hydrochloric acid, using phenolphthalein as an indicator, was 132.3 (calc. 132.1).

Two preparations of *pyruvic acid* were used for the measurements:

A. Pyruvic acid prepared according to *Organic Syntheses* [7] and fractionated twice under reduced pressure. The fraction used distilled from 68.9 to 69.4° C at 17 mm Hg. The molecular weight found by alkalimetric titration was 0.2 per cent too low.

B. A Merck preparation which was further purified by two fractional distillations under reduced pressure, followed by two partial crystallisations of the constant-boiling fraction. The final product had the melting point 11° C and a molecular weight found by alkalimetric titration which was 0.6 per cent too high.

The other chemicals used were of analytical purity. Redistilled water was used to make up the solutions.

EXPERIMENTAL METHOD

The procedure was essentially the same as that used by the author in earlier papers [8–10]. Glass electrodes with a plane membrane made of Corning glass no. 015 were applied. The electrodes were at room temperature immersed into the solutions to be measured, and the cells were placed in an electrically heated liquid-paraffin thermostat for temperature adjustment. The e.m.f. was measured by means of a valve potentiometer. No hysteresis effect owing to the

cooling of the electrodes to room temperature between the measurements was observed (*cf.* Irving and Williams [11]). Only such electrodes were used which, with sufficient accuracy, showed the same potential change as the hydrogen electrode when transferred from the solution 0.01 molar HCl,0.09 molar NaCl to a phosphate buffer of pH 6.81.

The cells examined had the composition

$$\text{Pt} \mid \text{S, quinhydrone} \mid \text{glass} \mid \text{X} \mid 3.5\ M\ \text{KCl} \mid \text{S, quinhydrone} \mid \text{Pt}$$

where S denotes the solution 0.00999 M HCl, 0.0900 M NaCl and X the solution under investigation. When a small asymmetry potential, measured daily by substituting S for X, was subtracted, the e.m.f. E volts of cells of the composition

$$\text{H}_2 \mid \text{X} \mid 3.5\ M\ \text{KCl} \mid \text{S} \mid \text{H}_2 \qquad (1)$$

was obtained. The solution X contained oxaloacetic or pyruvic acid (a molar), sodium or potassium chloride (s molar) and, sometimes, sodium hydroxide (b molar). a varied from 0.01 to 0.02; in a few cases, it was higher or lower. s varied from 0.02 to 0.3. The solutions were made up at room temperature, and no correction was applied for their expansion when they were heated to 25 or 37° C. If a correction were applied, all concentrations and dissociation constants given in this paper for the temperature 37° C should be diminished by 0.5 per cent, while 0.002 should be added to the values of $-\log K$. In the measurements at 25° C, the correction is without any importance.

The hydrogen ion concentration $[\text{H}^+]$ of solution X may be found from the measured values of E by means of the following equations when A is known:

$$-\log\,[\text{H}^+] = A + 16.90\ E \text{ (at } 25^\circ \text{ C)} \qquad (2)$$

$$-\log\,[\text{H}^+] = A + 16.25\ E \text{ (at } 37^\circ \text{ C)} \qquad (3)$$

A includes the salt effect and the liquid-liquid effect of the left half cell of scheme 1, together with a constant part representing the whole effect of the right half cell. For A was used an approximate value determined for a solution which did not contain the organic acid but, instead of the anions of this acid, an equivalent amount of chloride ions. E was therefore measured also for solutions X containing only hydrogen chloride (from 0.01 to 0.001 molar) and sodium or potassium chloride (from 0.3 to 0.02 molar). From the known hydrogen ion concentrations in these solutions, the values of A given in Table 1 were found by means of equations 2 and 3. When they are used for the solutions containing

Table 1. Values of A *in equations 2 and 3 for solutions of the composition:* h *molar hydrogen chloride,* s *molar sodium or potassium chloride.*

		h	A when s =				
			0.30	0.20	0.10	0.05	0.02
NaCl,	25°	0.0100	2.014	2.005	2.000	2.001	2.001
		0.0050	2.015	2.003	2.000	2.002	2.004
		0.0020	2.014	2.004	2.001	2.006	2.014
		0.0010	2.012	2.004	2.003	2.010	2.016
KCl,	25°	0.0100	1.979	1.977	1.983	1.992	1.997
		0.0050	1.978	1.978	1.987	1.992	2.001
		0.0020	1.981	1.981	1.986	1.998	2.009
		0.0010	1.983	1.982	1.987	2.002	2.012
NaCl,	37°	0.0100	2.014	2.006	2.000	2.001	2.000
		0.0080				2.004	2.003
		0.0060		2.007		2.006	2.002
		0.0050	2.016		2.002	2.008	2.008
		0.0020	2.017	2.005	2.003	2.011	2.016
		0.0010	2.015	2.006	2.006	2.012	2.023
KCl,	37°	0.0100	1.978	1.978	1.984	1.992	1.999
		0.0080			1.984	1.994	2.002
		0.0050	1.980	1.980	1.990	1.998	2.008
		0.0020	1.980	1.981	1.989	2.002	2.014
		0.0010	1.983	1.984	1.993	2.001	2.015

the organic acids, they give a better approximation the greater the concentration s of salt added. The error may be divided into two parts. The first part is due to the change of the ideal diffusion potential (Guggenheim [12]), the second to the change of activity coefficients when the organic acid is replaced by hydrochloric acid. The magnitude of the first part may be estimated by means of Henderson's formula [12,8]. It was found that it, in the most unfavourable cases, corresponds to an error in $-\log [H^+]$ of about 0.002. The magnitude of the second part of the error cannot be estimated beforehand, but its possible influence will be discussed later.

THE DISSOCIATION CONSTANT OF PYRUVIC ACID

The composition of the solutions measured and the results obtained are given in Tables 2 and 3 for the temperatures 25 and 37° C, respectively. The solutions were made from 0.2 molar standard solutions of pyruvic acid (either

Table 2. The dissociation constant K *of pyruvic acid at 25° C in the solution:* a *molar pyruvic acid,* b *molar sodium hydroxide,* s *molar sodium chloride. Preparation A of pyruvic acid was used except for the data in the column marked B, which were obtained from measurements with preparation B.*

a	*b*	*s*	*E*	$-\log[H^+]$	$-\log K$	$-\log K$ B	$\triangle 10^3$
0.02003	0	0.300	0.00275	2.060	2.174	2.173	+ 22
0.02003	0.00505	0.300	0.01385	2.248	2.189	2.189	+ 6
0.02003	0.00999	0.300	0.0278	2.484	2.191	2.188	+ 6
0.01002	0	0.300	0.01455	2.260	2.176	2.181	+ 18
0.01002	0.00500	0.300	0.0367	2.634	2.200	2.200	− 5
0.02003	0	0.200	0.0040	2.073	2.210		− 2
0.02003	0.00505	0.200	0.0151	2.259	2.212		− 4
0.02003	0.00997	0.200	0.0291	2.497	2.215		− 8
0.01002	0	0.200	0.01575	2.270	2.208		+ 2
0.01002	0.00499	0.200	0.03795	2.645	2.226		− 17
0.02003	0	0.100	0.0050	2.084	2.241		+ 10
0.02003	0.00505	0.100	0.0164	2.277	2.249		+ 1
0.02003	0.00999	0.100	0.0308	2.521	2.254		− 7
0.01002	0	0.100	0.01685	2.285	2.254		− 1
0.01002	0.00500	0.100	0.0391	2.662	2.260		− 9
0.02003	0	0.050	0.00575	2.098	2.277		+ 19
0.02003	0.00505	0.050	0.0173	2.294	2.284		+ 10
0.02003	0.00999	0.050	0.0318	2.541	2.287		+ 4
0.01002	0	0.050	0.0173	2.294	2.281		+ 19
0.01002	0.00500	0.050	0.0399	2.679	2.294		+ 4
0.02003	0	0.020	0.0061	2.104	2.293	2.292	+ 52
0.02003	0.00505	0.020	0.01795	2.307	2.310	2.311	+ 31
0.02003	0.00999	0.020	0.03295	2.566	2.327	2.325	+ 9
0.01002	0	0.020	0.0178	2.305	2.315	2.314	+ 37
0.01002	0.00500	0.020	0.0408	2.702	2.340	2.336	+ 9

preparation A or B), which were standardised against sodium hydroxide solution. The measurements at 25° were mainly carried out with preparation A, those at 37° mainly with preparation B, but at both temperatures some measurements were repeated with solutions of nearly the same composition but made from the other preparation. In the next to the last column of the tables, $-\log K$ calculated from these measurements is given. The agreement is always satisfactory.

Table 3. The dissociation constant K *of pyruvic acid at 37° C in the solution:* a *molar pyruvic acid,* b *molar sodium hydroxide,* s *molar sodium chloride. Preparation B of pyruvic acid was used except for the data in the column marked A, which were obtained from measurements with preparation A.*

a	*b*	*s*	*E*	$-\log[H^+]$	$-\log K$	$-\log K$ A	$\Delta 10^3$
0.03974	0	0.300	−0.00995	1.852	2.114		+ 13
0.03974	0.00999	0.300	0.0028	2.060	2.112		+ 15
0.01987	0	0.300	0.00165	2.041	2.114		+ 13
0.01987	0.00505	0.300	0.01265	2.221	2.122		+ 5
0.01987	0.00999	0.300	0.0270	2.456	2.131		− 4
0.00994	0	0.300	0.0142	2.246	2.122		+ 5
0.00994	0.00500	0.300	0.03635	2.608	2.128		− 1
0.01987	0	0.200	0.0025	2.047	2.131	2.129	+ 7
0.01987	0.00505	0.200	0.0138	2.231	2.144	2.143	− 7
0.01987	0.00999	0.200	0.02805	2.463	2.144	2.142	− 7
0.00994	0	0.200	0.01495	2.250	2.135	2.143	− 1
0.00994	0.00500	0.200	0.03745	2.615	2.144	2.142	− 5
0.01987	0	0.100	0.0036	2.058	2.164		+ 14
0.01987	0.00505	0.100	0.0148	2.242	2.169		+ 7
0.01987	0.00999	0.100	0.0295	2.481	2.175		0
0.00994	0	0.100	0.01575	2.258	2.161		+ 19
0.00994	0.00500	0.100	0.0388	2.634	2.186		− 7
0.01987	0	0.050	0.00425	2.072	2.201		+ 22
0.01987	0.00505	0.050	0.01585	2.264	2.215		+ 6
0.01987	0.00999	0.050	0.0309	2.511	2.227		− 10
0.00994	0	0.050	0.01635	2.273	2.209		+ 18
0.00994	0.00500	0.050	0.0397	2.655	2.233		− 8
0.01987	0	0.020	0.0047	2.078	2.217		+ 54
0.01987	0.00505	0.020	0.01655	2.276	2.240		+ 27
0.01987	0.00999	0.020	0.03155	2.525	2.250		+ 11
0.00994	0	0.020	0.01705	2.284	2.244		+ 34
0.00994	0.00500	0.020	0.0403	2.671	2.266		+ 7

In order to analyse the results and make an attempt to extrapolate the values of $-\log K$ to zero ionic strength, $-\log K + 2a\sqrt{I}$, where a is the Debye-Hückel coefficient and I the ionic strength of the solution, was plotted against I (fig. 1). It is seen from the graph that the points corresponding to the two temperatures fit moderately well to the two straight lines drawn when s is 0.1

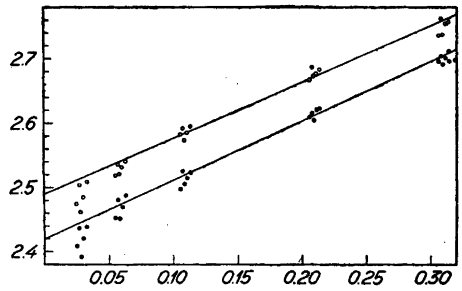


Fig. 1. Abscissa: the ionic strength I, ordinate: $-\log K + 2\alpha\sqrt{I}$, where α is the Debye-Hückel coefficient and K is the dissociation constant of pyruvic acid measured at 25° C (open circles) and at 37° C (solid circles).

or greater. When s is 0.02, the points fall, however, much below the straight lines. On the whole, the points are rather scattered, especially those corresponding to $s = 0.02$. This shows that the activity coefficients of the ions in the solutions are not with sufficient accuracy determined only by the ionic strengths of the solutions, but change perceptibly when a part of the chloride ions are substituted by an equal concentration of pyruvate ions and the concentration of undissociated pyruvic acid is increased. The salt effect error in A mentioned before may therefore have an influence on the values of $[H^+]$ and K determined. The scattering of the points is consequently explained partly by this error in K, partly by the fact that the true value of K depends not only on the ionic strength but also on the individual nature of the constituents of the solutions. Both effects will decrease when the concentration of sodium chloride increases. We shall therefore assume that the straight lines in fig. 1 represent the true values of K when the concentration of pyruvic acid is small compared with that of sodium chloride, and that their intercepts on the ordinate axis determine, with a fair degree of accuracy, the activity constants K_a at the two temperatures. As a result of these considerations, we obtain the following formulae for the dissociation constant of pyruvic acid:

$$(\text{at } 25^\circ\text{ C}) \quad -\log K = 2.490 - 1.013\sqrt{I} + 0.87\ I \tag{4}$$

$$(\text{at } 37^\circ\text{ C}) \quad -\log K = 2.420 - 1.038\sqrt{I} + 0.92\ I \tag{5}$$

It follows from the formulae that K_a is 3.24×10^{-3} at 25° C and 3.80×10^{-3} at 37° C. The difference $\triangle$ between $-\log K$ calculated from the formulae and found directly from the measurements is given in the last column of Tables 2 and 3. Böeseken, Hansen, and Bertram [3] have determined the dissociation constant of pyruvic acid at 25° C by conductance. They found 3.2×10^{-3}, a value which agrees well with that found in the present paper.

THE FIRST DISSOCIATION CONSTANT OF OXALOACETIC ACID

The solutions were prepared in the following way. The necessary amount of standard sodium or potassium chloride and, in some cases, sodium hydroxide were placed in a 100-ml graduated flask and diluted to about 90 ml. Oxaloacetic acid was weighed out in a small platinum bucket, which was dropped into the solution. When the acid was dissolved, water was added to the mark. Immediately after, the cell was made up and placed in the thermostat. Readings began as soon as temperature equilibrium had been attained. Although decarboxylation takes place with a considerable velocity (half of the acid is decomposed in about 100 minutes at 37°, in nearly 5 hours at 25°), the e.m.f. measured changed only very little with time. It was therefore easy to find the values of E corresponding to undecomposed oxaloacetic acid. They are given in Tables 4 and 5. In Table 5 are also given values of E_∞ found when the solu-

Table 4. The first dissociation constant K_1 *of oxaloacetic acid at 25° C in the solution:* a *molar oxaloacetic acid,* b *molar sodium hydroxide,* s *molar sodium chloride. Data for solutions containing potassium chloride instead of sodium chloride are given in the last column.*

a	b	s	E	$-\log[H^+]$	$-\log K_1$	$\Delta 10^3$	$-\log K_1$ (KCl)
0.02004	0	0.300	0.00315	2.067	2.219	+ 5	
0.01503	0	0.300	0.0078	2.146	2.220	+ 3	
0.01000	0	0.300	0.01465	2.263	2.228	− 4	
0.02002	0	0.200	0.00445	2.079	2.249	− 1	2.257
0.02000	0.00719	0.200	0.0202	2.346	2.250	− 2	
0.01500	0	0.200	0.0091	2.158	2.251	− 2	2.266
0.01003	0	0.200	0.0158	2.271	2.252	− 2	2.271
0.02000	0	0.100	0.00595	2.101	2.303	0	2.306
0.02000	0.00721	0.100	0.0223	2.377	2.298	+ 2	
0.01500	0	0.100	0.01055	2.178	2.302	+ 2	2.302
0.01000	0	0.100	0.01725	2.292	2.307	− 2	2.321
0.02000	0	0.050	0.00675	2.115	2.336	+ 18	2.335
0.02000	0.00719	0.050	0.0235	2.400	2.335	+ 15	
0.01501	0	0.050	0.01115	2.190	2.331	+ 25	2.347
0.01002	0	0.050	0.0179	2.305	2.341	+ 17	2.340
0.02000	0	0.020	0.0074	2.127	2.365	+ 43	
0.01502	0	0.020	0.0119	2.204	2.366	+ 45	
0.01002	0	0.020	0.0185	2.318	2.374	+ 40	

tion had been kept at 37° C for one day. At that time, all the oxaloacetic acid had been transformed into pyruvic acid. E_∞ is a little greater than E, from which it is concluded that the hydrogen ion concentration decreases slightly when the reaction goes on. Pyruvic acid is a little stronger than oxaloacetic acid, for which reason $[H^+]$ should increase, but this effect is more than compensated by a slight second dissociation of oxaloacetic acid, which tends to make $[H^+]$ greater at the beginning of the reaction. The values of E_∞ found here should correspond to solutions of pure pyruvic acid. They may therefore be compared with E in Table 3 for solutions of the same composition but prepared directly from pyruvic acid. E_∞ is on an average about 0.0002 volts greater than E in Table 3. The agreement may be considered satisfactory.

In the computation of the first dissociation constant K_1 of oxaloacetic acid from the hydrogen ion concentrations measured, a correction has been made for the concentration of divalent oxaloacetate ions by means of the formulae 10—12 for the second dissociation constant K_2. The results obtained for solutions containing sodium chloride are given in the first six columns of Tables

Table 5. The first dissociation constant K_1 *of oxaloacetic acid at 37° C in the solution:* a *molar oxaloacetic acid,* b *molar sodium hydroxide,* s *molar sodium chloride. Data for solutions containing potassium chloride instead of sodium chloride are given in the last column.*

a	s	E	E_∞	$-\log[H^+]$	$-\log K_1$	$\Delta 10^3$	$-\log K_1$ (KCl)
0.01999	0.300	0.00115	0.0019	2.033	2.122	+ 17	
0.01500	0.300	0.0063	0.0070	2.117	2.134	+ 5	
0.01001	0.300	0.01365	0.0142	2.237	2.146	− 7	
0.02001	0.200	0.00255	0.0029	2.047	2.161	− 6	2.146
0.01500	0.200	0.0075	0.00775	2.128	2.164	− 8	2.153
0.01003	0.200	0.01435	0.01495	2.240	2.154	+ 2	2.151
0.01999	0.100	0.00365	0.0039	2.059	2.189	+ 12	2.189
0.01499	0.100	0.0085	0.0089	2.139	2.190	+ 12	2.209
0.01000	0.100	0.0157	0.0159	2.257	2.198	+ 6	2.217
0.02001	0.050	0.0044	0.0046	2.075	2.230	+ 19	2.221
0.01500	0.050	0.0092	0.0092	2.154	2.230	+ 21	2.236
0.01000	0.050	0.01635	0.01645	2.273	2.242	+ 12	2.250
0.02000	0.020	0.00485	0.00495	2.082	2.245	+ 54	
0.01502	0.020	0.0098	0.0099	2.162	2.250	+ 53	2.259
0.01001	0.020	0.01705	0.0172	2.284	2.271	+ 36	

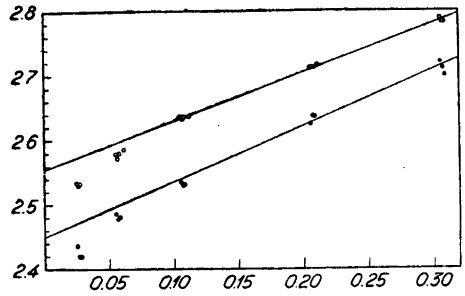


Fig. 2. Abscissa: the ionic strength I, ordinate: $-\log K_1 + 2a\sqrt{I}$, where a is the Debye-Hückel coefficient and K_1 is the first dissociation constant of oxaloacetic acid measured at 25° C (open circles) and at 37° C (solid circles) in solutions containing sodium chloride.

4 and 5. In fig. 2, $-\log K_1 + 2a\sqrt{I}$ has been plotted against I for the same solutions. The two straight lines have been drawn so as to fit best to the points when s is 0.1 or more. When s is lower than 0.1, the points fall below the straight lines. The picture is similar to that found earlier for pyruvic acid, and the discussion given there may also be applied here. If we assume that the straight lines represent the true values of K_1 when the concentration of oxaloacetic acid is small compared with that of sodium chloride, we obtain the following formulae

$$\text{(at 25° C)} \quad -\log K_1 = 2.555 - 1.013\sqrt{I} + 0.75\ I \tag{6}$$

$$\text{(at 37° C)} \quad -\log K_1 = 2.450 - 1.038\sqrt{I} + 0.86\ I \tag{7}$$

It follows from the formulae that the activity constant K_{1a} is 2.79×10^{-3} at 25° C and 3.55×10^{-3} at 37° C. The difference between $-\log K_1$ calculated from the formulae and found directly from the measurements is given in the next to the last column of Tables 4 and 5. In the last column of the tables are given values of $-\log K_1$ found for solutions containing potassium chloride instead of sodium chloride. They do not deviate much from those for sodium chloride, but they are apparently somewhat less regular.

In a preliminary paper [4] on the kinetics of the decarboxylation of oxaloacetic acid, K_1 was computed exclusively from kinetic data for solutions containing potassium chloride. The following comparison shows that there is a satisfactory agreement between the results obtained by the two methods.

I	0.05	0.10	0.20
$K_1 \times 10^3$ from kinetic data	5.44	6.10	6.75
$K_1 \times 10^3$ from formula 7	5.48	6.21	6.95

No precise measurements of the dissociation constant of oxaloacetic acid are found in the earlier literature. Wohl and Claussner [2] found by conductance at 17° C for two samples of oxaloacetic acid, considered by them to be respectively oxyfumaric and oxymaleic acid, for K_1 the values 2.76×10^{-3} and 2.50×10^{-3}.

THE SECOND DISSOCIATION CONSTANT OF OXALOACETIC ACID

The solutions were prepared in the same way as those used for the determination of the first dissociation constant but more sodium hydroxide was added (see Table 7). The e.m.f. rose here rapidly with time. It follows from the kinetic study of the reaction [13] that the concentration of the divalent oxaloacetate ion Ox^{--} remains constant until nearly all the univalent ion HOx^- has disappeared, and that

$$-\frac{d[HOx^-]}{dt} = k_1[HOx^-] + k_2[Ox^{--}]$$

If we divide by $[HOx^-]$ and introduce decadic logarithms, we obtain

$$-\frac{d\log[HOx^-]}{dt} = k_1^* + k_2^* \frac{[Ox^{--}]}{[HOx^-]} \tag{8}$$

When we use

$$[HOx^-] = [H^+]\ [Ox^{--}] / K_2$$

and equation 3, we get, for the temperature 37° C,

$$-d\log\ [HOx^-] = -d\log\ [H^+] = 16.25\ dE$$

On introducing this result into equation 8, we find

$$16.25 \frac{dE}{dt} = k_1^* + k_2^* \frac{[Ox^{--}]}{[HOx^-]} \tag{9}$$

When the values of E observed for a solution were plotted against t, the points fell close to a straight line for a much greater part of the reaction than we should expect from equation 9. The slope was roughly independent of the

Table 6.

a	b	$\frac{dE}{dt} 10^3$ 25°	$\frac{dE}{dt} 10^3$ 37°	$-\frac{d\log[H^+]}{dt}$ 37°	$\frac{[Ox^{--}]}{[HOx^-]}$ 37°	$k_2^* 10^3$ min^{-1} 37°
0.200	0.333	0.215	1.035	0.0168	2.09	4.9
0.200	0.300	0.145	0.735	0.0119	1.10	4.9
0.200	0.266	0.107	0.553	0.0090	0.61	4.1

concentration of sodium or potassium chloride added. Average values of the slopes for all the solutions listed in Table 7 are given in Table 6. For the temperature 37° C, an estimate has been made of k_2^* by means of equation 9. For this purpose we have used the ratios of the initial concentrations of the ions $[Ox^{--}]$ and $[HOx^-]$ given in the table and $k_1^* = 6.5 \times 10^{-3}$ min^{-1} found earlier[4]. The values computed for k_2^* vary from 4.1 to 4.9 $\times 10^{-3}$ min^{-1}, while we from direct kinetic measurements[13] should expect about 2×10^{-3} min^{-1} for the uncatalysed decarboxylation of the divalent ion. The reaction is, however, catalysed by traces of certain metal ions, and it is not excluded that impurities of such ions have been present in the sodium hydroxide used.

In order to find the value E would have if no decomposition of the oxaloacetic acid had taken place, a suitable extrapolation was carried out by means of the straight line in the plot of E against t. The solutions were prepared at room temperature (about 21° C), and 3.5 to 4 minutes elapsed from the dissolution of the acid until the cells were placed in the thermostat. From a previous determination of the temperature-time curve for the cell in the thermostat and knowledge of the variation of $\frac{dE}{dt}$ with temperature, it was found that, in measurements at 37° C, extrapolation should be made to a time 1.5 minutes after, in those at 25° C, to a time 1.5 minutes before the cells were placed in the thermostat. The values of E given in Table 7 have been found by extrapolation in this way. In calculating $-\log [H^+]$ from E, equations 2 and 3 were used together with the values of A corresponding to $h = 0.01$ in Table 1. In the computation of the second dissociation constant K_2 of oxaloacetic acid from the hydrogen ion concentrations measured, a correction was made for the concentration of undissociated acid by means of the values of K_1 measured before. The ionic strengths of the solutions and the values of $-\log K_2$ found by this computation are given in the 6th and 7th column of Table 7. All the

Table 7. The second dissociation constant K_2 *of oxaloacetic acid at 25 and 37° C in the solution:* a *molar oxaloacetic acid,* b *molar sodium hydroxide,* s *molar sodium chloride. In the last two measurements (marked *), the solution contained potassium chloride instead of sodium chloride.*

a	*b*	*s*	*E*	$-\log[H^+]$	*I*	$-\log K_2$	$-\log K_2$ calc.
At the temperature 25° C:							
0.02000	0.03328	0.200	0.1219	4.066	0.2468	3.746	3.750
0.02000	0.03004	0.200	0.1063	3.801	0.2406	3.750	3.754
0.02000	0.02659	0.200	0.09175	3.556	0.2343	3.763	3.757
0.02000	0.03328	0.100	0.12665	4.142	0.1468	3.824	3.824
0.02000	0.03004	0.100	0.11095	3.877	0.1406	3.831	3.831
0.02000	0.02659	0.100	0.0961	3.626	0.1342	3.840	3.839
0.02000	0.03328	0.050	0.13085	4.216	0.0968	3.901	3.891
0.02000	0.03004	0.050	0.1145	3.940	0.0905	3.898	3.903
0.02000	0.02659	0.050	0.0993	3.683	0.0841	3.904	3.914
0.02000	0.03004	0.000	0.12135	4.066	0.0404	4.031	4.026
0.01000	0.01502	0.015	0.12085	4.057	0.0353	4.013	4.044
At the temperature 37° C:							
0.01999	0.03003	0.250	0.10705	3.752	0.2906	3.703	3.708
0.02001	0.02660	0.250	0.09155	3.500	0.2843	3.710	3.710
0.02001	0.03331	0.200	0.12505	4.040	0.2469	3.720	3.725
0.02000	0.03003	0.200	0.10875	3.775	0.2406	3.728	3.728
0.02002	0.02662	0.200	0.09365	3.529	0.2343	3.743	3.731
0.02003	0.03004	0.100	0.1132	3.844	0.1405	3.805	3.807
0.01999	0.02658	0.100	0.0982	3.601	0.1341	3.824	3.815
0.02000	0.03004	0.050	0.1172	3.912	0.0905	3.873	3.881
0.02001	0.02664	0.050	0.10175	3.661	0.0841	3.888	3.892
0.02001	0.03003	0.020	0.12115	3.981	0.0604	3.948	3.946
0.02000	0.03010	0.000	0.1245	4.041	0.0405	4.004	4.006
0.01999	0.03007	0.250 *	0.10985	3.771	0.2907	3.720	3.720
0.02001	0.02664	0.250 *	0.0937	3.509	0.2844	3.718	3.721

values agree well with the following formulae where the square-root term has been calculated according to Debye and Hückel.

$$\text{(at 25° C, NaCl)} \quad -\log K_2 = 4.370 - 2.026\sqrt{I} + 1.57\ I \qquad (10)$$

$$\text{(at 37° C, NaCl)} \quad -\log K_2 = 4.359 - 2.076\sqrt{I} + 1.61\ I \qquad (11)$$

$$\text{(at 37° C, KCl)} \quad -\log K_2 = 4.359 - 2.076\sqrt{I} + 1.65\ I \qquad (12)$$

In the last column of Table 7 are given the values of $-\log K_2$ calculated by means of these formulae. It follows from the formulae that the activity constant K_{2a} is 4.27×10^{-5} at 25° C and 4.38×10^{-5} at 37° C.

SUMMARY

A description is given of a method for the determination of concentration dissociation constants of weak and moderately strong acids in salt solutions by means of electrometric measurements of cells with liquid junction. The limited usefulness of the method is discussed.

The method was used for the determination of the dissociation constant of pyruvic acid and the first and second dissociation constants of oxaloacetic acid in sodium and, in some cases, potassium chloride solutions at the temperatures 25 and 37° C. The results for pyruvic acid are summarised in the formulae 4—5, those for oxaloacetic acid in the formulae 6—7 and 10—12.

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On the Oxygen Electrode in Molten Salts

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The oxygen ion is one of the chief components of the common salts, glasses and slags. Reactions involving oxides may be described as a transfer of oxygen ions from one state of polarization to another *Cf.* [1]. An acid-base definition based on the oxygen ion [2] (analogous to the hydrogen ion in the Brönsted acid-base definition) may therefore give a uniform picture of many different reactions and facilitate the correlation between them. The determination of an acid-base equilibrium in a melt may in principle be regarded as an examination of oxygen ion activities. The experimental determination of oxygen ion activity has therefore recently become the object of considerable interest.

Electrometric determination of oxygen ion activities should be possible by means of an electrode on which the potential is determined by a reaction like

$$O^{2-} = O + 2\,e$$

It is the purpose of the present work to examine the question whether such a reversible oxygen electrode can be established in melts.

Several attempts have already been made to measure electromotive forces — EMF — in galvanic cells with molten oxides as electrolytes. Derge and Lo Ching Chang [3] tried to determine the SiO_2-activity in silicate melts by means of EMF-measurements of the cell

C, CO(g) ; Silicate melt ; SiC, CO(g)

They were, however, unable to give a reasonable cell reaction, which could explain the variation of the EMF with the silicate composition, gas pressure etc.

Csaki and Dietzel [4] have measured cells like

(Pt) O_2; Boric oxide: Borates; O_2 (Pt)

with elctrolytes of fused boric oxide and borates of different alkali ions. Their theoretical considerations are open to severe criticism, and the authors have been unable to give any quantitative evidence as to what kind of reaction is potential determining. It is interesting to note, however, that they claim to have measured well-defined EMF in this type of cells.

The work of Rose, Davis and Ellingham [5] on the reducibility of oxides indicates that an oxygen electrode really works reversibly in a melt mainly composed of sodium hydroxide at temperatures from 400 to 700° C. These investigators did not, however, primarily focus their attention on the oxygen electrode, and they therefore did not investigate the EMF as a function of the composition of the melt. (Compare also the overvoltage investigations in fused NaOH by Agar and Bowden [6]).

The investigations of Lux are the only ones which directly aim at establishing an oxygen electrode in a melt, in order to determine the "oxygen ion concentration". We shall here only briefly consider Lux's first paper [2], on which his later work [7] is based. Lux tries to establish a cell of the type:

$$(\mathrm{Pt})\mathrm{O_2}\,;\begin{matrix}\mathrm{Na_2SO_4}\\ \mathrm{K_2SO_4}\\ \mathrm{Na_2O\ (N)}\end{matrix}\Big\}\ \text{eutectic}\ :\begin{matrix}\mathrm{Na_2SO_4}\\ \mathrm{K_2SO_4}\\ \underbrace{\mathrm{Na_3PO_4 + NaPO_3}}_{\text{reference electrode}}\end{matrix}\Big\}\ \text{eutectic}\ ;\ \mathrm{O_2(Pt)}$$

$$(t = 950^\circ\ \mathrm{C})$$

The sodium oxide concentration in the left half cell is varied, and is expected to determine the potential. A weak point in his investigations seems to be that the actual sodium oxide concentration has not been analytically checked, in spite of rapid evaporation of alkali oxide and attack on the alumina crucible during the EMF-measurement. In consequence of the rapid change of EMF with the time, Lux is forced to extrapolate the potential to zero time (that is the point of time when a known amount of sodium oxide was added to the melt).

The result of the measurements is given in a diagram, where this EMF is plotted against the logarithm of the sodium oxide concentration. The curve is very far from linear, and in the middle part a tangent would correspond to the value RT/F. If, however, the electrode process is

$$\mathrm{O^{2-}} = \tfrac{1}{2}\ \mathrm{O_2} + 2\ \mathrm{e}$$

then the coefficient should be $RT/2F$.

As an explanation Lux suggests that the potential is determined by the process

$$\mathrm{Na} = \mathrm{Na^+} + \mathrm{e}$$

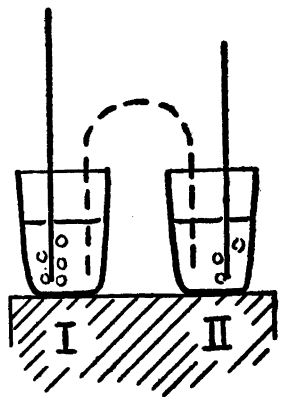


Fig. 1. Type of cell arrangement.

where the sodium is formed by a thermal decomposition of Na_2O according to

$$2Na_2O = 2\ Na + Na_2O_2$$

A proportionality between C_{Na_2O} and C_{Na}, however, demands a constant peroxide concentration independent of C_{Na_2O}, which is impossible to realize at these temperatures (the oxide is added as peroxide!) On the contrary, the equilibrium

$$Na_2O_2 = Na_2O + \tfrac{1}{2}\ O_2$$

i.e., the thermal decomposition of sodium peroxide, demands a proportionality between the oxide and peroxide concentrations. This brings the theoretical coefficient of EMF back to $RT/2F$.

We are inclined to consider the results as a consequence of an unfavourable cell arrangement combined with a not very fortunate choice of salt system (vaporization of K_2O and no buffer capacity with respect to the oxygen ion).

As a preparation for the present work some investigations on the oxygen overpotential in fused salts were made by Flood and Förland [8]. The results seemed to demonstrate that the overpotential at low current densities was not of the activated type. This indicated that the oxygen electrode would not be hindered in its reversible action.

PRINCIPLES, AND CHOICE OF SALT SYSTEM

Three main factors have to be considered: The type of cell, the salt system and the design of the apparatus.

The galvanic cell. A "formation cell", the type used *e.g.* by Hildebrand and Salstrom [9,10], in their electrometric studies of several halogenide mixtures,

has the advantage of not involving any liquid junction potential. To establish a "formation cell" at high temperatures is in general difficult. For this reason we chose instead a concentration cell with transference, introducing no other electrode than the oxygen electrode, but involving an unknown liquid junction potential. The cell, then, in principle should consist of two crucibles with melts of different but fixed oxygen ion activities. In each crucible there is placed an inert electrode (platinum) with oxygen bubbling past it. Between the melts there should be a salt bridge to give electrolytic contact. (Fig. 1).

The liquid junction potential in concentration cells containing fused salts as electrolytes has not been the subject of any extensive treatment in the literature. An attempt at quantitative treatment of transport numbers and liquid junction potential in fused salts has been made by Schwarz [11]. However, the assumptions on which his treatment rests disagree with the observations subsequently made by Karpachev and Pal'guev [12].

At present it is hard to predict how much the measurements in our cell will be affected by liquid junction potentials. The following extreme cases might give some idea of this. Liquid junction potential does not have to be taken into account if:

1. The conductivity of the salt bridge in our cell is due to the migration of cations, *i.e.*, the transport number of cations is large compared with that of the anions.

2. The solvent electrolyte alone is responsible for the conductivity of the melt, and there is a considerable excess of the solvent electrolyte.

If one of these conditions is fulfilled, the EMF will be given by the equations derived below, and the transport numbers will not appear in the equations.

The salt system should possess a buffer capacity with respect to the oxygen ion activity, and this activity should preferably vary in a known manner with the composition of the melt. Two salt systems have been dealt with, which were thought to fulfil these requirements.

The first system tried, was fused *sodium sulphate-sodium carbonate mixtures in equilibrium with a constant carbon dioxide pressure.* The sodium carbonate will dissociate into sodium oxide and carbon dioxide. (The corresponding dissociation of the sulphate will at the same temperature be negligible, see below). The dissociation of the carbonate can be written in terms of ions

$$CO_3^{2-} = O^{2-} + CO_2 \text{ (g)}$$

with the equilibrium constant

$$K = \frac{a_{O^{2-}} \cdot p_{CO_2}}{a_{CO_3^{2-}}} \text{ or } a_{O^{2-}} = K \cdot \frac{a_{CO_3^{2-}}}{p_{CO_2}}$$

where a is activity and p gas pressure. The oxygen ion activity is thus fixed by definite carbon dioxide pressure and carbonate activity. Further, investigations of equilibria in sodium borate-carbonate-sulphate-melts [13] indicated that melts of sodium carbonate-sulphate behaved very close to ideal, *i.e.*, activity equals mole fraction N. (In this system as well as in the chromate-dichromate system the mole fraction equals the ionic fraction as defined by Temkin [14]). With a constant partial pressure of carbon dioxide in the atmosphere we thus have

$$a_{O^{2-}} = \text{const. } N_{Na_2Co_3}$$

Hence the electromotive force of the cell should be

$$E = \frac{RT}{2F} \ln \frac{a_{O^{2-}\,(II)}}{a_{O^{2-}\,(I)}} = \frac{RT}{2F} \ln \frac{N_{II}}{N_I}$$

where N_I and N_{II} denote the mole fraction of carbonate in the crucible I and II respectively.

The equilibrium of the thermal decomposition of sodium carbonate has not yet been properly measured. All previous investigators have measured the "carbon dioxide pressure" at high temperatures, without paying regard to the possibility that the oxide is soluble in the fused carbonate. From thermodynamic data, however, we calculate for the reaction

$$Na_2CO_3\ (l) = Na_2O\ (l) + CO_2(g)$$

$$\Delta G^\circ_{1200^\circ K} = 40\ (\pm 5)\ \text{kcal}$$

hence

$$N_{Na_2O} / N_{Na_2CO_3} = 10^{-7\ (\pm 1)} \text{ at } 900^\circ C \cdot (p_{CO_2} = 1 \text{ atm.})$$

From this it is seen that the oxygen ion concentration is very small compared with the carbonate concentration.

For the corresponding thermal dissociation of sodium sulphate we calculate

$$\Delta G^\circ_{1200\,^\circ K} = 84\ (\pm 5)\ \text{kcal}$$

Hence we have at 1200° K roughly

$$p_{SO_2} + p_{SO_3} / p_{CO_2} \simeq 10^{-8} \cdot N_{Na_2SO_4} / N_{Na_2CO_3}$$

After it had been confirmed that the oxygen electrode worked satisfactorily in the carbonate-sulphate mixtures, it was of interest to find out whether the electrode would also work reversibly in salt systems with greater acidity. Some experiments were therefore carried out with mixtures of *potassium chromate-*

potassium dichromate. In this system it might be expected that the oxygen ion activity would be determined by the equilibrium

$$2\ CrO_4^{2-} = Cr_2O_7^{2-} + O^{2-}$$

with the equilibrium constant

$$K = \frac{a_{Cr_2O_7^{2-}} \cdot a_{O^{2-}}}{a^2_{CrO_4^{2-}}}$$

Here again, earlier measurements [15] indicated that the deviation from ideal mixture is comparatively small (though not entirely negligible.) Thus we have

$$a_{O^{2-}} \cong K \cdot \frac{N^2_{CrO_4^{2-}}}{N_{Cr_2O_7^{2-}}}$$

If we denote chromate by subscript c and dichromate by d, we have for the electromotive force

$$E = \frac{RT}{2F} \ln \frac{a_{O^{2-}\ (II)}}{a_{O^{2-}\ (I)}} \cong \frac{RT}{2F} \ln \frac{N^2_{c\ (II)}\ N_{d\ (I)}}{N^2_{c\ (I)}\ N_{d\ (II)}}$$

the liquid junction potential being neglected. The oxygen ion activity in these mixtures is definitely smaller than in the carbonate-CO_2 systems. The ratio of $a_{O^{2-}}$ in the two systems is estimated to be of the order of magnitude 10^{-5}. (Entropy data for the alkali chromates are not available).

EXPERIMENTAL

The cell is shown diagrammatically in Fig. 1. the apparatus in Fig. 2.

The salt bridge should consist of a non-conducting, insoluble, porous material, which can act as a sort of wick for the fused, highly conductive salts. For the sulphate-carbonate melts magnesium oxide proved to be a very satisfactory material. For the dichromate-chromate melts sintered alumina worked satisfactorily. In most of the runs the salt bridge and gas tubes were combined, see Fig. 2.

Reliable electric insulation is essential in measurements of this kind. When the temperature is raised to 1 000° C or more, most refractories get perceptible conductivity, this may be still more the case if they are exposed to alkali vapors or the sputtering from fused salts. The ceramic refractories will have the character of electrolytic conductors. Poor insulation between the two crucibles and between the electrodes will therefore not only give a leak current, but may introduce a superimposed EMF on the contact metal-refractory. When carrying out measurements in our apparatus with the salt bridge

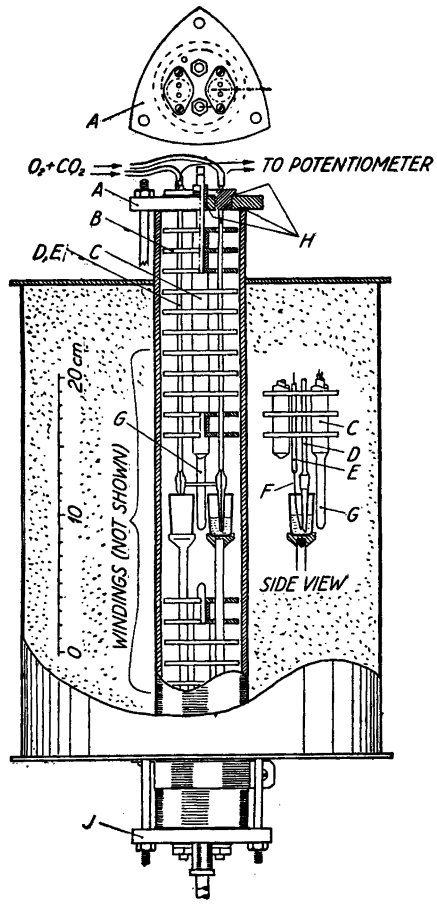


Fig. 2. A: Brass top. B: Alundum radiation shields. C: Mullite rings separating the shields. D: Mullite tubes (3 mm dia.) for gas inlet, prolonged with magnesia or alumina tubes. E: Mullite tubes (3 mm dia.) with internal platinum leads. F: Platinum electrodes. G: Mullite thermocouple sheath. H: Rubber gaskets. J: Brass bottom.

removed, we often found EMF of the order of 1 volt, *i. e.* ten or more times the EMF of the cell (but with definitely smaller sensitivity on the galvanometer).

If E_1 and R_1 represent EMF and internal resistance in the galvanic cell, respectively and E_2 and R_2 the same in the insulation, the error in the EMF measurement will be

$$\triangle E = (E_2 - E_1)\,\frac{R_1}{R_1 + R_2} \cong E_2 \cdot \frac{R_1}{R_2}$$

If, for instance, $E_2 = 1$ volt, and $R_1/R_2 = 1/1\,000$, then $\triangle E = 1$ mv. As the measured internal cell resistance R_1 was of the order 10 000 to 1 000 ohms (depending upon temperature *etc.*) the insulation resistance ought then to be at least some megohms.

Proper insulation was ensured by the arrangement shown in Fig. 2. The ceramic tubes supporting the crucibles, as well as the tubes for gas currents and electrode leads, are not in contact with any solid material in the hot zone of the furnace. (During the carbonate runs the insulation between the crucible supporters was not quite so good as indicated in Fig. 2, the measured value being just about $R_1/R_2 = 1/1\,000$).

The furnace was an ordinary resistance heated vertical tube furnace wound with Kanthal A. The windings were bifilar, and were closer spaced towards the ends of the furnace, to compensate for the extra loss of heat at the ends. A voltage stabilizer was used for the heating current. The furnace tube was internally heat insulated towards the ends by piles of alundum cement discs, with appropriate holes for the internal tubes. In order to get the platinum crucibles in correct position relatively to the electrodes *etc.*, a stable mechanical device was used for lowering and elevating the lower furnace lid. The brass lids were provided with rubber gaskets and were clamped in position by screws to secure a gas-tight sealing. The upper lid was cooled by water. The temperature was measured with a Pt-Pt 10 % Rh thermocouple. The EMF of cell and thermocouple were measured with an Otto Wolff potentiometer.

The atmosphere in the furnace proved to be of major importance. With a more or less uncontrolled atmosphere — *i. e.* the composition of the gas in the furnace differing somewhat from the composition of the gas bubbling on the electrodes, no stable potentials were obtained. The EMF then oscillated in time with each single bubble (a fact which suggests that the time necessary to establish the potential is only a fraction of a second at these high temperatures). Under such conditions the EMF also depended upon the velocity of the gas currents on the electrodes.

The procedure was usually as follows: Prior to the runs, melts of the wanted compositions were mixed, fused in platinum basins, cooled and crushed. (All the reagents were Merck p. a., dried at proper temperatures). Well in advance, the furnace was heated to the proper temperature. About 5 g of the salt mixture were weighed into each of the cell crucibles. These were placed on their refractory supporters, and the lower lid raised to a point about 2 cm from its upper position. After some fifteen minutes the salts were assumed to be fused and the lower lid was fully raised, thus causing the electrodes etc. to dip into the melts. From this point onwards readings of temperature and EMF were taken at regular intervals. The lids were tightened by the screws, and the furnace was flushed by a gas current of approx. 1.5 l/min. maintained for half an hour. The gas currents on the electrodes were maintained at 1.5—3 ml/min. (all gas volumes measured at room temperature) *i. e.* approx. 1—2 bubbles per second in the crucibles. Occasionally the electrode gas currents were varied between 1 and 8 ml/min.; this proved to have little or no influence on the EMF.

At the end of the run the lower furnace lid was lowered and the crucibles allowed to cool in a desiccator. The total time of the run varied from 2 to 8 hours.

After being weighed the crucibles were soaked in water, and the contents analysed. The analyses were necessary, because the composition of the melt changed somewhat during a run. This was due in some degree to volatilization of alkali. A more significant cause was the contamination by melts from the previous run, which were left as droplets on the electrodes etc. But the dominating factor was that the salt bridge acted as a siphon for the melt. Very frequently there was found a weight loss in one crucible and a corresponding increase in the other. This was difficult to avoid, even when taken into consideration by proper charging of the crucibles. Thus the analyses were indispensable. (It may be mentioned that in run no. 9, where the titrations are lacking, there was as a matter of fact no perceptible siphon action). The titration of carbonate in presence of larger amounts of sulphate has to be performed with an indicator having a pI close to pH = 7 because of the possible co-titration of a fraction of the sulphate. It was found that the best procedure was to titrate with phenol red in constantly boiling liquid (as recommended for the standardization of $N/100$ hydrochloric acid).

Still it proved necessary to apply a correction, especially for the lowest carbonate contents. If the same proportion of the amount of sulphate is cotitrated each time, the correction should take the form of a constant deduction from the total volume of titration, regardless of the magnitude of this volume. By titration of samples with known quantities of sulphate, the value of the correction was found to be — 0.048 ml $N/10$ HCl per 5 g sulphate. (Volume of titration about 150 ml).

No free alkali (or acid) could be detected in the pure sulphate, (*i. e.*, less than corresponding to 1 drop $N/100$ HCl = 0.005 ml $N/10$ HCl per 5 g sulphate).

The dichromate-chromate mixtures were analysed by determining the total amount of chromium by means of ordinary iodometric titration. Then the dichromate could be determined by acidimetric titration in the following way: To the water solution an excess of $N/10$ NaOH was added. The solution was heated to boiling, and $BaCl_2$ was added, whereby the chromate precipitated as $BaCrO_4$. The excess of NaOH was then titrated back with $N/10$ HCl.

RESULTS AND DISCUSSION

The system Na_2SO_4—Na_2CO_3—CO_2

The results from the sulphate — carbonate — carbon dioxide system are shown in Table 1. The values of E_{calc} are calculated from the formula

$$E = \frac{RT}{2F} \ln \frac{N_{II}}{N_I}$$

where N is the mole per cent of carbonate in the salt mixture as determined by analysis. It is evident that the formula is valid to a high grade of accuracy.

This is also shown in the diagram Fig. 3, where E is plotted against the logarithm of the carbonate mole fraction in the melt. The experimental data are demonstrated in the way that the point for one composition (marked with a vertical bar) has been plotted on a straight line of the theoretical slope $RT/2F$. The other point for the same run (marked with a small circle) is then fixed by the other composition and the observed EMF (adjusted to the temperature of the straight line by multiplying with the factor $T_{obs}/1223$).

It is evident that the experiments fit the theoretical slope very satisfactorily. This means that the electrode really behaves as an oxygen (oxygen ion) electrode in the melt.

Regarding the difference between the calculated and the observed EMF, $-\Delta E$ in Table 1, — it should be noted that 1 relative per cent error in *one* of the titrations corresponds to an error of 0.5 mV in $E_{calc.}$, and that a number of the titration volumes were very small. For the greater part of the runs the discrepancy thus lies within the analytical uncertainty. The maximum difference (3 mV) between observed and calculated EMF corresponds to 0.02 pO (pO = — log $a_{O^{2-}}$), and the average deviation of observed and calculated pO is less than 0.01.

Table 1. E.M.F. measurements in concentration cells with the system sodium sulphate — sodium carbonate — carbon dioxide.

Run No.	Gas	Start Mol % Na_2CO_3		End (analysed) Mol % Na_2CO_3		T_{obs} °K	E_{obs} mV	E_{calc} mV	ΔE mV	Time h
		N_I	N_{II}	N_I	N_{II}					
1	B	1	5	1.135	4.595	1227	74.5	73.9	+ 0.6	8
2	B	0.5	5	0.489	4.732	1222	122.3	119.4	+ 2.9	2 ½
3	B	2	10.4	1.937	10.00	1224	86.8	86.5	+ 0.3	4
4	A	2	30	1.954	28.96	1223	144.1	142.0	+ 2.1	5
5	A	2	20	1.966	19.40	1225	121.1	120.8	+ 0.3	5 ½
6	A	2	20	1.967	19.13	1223	120.5	119.8	+ 0.7	5 ½
7	D	2	20	1.992	19.49	1223	120.6	120.1	+ 0.5	5
8	C	2	5	1.990	4.983	1224	48.0	48.4	− 0.3	5 ½
9	C	0.5	2	—	—	1222	73.5	(73.0)	+ 0.5	4
10	C	0.5	2	0.483	2.007	1224	76.0	75.1	+ 0.9	7
11	C	0.00	1	0.0412	0.991	1223	166.8	167.5	− 0.7	3
12	D	5	—	4.879	57.11	1224	131.2	129.8	+ 1.4	4
13	D	2	100	1.953	93.96	1224	206.2	204.2	+ 2.0	2 ½
14	D	0.1	2	0.1374	2.474	1224	154.4	152.4	+ 2.0	3 ½
15	C	2	10.4	1.919	10.089	1324	97.6	94.6	+ 3.0	3
16	C	2	10.4	1.902	9.755	1321	93.1	93.0	+ 0.1	3 ½
17	C	1	5	0.947	4.758	1422	98.0	98.9	− 0.9	2 ½

Composition of gas mixtures:

A:	35.6 % CO_2	62.6 % O_2	1.8 % N_2
B:	57 » »		
C:	70.1 » »	29.1 » »	0.8 » »
D:	95.0 » »	4.6 » »	0.4 » »

As the equation above is valid also up to the highest carbonate contents it may be concluded that the system Na_2SO_4-Na_2CO_3 forms an ideal mixture in the fused state. Further it may be concluded that the liquid junction potential is negligible in a cell containing Na_2SO_4-Na_2CO_3 mixtures of different compositions.

The two conclusions are obviously reciprocally dependent, but they are reasonable as the simplest explanation to the observations.

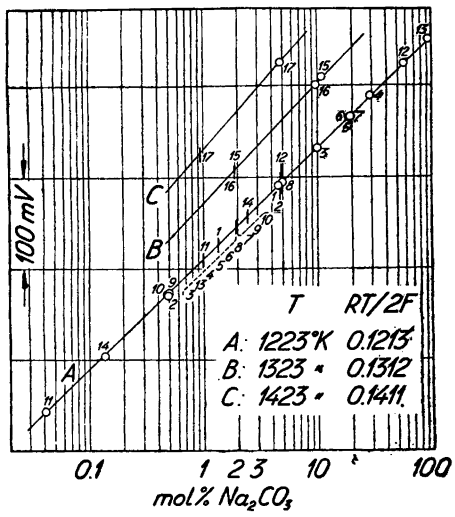


Fig. 3. Logarithmic diagram of EMF between oxygen electrodes as a function of the carbonate contents (in mol %) for concentration cells with the system sodium sulphate — sodium carbonate — carbon dioxide.

The system $K_2CrO_4-K_2Cr_2O_7$

The experiments in the chromate system were of a more preliminary character. Their purpose was to give information about the possibility of using the oxygen electrode in systems with a greater acidity.

The results are given in Table 2. As will be seen from the table, the observed potentials were about 15 % lower than calculated from the theoretical formula

$$E = \frac{RT}{2F} \ln \frac{N^2_{c\,(II)} \cdot N_{d\,(I)}}{N^2_{c\,(I)} \cdot N_{d\,(II)}}$$

Hence the deviation seems to be proportional to the absolute value of E.

As follows from the equilibrium experiments [15] the deviations from ideality in these mixtures are not of such a magnitude as to explain the observed deviation of EMF. At present it is difficult to give any definite explanation of the deviations. One possibility is that they are due to a liquid junction potential. Compared with the sulphate-carbonate system, in which no liquid junction potential was found, two factors have to be considered. The cation K^+ is larger, and therefore has a lower transport number, than Na^+. The oxygen ion may contribute considerably to the conductivity on account of a possible relay mechanism, analogous to the H^+ transport in water.

Another possibility must also be taken into account. The chromate-dichromate mixture might have a partial electronic conductivity (compare its dark metallic colour). This would to some extent short-circuit the cell, thus reducing the EMF.

Table 2. E.M.F. Measurements in concentration cells with the system potassium chromate — potassium dichromate.

Run No.	Start Mol % $K_2Cr_2O_7$		End (analysed) Mol % $K_2Cr_2O_7$		T_{obs} °K	E_{obs} mV	E_{calc} mV		E_{calc}/E_{obs}
	$N_{d\,(I)}$	$N_{d\,(II)}$	$N_{d\,(I)}$	$N_{d\,(II)}$			Start	End	
1	20.6	3.9			1265	101	111		1.10
2	20.8	8.4			1257	52	60		1.15
3	20.8	5.1			1263	84	97		1.15
4	22.4	9.6			1259	53	63		1.19
5	21.8	9.7			1258	51	60		1.18
6	41.3	5.2			1258	173	197		1.14
7	22.8	10.4			1251	48	56		1.17
8	22.1	10.1			1248	51	57		1.12
9	21.8	9.9	21.3	9.7	1248	51	58	56	1.14 (1.10)
10	45.6	10.6	45.9	11.5	1248	115	131	127	1.14 (1.10)

Gas: 98 % O_2 2 % N_2

Note: In the chemical analysis of runs 1—8, the method employed (after addition of $BaCl_2$ direct titration of dichromate with $N/10$ NaOH) was found to give uncertain values, due to occlusion phenomena. As, however, the difference between start- and endpoint compositions seems to be rather unimportant, compared with the relatively large deviations between E_{calc} and E_{obs}, we have laid no stress on reproducing the measurements with correct analysis.

SUMMARY

The investigations deal with the determination of oxygen ion activities in fused salts by means of an oxygen electrode. It is shown that the oxygen ion activity in melts of sodium sulphate-carbonate mixtures in equilibrium with carbon dioxide can be determined very accurately by means of an electrode consisting of platinum in an atmosphere of an oxygen-carbon dioxide-mixture.

In the more acid system, fused chromate — dichromate — mixtures, the measured EMF is about 15 % lower than calculated. Possible reasons for this are discussed.

Our investigations have been carried out with aid of grants from *Statsminister Gunnar Knudsen og hustru Sophie f. Cappelens Familielegat (Borgestad Legat IV)*, for which we wish to express our sincere gratitude.

The experiments were carried out at the laboratories of Institutt for uorganisk kjemi, Norges Tekniske Høgskole. We want to express our gratitude to the director, Professor dr. M. Ræder.

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The Acidic Dissociation Constants of Glucose, Mannitol and Sorbitol, as Measured by Means of the Hydrogen Electrode and the Glass Electrode at 0° and 18° C.

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The subject of the present investigation has been a determination of the acidic strength of glucose, mannitol and sorbitol at 0° C. Simultaneously I took the opportunity to determine also the dissociation constants at 18° C.

The acid qualities of the sugars were first observed by Kullgren [1]

$$ROH = RO^- + H^+ \quad (1)$$

where ROH represents the sugar and RO^- the saccharate ion; in 1898 he was able to show that sucrose diminishes the velocity of the saponification of ethyl acetate, and that consequently sucrose is to a certain extent able to neutralize hydroxyl ions

$$ROH + OH^- = RO^- + H_2O \quad (2)$$

It should be noted that Madsen [2] in 1901 determined the equilibrium constant of reaction (2) for glucose.

In 1912 Michaelis and Rona [3], determining the pH in solutions of sodium hydroxide and alcohols such as glycerol and mannitol, observed that these, too, will neutralize hydroxyl ions, though to a considerably lesser degree; *i. e.* these, too, are acids, though extremely weak ones. For the determination of the value of the dissociation constants were used not only the two methods mentioned above, but also determination of electrical conductivity and freezing point depression [4–6] of solutions of sodium hydroxide and alcohols. These methods may also supply information on the consumption of hydroxyl ions, since the conductivity of OH^- is greater than that of the alcoholate ion, and

since two dissolved molecules by neutralization are converted to one as may be seen from equation (2).

The values of the acidic dissociation constants for glucose, mannitol and sorbitol reported in the literature are, however, diverging to no small extent.

THE PRINCIPLE OF THE CALCULATION

The equilibrium constant of reaction (1) is

$$\frac{a_{RO^-} \cdot a_{H^+}}{a_{ROH}} = K_S$$

which, the activity coefficient of the non-electrolyte ROH being fixed at 1, may be converted to

$$\frac{a_{RO^-} \cdot a_{H^+}}{c_{ROH}} = K''_S$$

Furthermore, in solutions of equal ionic strength the activity coefficient of the monovalent ion RO^- will be approximately equal to that of OH^-, *i. e.*

$$\frac{c_{RO^-} \cdot f_{OH^-} \cdot a_{H^+}}{c_{ROH}} = K'_S$$

Finally, according to (2):

$$c_{ROH} = c_{\text{total alcohol}} - c_{RO^-}$$

$$c_{RO^-} = c_{\text{total sodium hydroxide}} - c_{OH^-}$$

c_{OH^-} being calculated from $a_{H^+} \cdot c_{OH^-} \cdot f_{OH^-} = K_{H_2O}$.

The problem has thus been reduced to that of determining a_{H^+} and f_{OH^-} in solutions of NaOH and ROH of known concentrations.

a_{H^+} was measured electrometrically, by means of the hydrogen electrode as well as by means of the glass electrode. To determine f_{OH^-}, *e. g.* of a solution of the ionic strength 0.05, pa_H was determined electrometrically in a pure 0.05 *M* solution of NaOH. As in such a solution c_{OH^-} is known, f_{OH^-} may be calculated directly from the equation

$$a_{H^+} \cdot c_{OH^-} \cdot f_{OH^-} = K_{H_2O}$$

K_{H_2O} having been fixed at $10^{-14,93}$ and $10^{-14,22}$, respectively.

EXPERIMENTAL

As already mentioned the determinations were carried out by means of a hydrogen electrode as well as a glass electrode. The hydrogen ion activity of the solutions was determined by means of the hydrogen electrode by measuring the electromotive force of the following cells:

I. Hg | HgCl 0.1 *M* KCl | 3.5 *M* KCl | NaOH | H_2Pt

II. Hg | HgCl 0.1 *M* KCl | 3.5 *M* KCl | NaOH + ROH | H_2Pt

The hydrogen electrodes were platinized platinum electrodes, saturated with hydrogen of atmospheric pressure from a hydrogen flask. The hydrogen was bubbled through pyrogallol-potassium hydroxide, through water, and finally through a test solution.

The potentials measured were converted to those at 760 mm hydrogen pressure by adding, the quantity

$$E_{\text{Bar.}} = \frac{RT}{2F \,.\, \log e} \log \frac{760}{B-p}$$

B = barometric height, p = water vapour pressure, $p_{0°C} = 4.6$ mm Hg, $p_{18°C} = 15.5$ mm Hg.

The potentials thus converted to one atmosphere of hydrogen pressure are presented as E_{760}.

The electrode vessels were of the shape indicated in Fig. 1 and were placed in a mixture of crushed ice and water for the experiments carried out at 0°C, and in a water thermostat for those carried out at 18° C.

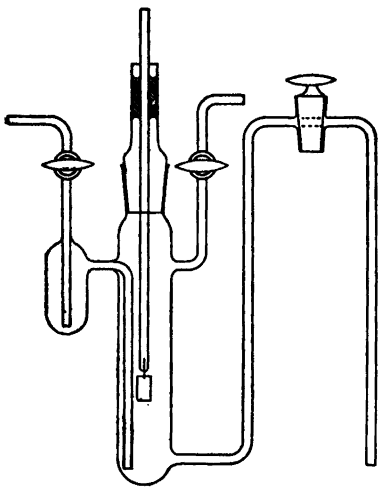


Fig. 1. The shape of electrode vessels.

The reference electrodes used were 0.1 *M* calomel electrodes, made according to the directions of Gjaldbæk [8]. The electrodes were controlled frequently against a 3.5 *M* calomel electrode. During the period of experiments they showed a constant potential of 0.0832 * volt against this electrode at 18° C.

For the liquid junction was used 3.5 *M* potassium chloride. The experimental arrangement as well as the preparation of the interface between the liquids were as stated by Bjerrum and Unmack [7].

The determinations of the potentials were carried out by means of a compensation apparatus from Hartmann and Braun and a galvanometer (N. C. Jensen). The standard cell used was a normal Weston cell.

* J. K. Gjaldbæk indicates for 18°C: 0.08313.

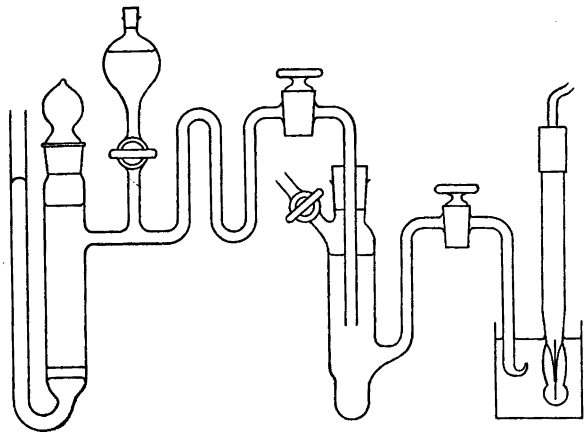


Fig. 2. The arrangement for the measurements by the glass electrode.

By means of the glass electrode the hydrogen ion activity was determined by measuring the electromotive force for the following cells:

I. Hg | HgCl 0.1 *M* KCl | 3.5 *M* KCl | NaOH | glass electrode Tp.E.

II. Hg | HgCl 0.1 *M* KCl | 3.5 *M* KCl | NaOH + ROH | glass electrode Tp.E

The arrangement for the experiments is indicated in Fig. 2, and was placed in an air thermostat at 0° and 18° C. The glass electrode was a Beckmann glass electrode [9] "Type E": a glass electrode made from a special kind of glass not giving the deflection of the potential in basic solution found by ordinary glass electrodes. The determinations of the potentials with the glass electrode were carried out by means of the potentiometer "Radiometer Tp. PHM3h".

The solutions used were prepared by the mixing of adequate amounts of carbonate-free 0.1 *M* NaOH and 0.5 *M* solutions of glucose, mannitol and sorbitol, respectively, and water. Carbon dioxide-free water was used in the preparation of all solutions.

The glucose used was "Dextropur", Corn Products Co. Ltd., Copenhagen. Upon analysis it was found to contain 91.2 % glucose and 8.8 % water, thus corresponding very closely $C_6H_{12}O_6$, H_2O.

The mannitol was *d*-mannitol Pfanstiehl Chemical Co., as well as mannitol The British Drug Houses Ltd. Both products were stated to be pure, and melted at 166—167° C. In the experiments they gave identical results. The melting point of mannitol is indicated to be 166° C.

The sorbitol was *d*-sorbitol Atlas Powder Co.*, stated to be of great purity. The melting point, indicated to be 97.7° C for the stable form, was determined to be 96—97° C.

* The sorbitol used was supplied through the generosity of the Atlas Powder Company, Wilmington 99, Del., U. S. A.

RESULTS

pa_H may be calculated from the potential of a hydrogen electrode against a 0.1 *M* calomel electrode by means of the following equation

$$\frac{(E-E_0)\,F\log e}{RT} = -\log a_{H^+} = pa_H$$

E being E_{760} corrected for the diffusion potential. E_0 is the potential of a normal hydrogen electrode ($a_{H^+} = 1$ and $p_{H_2} = 760$ mm) against a 0.1 *M* calomel electrode. For the calculations were used the values of E_0 given by Bjerrum and Unmack [7]: 0.3351 volts at 0° C. and 0.3360 volts at 18° C.

$\frac{RT}{F\log e} = 0.0542$ at 0° C. and 0.0577 at 18° C.

The results of the measurements in pure solutions of sodium hydroxide are presented in Tables 1 and 2.

In the first column are listed the concentrations of NaOH in moles per liter. E_{760} signifies the mean value of the experimental values of E_{760}. In column 3

Table 1. Hg | HgCl 0.1 mol. KCl | 3.5 mol. KCl | NaOH | H_2Pt at 0° C.

c_{NaOH}	$E_{760\,m}$		E_D	E	E_{Gm}		pa_H	f_{OH^-}
0.05	1.0676	8; 0.0005 1.0672; 1.0688	0.0017	1.0693	0.4233	4; 0.0009 0.4220; 0.4240	13.55	0.83
0.03	1.0563	7; 0.0003 1.0557; 1.0566	0.0012	1.0575	0.4154	2; 0.0009 0.4147; 0.4160	13.33	0.83
0.02	1.0483	10; 0.0009 1.0464; 1.0494	0.0008	1.0491	0.4061	2; 0.0008 0.4055; 0.4066	13.17	0.87
0.015	1.0428	5; 0.0005 1.0434; 1.0422	0.0005	1.0433	0.3996	2; 0.0009 0.3990; 0.4002	13.07	0.91
0.05 *M* glycine 0,05 *M* NaOH	0.9832	2; 0.0006 0.9828; 0.9836	0.0002	0.9834	0.3459	2; 0.0005 0.3454; 0.3464	11.96	
0,03 *M* borate 0.04 *M* NaOH	0.8901	2; 0.0002 0.8899; 0.8902	?	0.8901	0.2530	12; 0.0011 0.2518; 0.2553	10.24	

Table 2. Hg | HgCl 0.1 mol. KCl | 3.5 mol. KCl | NaOH | H_2Pt at 18° C.

c_{NaOH}	$E_{760\,m}$		E_D	E	E_{Gm}		pa_H	f_{OH^-}
0.05	1.0750	10; 0.0004 1.0746; 1.0758	0.0016	1.0766	0.4306	4; 0.0005 0.4311; 0.4300	12.84	0.83
0.03	1.0635	7; 0.0003 1.0632; 1.0639	0.0011	1.0646	0.4192	5; 0.0008 0.4179; 0.4200	12.63	0.85
0.02	1.0541	16; 0.0005 1.0527; 1.0550	0.0008	1.0549	0.4099	8; 0.0006 0.4091; 0.4108	12.46	0.87
0.015	1.0473	7; 0.0002 1.0472; 1.0476	0.0005	1.0478	0.4037	4; 0.0001 0.4036; 0.4038	12.34	0.88
0.05 *M* glycine 0.05 *M* NaOH	0.9905	1; 0.9905;	0.0003	0.9908	0.3460	1; 0.3460;	11.35	
0.03 *M* borate 0.04 *M* NaOH	0.9133	8; 0.0004 0.9130; 0.9142	?	0.9133	0.2700	11; 0.0010 0.2688; 0.2713	10.01	
0.0153 +0.1 *M* KCl	0.9576 + 0.0875	Michaelis, Rona (17°—19° C.)	0.0005	1.0456		$pK_{H_2O} = 14.22$	12.30	0.78
0.0150 +0.1 *M* KCl	0.9554 + 0.0875		0.0005	1.0434			12.26	0.73
0.095	0.7545 + 0.3360	Urban, Shaffer 17° C.	0.0011	1.0916		$pK_{H_2O} = 14.26$	13.13	0.78
0.190	0.7700 + 0.3360		0.0023	1.1083			13.43	0.78

are indicated the number of determinations, the mean error of single determinations, and the highest and the lowest potential measured. E_D signifies the correction for the two diffusion potentials of the cell, calculated from Henderson's formula. The potential: sample solution | 3.5 *M* KCl was calculated in the manner of Bjerrum and Unmack [7] from the expression

$$\frac{RT}{F \log e}\left(-1.81 - \sum \frac{uc}{m} + \sum \frac{vc'}{m'}\right)\left(\frac{\log 302 - \log (\Sigma uc + \Sigma vc')}{302 - (\Sigma uc + \Sigma vc')}\right)$$

Table 3. Hg | HgCl 0.1 mol.KCl | 3.5 mol.KCl | NaOH + glucose | H_2Pt at 0° C.

c_{NaOH}	$c_{glucose}$	$E_{760\ m}$		E_{Gm}		pa_H	pa_H G.	c_{OH^-}	c_{RO^-}	c_{ROH}	$pK'_{glucose}$	
0.05	0.2	1.0014	3; 0.0001 1.0013; 1.0014	0.3634	2; 0.0008 0.3628; 0.3639	12.29	12.34	0.0025	0.0475	0.1525	12.87	
0.03	0.2	0.9872	2; 0.0001 0.9871; 0.9873	0.3505	2; 0.0007 0.3500; 0.3510	12.03	12.09	0.0013	0.0287	0.1713	12.88	
0.02	0.2	0.9769	4; 0.0002 0.9767; 0.9771	0.3389	2; 0.0017 0.3376; 0.3401	11.84	11.87	0.0009	0.0191	0.1809	12.88	12.88
0.015	0.2	0.9703	3; 0.0006 0.9698; 0.9709	0.3319	2; 0.0013 0.3310; 0.3328	11.72	11.74	0.0005	0.0145	0.1855	12.87	
0.05	0.1	1.0257	6; 0.0007 1.0251; 1.0269	0.3853	2; 0.0003 0.3850; 0.3855	12.74	12.76	0.0076	0.0424	0.0576	12.95	
0.03	0.1	1.0085	4; 0.0005 1.0080; 1.0089	0.3691	2; 0.0003 0.3689; 0.3693	12.42	12.45	0.0035	0.0265	0.0735	12.95	
0.02	0.1	0.9963	4; 0.0004 0.9960; 0.9968	0.3573	2; 0.0008 0.3567; 0.3578	12.20	12.22	0.0022	0.0178	0.0822	12.92	12.93
0.015	0.1	0.9884	2; 0.0001 0.9883; 0.9885	0.3495	2; 0.0019 0.3481; 0.3508	12.05	12.07	0.0013	0.0137	0.0863	12.89	
0.05	0.05	1.0474	6; 0.0007 1.0463; 1.0481	0.4050	2; 0.0008 0.4044; 0.4055	13.14	13.14	0.0193	0.0307	0.0193	13.02	
0.03	0.05	1.0274	2; 0.0002 1.0272; 1.0275	0.3873	2; 0.0010 0.3866; 0.3880	12.77	12.80	0.0081	0.0219	0.0281	12.96	
0.02	0.05	1.0151	5; 0.0003 1.0147; 1.0155	0.3749	2; 0.0010 0.3741; 0.3756	12.55	12.57	0.0048	0.0152	0.0348	12.97	12.97
0.015	0.05	1.0056	2; 0.0001 1.0055; 1.0057	0.3660	2; 0.0016 0.3649; 0.3671	12.37	12.39	0.0029	0.0121	0.0379	12.91	

Mean 12.92

Table 4. Hg | HgCl, 0.1 mol.KCl | 3.5 mol.KCl | NaOH + glucose | H_2Pt at 18° C.

NaOH	$c_{glucose}$	$E_{760\ m}$		E_{Gm}		pa_H	pa_H G.	c_{OH^-}	c_{RO^-}	c_{ROH}	pK'-glucose	
).05	0.2	1.0154	5; 0.0008 1.0146; 1.0164	0.3739	2; 0.0001 0.3738; 0.3740	11.77	11.83	0.0040	0.0460	0.1540	12.38	
).03	0.2	1.0013	4; 0.0004 1.0009; 1.0015	0.3586	2; 0.0001 0.3585; 0.3586	11.53	11.56	0.0021	0.0279	0.1721	12.40	12.40
).02	0.2	0.9911	5; 0.0007 0.9900; 0.9918	0.3474	3; 0.0006 0.3470; 0.3479	11.35	11.36	0.0015	0.0185	0.1815	12.40	
).015	0.2	0.9839	3; 0.0002 0.9837; 0.9841	0.3404	2; 0.0000 0.3404	11.23	11.24	0.0011	0.0139	0.1861	12.41	
).05	0.1	1.0375	4; 0.0006 1.0368; 1.0381	0.3930	2; 0.0006 0.3925; 0.3934	12.16	12.17	0.0102	0.0398	0.0602	12.42	
).03	0.1	1.0217	4; 0.0003 1.0214; 1.0220	0.3776	2; 0.0001 0.3775; 0.3777	11.88	11.90	0.0052	0.0248	0.0752	12.44	12.44
).02	0.1	1.0103	3; 0.0001 1.0102; 1.0104	0.3656	2; 0.0001 0.3655; 0.3657	11.69	11.69	0.0034	0.0166	0.0834	12.45	
).015	0.1	1.0023	2; 0.0002 1.0021; 1.0024	0.3575	2; 0.0003 0.3573; 0.3578	11.55	11.54	0.0024	0.0126	0.0874	12.44	
).05	0.05	1.0560	5; 0.0007 1.0553; 1.0569	0.4107	2; 0.0001 0.4106; 0.4107	12.48	12.48	0.0217	0.0283	0.0217	12.45	
).03	0.05	1.0392	6; 0.0007 1.0385; 1.0401	0.3933	3; 0.0005 0.3928; 0.3938	12.19	12.18	0.0107	0.0193	0.0307	12.47	12.46
).02	0.05	1.0264	3; 0.0002 1.0262; 1.0266	0.3821	2; 0.0004 0.3818; 0.3823	11.97	11.98	0.0064	0.0136	0.0364	12.46	
).015	0.05	1.0180	2; 0.0002 1.0179; 1.0181	0.3731	2; 0.0001 0.3730; 0.3732	11.82	11.82	0.0045	0.0105	0.0395	12.46	

Mean 12.43

0.0153 +0.1 *M* KCl	1.0	0.8338 + 0.0875	Michaelis, Rona 17°—19° C.	10.14		0.0000	0.0153	0.9847	12.05
0.0153 +0.1 *M* KCl	0.5	0.8627 + 0.0875		10.64		0.0003	0.0150	0.4850	12.26
0.0153 +0.1 *M* KCl	0.2	0.8868 + 0.0875		11.06		0.0008	0.0145	0.1855	12.28
0.0153 +0.1 *M* KCl	0.1	0.9090 + 0.0875		11.45		0.0021	0.0132	0.0868	12.38
0.095	0.2	0.7019 + 0.3360	Urban, Shaffer 17° C.	12.21		0.0114	0.0836	0.1164	12.47
0.190	0.2	0.7358 + 0.3360		12.80		0.0449	0.1451	0.0549	12.49

where c is the number of gram-equivalents of a cation, m its valency, u its equivalent conductivity. c', m', and v, are the corresponding quantities for an anion. The equivalent conductivities needed were obtained from Landolt Börnstein: *Physikalisch Chemische Tabellen*, 1105, 5. Auflage (1923).

E_{Gm} is the mean value of the potential as measured by means of the glass electrode. Column 7 is analogous to column 3. pa_H in column 8 was calculated from the measurements with the hydrogen electrode. In the last column are listed the f_{OH^-} values. In the tables are further presented measurements with hydrogen electrode and glass electrode in a glycine buffer and a borate buffer. All of the pa_H-values listed in tables 1 and 2 were used for adjusting the glass electrode (Fig. 3)

In Tables 3—8 are given the results of the measurements in solutions of NaOH + ROH.

In columns 1 and 2 are listed the concentrations of NaOH and ROH, respectively, in the initial solutions. Columns 3—6 are analogous to the corresponding columns of the Tables 1 and 2.

The pa_H-values of column 7 originate from the hydrogen electrode measurements and have been calculated directly from E_{760}, a possible diffusion potential having been discarded. To ascertain the order of magnitude of the

Table 5. Hg | HgCl 0.1 mol.KCl | 3.5 mol.KCl | NaOH + mannitol | H_2Pt at 0° C.

c_{NaOH}	c_{mannit}	$E_{760\,m}$		E_{Gm}		pa_H	pa_H G.	c_{OH^-}	c_{RO^-}	c_{ROH}	$pK'_{mannitol}$
0.05	0.2	1.0523	8; 0.0006 1.0514; 1.0531	0.4091	3; 0.0008 0.4085; 0.4101	13.23	13.25	0.0239	0.0261	0.1739	14.13
0.02	0.2	1.0308	6; 0.0005 1.0302; 1.0316	0.3905	3; 0.0130 0.3880; 0.3905	12.84	12.86	0.0093	0.0107	0.1893	14.15
0.015	0.2	1.0238	6; 0.0006 1.0229; 1.0244	0.3829	3; 0.0011 0.3817; 0.3840	12.71	12.72	0.0064	0.0086	0.1914	14.10
0.05	0.1	1.0584	6; 0.0006 1.0579; 1.0595	0.4158	3; 0.0009 0.4149; 0.4166	13.35	13.35	0.0314	0.0186	0.0814	14.07
0.02	0.1	1.0385	8; 0.0009 1.0373; 1.0393	0.3966	3; 0.0009 0.3960; 0.3976	12.98	12.98	0.0129	0.0071	0.0929	14.16
0.015	0.1	1.0323	7; 0.0011 1.0304; 1.0340	0.3899	3; 0.0010 0.3888; 0.3907	12.87	12.86	0.0094	0.0056	0.0944	14.13
0.05	0.05	1.0626	6; 0.0005 1.0620; 1.0633	0.4197	3; 0.0010 0.4191; 0.4209	13.42	13.43	0.0370	0.0130	0.0370	13.96
0.02	0.05	1.0425	8; 0.0011 1.0410; 1.0437	0.4011	3; 0.0006 0.4005; 0.4017	13.05	13.06	0.0152	0.0048	0.0452	14.09
0.015	0.05	1.0365	7; 0.0007 1.0355; 1.0374	0.3948	3; 0.0012 0.3935; 0.3955	12.94	12.95	0.0110	0.0040	0.0460	14.04

Mean 14.09

error thus committed, the potentials were determined for some of the solutions with 3.5 *M* KCl, and subsequently with 1.75 *M* KCl, as liquid junction. Provided the diffusion potential against 1.75 *M* KCl is twice the one against 3.5 *M* KCl, we have, according to Bjerrum [10]:

$$E_{corr} = E_{3,5} - \Delta E$$

$$\Delta E = E_{1,75} - E_{3,5}$$

Table 6. Hg | HgCl 0.1 mol.KCl | 3.5 mol.KCl | NaOH + mannitol | H_2Pt at 18° C.

c_{NaOH}	c_{mannit}	$E_{760\,m}$		E_{Gm}		pa_H	$pa_HG.$	c_{OH^-}	c_{RO^-}	c_{ROH}	$pK'_{mannitol}$
0.05	0.2	1.0608	2; 0.0001 1.0607; 1.0609	0.4162	2; 0.0011 0.4154; 0.4169	12.56	12.58	0.0261	0.0239	0.1761	13.51
0.02	0.2	1.0388	2; 0.0000 1.0388; 1.0388	0.3948	2; 0.0011 0.3940; 0.3956	12.18	12.20	0.0105	0.0095	0.1905	13.54
0.015	0.2	1.0316	2; 0.0001 1.0315; 1.0316	0.3881	2; 0.0010 0.3874; 0.3888	12.06	12.09	0.0078	0.0072	0.1928	13.55
0.05	0.1	1.0673	2; 0.0000 1.0673; 1.0673	0.4224	2; 0.0014 0.4214; 0.4233	12.67	12.70	0.0337	0.0163	0.0837	13.46
0.02	0.1	1.0455	2; 0.0001 1.0454; 1.0455	0.4017	2; 0.0010 0.4010; 0.4024	12.30	12.31	0.0138	0.0062	0.0938	13.54
0.015	0.1	1.0386	2; 0.0002 1.0384; 1.0387	0.3949	2; 0.0013 0.3940; 0.3958	12.18	12.21	0.0103	0.0047	0.0953	13.55
0.05	0.05	1.0707	2; 0.0004 1.0704; 1.0709	0.4260	2; 0.0014 0.4250; 0.4270	12.73	12.76	0.0388	0.0112	0.0388	13.35
0.02	0.05	1.0496	2; 0.0001 1.0495; 1.0497	0.4057	2; 0.0011 0.4049; 0.4065	12.37	12.39	0.0162	0.0038	0.0462	13.52
0.015	0.05	1.0425	2; 0.0001 1.0424; 1.0426	0.3989	2; 0.0016 0.3978; 0.4000	12.24	12.28	0.0119	0.0031	0.0469	13.48

Mean 13.50

0.015 +0.1 *M* KCl	0.2	0.9400 + 0.0875	Michaelis, Rona 17°–19° C.			11.94		0.0071	0.0079	0.1921	13.46
0.015 +0.1 *M* KCl	0.1	0.9470 + 0.0875				12.06		0.0094	0.0056	0.0944	13.42
0.015 +0.1 *M* KCl	0.05	0.9505 + 0.0875				12.12		0.0108	0.0042	0.0458	13.29

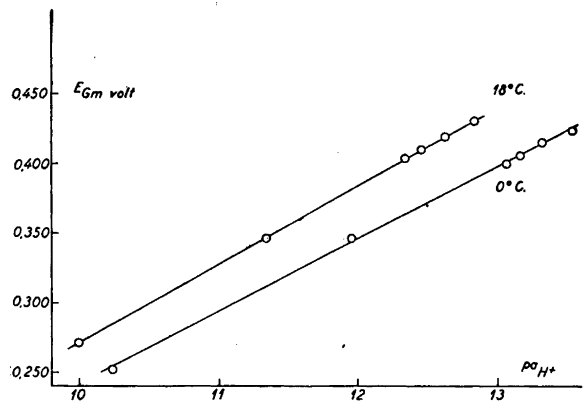


Fig. 3. Plot of the electromotive force as measured by the glass electrode in pure solutions of sodium hydroxide and buffer solutions against pa_H.

Table 7. Hg | HgCl 0.1 mol. KCl | 3.5 mol. KCl | NaOH + sorbitol | H_2Pt at 0° C.

c_{NaOH}	c_{sorbit}	$E_{760\,m}$		E_{Gm}		pa_H	pa_H G.	c_{OH^-}	c_{RO^-}	c_{ROH}	$pK'_{sorbitol}$
0.05	0.2	1.0539	4; 0.0005 1.0534; 1.0544	0.4126	2; 0.0006 0.4122; 0.4130	13.26	13.29	0.0255	0.0245	0.1755	14.19
0.02	0.2	1.0320	4; 0.0009 1.0311; 1.0328	0.3924	2; 0.0002 0.3922; 0.3925	12.86	12.90	0.0098	0.0102	0.1898	14.19
0.05	0.1	1.0597	4; 0.0005 1.0592; 1.0603	0.4190	2; 0.0001 0.4189; 0.4190	13.37	13.42	0.0329	0.0171	0.0829	14.14
0.02	0.1	1.0387	4; 0.0002 1.0384; 1.0389	0.3989	2; 0.0008 0.3983; 0.3994	12.98	13.03	0.0129	0.0071	0.0929	14.16
0.05	0.05	1.0629	4; 0.0005 1.0625; 1.0636	0.4224	2; 0.0004 0.4221; 0.4226	13.43	13.48	0.0378	0.0122	0.0378	14.01
0.02	0.05	1.0433	4; 0.0004 1.0428; 1.0439	0.4029	2; 0.0006 0.4025; 0.4033	13.07	13.10	0.0159	0.0041	0.0459	14.17

Mean 14.14

Table 8. Hg | HgCl 0.1 mol. KCl | 3.5 mol. KCl | NaOH + sorbitol | H_2Pt at 18° C.

c_{NaOH}	c_{sorbit}	$E_{760\,m}$		E_{Gm}		pa_H	pa_H G.	c_{OH^-}	c_{RO^-}	c_{ROH}	$pK'_{sorbitol}$
0.05	0.2	1.0618	2; 0.0005 1.0614; 1.0621	0.4187	2; 0.0001 0.4186; 0.4188	12.58	12.63	0.0273	0.0227	0.1773	13.56
0.02	0.2	1.0408	2; 0.0001 1.0407; 1.0408	0.3968	2; 0.0006 0.3964; 0.3972	12.21	12.24	0.0113	0.0087	0.1913	13.62
0.05	0.1	1.0684	3; 0.0003 1.0683; 1.0685	0.4246	3; 0.0003 0.4244; 0.4248	12.69	12.73	0.0353	0.0147	0.0853	13.53
0.02	0.1	1.0470	2; 0.0001 1.0469; 1.0470	0.4029	2; 0.0008 0.4023; 0.4035	12.32	12.35	0.0145	0.0055	0.0945	13.62
0.05	0.05	1.0718	2; 0.0001 1.0717; 1.0718	0.4274	2; 0.0006 0.4270; 0.4278	12.75	12.79	0.0406	0.0094	0.0406	13.47
0.02	0.05	1.0504	2; 0.0002 1.0502; 1.0505	0.4067	2; 0.0006 0.4063; 0.4071	12.38	12.41	0.0167	0.0033	0.0467	13.59

Mean 13.57

0.0153 +0.1 *M* KCl	0.2	0.9430 + 0.0875	Michaelis, Rona 17°–19° C.			12.03		0.0082	0.0071	0.1929	13.58
0.0153 +0.1 *M* KCl	0.1	0.9495 + 0.0875				12.15		0.0108	0.0045	0.0955	13.59

where $E_{1.75}$ and $E_{3.5}$ are equal to E_{760m} measured with 1.75 *M* KCl and, respectively, 3.5 *M* KCl as liquid junction. The correction obtained in this way appeared to be of the same order of magnitude as was the correction calculated from Henderson's formula into which was introduced the activity of free hydroxyl ions as calculated from the uncorrected potential E_{760m}.

Concerning the experiments with the glucose, the corrections were insignificant owing to the neutralization of the greater part of the sodium hydroxide.

As for the experiments with mannitol and sorbitol the corrections were somewhat greater, amounting to about 0.0005 volts, but could still be discarded provided the accuracy desired did not exceed 0.001 volts on each potential.

The pa_H-values of column 8 originate from the glass electrode measurements, and were read from Fig. 3 on the basis of the E_{Gm} values of column 5. In column 9 are given the concentrations of free hydroxyl ions, c_{OH^-}. In column 10 is listed c_{RO^-}, and in column 11 c_{ROH}. In the last column is listed pK', calculated from the mass action expression.

By comparison of the pa_H-values of the columns 7 and 8 it is found that they agree well in most cases; in a few cases they differ as much as 0.06 units of pa_H, but the deviations may be ascribed to experimental errors. From the data it may be estimated that the hydrogen of the hydrogen electrode has exhibited no reducing effect on either aldehyde or alcohol-groups.

The following mean values were calculated from the pK'-values:

	0° C.	18° C.
Glucose	12.92	12.43
Mannitol	14.09	13.50
Sorbitol	14.14	13.57

It should be noted, however, that a slight increase of the pK'-values occurs with increasing dilution of the solutions, particularly in the case of glucose.

COMPARISON WITH PREVIOUS RESULTS

In some of the earlier works on the determination on K_s on the basis of hydrogen electrode measurements of the hydrogen ion concentration in solutions of sodium hydroxide and, respectively, glucose, mannitol, and sorbitol, sufficient data are reported to allow for a recalculation of pK' according to the method employed by me. This applies to the works of Michaelis and Rona [3], and of Urban and Schaffer [11], both of which were carried out at about 18° C.

Michaelis and Rona in their summary state the pK of glucose, mannitol and sorbitol, as 12.18, 13.47 and 13.62, respectively. Urban and Schaffer indicate for glucose pK 12.34. The recalculated values, too, are listed in the tables 3 to 8 for comparison with the values found in the present investigation.

SUMMARY

The acidic dissociation constants for glucose, mannitol and sorbitol at 0° and 18° C. were determined by measuring the pa_H in solutions of these substances added NaOH. The measurements were carried out by means of the

hydrogen electrode as well as the glass electrode, the two kinds of electrodes giving practically identical results.

The following mean values were calculated for the pK'-values:

	0° C.	18° C.
Glucose	12.92	12.43
Mannitol	14.09	13.50
Sorbitol	14.14	13.57

It should be noted, however, that a slight increase of the pK'-values occurs with increasing dilution of the solutions, particularly in the case of glucose.

The experimental material, on the basis of which Michaelis and Rona, and Urban and Schaffer, calculated their pK-values at about 18° C for glucose, mannitol and sorbitol, was used by me for the calculation of pK' according to the method employed in the present work. The recalculated values and those of my own agree reasonably well.

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The Uncatalysed and the Metal-Ion Catalysed Decarboxylation of Oxaloacetic Acid

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The decomposition of oxaloacetic acid in aqueous solution into pyruvic acid and carbon dioxide is an example of the ketonic decomposition exhibited by β-keto carboxylic acids in general. From a kinetic study of the decarboxylation of α,α-dimethyl acetoacetic acid [1], the author of the present paper concluded that, in the decomposition of β-keto carboxylic acids, the keto-form of the acid (or its anion) decomposes spontaneously into carbon dioxide and an enol (or enolate ion) which, by a consecutive reaction, is transformed into the stable ketone. In the decarboxylation of oxaloacetic acid, we should therefore expect that the keto-form of the acid (or its uni- or divalent anion) decomposes spontaneously into carbon dioxide and an enol-form of pyruvic acid (or a corresponding anion) which, by a consecutive reaction, is transformed into ordinary pyruvic acid:

$$HOOC-CH_2-CO-COOH \rightarrow CO_2 + CH_2=COH-COOH \quad (1)$$

$$CH_2=COH-COOH \rightarrow CH_3-CO-COOH \quad (2)$$

Steinberger and Westheimer [2] have confirmed this mechanism for the decarboxylation of α,α-dimethyl oxaloacetic acid.

The decarboxylation of oxaloacetic acid has been studied by biochemists [3-10] who have found that the rate of the spontaneous reaction depends upon the pH of the solution and has a maximum when pH is about 4, and that the reaction is catalysed (1) by enzymes, (2) by primary amines (like the decarboxylation of all other β-keto carboxylic acids), and (3) by certain metal ions (unlike the decomposition of β-keto monocarboxylic acids, but like that of some di- and tri-carboxylic acids, e.g. acetonedicarboxylic acid and oxalosuccinic acid).

In the present paper, the decarboxylation of oxaloacetic acid has been studied from a purely physico-chemical point of view. It was the object of the work to find out which molecular species (undissociated acid, uni- and divalent

oxaloacetate ion, and metal complexes of these ions) decompose spontaneously, and to give a quantitative interpretation of the rates from velocity constants for each of the unstable molecules. For that purpose, the uncatalysed reaction was examined in solutions of potassium and hydrogen chloride and in acetate buffers. The metal-ion catalysis was studied in solutions of potassium and hydrogen chloride containing in addition zinc chloride or cupric nitrate. In order to interpret the results in a satisfactory way, it was desirable to have better data for the dissociation constants of oxaloacetic and pyruvic acid than those already available. The dissociation constants of the two acids were therefore measured by means of the glass electrode. The results of these measurements have recently been published [11]. A preliminary report of some of the results of the kinetic study and the conclusions drawn from them has already been given [12].

Materials and experimental method. Oxaloacetic acid was prepared and purified in the same way as in the paper on the electrometric determination of the dissociation constants [11]. The molecular weight found by alkalimetry varied from 132.1 to 132.5 (calc. 132.1). The other chemicals were of analytical purity. The water, hydrochloric acid, and acetic acid were redistilled in an all-glass apparatus. Sodium acetate and cupric nitrate were recrystallised. The kinetic measurements were carried out at the temperature 37.0° C. The reaction was followed by observing the pressure above the solution by means of an apparatus described earlier [13]. Since P, the difference between the final pressure reading and the reading at the time t minutes after the start, is proportional to the concentration x of oxaloacetic acid present in the solution at the time t, we may write $P = \varphi\, x$. The constant φ may be calculated by means of the equation $P_o = \varphi\, x_o$, where P_o and x_o are the initial values of P and x.

UNCATALYSED DECOMPOSITION IN SOLUTIONS OF HYDROGEN AND POTASSIUM CHLORIDE

Three series of measurements were carried out. Within each series the total concentration s of hydrogen and potassium chloride was kept constant at 0.050, 0.100, and 0.200 molar, respectively, while the concentration h of hydrogen chloride was varied between s and zero. The oxaloacetic acid is in these solutions present partly in the form of undissociated acid (H_2Ox) and partly as univalent ion (HOx^-) and divalent ion (Ox^{--}). The velocity of decarboxylation may be written as follows

$$-\frac{dx}{dt} = k_o[H_2Ox] + k_1[HOx^-] + k_2[Ox^{--}]$$
$$= [k_o + (k_1 - k_o)\alpha_1 + (k_2 - k_o)\alpha_2]x$$

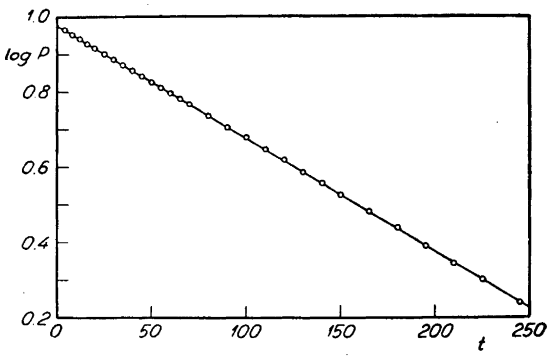


Fig. 1. Decomposition of oxaloacetic acid in 0.05 molar potassium chloride at 37.0° C. The diagram shows that the reaction obeys the first-order law.

where k_o, k_1, and k_2 are velocity constants for the spontaneous decarboxylation of the molecules H_2Ox, HOx^-, and Ox^{--}, while α_1 and α_2 are degrees of dissociation into the ions HOx^- and Ox^{--}, respectively. If we presuppose that the velocity constants k_o, k_1, and k_2 are not equal, the reaction will be of first order only if α_2 and α_1, and consequently also the hydrogen ion concentration, are constant during the reaction. In that case, the first-order constant for the total reaction will be

$$k = k_o + (k_1 - k_o)\alpha_1 + (k_2 - k_o)\alpha_2 \tag{3}$$

The measurements showed that the reaction followed the first-order law for all the solutions listed in Table 1. In order to demonstrate this we take as an example one of the solutions where the greatest deviation from the first-order law might be expected, namely 0.050 molar potassium chloride without hydrogen chloride. In Fig. 1, log$P$ for the reaction in this solution has been plotted against t. The first-order law is obeyed since all the points fall close to a straight line. The numerical value of the slope is $k^* = 0.4343\ k$.

In Table 1 are given the composition of the solutions examined, their ionic strengths I, and the degrees of dissociation of oxaloacetic acid calculated by means of the dissociation constants. The subscripts o and ∞ denote that the values are, respectively, initial and final (that is, corresponding to a solution x_o molar with respect to the *keto*-form of pyruvic acid). In calculating the dissociation constants of oxaloacetic acid (K_1 and K_2) and pyruvic acid (K_{HPy}), the following formulae have been used:

$$-\log K_1 = 2.450 - 1.038\sqrt{I} + 0.86\ I \tag{4}$$

$$-\log K_2 = 4.359 - 2.076\sqrt{I} + 1.61\ I \tag{5}$$

$$-\log K_{HPy} = 2.420 - 1.038\sqrt{I} + 0.92\ I \tag{6}$$

The formulae have been found to hold [11] at the temperature 37° C for sodium chloride solutions, but it has been shown that it makes very little difference whether the solutions contain sodium or potassium chloride. It is seen from Table 1 that the initial and final values of α_1 are practically the same. The magnitude of α_1 during the reaction cannot be calculated since both the dissociation constant and the life-time of the enol-form of pyruvic acid are unknown,

Table 1. Decomposition of oxaloacetic acid (initial concentration x_0 molar) at 37.0° C in the solvent: h molar hydrogen chloride, (s−h) molar potassium chloride.

x_0	h	I	a_{10}	$a_{1\infty}$	a_2	k^*10^3	k^*10^3 calc.
Series 1 (s = 0.050):							
0.01514	0.0499	0.0515	0.0969	0.0968	0.0002	0.764	0.765
0.01514	0.03985	0.0518	0.1170	0.1168	0.0003	0.900	0.893
0.01533	0.02496	0.0526	0.1671	0.1666	0.0006	1.206	1.213
0.01515	0.00996	0.0543	0.2799	0.2782	0.0021	1.926	1.932
0.01514	0.00498	0.0554	0.3486	0.3466	0.0037	2.370	2.371
0.01508	0.00000	0.0570	0.4446	0.4432	0.0071	2.999	2.992
Series 2 (s = 0.100):							
0.01508	0.0998	0.1009	0.0581	0.0581	0.0001	0.523	0.521
0.01515	0.0499	0.1016	0.1077	0.1076	0.0003	0.845	0.839
0.01521	0.03985	0.1020	0.1294	0.1292	0.0004	0.969	0.978
0.01510	0.01994	0.1033	0.2118	0.2112	0.0012	1.487	1.506
0.01508	0.00498	0.1058	0.3681	0.3671	0.0048	2.517	2.513
0.01506	0.00000	0.1073	0.4612	0.4610	0.0089	3.133	3.120
Series 3 (s = 0.200):							
0.01518	0.1995	0.2005	0.0336	0.0336	0.0000	0.374	0.369
0.01514	0.1493	0.2007	0.0443	0.0443	0.0001	0.443	0.437
0.01518	0.0998	0.2010	0.0646	0.0646	0.0001	0.568	0.568
0.01512	0.0664	0.2014	0.0932	0.0932	0.0002	0.747	0.752
0.01512	0.0499	0.2018	0.1188	0.1187	0.0004	0.914	0.916
0.01537	0.03316	0.2026	0.1633	0.1630	0.0008	1.182	1.202
0.01520	0.02492	0.2031	0.1992	0.1989	0.0012	1.413	1.434
0.01515	0.01246	0.2045	0.2905	0.2900	0.0031	2.027	2.022
0.01534	0.00499	0.2062	0.3844	0.3844	0.0061	2.676	2.632
0.01500	0.00249	0.2068	0.4287	0.4295	0.0082	2.942	2.923
0.01525	0.00000	0.2077	0.4744	0.4764	0.0110	3.215	3.226
0.00762	0.00000	0.2049	0.5819	0.5875	0.0214	3.807	3.946
0.01223	0.00000	0.2067	0.5088	0.5117	0.0136	3.406	3.454
0.02041	0.00000	0.2093	0.4305	0.4316	0.0083	3.012	2.935
0.03015	0.02490	0.2057	0.1857	0.1851	0.0011	1.401	1.345
0.03015	0.00000	0.2118	0.3742	0.3739	0.0057	2.662	2.565

but from the fact that the first-order law holds we conclude that α_1 is practically the same all the time. It may be added that it has been found [11] that solutions of oxaloacetic acid which had been left for 24 hours at 37° C to complete decarboxylation, when measured with the glass electrode, gave the same e.m.f. (average deviation 0.0002 volts) as solutions of the same composition prepared directly from pyruvic acid. As seen from Table 1, α_2 is, for all the solutions examined, very small compared with α_1. Its variation during the reaction is therefore negligible.

The velocity constants k^* found from the measurements are given in the next to the last column of Table 1. In order to test equation 3 we calculate the last and very small term of this equation from $k_2^* = 1.8 \times 10^{-3}$ found in the next part of this paper and a preliminary value of k_0^*. We then plot $k^* - (k_2^* - k_0^*)\alpha_2$ against α_1 for all the solutions within each of the three series. The points fall for each series close to a straight line from which the constants k_0^* and $(k_1^* - k_0^*)$ are determined. The values of k_0^* and k_1^* found in this way are given in Table 2. It is seen that both velocity constants are nearly inde-

Table 2. Velocity constants for the spontaneous decomposition of undissociated oxaloacetic acid (k_0^*) and the univalent oxaloacetate ion (k_1^*) at the temperature 37.0° C.

Series	s	$k_0^* 10^3$	$k_1^* 10^3$
1	0.050	0.147	6.53
2	0.100	0.148	6.56
3	0.200	0.153	6.57

pendent of the salt concentration, and that the univalent ion decomposes about 44 times as fast as the undissociated acid. When the values of k_0^* and k_1^* given in Table 2 and $k_2^* = 1.8 \times 10^{-3}$ are introduced into equation 3 we calculate the values of k^* presented in the last column of Table 1. The agreement with k^* found directly from the measurements is good when x_0 is about 0.015. For the last five solutions in the table, where x_0 is either smaller or greater than 0.015, the difference between the calculated and measured values of k^* is somewhat greater than that corresponding to the usual experimental error.

UNCATALYSED DECOMPOSITION IN ACETATE BUFFER SOLUTIONS

Experiments were carried out in solutions of acetic acid (a molar) and sodium acetate (b molar). In three series of measurements, b was nearly 0.1, 0.2, and 0.3, respectively, while a within each series varied from about 0.1 to a little more than 0.6. In these solutions, the oxaloacetic acid is nearly completely dissociated into uni- and divalent ions; only from 0 to 2 % is undisso-

ciated. Since, further, k_o is much smaller than k_1 and k_2, the contribution of the undissociated acid to the total rate of decomposition is negligible. We may therefore express the velocity as follows

$$-\frac{dx}{dt} = (k_1\alpha_1 + k_2\alpha_2)\ x \tag{7}$$

The reaction does not obey the first-order law since α_1 and α_2 vary during the decomposition. We shall attempt to compute the constant

$$k_\infty = k_1\alpha_{1\infty} + k_2\alpha_{2\infty} \tag{8}$$

from the measurements.

The degrees of dissociation α_1 and α_2 may be calculated when the dissociation constants of acetic acid (K_{HAc}), oxaloacetic acid, and pyruvic acid for the respective solutions are known. It is, however, sufficient to know the ratio

Table 3. Dissociation constants of acetic acid (K_{HAc}), pyruvic acid (K_{HPy}), and oxaloacetic acid (K_1 and K_2) at 37° C in s molar sodium chloride.

s	$K_{HAc}10^5$	$K_{HPy}10^3$	$K_1 10^3$	$K_2 10^4$	K_{HAc}/K_2
0.1	2.79	6.55	6.19	1.37	0.204
0.2	3.06	7.24	6.95	1.77	0.173
0.3	3.20	7.45	7.24	1.97	0.162

Table 4. Decomposition of oxaloacetic acid (initial concentration x_0 molar) at 37° C in the solvent: a molar acetic acid, b molar sodium acetate.

x_0	a	b	a_{10}	$a_{1\infty}$	a_{20}	$a_{2\infty}$	$k_\infty{}^*10^3$
Series 1:							
0.01526	0.6257	0.0996	0.609	0.594	0.368	0.386	4.745
0.01503	0.4173	0.0997	0.526	0.503	0.461	0.486	4.170
0.01503	0.2084	0.0997	0.382	0.348	0.612	0.648	3.407
0.01510	0.1042	0.0997	0.264	0.222	0.734	0.776	2.870
0.02004	0.1042	0.0997	0.298	0.240	0.699	0.758	2.892
0.01015	0.1042	0.0997	0.232	0.206	0.766	0.793	2.824
Series 2:							
0.01522	0.6263	0.1994	0.389	0.374	0.605	0.620	3.794
0.01503	0.4171	0.1994	0.304	0.288	0.692	0.710	3.285
0.01503	0.2086	0.1994	0.191	0.173	0.808	0.826	2.725
0.01501	0.1042	0.1992	0.118	0.101	0.882	0.899	2.331
Series 3:							
0.01501	0.6257	0.2991	0.278	0.267	0.720	0.731	3.329
0.01518	0.3128	0.2991	0.169	0.157	0.831	0.842	2.690
0.01526	0.1042	0.2991	0.074	0.064	0.926	0.936	2.210

K_{HAc}/K_2 accurately; the other constants are of minor importance in the calculation. Since the constants are not known accurately enough for a complete analysis of the results, we shall use them only where no great accuracy is essential. The initial and final values of α_1 and α_2 given in Table 4 have been computed from the dissociation constants presented in Table 3. The constants listed pertain to sodium chloride solutions with the same sodium ion concentration as the acetate buffers. K_1, K_2, and K_{HPy} have been calculated from formulae 4—6, while K_{HAc} has been estimated on the basis of the determinations of Harned and Hickey[14].

By means of equation 8, equation 7 may be written as follows

$$-\frac{dx}{dt} = (k_\infty + k_1(\alpha_1 - \alpha_{1\infty}) - k_2(\alpha_{2\infty} - \alpha_2))\,x \tag{9}$$

We introduce the approximation that α_1 and α_2 vary linearly with x:

$$\alpha_1 - \alpha_{1\infty} = \beta_1 x \quad \text{and} \quad \alpha_{2\infty} - \alpha_2 = \beta_2 x$$

where the constants β_1 and β_2 may be calculated from the equations

$$\alpha_{10} - \alpha_{1\infty} = \beta_1 x_0 \quad \text{and} \quad \alpha_{2\infty} - \alpha_{20} = \beta_2 x_0$$

When we further use the abbreviation

$$\gamma = (\beta_1 k_1 - \beta_2 k_2)/k_\infty \varphi \tag{10}$$

equation 9 may be written as follows

$$-\frac{dx}{dt} = k_\infty (1 + \varphi\gamma x)\,x$$

By integration and change to decadic logarithms is obtained

$$-k_\infty^* t = \log x - \log(1 + \varphi\gamma x) + \text{const.}$$

If we introduce $P = \varphi x$, we obtain

$$-k_\infty^* t = \log P - \log(1 + \gamma P) + \text{const.} \tag{11}$$

In the calculation of γ by means of equation 10, we use k_1^* found in the previous part of this paper and preliminary values of k_2^* and k_∞^*. The constant

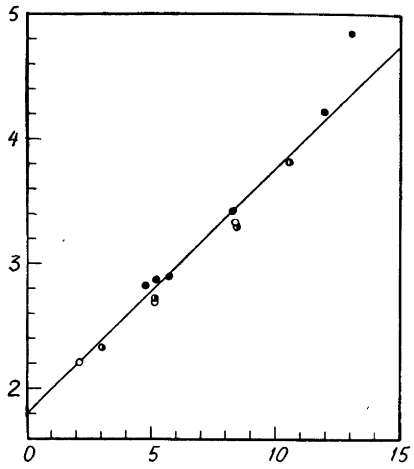


Fig. 2. Decomposition in acetate buffer solutions. Abscissa: X defined by equation 14; ordinate: $k^*_\infty/(a_{1\infty} + a_{2\infty})$. Filled circles refer to series 1, half-filled circles to series 2, and open circles to series 3.

k^*_∞ is finally found graphically by means of equation 11. The results are given in the last column of Table 4.

We shall now test equation 8 and evaluate $k_2{}^*$. We assume that the ratio K_{HAc}/K_2 is constant within each of the three series of measurements, but make no assumption of its exact numerical value. When we remember that

$$\frac{K_{\mathrm{HAc}}}{K_2} = \frac{[\mathrm{Ac}^-]_\infty}{[\mathrm{HAc}]_\infty}\,\frac{a_{1\infty}}{a_{2\infty}} \tag{12}$$

we may show that equation 8 is identical with the following equation

$$\frac{k^*_\infty}{a_{1\infty} + a_{2\infty}} = k_2{}^* + \frac{K_{\mathrm{HAc}}}{K_2}\,X \tag{13}$$

where

$$X = \frac{[\mathrm{HAc}]_\infty}{[\mathrm{Ac}^-]_\infty}\left(k_1{}^* - \frac{k^*_\infty}{a_{1\infty} + a_{2\infty}}\right) \tag{14}$$

According to equation 13, a plot of $k^*_\infty/(a_{1\infty} + a_{2\infty})$ against X should, for each series, give a straight line from which $k_2{}^*$ may be obtained by extrapolation to $X = 0$ while the slope is equal to K_{HAc}/K_2. In the calculation of $k^*_\infty/(a_{1\infty} + a_{2\infty})$, it is accurate enough to use the values of $a_{1\infty}$ and $a_{2\infty}$ given in Table 4 since their sum is nearly unity. The plot is shown in Fig. 2 where the filled circles represent series 1, the half-filled circles series 2, and the open circles series 3. All the points except one (corresponding to the first solution

in Table 4) fall rather close to the straight line drawn, from which we obtain by extrapolation $k_2^* = 1.80 \times 10^{-3}$. The slope of the straight line is $K_{HAc}/K_2 = 0.197$. The points for series 1 alone would give a little more, those for series 2 and 3 a little less than this value. There is a moderately good agreement between the values of the ratio determined from kinetic measurements on acetate buffers and those estimated for sodium chloride solutions and presented in Table 3.

CATALYSIS BY ZINC IONS

Experiments were carried out in solutions of hydrogen chloride, potassium chloride, and zinc chloride (c molar). The total ionic strength was always 0.200. The reaction followed the first-order law. The composition of the solutions examined and the velocity constants k^* found are given in Table 5. The hydrogen ion concentrations and degrees of dissociation α_1 and α_2 have been computed by means of dissociation constants calculated from equations 4—6 ($I = 0.200$). The kinetic results are shown graphically in Fig. 3. It is seen that there is a linear relation between the velocity constant and the concentration of zinc chloride when the hydrogen ion concentration is constant, and that the effect of the zinc ions increases rapidly with decreasing hydrogen ion concentration. The zinc ion catalysis may be explained quantitatively

Table 5. Decomposition of oxaloacetic acid (initial concentration x_0 molar) at 37° C in the solvent: h molar hydrogen chloride, c molar zinc chloride, $(0.200 - h - 3c)$ molar potassium chloride.

x_0	h	$c10^3$	$[H^+]$	α_1	α_2	k^*10^3	k^*10^3 calc.
0.01506	0.1798	3.32	0.1804	0.0371	0.0000	0.415	0.414
0.01514	0.1798	6.64	0.1804	0.0371	0.0000	0.445	0.436
0.01523	0.1498	16.61	0.1505	0.0441	0.0001	0.592	0.588
0.01521	0.0998	6.64	0.1008	0.0645	0.0001	0.686	0.686
0.01514	0.0998	13.27	0.1008	0.0645	0.0001	0.799	0.805
0.01514	0.0999	19.96	0.1008	0.0645	0.0001	0.921	0.925
0.01519	0.0999	26.58	0.1008	0.0645	0.0001	1.026	1.044
0.01509	0.0500	6.64	0.0518	0.1183	0.0004	1.282	1.291
0.01517	0.0500	13.27	0.0518	0.1182	0.0004	1.676	1.669
0.01523	0.02500	6.63	0.02809	0.1984	0.0012	2.541	2.529
0.01502	0.02500	13.27	0.02805	0.1986	0.0012	3.630	3.635
0.01525	0.00998	6.64	0.01493	0.3164	0.0037	5.38	5.38
0.01504	0.00999	13.27	0.01489	0.3171	0.0037	8.47	8.59
0.01512	0.00499	1.361	0.01099	0.3851	0.0060	3.75	3.70
0.01517	0.00499	3.32	0.01101	0.3846	0.0060	5.28	5.23
0.01529	0.00500	6.64	0.01105	0.3838	0.0060	7.85	7.79

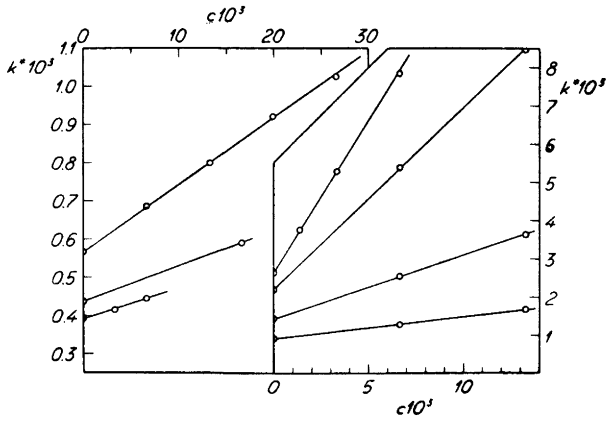


Fig. 3. Catalysis by zinc ions (c molar) and its dependence upon the hydrogen ion concentration. In the seven groups of solutions shown in the diagram, $[H^+]$ was, respectively, (left side of the diagram, taken from the bottom) 0.180, 0.150, and 0.101, (right side, from the bottom) 0.0518, 0.0281, 0.0149, and 0.0110.

when it is assumed that unstable complexes of the composition ZnOx and $ZnHOx^+$ are formed in the solutions. The velocity of decarboxylation will then be given by the expression

$$k_0\ [H_2Ox] + k_1[HOx^-] + k_2[Ox^{--}] + k_3[ZnOx] + k_4[ZnHOx^+] \qquad (15)$$

The concentrations of the unstable complexes are determined by the equilibria

$$Zn^{++} + HOx^- \rightleftharpoons ZnOx + H^+ \qquad (16)$$

$$Zn^{++} + HOx^- \rightleftharpoons ZnHOx^+ \qquad (17)$$

When K_3 and K_4 denote the mass-action constants for the equilibria 16 and 17, respectively, the two last terms of expression 15 may be written

$$k_3K_3[Zn^{++}][HOx^-][H^+]^{-1} + k_4K_4[Zn^{++}][HOx^-]$$

If [ZnOx] and $[ZnHOx^+]$ are negligible compared with c, we may set $[Zn^{++}] = c$. From expression 15 we then obtain

$$k = k_u + k_3K_3c\alpha_1[H^+]^{-1} + k_4K_4c\alpha_1 \qquad (18)$$

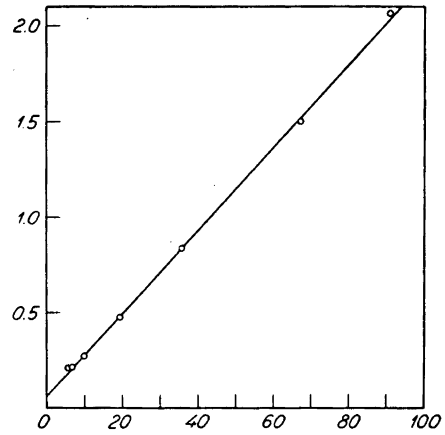


Fig. 4. Catalysis by zinc ions. Abscissa: $[H^+]^{-1}$*; ordinate:* $(k^*-k_u^*)/c\,a_1$

where k_u is the contribution to k from the uncatalysed reaction:

$$k_u = k_0 + (k_1-k_0)\alpha_1 + (k_2-k_0)\alpha_2 \qquad (19)$$

Equation 18 may also be written in the following way

$$(k^*-k_u^*)/c\alpha_1 = k_3^*K_3[H^+]^{-1} + k_4^*K_4 \qquad (20)$$

In Fig. 4, mean values of $(k^*-k_u^*)/c\alpha_1$ for each of the seven groups of solutions examined have been plotted against $[H^+]^{-1}$. The points fall close to a straight line which shows that the explanation given above, including the assumption that $[Zn^{++}] = c$, is valid. From the straight line we find

$$k_3^*K_3 = 0.0217 \quad \text{and} \quad k_4^*K_4 = 0.063$$

Since the concentrations of the unstable zinc complexes are small compared with c, we can only determine the products of the velocity and complexity constants but not the constants separately. In the last column of Table 5 are given the values of k^* calculated by means of equation 18 when the values found for $k_3^*K_3$ and $k_4^*K_4$ are introduced and k_u^* is computed by means of equation 19 and the values of k_0^*, k_1^*, and k_2^* found earlier. There is a good agreement between k^* calculated from the formula and k^* found directly from the measurements.

CATALYSIS BY CUPRIC IONS

Experiments were carried out in solutions of hydrogen chloride, potassium chloride, and cupric nitrate (c molar). The sum of the concentrations of hydrogen and potassium chloride was in three series of experiments 0.050, 0.100, and 0.200, respectively. Table 6 and 7 show the composition of the solutions examined, their ionic strengths and hydrogen ion concentrations, and the degrees of dissociation α_1 and α_2 of the oxaloacetic acid, computed by means of the dissociation constants calculated from equations 4—6. It was found that cupric ions have a much greater catalytic effect than zinc ions, and that the reaction follows the first-order law only when the hydrogen ion concentration is sufficiently high. It will be shown that the kinetic results may be explained quantitatively when it is assumed that unstable complexes of the composition CuOx and $CuHOx^+$ are formed in the solutions. The velocity of decarboxylation may then be written in the following way

$$-\frac{dx}{dt} = k_u x + k_3[CuOx] + k_4[CuHOx^+] \tag{21}$$

where k_u is defined by equation 19. The concentrations of the unstable complexes are given by the mass-action expressions

$$[CuOx] = K_3\,[Cu^{++}]\,[HOx^-]\,[H^+]^{-1} = K_3\,[Cu^{++}]\,\alpha_1 x\,[H^+]^{-1} \tag{22}$$

$$[CuHOx^+] = K_4\,[Cu^{++}]\,[HOx^-] = K_4\,[Cu^{++}]\,\alpha_1 x \tag{23}$$

From equations 22 and 23, we find

$$[Cu^{++}] = c/(1 + \beta x) \tag{24}$$

where we have used the abbreviation

$$\beta = K_3\,\alpha_1[H^+]^{-1} + K_4\,\alpha_1 \tag{25}$$

As x approaches zero, $[Cu^{++}]$ will approach c, and the reaction will approach a first-order course with the velocity constant

$$k = k_u + k_c \tag{26}$$

where

$$k_c = k_3 K_3 c\alpha_1[H^+]^{-1} + k_4 K_4 c\alpha_1 \tag{27}$$

We shall find an expression for k by means of the pressures observed during the reaction. The differential equation 21 may, by means of equations 23—26, be written as follows

$$-\frac{dx}{dt} = \left(k_u + \frac{k_c}{1+\beta x}\right)x = kx\left(1 - \frac{k_c}{k}\frac{\beta x}{1+\beta x}\right)$$

Upon integration, it is found that

$$kt = \ln x + \frac{k_c}{k_u}\ln\left(1 + \frac{k_u}{k}\beta x\right) + \text{const.}$$

or, introducing $P = \varphi x$ and changing to decadic logarithms,

$$k^*t = \log P + \frac{k_c}{k_u}\log\left(1 + \frac{k_u\beta}{k\varphi}P\right) + \text{const.} \tag{28}$$

Since $P = P_0$ when $t = 0$, we obtain from equation 28

$$k^*t = \log\frac{P_0}{P} + \frac{k_c}{k_u}\log Y \tag{29}$$

where

$$Y = \frac{1 + \frac{k_u\beta}{k\varphi}P_0}{1 + \frac{k_u\beta}{k\varphi}P} \tag{30}$$

Instead of equations 29 and 30, we may write

$$\log Y = \frac{k_u}{k_c}\left(k^*t - \log\frac{P_0}{P}\right) \tag{31}$$

$$Y - 1 = \frac{k_u\beta}{k\varphi}(P_0 - PY) \tag{32}$$

When β is sufficiently small, that is (since, as we shall see, the last term of equation 25 is small) when $[H^+]$ is sufficiently high, equation 28 may be written as follows

$$k^*t = \log P + 0.4343\frac{k_c\beta}{k\varphi}P + \text{const.} \tag{33}$$

Table 6. Decomposition of oxaloacetic acid (initial concentration x_0 molar) at 37.0° C in the solvent: h molar hydrogen chloride, c molar cupric nitrate, (s−h) molar potassium chloride.

x_0	h	$c10^3$	I	$[H^+]$	a_1	a_2	k^*10^3	k^*10^3 calc.
Series 1 (s = 0.050):								
0.01490	0.0499	0.100	0.0518	0.0513	0.0971	0.0002	1.338	1.356
0.01517	0.0499	0.249	0.0522	0.0514	0.0972	0.0002	2.243	2.227
0.01519	0.0499	0.498	0.0530	0.0514	0.0973	0.0002	3.700	3.676
0.01514	0.0499	0.996	0.0543	0.0514	0.0977	0.0002	6.57	6.567
0.01495	0.0250	0.100	0.0529	0.0275	0.1673	0.0006	3.026	3.082
Series 2 (s = 0.100):								
0.01510	0.0998	0.100	0.1012	0.1007	0.0581	0.0001	0.668	0.670
0.01510	0.0998	0.599	0.1027	0.1007	0.0583	0.0001	1.410	1.413
0.01504	0.0998	0.997	0.1039	0.1007	0.0585	0.0001	2.018	2.004
0.01506	0.0998	1.996	0.1069	0.1007	0.0586	0.0001	3.456	3.466
0.01515	0.0499	0.996	0.1046	0.0515	0.1082	0.0003	6.125	6.124
Series 3 (s = 0.200):								
0.01493	0.1995	0.100	0.2008	0.2000	0.0336	0.0000	0.410	0.404
0.01512	0.1996	0.996	0.2035	0.2001	0.0337	0.0000	0.719	0.716
0.01517	0.1996	1.996	0.2065	0.2001	0.0337	0.0000	1.049	1.062
0.01516	0.1493	0.100	0.2010	0.1500	0.0443	0.0000	0.501	0.499
0.01517	0.1494	0.996	0.2037	0.1501	0.0444	0.0000	1.041	1.039
0.01508	0.1494	1.996	0.2067	0.1501	0.0444	0.0000	1.649	1.640
0.01507	0.0998	0.100	0.2013	0.1008	0.0647	0.0001	0.686	0.699
0.01514	0.0998	0.204	0.2016	0.1008	0.0647	0.0001	0.834	0.834
0.01512	0.0998	0.498	0.2025	0.1008	0.0647	0.0001	1.220	1.215
0.01516	0.0998	0.997	0.2040	0.1008	0.0647	0.0001	1.865	1.858
0.01516	0.0998	1.993	0.2070	0.1008	0.0648	0.0001	3.116	3.142

The solutions examined may, according to their hydrogen ion concentrations, be divided into two groups, presented in Tables 6 and 7, respectively. In the first group, the hydrogen ion concentration is so high that the reaction approximately follows the first-order law, while there is a pronounced deviation from this law in the second group. In the first group, the velocity constants k^* are calculated by means of formula 33, where the small second term on the right side is estimated from preliminary values of k^*, k_c^*, and β. The constants k^* found in this way are given in the next to the last column of Table 6. The velocity constants k_u^* for the uncatalysed reaction in the same solutions are calculated by means of equation 19 and the values of k_0^*, k_1^*, and k_2^* found earlier in this paper. When k_u^* is subtracted from k^*, we

Table 7. Decomposition of oxaloacetic acid (initial concentration x_0 molar) at 37.0° C in the solvent: h molar hydrogen chloride, c molar cupric nitrate, (s−h) molar potassium chloride. The complexity constant K_3 has been computed from the kinetic data.

x_0	h	$c10^3$	I	$[H^+]$	α_1	α_2	k^*10^3 calc.	K_3
Series 1 (s = 0.050):								
0.01527	0.00996	0.0249	0.0544	0.01434	0.2791	0.0021	3.400	2.16
0.01504	0.00996	0.0996	0.0546	0.01427	0.2801	0.0021	7.869	2.03
0.01523	0.00498	0.0996	0.0557	0.01042	0.3474	0.0037	12.36	1.98
0.01515	0.00498	0.1991	0.0560	0.01040	0.3483	0.0037	22.38	2.04
Series 2 (s = 0.100):								
0.01508	0.00996	0.0996	0.1049	0.01459	0.2992	0.0028	7.176	1.74
0.01507	0.00996	0.1992	0.1052	0.01460	0.2989	0.0028	12.24	1.83
0.01506	0.00498	0.0996	0.1061	0.01069	0.3680	0.0048	11.04	1.66
Series 3 (s = 0.200):								
0.01515	0.01246	0.0498	0.2046	0.01697	0.2906	0.0031	3.697	1.28
0.01515	0.01246	0.0996	0.2048	0.01697	0.2906	0.0031	5.376	1.32
0.01506	0.00996	0.0996	0.2053	0.01488	0.3181	0.0038	6.384	1.26
0.01514	0.00499	0.0100	0.2062	0.01101	0.3856	0.0061	3.314	1.16
0.01515	0.00499	0.0249	0.2062	0.01101	0.3856	0.0061	4.341	0.94
0.01514	0.00499	0.0498	0.2063	0.01101	0.3856	0.0061	6.055	1.22
0.01515 [a]	0.00498	0.0996	0.2064	0.01101	0.3856	0.0061	9.49	1.28
0.01525	0.00498	0.1991	0.2067	0.01104	0.3850	0.0061	16.27	1.16
0.01523	0.00249	0.0249	0.2069	0.00923	0.4270	0.0082	5.152	1.05

[a] The kinetic data for this solution are shown in Fig. 5.

obtain k_c^*. In order to test equation 27, we proceed in the same way as in the discussion of the zinc ion catalysis. When $k_c^*/c\alpha_1$ is plotted against $[H^+]^{-1}$, the points fall, for each of the three series of solutions with nearly constant ionic strength, close to a straight line whose intercept on the ordinate axis has a small positive value. This shows that the cupric ion catalysis can nearly completely be explained by the spontaneous decarboxylation of the complex CuOx, while decarboxylation of the complex $CuHOx^+$ gives only a small, if any, contribution to the catalysis. A further analysis of the data shows that we get the best agreement with equation 27 when we for $k_3^*K_3$ and $k_4^*K_4$ choose the values given in Table 8 at the ionic strengths stated. When they are used for the calculation of k^* by means of equations 26 and 27, the values given in the last column of Table 6 are obtained. In the calculation, $k_3^*K_3$

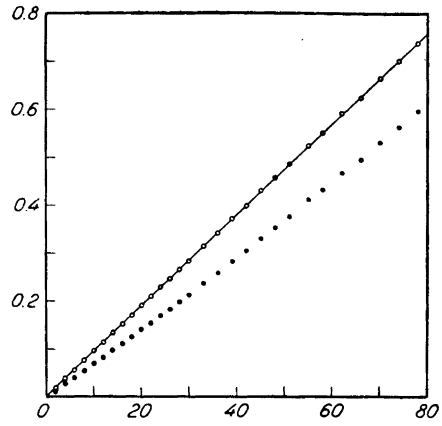


Fig. 5. Catalysis by cupric ions. Kinetic data for the solution marked a in Table 7. Abscissa: t in minutes; ordinate: (filled circles) log (P_0/P), (open circles) log $(P_0/P) + (k_c/k_u)\log Y$.

has been corrected for the effect of small differences in ionic strength by means of the following formula derived from the data in Table 8:

$$-\Delta\log\,(k_3{}^*K_3) = 2.076\;\Delta\sqrt{I} - 1.80\,\Delta\,I$$

A comparison of the last two columns of Table 6 shows that there is a satisfactory agreement between k^* calculated by means of formulae 26 and 27 and k^* found directly from the measurements.

The composition of the second part of the solutions examined, those with the lowest hydrogen ion concentrations, is given in Table 7. If the cupric ion concentration were constant during the decomposition and equal to c, the reaction would follow the first-order law with the velocity constant k^*. Values of k^*, calculated by means of equations 26 and 27 and the constants found above, are given in the next to the last column of Table 7. The actual rate was always lower than that calculated from the assumption that $[Cu^{++}] = c$. This is shown for one of the solutions in Fig. 5, where the filled circles represent $\log (P_0/P)$ plotted against the time t in minutes. If the assumption were valid, $\log (P_0/P)$ should be equal to k^*t and the points fall close to the straight line shown in the diagram. From the deviation, it is possible to estimate K_3. We calculate Y by means of equation 31 and plot $Y-1$ against PY. In agreement with equation 32, the points fall close to a straight line. From the slope of this line, we compute β. K_3 is finally calculated by means of equation 25 where we neglect the last term. It is reasonable to do this, since, as we have seen, $k_4{}^*K_4$ is much smaller than $k_3{}^*K_3\,[H^+]^{-1}$ while $k_4{}^*$ is probably at least of the same order of magnitude as $k_3{}^*$. On proceeding in this way, we find for the experiment shown in Fig. 5 that $\beta = 44.8$ and $K_3 = 1.28$. When we, for the same experiment, calculate Y by means of equation 30, setting $\beta = 44.8$,

Table 8. Cupric and zinc ion catalysis in the decarboxylation of oxaloacetic acid at 37.0° C.

Catalyst	Series	I	$k_3{}^*K_3$	$k_4{}^*K_4$	K_3	$k_3{}^*$	$\frac{(CuOx)}{(Cu^{++})\ (Ox^{--})}$
Cu^{++}	1	0.053	3.05	0.7	2.03	1.50	1.88×10^4
Cu^{++}	2	0.104	2.50	0.57	1.74	1.44	1.25×10^4
Cu^{++}	3	0.206	1.96	0.50	1.20	1.63	0.67×10^4
Zn^{++}		0.200	0.0217	0.063			

introduce the values found for Y into equation 29, and plot the sum of the two terms on the right side of the equation against t, we obtain the open circles shown in Fig. 5. They all fall close to the straight line already drawn. This shows that the value 1.28 for the complexity constant K_3 satisfactorily explains the deviations from the course we should expect if the cupric ion concentration were all the time equal to c. The values of K_3 found for the respective solutions are given in the last column of Table 7. Average values of K_3 for each of the three series of experiments are given in Table 8 together with $k_3{}^*$ calculated by means of K_3 and the known values of $k_3{}^*K_3$. As we should expect, K_3 decreases considerably with increasing ionic strength. The accuracy of K_3 is, however, too small, and the salt concentration too high, to justify an extrapolation to zero ionic strength by means of Debye-Hückel's law. We should expect $k_3{}^*$, the velocity constant for the spontaneous decarboxylation of the complex CuOx, to be nearly independent of the salt concentration. The irregular variations found are probably due to inaccuracy in the determination. The mean value for all the three series, $k_3{}^* = 1.52$, corresponds to a time of half-completion amounting to 12 seconds. The mass-action constant for the equilibrium

$$Cu^{++} + Ox^{--} \rightleftharpoons CuOx$$

is equal to K_3/K_2. In the last column of Table 8 are given the values computed for it. In the last line of the table are shown the values of $k_3{}^*K_3$ and $k_4{}^*K_4$ found in the zinc ion catalysis.

THE MECHANISM OF THE METAL-ION CATALYSIS

Metal-ion catalysed decarboxylation has been found only for di- and tricarboxylic acids. The decarboxylation of monocarboxylic acids may even be retarded by metal ions. Let us compare the decarboxylations of oxaloacetic and nitroacetic acid. It has been shown [15] that the mechanism of the latter reaction is analogous to that of the decarboxylation of β-keto carboxylic acids.

The *nitro*-form of the univalent nitroacetate ion decomposes spontaneously into carbon dioxide and the anion of *aci*-nitromethane. It was suggested [15] that an electron displacement in the molecule towards the nitro (or keto) group is the reason why the bond is broken and carbon dioxide set free. The effect of metal ions on the decarboxylation of nitroacetic acid has been studied by the author of this paper [16]. It was found that both the undissociated nitroacetic acid and metal ion complexes of the nitroacetate ion are stable. It is reasonable to assume that a metal ion bound directly to the carboxylate group will hinder the displacement of electrons towards the nitro group and therefore stabilize the molecule. A divalent oxaloacetate ion, on the other hand, may bind a metal ion either to the carboxyl group having the keto group in β-position or to the stable carboxyl group in the other end of the molecule. In both cases, the metal ion will probably also be linked to the keto group forming a chelate compound. While the metal ion in the former case stabilizes the molecule, it will, in the latter case, owing to the electric attraction emanating from it, enlarge the displacement of electrons from the unstable carboxylate group towards the keto group, and therefore make the molecule less stable. Since the probability for a decarboxylation in this way may be increased a great number of times, it is not surprising that it greatly outweighs the stabilizing effect.

An important contribution to the understanding of the metal-ion catalysis in the decarboxylation of dicarboxylic acids has been given by Steinberger and Westheimer [2] who have examined the decomposition of dimethyloxaloacetic acid, $HOOCCOC(CH_3)_2COOH$, and its monoester, $C_2H_5OOCCOC(CH_3)_2COOH$. They have shown that heavy metal ions catalyse the former, but not the latter reaction. Their explanation of the mechanism of the metal-ion catalysis is essentially the same as that given above.

SUMMARY

The uncatalysed decarboxylation of oxaloacetic acid was examined at 37.0° C in solutions of potassium and hydrogen chloride and in acetate buffer solutions. It was found that both undissociated oxaloacetic acid (H_2Ox) and the uni- and divalent ion (HOx^- and Ox^{--}) decompose spontaneously. The velocity may therefore be expressed by the sum

$$v_u = k_0\,[H_2Ox] + k_1\,[HOx^-] + k_2\,[Ox^{--}]$$

The velocity constants k_0, k_1, and k_2 were determined.

The zinc and cupric ion catalysis in the decarboxylation of oxaloacetic acid was examined at 37.0° C in solutions of potassium and hydrogen chloride containing in addition zinc chloride or cupric nitrate. It was shown that the catalysis may, for the greatest part, be explained by the spontaneous decarboxylation of a complex of the composition MeOx (where Me is either Zn or Cu), but also the spontaneous decarboxylation of the complex $MeHOx^+$ contributes to the reaction. When K_3 and K_4 denote mass-action constants for the equilibria

$$Me^{++} + HOx^- \rightleftharpoons MeOx + H^+ \text{ and } Me^{++} + HOx^- \rightleftharpoons MeHOx^+$$

the velocity may be expressed by the following sum

$$\begin{aligned} v &= v_u + k_3 [MeOx] + k_4 [MeHOx^+] \\ &= v_u + k_3K_3 [Me^{++}] [HOx^-] [H^+]^{-1} + k_4K_4 [Me^{++}] [HOx^-] \end{aligned}$$

The products k_3K_3 and k_4K_4 were determined both for the zinc and the cupric ion catalysis. For the latter, the two constants k_3 and K_3 were also determined separately.

The mechanism of the uncatalysed and the metal-ion catalysed decarboxylation was discussed, and it was explained why metal ions catalyse the decarboxylation of di-, but not of mono-carboxylic acids.

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Short Communications

On the Mechanism of Ribose Synthesis in the Rat

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The major metabolic pathway to ribose has previously been considered to be a more direct conversion from hexoses [1–3]. Recently, however, it has been proposed that the synthesis of ribose proceeds instead over two- and three-carbon units [4]. This theory is supported by an investigation by Bernstein [5] in which chicks were fed with sodium acetate containing C^{14} in the carboxyl group. A comparison of the isotope content in the different carbon atoms of glycogen and of ribose indicated that a direct conversion of hexose to ribose is not the major synthetic pathway.

In connection with this it is of interest to report certain experiments performed in this laboratory in which acetate labeled with C^{14} in the carboxyl group and with C^{13} in the methyl group was administered to rats.

Ten rats were injected intraperitoneally with two mM of labeled acetate per 100 g body weight. This was divided into four doses given at three hours intervals. The animals were sacrificed two hours after the last injection, and the liver and intestines dried immediately with alcohol and ether. The polynucleotides were prepared according to Hammarsten [6]. The ribose from ribonucleic acid was converted to furoic acid as described earlier [7]. The carbon was isolated as barium carbonate; the activity was measured in a Geiger-Müller counter and the excess of C^{13} determined in a mass spectrometer. The results can be seen in the table.

	Atom % excess C^{13}	Counts per min C^{14}
Acetate injected	14.5	30 000
Furoic acid isolated	0.15	60

These values show that the carbon from the methyl group of acetic acid is incorporated into ribose to a much greater extent than is the carbon from the carboxyl group (dilution of C^{13} from methyl one-fifth that of C^{14} from carboxyl). This might indicate that acetate is not incorporated into ribose as an intact molecule and is, therefore, not an immediate precursor, but may enter the ribose by some indirect route.

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Determination of Sulphate by Reduction with Stannous Chloride. II

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In a previous paper [1] was shown that sulphate may be determined by reduction with stannous chloride, when a solution of sodium sulphate, stannous chloride and phosphoric acid is boiled down.

By checking the method with another apparatus but the same experimental set-up, it proved convenient to heat the reacting mixture a little more cautiously and to reduce the rate of the current of carbon dioxide. Otherwise there seemed to be a tendency to attain an incomplete absorption of the hydrogen sulphide formed.

Nitric acid present. As to be expected the presence of nitric acid prohibited the method. However, experiments have shown that the determination could be carried out to complete satisfaction, when the nitric acid was removed by boiling down with phosphoric acid before the stannous chloride was added.

Mercury salts present. As to be expected the method failed, when mercury salts were present in the sample.

Barium salts present. A standard solution of sodium sulphate containing 1.336 % SO_4 was used in the following experiments. This solution was controlled gravimetrically as barium sulphate and by evaporation as sodium sulphate.

Introductory experiments were carried out as follows: Barium chloride in excess was added to a weighed portion of the sodium sulphate sample, and the mixture was evaporated towards dryness. Now 1) 20 ml water, 2) 4 g stannous chloride, and 3) 10 ml phosphoric acid (85 %) were added. However, the method could not be carried through to satisfaction (and the results were a few percent too low), because an almost solid mass was formed. Therefore the method was altered: 20 ml phosphoric acid was used (instead of 10 ml), and then the reduction was carried through without difficulty and in usual time.

Table 1. Determination — by reduction with stannous chloride — of sulphate in a sodium sulphate solution which contains 1.336 % SO_4. Before the reduction an excess of barium chloride was added. The mentioned volumes of sodium thiosulphate solution and potassium iodate solution are recalculated to 0.1000 N.

Sample g	$Na_2S_2O_3$ ml	KIO_3 ml	% SO_4 found
6.7692	6.07	18.91	1.342
6.8949	5.75	19.23	1.340
6.9159	5.68	19.30	1.340
7.6388	3.66	21.32	1.341
7.3163	4.53	20.45	1.343
6.1333	7.81	17.17	1.345
6.1246	7.84	17.14	1.344
6.2188	7.63	17.35	1.340
6.1162	7.91	17.07	1.341

The experimental results are given in Table 1. It may be added that no loss of iodine, caused by the carbon dioxide current, could be detected, and the results were not corrected for an eventual loss of iodine (see previous paper [1]). The mean value was 1.342 % SO_4 in good accordance with the 1.336 % SO_4. If we assume that the results are 3 ‰ too high (due to loss of iodine), we get the corrected value 1.338 %.

When carrying out the reduction of the slightly soluble barium sulphate it was seen that the reacting mixture gradually became completely limpid which showed that the precipitated sulphate was now reduced. A few experiments were interrupted when the reacting mixture had just become limpid, and it was found that hydrogen sulphide corresponding to 1.315 and 1.318 % SO_4 had distilled over. If the remains of hydrogen sulphide have to be forced over into the iodine solution, the boiling must be continued as described in the earlier published method.

Table 2. Determination — by reduction with stannous chloride and simultaneous presence of cupric oxide — of sulphate in potassium sulphate pro analysi. 20.00 ml about 0.1 N potassium iodate solution were used in each of the first 6 experiments and 50.00 ml in each of the last 3 experiments. The volumes of potassium iodate solution are recalculated to 0.1000 N.

K_2SO_4 weighed g	KIO_3 ml	K_2SO_4 found g
0.1069	12.19	0.1062
0.1114	12.78	0.1114
0.1126	12.96	0.1129
0.1064	12.11	0.1055
0.1072	12.33	0.1074
0.1115	12.76	0.1112
0.3449	39.59	0.3450
0.3080	35.18	0.3065
0.3062	35.18	0.3065

Presence of other elements which form slightly soluble sulphates. In the same way as mentioned in the previous section, it was investigated if presence of calcium or lead influenced the method. This was not the case. It may be added that the presence of lead never caused formation of visible lead sulphide in the boiling flask.

Presence of elements which form slightly soluble sulphides. An example of such an element is lead which is mentioned above. Further it was tried to add copper salts: To the weighed sample was added about 0.5 g of diverse copper salts. Surprisingly, it now appeared that no hydrogen sulphide distilled over, but sulphur dioxide only, though copper sulphide was formed as an intermediate in the boiling flask.

This fact led up to the possibility of altering the earlier published method so that sulphur dioxide only is produced by the reduction of sulphate. The following method was tried with a favourable result: To a weighed portion of the sample were added 1) 4 g stannous chloride, 2) 1 g large-grained cupric oxide, and 3) 20 ml phosphoric acid (85 %). An ordinary Bunsen burner was used for heating. Further 1) standard potassium iodate solution (about 0.1 *N*) was pipetted off into the absorption system, and 2) 2 g potassium iodide, and 3) 10 ml 2 *M* sodium hydroxide were added. According to this method the sulphate content in potassium sulphate pro analysi was determined; the results are given in Table 2.

Introductory experiments indicate that sulphate may be determined also by boiling down with phosphoric acid using cuprous chloride or zinc as reductant. Investigations of these problems will be continued.

1. Rancke-Madsen, E. *Acta Chem. Scand.* **3** (1949) 773.

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Tellurenyl Sulphur Compounds

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Whereas sulphenyl and selenenyl compounds, with the formulae RSX and RSeX where X is an electronegative atom or group, are well known [1–4], no tellurenyl compounds, RTeX, have been described in literature. We wish to report the syntheses of the first types of tellurenyl compounds, *viz.*, some *p*-methoxybenzenetellurenyl pseudohalides.

p-Anisyl telluritrichloride [5] reacts with sodium methanethiosulphonate to give *p*-methoxybenzenetellurenyl methanethiosulphonate:

$$ArTeCl_3 + 3\ CH_3SO_2SNa = ArTeS_2O_2CH_3 + \\ + S_2(SO_2CH_3)_2 + 3\ NaCl$$

The following procedure was employed: 6.8 g (0.02 mole) of *p*-anisyl telluritrichlor-

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$$ArTeCl_3 + 3\ CH_3SO_2SNa = ArTeS_2O_2CH_3 + \\ + S_2(SO_2CH_3)_2 + 3\ NaCl$$

The following procedure was employed: 6.8 g (0.02 mole) of *p*-anisyl telluritrichlor-

ide and 10 g (0.075 mole) of finely powdered sodium methanethiosulphonate in 25 ml of ethanol-free chloroform were triturated for a few minutes, first at room temperature and afterwards at boiling temperature of the solvent. The mixture was filtered while hot, and 20 ml of ether were added to the deep red filtrate. On standing, the tellurenyl methanethiosulphonate crystallized as orange red prisms. Yield, 4.7 g (68 %). The compound is stable, readily soluble in chloroform and fairly soluble in warm methanol.

The methanethiosulphonate group possesses pronounced pseudohalogen properties, the nucleophilic reactivity of the anion, in displacements on divalent sulphur, selenium and tellurium, being lower than that of aromatic thiosulphonate, thiosulphate, and thiocarbonyl anions [2,6]. Accordingly, such anions react with the tellurenyl methanethiosulphonate to displace the methanethiosulphonate group. In methanol solutions potassium benzenethiosulphonate reacts as follows:

$$ArTeS_2O_2CH_3 + C_6H_5SO_2S^- = ArTeS_2O_2C_6H_5 + CH_3SO_2S^-$$

The resulting *p*-methoxybenzenetellurenyl benzenethiosulphonate forms stable, light orange red crystals, readily soluble in warm chloroform, sparingly soluble in ether.

The tellurenyl thiosulphonates react with aqueous sodium thiosulphate to produce aqueous solutions of a compound, presumably sodium *p*-methoxybenzenetellurenyl thiosulphate, having in dilute solutions a greenish yellow colour and in more concentrated solutions a reddish colour (R = methyl or phenyl):

$$ArTeS_2O_2R + S_2O_3^{--} = ArTeS_2O_3^- + RSO_2S$$

The tellurenyl thiosulphate solutions react with aqueous potassium methylxanthate to give *p*-methoxybenzenetellurenyl methylxanthate:

$$ArTeS_2O_3^- + CH_3OCS_2^- = ArTeS_2COCH_3 + S_2O_3^{--}$$

The same compound was obtained directly from the tellurenyl thiosulphonates on triturating these with an excess of potassium methylxanthate suspended in warm ether. On filtering, and partial evaporation of the ether, the tellurenyl methylxanthate crystallized as stable, orange red plates or prisms.

Compound (Ar = *p*-anisyl)	M.p. °C	% Te Calc.	% Te Found
$ArTeS_2O_2CH_3$	107	36.9	37.1
$ArTeS_2O_2C_6H_5$	110	31.3	31.4
$ArTeS_2COCH_3$	78	37.3	37.4

Further researches on tellurenyl pseudohalides are being made as part of a program for the study of the relative electrophilic reactivities of divalent sulphur, selenium and tellurium.

The author is indebted to *Styret for Nansenfondet og de dermed forbundne fond* for a grant.

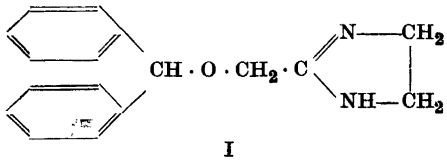
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2-(Benzohydrylmercaptomethyl)-imidazoline and Some Related Compounds

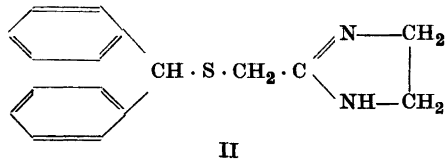
RICHARD DAHLBOM

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Following the synthesis of 2-(benzohydryloxymethyl)-imidazoline (I) some years ago [1] it was considered of interest to investigate the pharmacological properties of its sulphur analogue (II).



I



II

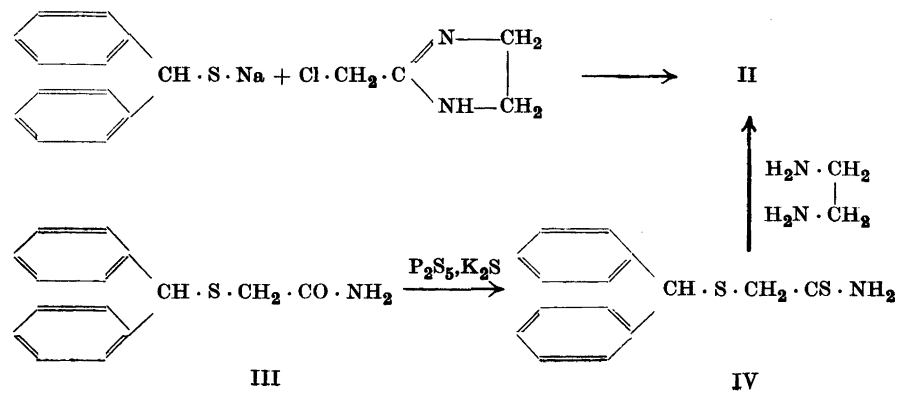
This compound was prepared by the reaction of benzohydryl mercaptan with 2-(chloromethyl)-imidazoline in the presence of sodium ethoxide. Alternatively it could be obtained by the treatment of (benzohydrylmercapto)-acetamide with phosphorus pentasulphide and potassium sulphide and reaction of the resulting thioamide with ethylenediamine.

The new imidazoline was tested for antihistaminic and antispasmodic properties on isolated guinea pig intestine. Its effect on spasms induced by histamine and acetyl choline was only 1/20 and 1/2 respectively of that of the oxygen analogue (I).

In connection with the synthesis of the compound II (benzylmercapto)-thioacetamide, 2-(benzylmercaptomethyl)-imidazoline and the ethyl ester and amide of (triphenylmethylmercapto)-acetic acid were prepared and are recorded in this note.

EXPERIMENTAL. *2-(Benzohydrylmercaptomethyl)-imidazoline (II).*

Method A. Benzohydrylmercaptane [2] (12.5 g, 80 %) and 2-(chloromethyl)-imidazoline hydrochloride [3] (7.75 g) were added to a solution of sodium (2.3 g) in absolute ethanol (100 ml). The mixture was refluxed with stirring for one hour and filtered and an ethereal solution of oxalic acid was added until precipitation ceased. The crude *oxalate* (14.2 g) was recrystallised twice from ethyl acetate; m.p. 171–171.5°. (Found: C 61.0; H 5.43. $C_{19}H_{20}N_2O_4S$ (372.4) requires C 61.3; H 5.41 %).



III IV

The *free base*, obtained by treatment of an aqueous suspension of the oxalate with dilute sodium hydroxide, melted at 115—116° after recrystallisation from light petroleum. (Found: C 72.6; H 6.29; S 11.2. $C_{17}H_{18}N_2S$ (282.4) requires C 72.3; H 6.42; S 11.4 %). Its *hydrochloride* melted at 189.5—191° (from acetone-ethanol 3 : 1). (Found: C 64.2; H 5.86; Cl 11.3. $C_{17}H_{19}ClN_2S$ (318.9) requires C 64.0; H 6.00; Cl 11.1 %).

Method B. A mixture of (benzohydrylmercapto)-acetamide [4] (5.2 g), phosphorus pentasulphide (1.8 g), potassium sulphide (1.7 g) and toluene (20 ml) was heated on the water bath for one hour with occasionally shaking. The supernatant liquid was decanted and the residue was extracted with hot toluene (20 ml). The combined toluene solutions yielded on cooling yellow crystals (4.5 g), m.p. 72—73°. Recrystallisation from toluene removed the colour, but the m.p. was not raised. Analysis indicated, that the reaction product was an addition compound containing approximately one mole of the initial amide (III) and one mole of the expected *thioamide* (IV). (Found: C 66.7; H 5.71; S 18.4. $C_{30}H_{30}N_2OS_3$ (530.7) requires C 67.9; H 5.70; S 18.1 %).

It should be noted, that (benzohydryloxy)-acetamide and the corresponding thioamide form a similar addition compound [5].

The amide-thioamide product obtained above (3.1 g) was dissolved in toluene (25 ml) and refluxed with anhydrous ethylenediamine (0.75 g) for three hours. After cooling, the toluene solution was extracted with aqueous sodium carbonate and with water, and the 2-(benzohydrylmercaptomethyl)-imidazoline was isolated as the oxalate by the addition of ethereal oxalic acid. The crude oxalate (1.5 g) was recrystallised twice from ethyl acetate; m.p. 171—172° undepressed on admixture with the oxalate prepared by method A above. (Found: C 60.9; H 5.50. $C_{19}H_{20}N_2O_4S$ (372.4) requires C 61.3; H 5.41 %).

(Benzylmercapto)-thioacetamide. (Benzylmercapto)-acetamide [6] (10 g) was treated with phosphorus pentasulphide (5.0 g) and potassium sulphide (4.7 g) in toluene (55 ml) as described above. The crude thioamide (3.3 g) was recrystallised from ethanol; m.p. 78—78.5°. (Found: N 6.92; S 32.2. $C_9H_{11}NS_2$ (197.3) requires N 7.10; S 32.5 %).

2-(Benzylmercaptomethyl)-imidazoline oxalate. This compound was prepared from (benzylmercapto)-thioacetamide (3.0 g) and ethylenediamine (1.0 g) in the same manner as the corresponding benzohydryl compound (method B). The crude oxalate (1.5 g) was recrystallised from acetone; m.p. 138—139°. (Found: C 53.0; H 5.58; N 9.50. $C_{13}H_{16}N_2O_4S$ (296.3) requires C 52.7; H 5.44; N 9.45 %).

Ethyl (triphenylmethylmercapto)-acetate. (Triphenylmethylmercapto)-acetic acid [7] (20 g) was dissolved in hot ethanol (50 ml) and conc. sulphuric acid (5 ml) was added. A vigorous reaction occurred, and after a few minutes the ester separated as an oil. The reaction mixture was poured into water (500 ml) and the ester was extracted with ether. The ether extract was dried and the solvent evaporated giving a crystalline residue (19.0 g) which was recrystallised from ethanol; m.p. 93—94°. (Found: C 76.1; H 6.12; S 8.73. $C_{23}H_{22}O_2S$ (362.5) requires C 76.2; H 6.12; S 8.84 %).

(Triphenylmethylmercapto)-acetamide. The ethyl ester obtained above (6.4 g) was dissolved in ethanol (40 ml), and the solution was saturated with ammonia at − 20° and heated in a sealed vessel at 100° overnight. On cooling, the amide (4.7 g) separated; m.p. 145—146° after recrystallisation from ethanol. (Found: N 4.26; S 9.55. $C_{21}H_{19}NOS$ (333.4) requires N 4.20; S 9.62 %).

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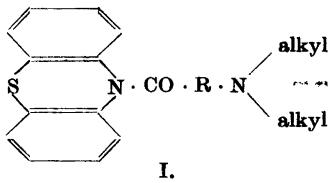
Received February 1, 1952.

Some 9-Aminoacylcarbazoles

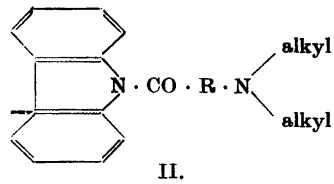
RICHARD DAHLBOM

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The interesting spasmolytic properties of a series of 10-dialkylaminoacylphenothiazines (I) prepared in this laboratory [1,2] prompted us to study the effect of the replacement of the phenothiazine moiety of the molecule by carbazole. For this reason some 9-dialkylaminoacylcarbazoles (II) were synthesised by essentially the same method as was used for the



I.



II.

phenothiazine compounds. The new carbazole derivatives were isolated as oxalates, which were also used in the pharmacological tests. These were carried out on isolated guinea pig intestine (S. Wiedling), and it was found that the aminoacylcarbazoles were devoid of spasmolytic and antihistaminic activity. It is of interest to note that an analogous replacement of the phenothiazine nucleus by carbazole in the aminoalkylphenothiazine series greatly reduced the strong antihistaminic activity of the parent compounds [3,4].

EXPERIMENTAL. *9-(Chloroacetyl)-carbazole.* Chloroacetyl chloride (11.3 g, 0.1 mole) in toluene (20 ml) was added to a solution of carbazole (16.7 g, 0.1 mole) in boiling toluene (250 ml). The mixture was refluxed for twenty-four hours, most of the solvent was evaporated, and the residue was cooled. The precipitate which formed (18.4 g, 76 % yield) was collected and recrystallised from ethanol; m.p. 98–100°. (Found: C 68.7; H 4.11; Cl 14.4. $C_{14}H_{10}ClNO$ (243.7) requires C 69.0; H 4.14; Cl 14.6 %).

9-(Diethylaminoacetyl)-carbazole oxalate. The above chloro compound (2.43 g, 0.01 mole) was dissolved in benzene (20 ml) and refluxed with diethylamine (1.9 g, 0.026 mole) for four hours. The reaction mixture was filtered and washed with water and the oxalate of the reaction product was precipitated by the addition of an ethereal solution of oxalic acid. The crude oxalate (1.9 g, 46 %) was recrystallised from acetone; m.p. 155–157° (dec.). Analysis indicated that three moles of oxalic acid were associated with two moles of the base. (Found: C 60.9; H 5.72; N 6.82; $H_2C_2O_4$ 33.0. $2C_{18}H_{20}N_2O \cdot 3H_2C_2O_4$ (830.8) requires C 60.7; H 5.58; N 6.74; $H_2C_2O_4$ 32.5 %).

The percentage of oxalic acid was determined as follows: A sample of the oxalate was decomposed with alkali, the base was removed by extraction with ether, and the aquous solution was acidified and titrated with permanganate.

9-(Piperidinoacetyl)-carbazole oxalate. This compound was prepared in 67 % yield from 9-(chloroacetyl)-carbazole and piperidine in an analogous way to the diethylamino compound. M.p. 228–229° (dec.) after recrystallisation from acetone-water (1 : 1). (Found: C 66.0; H 5.70; N 7.36. $C_{19}H_{20}N_2O \cdot H_2C_2O_4$ (382.4) requires C 65.9; H 5.80; N 7.33 %).

9-(α-Diethylaminopropionyl)-carbazole oxalate. Prepared similarly, in 56 % yield, from 9-(α-bromopropionyl)-carbazole [5] and diethylamine. M.p. 174–175° (dec.) after recrystallisation from acetone. (Found: C 65.7; H 6.17; N 7.10. $C_{19}H_{22}N_2O \cdot H_2C_2O_4$ (384.4) requires C 65.6; H 6.29; N 7.29 %).

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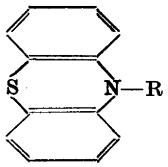
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10-Hydroxyalkylphenothiazines

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In a previous communication on antihistamine agents the synthesis of 10-(β-hydroxypropyl)-phenothiazine (III) was described [1]. In connection with this work another two compounds of this class hitherto unreported (I, II) were prepared. Compound I was prepared by the addition of ethylene oxide to the sodium salt of



R =

I. $-CH_2 \cdot CH_2OH$
II. $-CH_2 \cdot CH_2 \cdot CH_2OH$
III. $-CH_2 \cdot CHOH \cdot CH_3$

phenothiazine and compound II by the reduction of β-(10-phenothiazine)-propionic acid with lithium aluminium hydride. Attempts were also made to obtain 10-(hydroxymethyl)-phenothiazine by the reduction of methyl phenothiazine-10-carboxylate. However, this compound decomposed when treated with lithium aluminium hydride giving phenothiazine as the only identifiable product. Phenothiazine derivatives with a carbonyl group attached to the nitrogen atom in the 10-position have previously been shown to be sensitive to lithium aluminium hydride [2].

EXPERIMENTAL. *10-(β-Hydroxyethyl)-phenothiazine (I).* Phenothiazine (39.8 g, 0.2 mole) was added to a suspension of sodium amide (prepared from sodium (4.6 g, 0.2 mole) and liquid ammonia) in toluene (100 ml) and the mixture was refluxed with stirring for three hours. After cooling to room temperature, ethylene oxide (17.6 g, 0.4 mole) in toluene (50 ml) was added in portions, whereupon the temperature rose to 70°. The mixture was refluxed for one hour, cooled to room temperature and washed with water. The solvent was then evaporated and the residue was distilled *in vacuo* giving a viscous oil (26.9 g, 55 %) of b.p. 210–213°/0.3 mm. (Found: C 68.8; H 5.47; N 5.68. $C_{14}H_{13}NOS$ (243.3) requires C 69.1; H 5.39; N 5.76 %).

10-(γ-Hydroxypropyl)-phenothiazine (II). Lithium aluminium hydride (0.57 g, 0.015 mole) was suspended in dry ether (50 ml) and the mixture was stirred for half an hour. A solution of β-(10-phenothiazine)-propionic acid[3] (3.0 g, 0.011 mole) was added portionwise and the mixture was stirred at the reflux temperature for another half hour. Excess of hydride was destroyed with ethyl acetate (2 ml) and *N* hydrochloric acid (50 ml) was then cautiously added to the reaction mixture. The organic layer was separated, washed with water and dried and the solvent was evaporated. The residue was distilled *in vacuo* giving a very viscous oil (1.4 g, 50 %) boiling at 0.01 mm at 160° (bath temperature). (Found: C 70.2; H 5.68; N 5.57. $C_{15}H_{15}NOS$ (257.3) requires C 70.0; H 5.88; N 5.45 %).

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Received February 1, 1952.

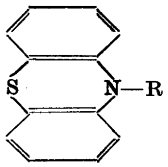
Studies on Sphagnum Peat
II.* Lignin in Sphagnum

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Although various groups of workers have sought to demonstrate the presence of lignin in *Sphagnum* species, their results have been rather indecisive. Thus, Stadnikow and Baryschewa [1] found 9 % of hydrochloric acid lignin in *S. parvifolium* but Holmberg [2] found only traces of thioglycolic acid lignin in *S. subsecundum* Nees. Lange [3] studied *Sphagnum* moss with the ultraviolet microscope and concluded that it probably contained lignin, while Kratzl and Eibl [4], recently, working on similar material, found about 7 % of Klason lignin, which, however, contained only 1 % of methoxyl. As nitrobenzene oxidation of the moss yielded a product, containing only 0.1 % of methoxyl, from which they were unable to isolate any vanillin, Kratzl and Eibl concluded that the moss contains no lignin, but does contain some partly methoxylated carbohydrates which easily

* Part I. *Svensk Kem. Tid.* In press.
Also part XIII in the series *Studies on Lignin.*



R =

I. $-CH_2 \cdot CH_2OH$
II. $-CH_2 \cdot CH_2 \cdot CH_2OH$
III. $-CH_2 \cdot CHOH \cdot CH_3$

phenothiazine and compound II by the reduction of β-(10-phenothiazine)-propionic acid with lithium aluminium hydride. Attempts were also made to obtain 10-(hydroxymethyl)-phenothiazine by the reduction of methyl phenothiazine-10-carboxylate. However, this compound decomposed when treated with lithium aluminium hydride giving phenothiazine as the only identifiable product. Phenothiazine derivatives with a carbonyl group attached to the nitrogen atom in the 10-position have previously been shown to be sensitive to lithium aluminium hydride [2].

EXPERIMENTAL. *10-(β-Hydroxyethyl)-phenothiazine (I).* Phenothiazine (39.8 g, 0.2 mole) was added to a suspension of sodium amide (prepared from sodium (4.6 g, 0.2 mole) and liquid ammonia) in toluene (100 ml) and the mixture was refluxed with stirring for three hours. After cooling to room temperature, ethylene oxide (17.6 g, 0.4 mole) in toluene (50 ml) was added in portions, whereupon the temperature rose to 70°. The mixture was refluxed for one hour, cooled to room temperature and washed with water. The solvent was then evaporated and the residue was distilled *in vacuo* giving a viscous oil (26.9 g, 55 %) of b.p. 210–213°/0.3 mm. (Found: C 68.8; H 5.47; N 5.68. $C_{14}H_{13}NOS$ (243.3) requires C 69.1; H 5.39; N 5.76 %).

10-(γ-Hydroxypropyl)-phenothiazine (II). Lithium aluminium hydride (0.57 g, 0.015 mole) was suspended in dry ether (50 ml) and the mixture was stirred for half an hour. A solution of β-(10-phenothiazine)-propionic acid[3] (3.0 g, 0.011 mole) was added portionwise and the mixture was stirred at the reflux temperature for another half hour. Excess of hydride was destroyed with ethyl acetate (2 ml) and *N* hydrochloric acid (50 ml) was then cautiously added to the reaction mixture. The organic layer was separated, washed with water and dried and the solvent was evaporated. The residue was distilled *in vacuo* giving a very viscous oil (1.4 g, 50 %) boiling at 0.01 mm at 160° (bath temperature). (Found: C 70.2; H 5.68; N 5.57. $C_{15}H_{15}NOS$ (257.3) requires C 70.0; H 5.88; N 5.45 %).

1 Dahlbom, R. *Acta Chem. Scand.* **3** (1949) 247.
2. Dahlbom, R., and Ekstrand, T. *Acta Chem. Scand.* **5** (1951) 102.
3. Smith, N. L. *J. Org. Chem.* **15** (1950) 1125.

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Studies on Sphagnum Peat
II.* Lignin in Sphagnum

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Although various groups of workers have sought to demonstrate the presence of lignin in *Sphagnum* species, their results have been rather indecisive. Thus, Stadnikow and Baryschewa [1] found 9 % of hydrochloric acid lignin in *S. parvifolium* but Holmberg [2] found only traces of thioglycolic acid lignin in *S. subsecundum* Nees. Lange [3] studied *Sphagnum* moss with the ultraviolet microscope and concluded that it probably contained lignin, while Kratzl and Eibl [4], recently, working on similar material, found about 7 % of Klason lignin, which, however, contained only 1 % of methoxyl. As nitrobenzene oxidation of the moss yielded a product, containing only 0.1 % of methoxyl, from which they were unable to isolate any vanillin, Kratzl and Eibl concluded that the moss contains no lignin, but does contain some partly methoxylated carbohydrates which easily

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form "humus substances" which are responsible for the values of Klason lignin found.

The publication of Kratzl and Eibl prompted us to record some results obtained during the investigation of *Sphagnum balticum* Russow and *Sphagnum fuscum* W. Klinggr. Both species gave about 5 % Klason lignin, containing only about 1 % of methoxyl. Both species were carefully extracted with organic solvents and water and then submitted to nitrobenzene oxidation. The resulting products were worked up, following the procedure used by Leopold and Malmström [5], and a 0.6 % yield of aldehydes (calculated as *p*-hydroxybenzaldehyde) was obtained. Paper chromatography revealed that the major constituent of the aldehyde fraction was, indeed, *p*-hydroxybenzaldehyde, and this compound was isolated in a pure state (corresponding to 90 % of the aldehyde groups). Traces of vanillin (about 4.0 %), syringic aldehyde (about 0.6 %) and formyl vanillin (about 0.1 %) were identified on the paper chromatograms, and three further substances as yet unidentified were present.

The investigations of Bland [6] and Leopold and Malmström [5] have shown that *p*-hydroxybenzaldehyde is obtained by nitrobenzene oxidation of lignin from various sources, and it follows that *p*-hydroxyphenyl groups occur in most lignins, although to a very small extent. Sphagnum species apparently contain a lignin which is built up chiefly from *p*-hydroxyphenyl units and thus differs from all lignins previously investigated. This investigation is being continued.

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2. Holmberg, B. *Ing. Vetenskapsakad. Handl.* **131** (1934) 26.
3. Lange, P. Private communication.
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Very Long Hydrocarbon Chains

I. The Synthesis of *n*-Dooctacontane and *n*-Hectane

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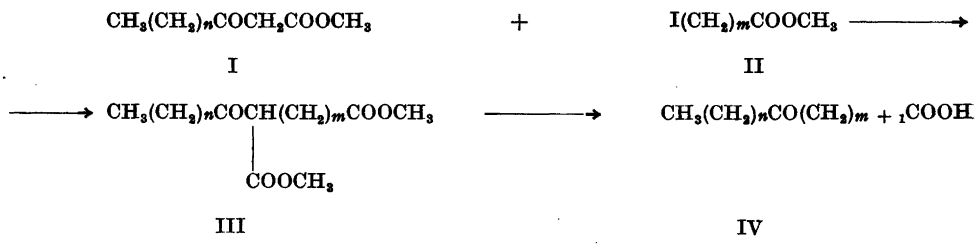
The normal hydrocarbon chain is one of the simplest types of chain polymers possible, and the properties of very long chain hydrocarbons are therefore of considerable theoretical interest. Bunn [1] has attempted to determine the shape of the $-CH_2-$ group from the powder diffraction patterns given by polythene obtained by thermal polymerization of ethylene under high pressure. He found serious discrepancies between the calculated and observed values of the intensities of the diffractions which were explained as partly due to anisotropic thermal motions of the atoms in the crystal, and partly due to a distortion of the electron clouds of the $-CH_2-$ groups. However, it has since been shown by infra-red spectroscopy that polythene contains a certain number of methyl side chains [2-5]. Polythene shows a band at 1 378 cm^{-1} which lies at the position characteristic of the symmetrical deformation vibration of the methyl group, and from a comparison of the intensity of this band in polythene and in branched chain hydrocarbons of known structure, Thompson and Torkington [3] estimate that approximately one methyl group is present per fifty methylene groups. They further found a weak absorption at about 1 720 cm^{-1}, indicating the presence of carbonyl groups. Ethylenic bonds and hydroxyl groups may also be present [5]. As the crystal behaviour of long chain hydrocarbons is markedly affected by the presence of methyl side chains [6], it seems possible that at least some of the discrepancies encountered by Bunn are due to the methyl side chains present.

It thus appears desirable to repeat the X-ray and spectroscopic studies using very long chain hydrocarbons of exactly known structure and chain length. One of the aims of the present work was to develop methods for the synthesis of such compounds.

The chain-lengthening method involving the use of long chain β-keto esters has been used in this laboratory for the synthesis of a large number of long chain compounds of different types. The method has yielded satisfactory results in the synthesis of ketones [6] and keto-acids [7,8] of fairly high molecular weight, and the yields in the case of keto-compounds with 30—36 carbon atoms have been at least as good as in the case of compounds with shorter chains. It was of interest to investigate the possibilities of the method for the preparation of very long carbon chains. The longest normal chain carboxylic acid so far described in the literature is *n*-hexatetracontanoic acid ($C_{46}H_{92}O_2$) prepared by Francis, King and Willis [9]. This acid was prepared by Clemmensen reduction of the corresponding 13-keto-acid, which was prepared by the modified Robinson — Robinson method [10]. The yield of the keto-acid was only 7 % of the theoretical, however.

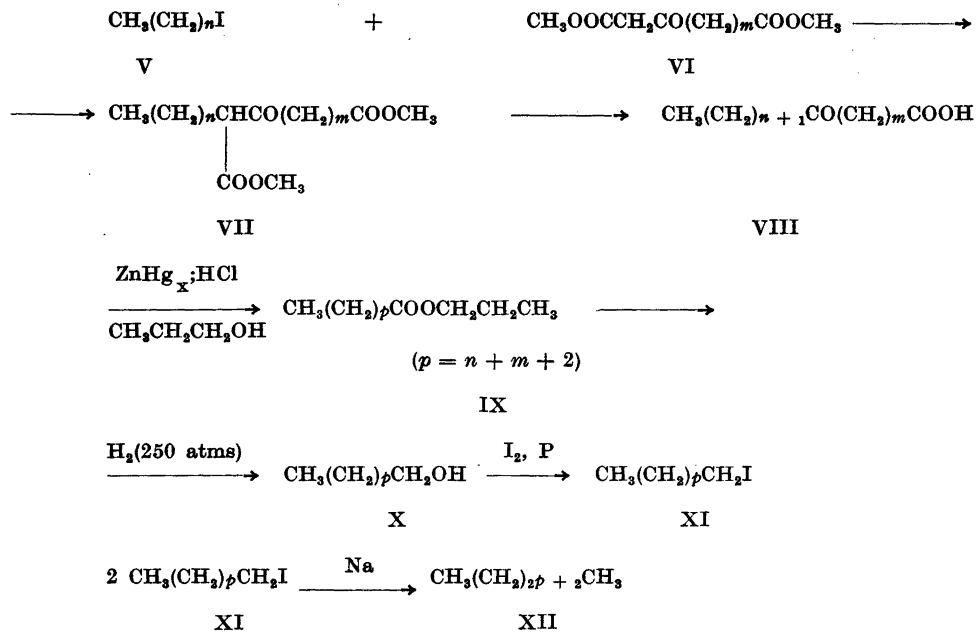
The separation of very long chain compounds in which the chain lengths differ only by a few. Carbon atoms is usually very difficult. It is essential, therefore, to use homologue-free starting material, and to employ chain-lengthening methods that do not give rise to by-products of chain-lengths comparable to that of the desired product.

The long chain β-keto ester method can be used in two different ways in the synthesis of long chain keto-acids. A long chain ω-iodo ester (II) may be used to alkylate a long chain β-keto ester (I) and the resulting mono-alkylation product (III), after hydrolysis and decarboxylation, gives the keto-acid (IV).



The second route uses 1-iodoalkanes (V) and β-keto esters (VI) derived from a monoester of a dibasic acid, and gives in an analogous manner the alkylation product (VII) and the keto-acid (VIII).

Both routes have been used in the work to be described. It has been found possible to prepare β-keto esters of the type (I) with $n = 28$ but the difficulty of separating the methyl ester of the acid used as initial material in the preparation of the β-keto ester increases as n gets larger. The preparation of pure ω-iodoesters of the type (II) with m larger than 16 is also laborious. For these reasons it was found advantageous to employ the second route for the syn-



thesis of the long chain keto-acids described in this communication, using the comparatively easily accessible methyl 2-ketooctadecane-1,18-dicarboxylate (VI, $m = 16$) (8) as "chain extender". Alkylation of (VI) with 1-iodododecane (V, $n = 11$) and 1-iododocosane (V, $n = 21$) respectively gave, after hydrolysis and ketonic cleavage of the alkylation product (VII), 18-ketohentriacontanoic acid (VIII, $n = 11$, $m = 16$), m.p. 103.6—104.1°, and 18-ketohentetracosanoic acid (VII, $n = 21$, $m = 16$), m.p. 112.4—112.8°, in yields of 69 and 64 %. 1-Iodononacosane (V, $n = 28$) and 1-iodohentriacontane gave in an analogous manner 18-ketooctatetracontanoic acid (VIII, $n = 28$, $m = 16$), m.p. 116.0—116.3°, and 18-ketopentacontanoic acid (VIII, $n = 30$, $m = 16$), m.p. 116.5—117.3°, respectively, in yields of 75—80 % of the theoretical.

The keto-acids crystallize with vertical double molecules. They may be obtained from solvents such as glacial acetic acid in the form of small, well-developed rhombic plates *Cf.* [12] with an obtuse angle of 113°. They give excellent X-ray diffraction patterns showing many $00l$ reflexions. No polymorphism has been observed.

In order to prepare long chain primary alcohols (X) the keto-acids of the type (VIII) had first to be reduced to the corresponding n-acids and the latter further reduced to the alcohols. Considerable work had to be performed in

order to get a satisfactory procedure for the reduction of the carbonyl group of the keto-acids. Various modifications of both the Wolff-Kishner [13] and Clemmensen method [14] were tried. The convenient modification of the former method due to Huang-Minlon [15] could not be used as the potassium salts of the long chain keto-acids were too insoluble in the reaction mixture. It was found that the Clemmensen method using concentrated hydrochloric acid and glacial acetic acid (or propionic acid) did not give satisfactory results, and that an improvement could be effected by carrying out the reduction in *n*-propanol saturated with dry hydrogen chloride. The Clemmensen reduction performed in this manner gives the *n*-propyl esters (IX) of the reduced acids. The two highest propyl esters were purified by molecular distillation and the yields of the pure distilled esters of *n*-hentetracontanoic acid and *n*-pentacontanoic acid were 70 and 73 % of the theoretical respectively.

The *n*-propyl esters are comparatively soft. Two crystalline modifications have been observed in each case but only a single melting point. In the case of the two highest esters, *viz.* *n*-propyl *n*-hentetracontanoate (IX, $p = 39$), m.p. 84.1—84.3°, and *n*-propyl *n*-pentacontanoate (IX, $p = 48$), m.p. 93.4—93.6°, crystallization from solvents gives a crystal form with long spacings of 60 and 70 Å respectively. It was found, however, that in both cases a very gradual transition took place on heating to a crystalline form with a shorter long spacing. The transition occurs over a large temperature interval (30° or more) and is complete just before the m.p. *n*-Propyl *n*-hentriacontanoate showed a different behaviour in that the melting point at 68.6—68.8° is that of the crystal form with the longer spacing.

The long chain *n*-propyl esters were converted into alcohols by high pressure hydrogenation over copper chromite catalyst. It was found that the best results were obtained when the hydrogenation was performed without solvent. The yield of *n*-pentacontanol-1 (X, $p = 48$) from *n*-propyl *n*-pentacontanoate was 87 % of the theoretical.

It is stated in the literature [16] that the melting points of the odd- and even-numbered higher *n*-alcohols lie on one curve, and that this indicates that the chains are vertical (A form) at the melting point. The odd-numbered alcohols crystallize in the A form at room temperature but the even-numbered alcohols crystallize with tilted chains (B form) and it is assumed that they adopt the A form near the m.p. Although this may be true for alcohols with less than 29 carbon atoms, we have found that it is not true for higher alcohols. Investigations with the recording high temperature camera [17] showed that the C_{29} and C_{31} alcohols prepared in the course of the present work, which were present in the A form at room temperature, changed to a tilted form, which we have called B′, just before the melting point. Furthermore, a specimen of the C_{32}

alcohol * was found to retain the B form up to the melting point. It seems likely, therefore, that the higher alcohols show alternation in the melting points, with those of the odd-alcohols falling on a curve lying very slightly below that of the even numbered ones. The behaviour of the *n*-alcohols near the m.p. should to be further studied.

n-Hentetracontanol-1 (X, $p = 39$) showed a transition at a temperature of 87° to a form with a shorter long spacing, which melted at 97.3—97.6°. The alcohol was found to melt at 96.3—96.5° on immediate reheating after solidification. *n*-Pentacontanol-1 (X, $p = 48$) showed a similar thermal behaviour with melting points at 104.5—104.7° and 103.4° respectively.

The iodides (XI) with 29, 31, 41 and 50 carbon atoms were synthesized from the corresponding alcohols by means of iodine and phosphorus in the usual manner. The yields were satisfactory, even in the case of the longest chain iodide so far prepared, *viz.* 1-iodopentacontane (XI, $p = 48$), for which the figure for the yield was 88 % of the theoretical.

1-Iodononacosane and 1-iodohentriacontane show a crystal behaviour similar to that of the lower homologues. They crystallize from solvents with tilted double molecules and the melting points of this form are 61.6—61.8° and 71.4—72.2° respectively. In both cases a lower melting modification has been observed, but, as has previously been found for the lower homologues (11), this form is only obtained after previous heating of the molten iodide to a temperature considerably above the m.p. of the higher melting modification.

1-Iodohentetracontane is also dimorphous with melting points at 84.0—84.3° and 81.8—82.0° respectively, the lower m.p. being observed for previously melted material. This iodide crystallizes in a manner quite different from that of the lower homologues, in that the higher-melting form crystallizes with tilted single molecules. The intensity distribution among the various 00*l* reflexions resembles that of a *n*-hydrocarbon. 1-Iodopentacontane also crystallizes with single molecules. Only one melting point, *viz.* 92.2—92.4°, has been observed in this case.

In 1889 Hell and Hägele [18] described the synthesis of dimyricyl from myricyl iodide. The iodide was prepared from myricylalcohol derived from carnauba wax. They considered their hydrocarbon to be *n*-hexacontane. The exact constitution of myricyl alcohol has been the subject of discussion. Gascard [19] was of the opinion that this alcohol contains 31 carbon atoms, and this is probably the reason why the dimyricyl of Hell and Hägele in a recent monograph dealing with hydrocarbons [20] is described as *n*-dohexacontane. However, the extensive studies of Chibnall, Piper *et al.*[21] indicate that

* We are indebted to Professor A. C. Chibnall, Cambridge, England, for this specimen.

the higher alcohols found in natural waxes are in most cases mixtures of closely related even-numbered homologues. Koonce and Brown [22] have recently shown that carnauba wax contains a mixture of the C_{28}, C_{30} and C_{32} alcohols, with the last mentioned predominating. Owing to the nature of the starting material the hydrocarbon of Hell and Hägele was thus not a pure compound, but their work showed that the Wurtz reaction could be used for the synthesis of hydrocarbons of very high molecular weight. The Wurtz reaction has since been used for the preparation of a large number of long chain hydrocarbons *Cf. e.g.* [19] and long chain ethers [23]. The longest *n*-hydrocarbon previously reported in the literature, *viz.* *n*-heptacontane, was prepared by the Wurtz reaction from decamethylene dibromide by Carothers, Hill, Kirby and Jacobson [24]. In this case the reaction products contains a series of *n*-hydrocarbons differing in chain length by 10 carbon atoms, but the isolation of hydrocarbons with more than 70 carbon atoms was not accomplished.

Ziegler, Weber and Gellert [23] have made a detailed study of the Wurtz reaction in order to find the conditions for optimum yield. Their work refers to the coupling of halogenalkyl-aryl ethers. They found that 1) iodides give better results than bromides, 2) potassium gives lower yields than sodium 3) up to a certain point an increase in the concentration of the halide improves the yield, and finally 4) when ethers are used as solvents an increase in the reaction temperature lowers the yield.

Our results are in general agreement with those of Ziegler *et al.* The presence of very long hydrocarbon chains is equivalent to dilution of the halide groups, with the result that the best yields were obtained when no solvent was used. Under these conditions, a fairly high reaction temperature (about 150°) together with vigorous shaking of the reaction vessel were necessary. The yields of practically pure hydrocarbons were 60—70 % of the theoretical.

n-Dooctacontane (XII, $p = 39$) and *n*-hectane (*n*-decacontane) (XII, $p = 48$) were thus obtained from 1-iodohentetracontane and 1-iodopentacontane respectively. Final purification of the hydrocarbons was carried out by treatment with hot sulphuric acid [21]. Although this treatment leads to considerable losses *Cf.* [25], it appeared the best way of purification available as the molecular weight was too large for molecular distillation. *n*-Dooctacontane melted at 110.3—110.4° and *n*-hectane at 115.1—115.4°.

A large number of empirical relations expressing the melting point as a function of the number of carbon atoms or the molecular weight of *n*-alkanes have been put forward [26,27]. The most satisfactory equation to date appears to be that due to Etessam and Sawyer [28]

$$T = \frac{414.5 \cdot M}{M + 94.4}$$

where T is the melting point in degrees Kelvin and M the molecular weight. The melting points of *n*-dooctacontane and *n*-hectane calculated with the aid of the above equation are 109.9° and 115.2° C respectively. These values agree well with those found experimentally.

The crystal behaviour of the hydrocarbons and some of the intermediates will be the subject of further study.

EXPERIMENTAL *

n-*Nonacosanol-1.* *n*-Nonacosanoic acid was prepared by Clemmensen reduction of 13-ketononacosanoic acid. The keto-acid of m.p. 102.8—103.0° was synthesized in 73 % yield from methyl 3-ketononadecanoate [29] and ethyl 11-iodohendecanoate in the manner described previously for the synthesis of 13-ketotetratriacontanoic acid [7]. The reduction of the keto-acid was carried out in the manner described for the C_{34} acid [7]. After crystallization from benzene, extraction of the crystalline material with hot acetone, and recrystallization from benzene, a 55 % yield of *n*-nonacosanoic acid of m.p. 90.1—90.4° was obtained. Francis and Piper [30] give m.p. 90.3°.

The acid was converted into the methyl ester by means of diazomethane, and the ester, after recrystallization from light petroleum (b. p. 40—60°), converted into alcohol by high pressure hydrogenation over copper chromite catalyst in the manner described below for the higher alcohols. After crystallization from ethanol, from chloroform, and from light petroleum (b.p. 40—60°), the alcohol formed lustrous plates melting at 84.5—84.7°. Piper, Chibnall and Williams [16] give m.p. 84.6—85.0°. At room temperature the alcohol gave a long spacing of 77.8 Å. Piper *et al.* give a value of 78.0 Å for the A form (with vertical chains) at room temperature. The diffraction pattern-temperature diagram (DPT-diagram *Cf.* [17]) showed that a transition from the A form to a form with tilted chains (B'), having a long spacing of 72 Å, occurred at 83°.

1-Iodononacosane. *n*-Nonacosanol-1 (2.42 g), iodine (0.80 g), and red phosphorus (0.06 g) were kept at a temperature of 145° for 5.5 hours. The iodide was extracted with benzene, and the benzene solution washed with water followed by 2 *N* sodium hydroxide solution. After washing with water again part of the benzene was distilled off. On cooling the benzene solution 1-iodononacosane (2.80 g or 92 % of the theoretical) crystallized out. The iodide had m.p. 67.5—68.0°. After recrystallization from benzene the iodide formed colourless lustrous plates of m.p. 67.5—67.8°. The long X-ray spacing at room temperature, calculated from a very good photograph, was 72.0 Å. The DPT-diagram showed that this crystal form persisted up to the m.p. just recorded. After heating the melt to about 85°, solidification occurred at 61.5°. On immediate reheating melting took place at 61.6—61.8°, indicating the existence of a low melting modification.

Calcd. for $C_{29}H_{59}I$ (534.7)	I	23.73 %,
Found	»	23.76 »

18-Ketooctatetracontanoic acid. 1-Iodononacosane (2.63 g), methyl 2-ketooctadecane-1,18-dicarboxylate (1.90 g *Cf.* [8]), dry potassium carbonate (2 g), and methyl-*n*-propyl ketone (7 ml, the ketone was distilled from dry potassium carbonate immediately before use)

* Melting points and crystal spacings were determined in the manner previously described *Cf. e.g.* [6]. The molecular still used was one made by Schott und Gen., Jena *Cf.* [6].

were refluxed (calcium chloride tube) for 24 hours. The mixture was acidified with dilute sulphuric acid and extracted with hot benzene. The hot benzene solution was washed with several portions of hot water. The benzene was distilled off, and the residue subjected to hydrolysis and ketonic cleavage by dissolving it in benzene (25 ml), and adding a solution of potassium hydroxide (8 g) in water (8 ml) and methanol (120 ml). The mixture was kept at a temperature of 55° for 60 hours with occasional shaking. Excess dilute hydrochloric acid and benzene were added, and the mixture heated to the boiling point for a few minutes. The lower clear water-methanol layer was removed. The upper (benzene) layer still contained undissolved material, and further portions of dilute hydrochloric acid and benzene (until the benzene layer had a volume of about 1 500 ml) were therefore added, and the mixture refluxed for 45 minutes. After this time both layers were clear. The benzene layer was separated and washed with hot water. Water and part of the benzene were distilled off from the benzene solution. On cooling, the benzene solution deposited crystals (2.87 g or 81.2 % of the theoretical) of 18-ketooctatetracontanoic acid, m.p. 115.5—116.2°. Crystallization from glacial acetic acid gave 2.80 g of m.p. 115.7—116.2°. Further crystallization of a portion of this material from chloroform gave the keto-acid in the form of lustrous, easily electrified crystals of m.p. 116.0—116.3°. Long X-ray spacing (pressed specimen) 126 Å.

Calcd. for $C_{48}H_{94}O_3$ (719.2)	C	80.15,	13.17 %
Found	»	80.13,	13.13 »

18-Ketohentriacontanoic acid was prepared in an analogous manner from 1-iodododecane (b.p. 118°, 2 mm) and methyl 2-ketooctadecane-1,18-dicarboxylate. The iodide was prepared from *n*-dodecanol-1 that had been distilled through a one metre electrically heated and packed column, the b.p. of the alcohol being 102°, 0.9 mm. The keto-acid was crystallized from light petroleum (b.p. 95—100°) and from glacial acetic acid. From the latter solvent the keto-acid was obtained in the form of lustrous plates melting at 103.6—104.1°. The yield of pure keto-acid was 69 % of the theoretical. Long X-ray spacing (pressed specimen) 82.4 Å.

Calcd. for $C_{31}H_{60}O_3$ (480.8)	C	77.44,	H 12.58 %
Found	»	77.31,	» 12.71 »

n-*Propyl* n-*hentriacontanoate*. The keto-acid just described was reduced by Clemmensen's method in *n*-propanol solution in a manner similar to that described below for the C_{50} homologue, the only difference being that benzene was used instead of xylene in the extractions. After crystallization from light petroleum (b.p. 40—60°), the ester formed small colourless, soft, lustrous plates melting at 68.6—69.0°. Solidification occurred at 68.4°, and remelting took place at 68.6—68.8°. Yield 82 % of the theoretical.

The X-ray diffraction pattern of a crystallized specimen at room temperature showed that two crystalline forms with long spacings of 45 and 40.5 Å respectively were present. The DPT-diagram showed that the form with the shorter spacing disappeared on heating at about 54°. A previously melted specimen showed the 45 Å spacing only.

Calcd. for $C_{34}H_{68}O_2$ (508.9)	C	80.24,	H 13.47 %
Found	»	80.44,	» 13.48 »

n-*Hentriacontanol-1* was prepared by high pressure hydrogenation of the ester just described, the procedure being similar to that described for the C_{50} alcohol. After crystallization from a mixture of chloroform and light petroleum (b.p. 95—100°), the alcohol formed colourless lustrous plates melting at 87.1—87.2°. Solidification occurred at 86.5°, and the alcohol remelted at 87.0—87.2°. The melting and solidification points were unchanged after further crystallization from a mixture of ether and benzene (1 : 1) and from

light petroleum (b.p. 95—100°). Long X-ray spacing (crystallized specimen at room temperature) 83.0 Å. The DPT-diagram showed that a transition occurred at 85.5° to a form with a long spacing of 76 Å. The m.p. of this form as read from the DPT-diagram was 87.4°, which agrees, within the experimental error, with that recorded above.

Calcd. for $C_{31}H_{64}O$ (452.8)	C	82.22,	H	14.25 %
Found	»	82.24,	»	14.23 »

1-*Iodohentriacontane* was prepared from the alcohol in the manner described for the C_{50} homologue. After crystallization from ethanol-light petroleum (b.p. 100—120°) (5:1) the iodide formed colourless lustrous plates of m.p. 71.4—72.2°. The yield was 89 % of the theoretical. The melting point was unchanged after crystallization from acetone. Solidification occurred at about 68° and the iodide remelted at the temperature just mentioned. After the melt had been heated to 95°, however, solidification occurred at 65.9° and remelting took place at 66.0—66.2°, showing the existence of a lower melting modification. Long X-ray spacing (pressed specimen) 75.8 Å.

Calcd. for $C_{31}H_{63}I$ (562.7)	I	22.56 %		
Found	»	22.25 »		

18-Ketohentetracontanoic acid was prepared in a manner analogous to that just described for the C_{48} acid, using *n*-docosyl iodide instead of *n*-nonacosyl iodide. The *n*-docosyl iodide used had m.p. 48.2—48.4° and s.p. 41.4° (Francis *et al.* [11] give m.p. 48.2°, s.p. 40.6°). After crystallization from propionic acid, the keto-acid (64 % of the theoretical) melted at 111.5—112.0°, but a small part of the material did not melt until at about 125°. On further crystallization from methyl-*n*-hexyl ketone and from light petroleum (b.p. 95°) the amount of higher melting material increased, and after three recrystallizations the m.p. of the high-melting material was 130—131°. On cooling, solidification in the form of fine needles began at 129.5°. The compound contained nitrogen (found: N, 1.25 %), and evidently consisted of the acid ammonium salt of the keto-acid (calcd: N, 1.11 %), ammonia having been taken up from the laboratory air. The long X-ray spacing was the same as that of the free keto-acid reported below.

After crystallization from glacial acetic acid, the keto-acid was obtained in the form of small rhombic plates melting at 112.2—112.6°. Solidification with the formation of well-developed plates in the melt began at 111.5°, and remelting occurred at 111.8—112.6°.

A pressed specimen of the keto-acid gave a good X-ray diffraction pattern with a long spacing of 108 Å.

Calcd. for $C_{41}H_{80}O_3$ (621.1)	C	79.29,	H	12.98 %
Found	»	78.75,	»	12.95 »

n-*Propyl* n-*hentetracontanoate* was prepared from 4.54 g of 18-ketohentetracontanoic acid in a manner exactly analogous to that described for *n*-propyl *n*-pentacontanoate. After crystallization from ethyl acetate the ester (4.4 g) melted at 84.0—84.5°, solidified at 83.5°, and remelted at 83.9—84.0°. After molecular distillation the m.p. of the main fraction (3.2 g) as obtained in the still was 84.2—84.3°. The ester solified without nucleus at 83.9° and remelted at 84.1—84.3°. After crystallization from *n*-propanol the ester was obtained in the form of very small lustrous plates melting at 83.9—84.4°. Solidification occurred at 83.9° and the ester remelted at 84.1—84.3°. The long X-ray spacing of a crystallized specimen at room temperature was 60 Å, and a melted specimen showed a spacing of 53 Å. The DPT-diagram showed that on heating a crystallized specimen with a long crystal spacing of 60 Å at room temperature, a gradual transition to the form with the shorter spacing occurred. The diagram showed no discontinuity in the lines.

The decrease of the long spacing took place continously from 60° upwards, and the transition to the form with the shorter spacing was complete just before the m.p.

Calcd. for $C_{44}H_{88}O_2$ (649.1) C 81.41, H 13.66 %
Found » 81.52, » 13.68 »

n-*Hentetracontanol-1* was obtained by high pressure hydrogenation of the above ester (14.43 g) in the manner described for the C_{50} alcohol. The yield of *n*-hentetracontanol-1 of m.p. 97.2—97.4° was 10.45 g or 79 % of the theoretical. After recrystallization from light petroleum (b.p. 96—100°) the alcohol was obtained in the form of lustrous plates of m.p. 97.3—97.6°. Solidification (without nucleus) occurred at 95.6° and on rapid reheating melting took place at 96.3—96.5°. The DPT-diagram for a specimen crystallized from light petroleum showed that the alcohol at room temperature possessed the A form with a long spacing of 108.2 Å. On heating a transition occurred at 87° to a form with a long spacing of 93 Å. The m.p. of this form as read from the diagram was 98.0°.

Calcd. for $C_{41}H_{84}O$ (593.1) C 83.03, H 14.28 %
Found » 83.04, » 14.28 »

1-Iodohentetracontane. The iodide was prepared from the alcohol in the manner described for the C_{50} homologue. The reaction mixture was extracted with hot xylene. After part of the xylene had been distilled off the solution was allowed to cool to room temperature. The iodide separated in the form of slightly brownish, lustrous plates. The crystals were dissolved in ethyl acetate and the solution filtered. On cooling, the iodide separated in the form of colourless lustrous plates of m.p. 84.0—84.3°. Solidification occurred at 81.6° and the iodide remelted at 81.8—82.0°. The mode of solidification of the low-melting modification was similar to that of the lower homologues just described, but in this case it was not necessary to heat the melt much above the m.p. in order to obtain the low-melting form on solidification. The iodide (crystallized from ethyl acetate) gave a beautiful diffraction pattern with a long spacing of 49.0 Å. The DPT-diagram showed that this crystal form persisted up to the higher of the two melting points recorded above. A previously melted specimen showed two sets of lines, corresponding to long spacings of 49.0 and 46.8 Å respectively.

Calcd. for $C_{41}H_{83}I$ (703.0) I 18.06 %
Found » 17.71 »

18-Ketopentacontanoic acid was prepared in the manner described for the lower homologues, starting from 1-iodohentriacontane and methyl 2-ketooctadecane-1,18-dicarboxylate. After crystallization from xylene the keto-acid (75 % of the theoretical) melted at 116.5—117.3°. The keto-acid is very insoluble in most organic solvents at room temperature. It is best crystallized from hot xylene, methyl-*n*-hexyl ketone, or propionic acid, and separates on cooling in the form of small rhombic plates. The keto-acid dissolves in concentrated sulphuric acid on slight warming to give colourless solutions, but on cooling the solution to temperatures below room temperature gelation takes place.

Long X-ray spacing (pressed specimen) 131 Å. No polymorphism has been observed.

Calcd. for $C_{50}H_{98}O_3$ (747.3) C 80.36, H 13.22
Found » 80.04, » 13.20

n-*Propyl* n-*pentacontanoate.* 18-Ketopentacontanoic acid (3.68 g), mossy amalgamated zinc (100 g, Baker's Analyzed) and *n*-propanol (150 ml) were placed in a round bottomed flask of 500 ml capacity, fitted with gas inlet tube and reflux condenser. The mixture was heated in order to dissolve the keto-acid, and the solution saturated with dry hydrogen chloride from a commercial cylinder. After refluxing on the sand bath for three hours, the solution was again saturated with hydrogen chloride, and this was repeated at three

hours intervals. After nine hours the solution was no longer homogeneous. In order to remove water and zinc chloride formed, the reduction was interrupted. Water was added and the organic material extracted with hot xylene. The xylene solution and the zinc used were washed with hot water, the xylene solution added to the zinc, and water and xylene removed by distillation under reduced pressure. Fresh amalgamated zinc was added to replace the metal used up, *n*-propanol added, the mixture saturated with dry hydrogen chloride and the reduction continued in the manner described above for nine hours, after which time water and zinc chloride were again removed in the manner just described. The reduction was then continued for a third period of 9 hours, the solution being saturated with hydrogen chloride at three hours intervals.

The organic material was extracted with hot xylene and the solution washed with hot water. The solvent was removed under reduced pressure, and the colourless residue dissolved in ethyl acetate. The solution was filtered hot in order to remove a small amount of undissolved material. On cooling, the filtrate gave 3.48 g of white, crystalline *n*-propyl ester of *n*-pentacontanoic acid. The m.p. of the ester obtained was 92.8—93.2°. The ester was distilled in the molecular still, the following fractions being taken:

I	0.50 g	93.4—93.6°
II	2.30 g	93.4—93.6°
III	0.07 g	93.2—93.4°

The yield of pure *n*-propyl *n*-pentacontanoate (fractions I + II) was 2.80 g or 73 % of the theoretical, calculated on the keto-acid used. After crystallization from light petroleum (b.p. 95—100°) the ester formed small lustrous plates melting at 93.4—93.6°. Solidification took place at 93.35° with the formation of small needles within the melt.

The long X-ray spacing of a crystallized specimen at room temperature was 70 Å. A melted specimen gave a spacing of 61.5 Å. The DPT-diagram of a crystallized specimen showed a gradual transition to the form with the shorter spacing, the transition being of the same type as in the case of the lower homologue.

Calcd. for $C_{53}H_{106}O_2$ (775.4)	C	82.09,	H	13.78 %
Found	»	82.17,	»	13.73 »

n-*Pentacontanol-1.* *n*-Propyl *n*-pentacontanoate (3.48 g) and copper chromite catalyst (2.5 g) were placed in a high pressure reaction vessel of 40 ml capacity. The vessel was filled with hydrogen at a pressure (at room temperature) of 150 kgs/sq. cm, and the hydrogenation carried out at a temperature of 250°. After 2.5 hours at 250° there was no further drop in the pressure. The reaction products were taken up in 500 ml of hot light petroleum (b.p. 96—100°). The solution was filtered to remove suspended catalyst, and on cooling 2.80 g (87 % of the theoretical) of *n*-pentacontanol-1 were obtained. The m.p. of the alcohol was 104.5—104.7°. Solidification occurred at 102.1°. On immediate reheating melting occurred at 103.4°, but after cooling down a few degrees followed by slow reheating the m.p. was 104.3—104.6°. The X-ray diffraction pattern of a crystallized specimen showed a long spacing of 131 Å (A form). A few weak lines corresponding to a second crystalline form were seen in the diffraction patterns of some specimens. The DPT-diagram showed that the latter form disappeared on heating and that the form with the 131 Å spacing persisted up to the melting point.

Calcd. for $C_{50}H_{102}O$ (719.3)	C	83.48,		14.29 %
Found	»	83.83,		14.40 »

1-Iodopentacontane. *n*-Pentacontanol-1 (2.0 g), iodine (0.4 g) and red phosphorus (0.03 g) were kept at a temperature of 148° for 9 hours. The iodide was extracted from the reaction mixture by warm xylene. The xylene solution was washed with water, fol-

lowed by 2 N sodium hydroxide solution and water again. Part of the xylene was distilled off *in vacuo* and, on cooling to room temperature, the remaining solution deposited yellow-greyish crystals of iodide. The material was dissolved in 400 ml of diisopropyl ether, and the hot solution treated with charcoal, filtered, and allowed to cool to room temperature. 2.0 Grams (88 % of the theoretical) of 1-iodopentacontane, m.p. 92.1—92.3°, s.p. 91.5°, were obtained in the form of small colourless lustrous plates. Remelting took place at 92.2—92.4°. After heating the melt to 113° the same solidification and remelting points were obtained. A crystallized specimen of the iodide gave a long spacing of 54 Å.

Calcd. for $C_{50}H_{101}I$ (829.2) I 15.31 %
Found » 15.11 »

n-*Dooctacontane*. 1-Iodohentetracontane (1.0 g) and sodium (0.5 g) were placed in a thick-walled glass tube of 25 ml volume. The tube was sealed off, placed in a shaking oven and shaken at a temperature of 155° for 34 hours. The temperature of the oven was kept constant within very close limits by a thermocouple and a temperature controller.

After cooling, the tube was opened and the solid cake dissolved in a mixture of xylene and ethanol. The mixture was heated and boiled until all sodium had disappeared. The solution was then washed twice with hot water, and the xylene layer, which now contained a suspended precipitate of hydrocarbon, separated and allowed to cool slightly. The precipitated material was filtered off and crystallized from light petroleum (B.D.H., b.p. above 120°), giving 0.48 g (58 % of the theoretical) of colourless *n*-dooctacontane melting at 109.6—110.0°. In order to remove unsaturated impurities, the hydrocarbon was treated with concentrated sulphuric acid at 110° for one hour. Water was added, and the dark-coloured hydrocarbon taken up in hot xylene. The xylene was distilled off and the residue dissolved in butyl acetate and treated with charcoal. After crystallization from butyl acetate the product was slightly yellow, and the charcoal treatment was therefore repeated. After a final crystallization from butyl acetate 0.20 g of *n*-dooctacontane was obtained in the form of small colourless lustrous plates. The m.p. was 110.3—110.4°. Solidification took place at 110.0° with the formation of long needles within the melt, and the hydrocarbon remelted at 110.3—110.4°.

Long X-ray spacing (pressed specimen) 106 Å.

Calcd. for $C_{82}H_{166}$ (1.152.1) C 85.48, H 14.52 %
Found » 85.50, » 14.52 »

n-*Hectane* (n-*Decacontane*). 1-Iodopentacontane (1.09 g) and sodium (0.5 g) were placed inside a thick-walled tube, which was sealed off and heated for 38 hours to a temperature of 154° in the shaking oven. After cooling, the tube was opened and the solid cake dissolved in a mixture of xylene and ethanol. The mixture was boiled gently until all sodium had disappeared. Owing to the low solubility of the hydrocarbon, it became necessary to add light petroleum (b.p. above 120°) to the xylene-ethanol mixture in order to get a clear solution. The solution was washed with hot water and allowed to cool, giving 0.78 g of crude hydrocarbon. Crystallization from butyl acetate gave 0.76 g of white microcrystalline material of m.p. (after previous melting) 111.7—112.5°. After two crystallizations from high-boiling (b.p. above 120°) light petroleum and one from butyl acetate, in each case using a solvent volume of about 200 ml and filtering off the crystals formed at a temperature of 50°, 0.50 g (61 % of the theoretical) of hydrocarbon was obtained. M.p. 113.8—114.2°. The hydrocarbon solidified at 113.6°. 0.3 Gram of this product was treated with concentrated sulphuric acid for one hour at 110°. After cooling, water was cautiously added, and the hydrocarbon extracted with hot light petroleum (b.p. 96—100°). The solution was washed twice with hot water. The light

petroleum layer was separated and allowed to cool. The hydrocarbon separated on cooling in microcrystalline form and was filtered off with some difficulty. The greyish mass obtained was dissolved in 100 ml of butyl acetate, and the still slightly greyish crystals obtained on cooling this solution treated with charcoal in hot butyl acetate solution and again crystallized from this solvent, giving 0.06 g of *n*-hectane in the form of small, colourless, lustrous plates. M.p. 115.1—115.4°. The hydrocarbon solidified at 114.9° and remelted at 115.1—115.4°. Long X-ray spacing (pressed specimen) 130 Å.

Calcd. for $C_{100}H_{202}$ (1.404.6)	C	85.50,	H	14.50	%
Found	»	85.45,	»	14.47	»

SUMMARY

The long chain β-keto ester method has been applied to the synthesis of a series of long chain keto-acids, up to and including 18-ketopentacontanoic acid. Alkylation of methyl 2-ketooctadecane-1,18-dicarboxylate with the appropriate long normal chain iodides gives, after hydrolysis and ketonic cleavage, 18-keto-acids in 63 to 81 % yield. The keto-acids are reduced by Clemmensen's method using *n*-propanol and dry hydrogen chloride. The propyl esters obtained in the reduction have been converted into the corresponding alcohols by high pressure hydrogenation over copper chromite. The alcohols have finally been converted into the iodides. The overall yield keto-acid → iodide is of the order of 50 % of the theoretical. The long chain compounds have been carefully purified, in many cases by molecular distillation, and their purity checked by means of thermal and X-ray investigations. It has been found that the crystal behaviour of *n*-alcohols and iodides with more than 40 carbon atoms is considerably different from that of the lower homologues.

1-Iodohentetracontane and 1-iodopentacontane give in the Wurtz reaction *n*-dooctacontane and *n*-hectane (*n*-decacontane) respectively. The latter hydrocarbon with a molecular weight of 1 404.6 and m.p. 115.1—115.4° is the longest chain *n*-hydrocarbon so far synthesized.

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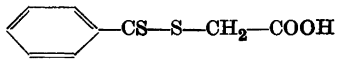
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On Carboxymethyl Dithiophenylacetate and Its Reactions with Amines, Amino Acids and Peptides

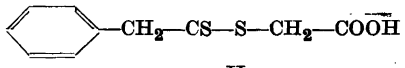
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In a previous communication [1] the applicability of carboxymethyl dithiobenzoate (I) as a means of introducing the thiobenzoyl-grouping in esters and amides of α-amino acids was demonstrated. The present paper describes the preparation of the heretofore unknown, analogous carboxymethyl dithiophenylacetate (II), which has proved to be a very convenient reagent for introducing the phenylthioacetyl-grouping in amines, amino acids and peptides.



I

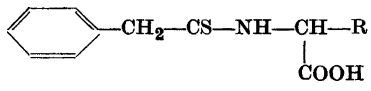


II

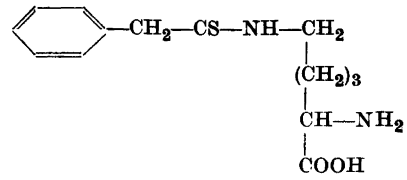
Phenylthioacetylation of amino acids has formerly been achieved by means of sodium dithiophenylacetate [2], although in poor yields (20–25 %). Better results were obtained when methyl dithiophenylacetate was employed [3], a procedure recently adopted by other authors [4,5].

Upon reaction of benzylmagnesium chloride with carbon disulphide and chloroacetic acid, carboxymethyl dithiophenylacetate (II) was produced as an intensely yellow compound, separating from hexane in a total yield of 60–70 %. The acid could be stored for months in a closed vessel with no signs of deterioration. Its reactions with ammonia, morpholine and aniline proceeded with great ease at room temperature, rendering the substituted thioamides in satisfactory yields. Similarly, reaction of the dithioacid with glycine, L- and DL-alanine, L-valine, DL-leucine, L-isoleucine and DL-methionine afforded the respective phenylthioacetyl-derivatives (III) as colourless crystalline compounds, while the corresponding oily reaction products of DL-phenylalanine, L-leucine and DL-valine were not obtained in crystalline form. No reaction was noticed with α-aminoisobutyric acid, the failure being

ascribable to sterically unfavorable conditions as suggested previously in the case of thiobenzoylation. When DL-lysine was treated with one mole of the dithioacid (II), a mono-thioacylated derivative was obtained for which the structure (IV) is tentatively suggested, on the basis of the finding that the substance produced a positive ninhydrin reaction. On repeating the experiment with three moles of dithioacid, an oil was obtained which yielded the *mono*-derivative (IV) as the only crystalline constituent.



III



IV

In order to extend the reaction to amino amides and peptides, glycinamide and L-tyrosinamide were successfully submitted to phenylthioacetylation. Again, well crystallizing derivatives were obtained from glycylglycine, glycylglycylglycine and DL-alanylglycine. Glycyl-L-tryptophane yielded a crystalline product, which could not be obtained in analytically pure form.

L-Asparagine gave an 80 % yield of the phenylthioacetyl-derivative, which was selected to prove the sterical homogeneity of the thioacylated compounds. Oxidative desulphurization with hydrogen peroxide in slightly alkaline solution provided N-phenylacetyl-L-asparagine, whose dextrorotation practically agreed with that of a synthetic specimen, prepared by phenacetylation of L-asparagine under non-racemizing conditions.

From the experimental evidence presented above, it may be concluded that carboxymethyl dithiophenylacetate can generally be used as a reagent for introducing the phenylthioacetyl-grouping in amines, amino acids and peptides. The reactions proceed without racemization at room temperature and in satisfactory yields.

EXPERIMENTAL *

Carboxymethyl dithiophenylacetate (II)

A Grignard-solution, prepared under nitrogen from 16.5 g of magnesium (0.68 mole), 82.2 g of benzyl chloride (0.65 mole) and 300 ml of dry ether, was added during one hour to a well agitated and ice-cooled solution of 76.5 g of dry carbon disulphide (1.01 mole)

* All melting points are uncorrected and determined in capillary tubes in an electrically heated bloc.

in 150 ml of dry ether. The mixture was kept overnight, thus acquiring room temperature. Then the solution was poured onto 400 g of crushed ice and the aqueous layer separated from the ethereal phase which contained a voluminous amorphous precipitate. A solution of 64.2 g of chloroacetic acid (0.68 mole) and 38 g of sodium carbonate in 250 ml of water was added in one portion to the aqueous phase, and within a few minutes, the mixture was transformed into a yellow mass of fine needles, consisting of the sodium salt of (II). After standing at 0° for 48 hours, a mixture of 45 ml of concentrated sulphuric acid (0.83 mole) and 50 ml of water was added dropwise to the crystal slurry in order to liberate the free dithioacid. A small amount of a brownish, oily substance separated and was taken up in ether. After thorough extraction with several portions of fresh ether, all ether extracts were pooled, dried over sodium sulphate and the ether removed, leaving the final product as a yellow crystalline mass. Recrystallized from ligroin, 87 g (64 %) of carboxymethyl dithiophenylacetate was obtained. M. p. 78—79°.

A sample for analysis was prepared by an additional crystallization from ligroin. M. p. 79—80°.

$C_{10}H_{10}O_2S_2$ (226.30)	Calc.	C 53.07	H 4.45	S 28.33
	Found	» 53.03	» 4.56	» 27.99

Neutralization equivalent found by microtitration (indicator : phenolphthalein) : 225.

Phenylthioacetylation of amines, α-amino acids, amino amides and peptides

Phenylthioacetamide. Separated as prisms from a solution of 595 mg of (II) in 10 ml of dilute aqueous ammonia. Yield 321 mg (81 %). M. p. 98°. (Lit.[6] 97.5—98°).

Phenylthioacetanilide. To a solution of 667 mg of (II) in 2.95 ml of 1 *N* NaOH was added 0.5 ml of aniline, whereupon an oil separated which rapidly became crystalline. Yield 641 mg (96 %). Recrystallized from benzene-hexane as pale yellow needles. M. p. 89°. (Lit.[7] 87°).

Phenylthioacetomorpholide. From a solution of 456 mg of (II) in 2.02 ml of 1 *N* NaOH containing 0.25 ml of morpholine, an oil separated which on cooling gave 434 mg (97 %) of crystalline material. Recrystallized from dilute methanol as compact, colourless rhombs. M. p. 79—80°. (Lit.[8] 79—80°).

N-Phenylthioacetylglycine (III, R = H). A mixture of 2.26 g of (II) and 0.75 g of glycine in 10.0 ml of 2 *N* NaOH after standing and acidification deposited 1.80 g (86 %) of pale yellow plates. Recrystallized from water, the colourless compound melted at 141—142°. (Lit.[2] 142°).

N-Phenylthioacetyl-L-alanine (III, R = CH_3). To 0.84 g of L-alanine, dissolved in 18.8 ml of 1 *N* NaOH was added 2.13 g of (II). Acidification gave an oil which only after prolonged cooling crystallized partly. Twice recrystallized from ether-petroleum ether, the substance melted at 121—122°.

$C_{11}H_{13}O_2NS$ (223.28)	Calc.	N 6.27	S 14.36
	Found	» 6.32	» 14.34

Neut.equiv. found 222 (microtitration).

Rotation: $[\alpha]_D^{23} = -2.07°$ (c = 2.5 g pr. 100 ml of solution; methanol).

N-Phenylthioacetyl-DL-alanine (III, R = CH_3). Prepared exactly as described for the L-isomeride. Yield 75 %. M. p. 121°. (Lit.[2] 121°).

N-Phenylthioacetyl-L-valine (III, R = *iso*-C_3H_7). From 0.53 g of L-valine and 1.02 g of (II) in the usual way. The oily, crude material crystallized on keeping, yielding 0.81 g

(72 %) of a yellowish product. Crystallized from ether-petroleum ether in irregular, colourless plates. M. p. 100—101°.

$C_{13}H_{17}O_2NS$ (251.34)	Calc.	N	5.70	S	12.76
	Found	»	5.67	»	12.79

Neut.equiv. found 250 (microtitration).

Rotation: $[\alpha]_D^{23} = -13.80°$ ($c = 1.5$; methanol).

The corresponding DL-isomeride could not be obtained in crystalline form during this investigation, but has been recorded previously [4] with m. p. 102—103°.

N-Phenylthioacetyl-DL-leucine (III, R = *iso*-C_4H_9). Prepared from 262 mg of DL-leucine and 452 mg of (II) in 4.00 ml of 1 *N* NaOH. After acidification, the oily product was extracted with ether, which after drying was removed by spontaneous evaporation, leaving 420 mg (78 %) of a crystalline product. This, recrystallized from ether-petroleum ether for analysis, had m. p. 111°.

$C_{14}H_{19}O_2NS$ (265.36)	Calc.	N	5.28	S	12.08
	Found	»	5.36	»	12.00

Neut.equiv. found 267 (microtitration).

N-Phenylthioacetyl-L-isoleucine (III, R = *sec.*-C_4H_9). From 456 mg of L-isoleucine and 790 mg of (II) in 7.00 ml of 1 *N* NaOH in the usual way. Yield 702 mg (76 %). Colourless, dense prisms from chloroform-petroleum ether. M. p. 95—96°.

$C_{14}H_{19}O_2NS$ (265.36)	Calc.	N	5.28	S	12.08
	Found	»	5.48	»	11.93

Neut.equiv. found 265 (microtitration).

Rotation: $[\alpha]_D^{23} = -7.70°$ ($c = 1.5$; methanol).

The corresponding DL-isomeride has previously been reported [4]; m. p. 95—96°.

N-Phenylthioacetyl-DL-methionine (III, R = $CH_3SCH_2CH_2$). Treated in the usual way, a mixture of 450 mg of DL-methionine and 682 mg of (II) in 6.05 ml of 1 *N* NaOH yielded on acidification 640 mg (75 %) of crystalline material. Colourless plates from chloroform-petroleum ether. M. p. 117—119°.

$C_{13}H_{17}O_2NS_2$ (283.40)	Calc.	N	4.94	S	22.63
	Found	»	5.03	»	22.55

Neut.equiv. found 282 (microtitration).

ε-(N-Phenylthioacetyl)-DL-lysine (IV). To a solution of 1.02 g of lysine monohydrochloride in 16.8 ml of 1 *N* NaOH was added 1.26 g of (II). On acidification, 0.88 g (56 %) of crystalline material separated after triturating the crude reaction mixture with hot ethanol. M. p. 250° (dec.). The compound produced an intensively violet colour on short heating with ninhydrin.

$C_{14}H_{20}O_2N_2S$ (280.38)	Calc.	N	9.99	S	11.44
	Found	»	9.86	»	11.37

N-Phenylthioacetyl-glycinamide. A smooth reaction ocurred upon mixing 0.90 g of glycinamide hydrogen sulphate [9] and 1.19 g of (II) in 15.7 ml of 1 *N* NaOH, immediately followed by the separation of 0.86 g (79 %) of practically colourless plates. Recrystallized twice from chloroform-petroleum ether for analysis. M. p. 104—105°.

$C_{10}H_{12}ON_2S$ (208.28)	Calc.	N	13.45	S	15.39
	Found	»	13.45	»	15.54

N-Phenylthioacetyl-L-tyrosinamide. To a solution of 442 mg of (II) in 2.00 ml of 1 *N* NaOH was added 351 mg of L-tyrosinamide dissolved in 3.5 ml of lukewarm water. After

a few minutes, 469 mg (77 %) of a crystalline powder could be collected. Separated as stout prisms from dilute methanol. M. p. 88—90°.

$C_{17}H_{18}O_2N_2S$ (314.39) Calc. N 8.91 S 10.20
Found » 8.86 » 10.14

Rotation: $[\alpha]_D^{23} = +77.69°$ ($c = 2.5$; methanol).

N-Phenylthioacetyl-glycylglycine. A mixture of 0.93 g of glycylglycine hydrochloride monohydrate and 1.13 g of (II) in 15.0 ml of 1 *N* NaOH was acidified, when 0.75 g (56 %) of crystals separated, which after recrystallization from chloroform-petroleum ether melted at 157—158° (dec.).

$C_{12}H_{14}O_3N_2S$ (266.31) Calc. N 10.52 S 12.04
Found » 10.48 » 11.93

Neut.equiv. found 268 (microtitration).

N-Phenylthioacetyl-glycylglycylglycine. Similarly, after dissolution of 0.84 g of triglycine and 1.00 g of (II) in 8.9 ml of 1 *N* NaOH, acidification yielded 0.93 g (65 %) of a crystalline product, which could be purified by recrystallization from chloroform-petroleum ether. M. p. 166—167° (dec.).

$C_{14}H_{17}O_4N_3S$ (323.36) Calc. N 12.99 S 9.92
Found » 12.96 » 9.71

Neut.equiv. found 321 (microtitration).

*N-Phenylthioacetyl-*DL*-alanylglycine.* From a solution of 420 mg of DL-alanylglycine and 650 mg of (II) in 5.74 ml of 1 *N* NaOH, addition of excess acid precipitated 570 mg (71 %) of the crude reaction product. Recrystallized once from chloroform-petroleum ether for analysis. M. p. 181—182° under evolution of gas.

$C_{13}H_{16}O_3N_2S$ (280.34) Calc. N 9.99 S 11.44
Found » 9.91 » 11.52

Neut.equiv. found 277 (microtitration).

*N-Phenylthioacetyl-*L*-asparagine* (III, R = CH_2CONH_2). To 1.62 g of L-asparagine monohydrate in 10.8 ml of 1 *N* NaOH, a solution of 2.44 g of (II) in 10.8 ml of 1 *N* NaOH was added. After standing for 20 hours, cooling and acidification yielded 2.42 g (84 %) of practically pure crystalline substance. Separated from ethyl acetate-petroleum ether in beautiful, colourless plates. Dried for 18 hours at room temperature over P_2O_5 for analysis. M. p. 139—140° under evolution of gas.

$C_{12}H_{14}O_3N_2S$ (266.31) Calc. N 10.52 S 12.04
Found » 10.46 » 11.99

Neut.equiv. found 265 (microtitration).

Rotation: $[\alpha]_D^{23} = +85.10°$ ($c = 1.1$; methanol).

Oxidative desulphurization of N-phenylthioacetyl-L-asparagine

To a solution of 266 mg of N-phenylthioacetyl-L-asparagine in 1.00 ml of 1 *N* NaOH was added dropwise with cooling and vigorous stirring 450 mg of 30 % hydrogen peroxide. After half an hour, the reaction mixture was acidified and 220 mg (88 %) of N-phenylacetyl-L-asparagine was collected as colourless crystals. Separated from water as glistening, thin plates. M. p. 189—190°. A non-analyzed preparation with m. p. 180—181° is recorded in the literature [10].

$C_{12}H_{14}O_4N_2$ (250.25) Calc. C 57.58 H 5.64 N 11.20
Found » 57.80 » 5.80 » 11.14

Neut.equiv. found 249 (microtitration).

Rotation: $[\alpha]_D^{23} = +8.95°$ ($c = 1.0$; methanol (supersat.)).

Phenacetylation of L-asparagine

When 2.00 g of L-asparagine was treated with 2.5 g of phenylacetyl chloride in a solution of potassium bicarbonate according to Schotten-Baumann, 1.65 g of N-phenacetyl-L-asparagine was obtained. Recrystallized twice from hot water. M. p. 189°.

Rotation: $[\alpha]_D^{23} = +9.01°$ ($c = 1.0$; methanol).

SUMMARY

Carboxymethyl dithiophenylacetate has been prepared, and its potentialities as a phenylthioacetylating agent towards amines, amino acids and peptides have been demonstrated.

The reactions proceed without racemization.

The microanalyses have been performed in this Laboratory by Mr. A. Grossmann.
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The Kinetics of Decomposition of Potassium Pentathionate in Alkaline Solution

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The velocity of the reaction

$$2\ S_5O_6^{--} + 6\ OH^- \rightarrow 5\ S_2O_3^{--} + 3H_2O \qquad (1)$$

has been found to be proportional to the concentrations of pentathionate and hydroxyl ions to the first power and to show a positive salt effect as to be expected from a rate-determining step involving two ions with one and two charges of the same sign, respectively.

The velocity is — *ceteris paribus* — independent of the presence of $S_2O_3^{--}$, $S_4O_6^{--}$, NH_3 or CH_3COO^-.

A discussion of these results shows that the first and rate-determining step in the sequence of reactions resulting in (1) is

$$S_5O_6^{--} + HO^- \rightarrow S_2O_3^{--} + HOSS_2O_3^-$$

An automatic device called a potentiostat is described.

Previous investigations (for references see Foss [1] and Goehring [2]) have shown that pentathionate ions in aqueous alkaline solution are decomposed into thiosulphate ions, free sulphur and tetrathionate ions.

At the beginning of the reaction the only changes which are observed are the disappearance of pentathionate and hydroxyl ions and the formation of thiosulphate. Then, very sudden liberation of free sulphur occurs which makes the solution opaque and, at the same time, an equivalent amount of tetrathionate is formed [2,4]. This reaction is catalysed by sulphur [4] and thiosulphate [4] *i.e.* it is autocatalytic. Analysing the reaction mixture before the occurrence of free sulphur Kurtenacker [4] showed that the initial reaction stoichiometrically agrees with

$$2\ S_5O_6^{--} + 6\ OH^- \rightarrow 5\ S_2O_3^{--} + 3\ H_2O \qquad (1)$$

We have been able to show that, within 0.5 per cent, this is also the case when the reaction is performed under the experimental conditions used by us, which

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allow us to measure the amount of base consumed and, by titration with iodine, of the thiosulphate formed. Also the consumption of pentathionate was found to agree stoichiometrically with the consumption of base within a few per cent.

Kurtenacker [4] concluded, from experiments by Foerster [5] and his own results, that reaction (1) proceeds through the following steps:

$$S_5O_6^{--} + 2\ OH^- \rightarrow S(OH)_2 + 2\ S_2O_3^{--} \quad (2)$$

$$2\ S(OH)_2 + 2\ OH^- \rightarrow S_2O_3^{--} + 3\ H_2O \quad (3)$$

The assumption of sulphoxylic acid $S(OH)_2$ as an intermediate and of its conversion into thiosulphate is strongly supported by the experiments of Goehring [2], by the reaction between pentathionate and piperidine to yield sulphur dipiperidide described by Foss [1] and, finally, by the experiments of Meuwsen and Gebhardt [6] on the reaction between sulphur diethoxide and base to form thiosulphate, alcohol and alcoholate. As precise kinetic measurements on reaction (1) have not been published we decided to undertake such measurements, in an attempt to elucidate its mechanism. In consequence of the side reaction producing sulphur and tetrathionate, reaction (1) could be followed only until about 20 per cent of the pentathionate was converted. For this reason the technique chosen consisted in making a number of experiments with different initial concentrations, continuing each experiment only long enough to determine the initial velocity with the necessary accuracy.

EXPERIMENTAL

Potassium pentathionate was prepared according to Goehring [3] and recrystallized four times. The product formed a clear solution with water and analysis by the method of Foss [1] gave 98.2 per cent assay. No traces of sulphate, sulphite, thiosulphate, trithionate, chloride or free sulphur were found. The remainder was tetrathionate. The reactions were performed in a thin-walled glass cylinder 4 cm high, 15 ml capacity, which was fitted with a stirrer of glass, glass electrode, calomel electrode, inlet capillary for the addition of the base and inlet and outlet tubes for passing nitrogen freed from carbon dioxide over the solution. This reaction vessel was immersed in a water thermostat, operating at $20.00 \pm 0.05°$ C. By means of the E.M.F. between the electrodes in the reaction mixture, an automatic device described below maintained the OH^- activity at a constant value by adding the required amounts of N or 0.1 N potassium hydroxide to the mixture.

The electrodes were connected to a pH-meter (valve potentiometer) with constant zero point reading and constant amplification factor (Radiometer's A.C. fed "PHM 22"), which instrument furnishes a current varying linearly with pH. Across a rheostat the current produces a voltage which is nearly compensated. The "unbalance" is sent

through a mirror galvanometer. When the pH reaches a certain value, the mirror galvanometer permits a light beam to act upon a photocell which in turn controls a pulse generator. The electrical pulses drive a relay which acts upon a micrometer screw through a saw-toothed gear to push in the glass piston in a hypodermic syringe, thus delivering the reagent to the reaction mixture. This addition of base changes the pH and causes the galvanometer mirror to turn the light beam away from the photocell. The pulses then cease until the pH value desired is again passed by the consumption of hydroxyl ions in the reaction with pentathionate ions. After calibration of the micrometer screw and syringe this device, called a pH-stat or potentio-stat, permits the measurement of the amount of base consumed which is a measure of the degree of conversion. The apparatus works satisfactorily only when care is taken to keep the glass electrode sufficiently constant from the very beginning of the experiment. For this reason the glass electrode was placed for some hours before each run in a dilute buffer solution with the required pH. Then it was wiped off with a piece of filtering paper (but not rinsed) before it was immersed in the reaction mixture, the pH of which had been previously adjusted by means of an empirically determined amount of base. The pH-meter readings were standardized at closely spaced values over the whole pH range investigated, by means of standard buffer solutions. The constancy of the pH during each run was $\pm$ 0.01 to $\pm$ 0.02 pH units. The reaction mixture was composed of measured volumes of freshly prepared potassium pentathionate, hydroxide and chloride solutions. In order to follow the reaction during the first few seconds it was necessary in case of the fastest reactions that the apparatus was in operation before the addition of pentathionate. The results for a typical experiment are given below.

Table 1.

Time minutes	Micrometer screw readings cm	
	exp.	calc.
0.00	(3.739) *	(3.739)
0.65	3.753	3.753
1.00	60	60
1.25	64	65
1.50	69	70
1.75	75	76
2.00	81	81
2.25	86	86
2.50	91	92
3.00	3.800	3.802
3.50	09	12
4.00	17	23
4.50	26	34
5.00	35	44

* extrapolated

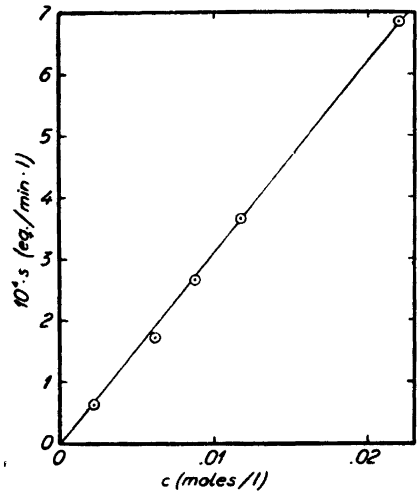


Fig. 1. The initial velocity s *measured in equivalents of potassium pentathionate per minute per litre reaction mixture versus the pentathionate concentration* c *in moles per litre.*

pH = 9.81; μ = 1.50; temperature 20.0° C; base: 0.100 N *Potassium hydroxide; total volume 10.0 ml.*

In the reaction vessel: 12 ml solution with $c_{S_5O_6^{--}} = 0.00897$, $\mu = 0.83$ (by means of KCl), pH = 10.50. Calibration of syringe: 1 cm = 0.418 ml. Titrator: 1.00 *N* KOH.

From a plot of micrometer screw readings *versus* time the initial velocity is found as the slope of a straight line fitting with the first 8 points. Slope = 0.0210 cm/min. = $8.78 \cdot 10^{-6}$ eq./min.

$s = 8.78 \cdot 10^{-6}/0.012 = 7.32 \cdot 10^{-4}$ eq./min. l; $k = s/3c_{S_5O_6^{--}} = 2.72 \cdot 10^{-2}$ min^{-1}.

RESULTS

1. From Fig. 1 it is seen that at constant pH and ionic strength the initial base consumption velocity s measured in equivalents of potassium hydroxide per minute per liter reaction mixture is proportional to the pentathionate concentration. So we may write

$$s = k_1 \cdot c_{S_5O_6^{--}}$$

From (1) we derive, at constant c_{OH^-}

$$-\frac{dc_{S_5O_6^{--}}}{dt} = \frac{1}{3}s$$

or

$$-\frac{dc_{S_5O_6^{--}}}{dt} = k \cdot c_{S_5O_6^{--}}$$

where

$$k = \frac{1}{3}k_1 = \frac{s}{3\,c_{S_5O_6^{--}}} = \text{a constant}$$

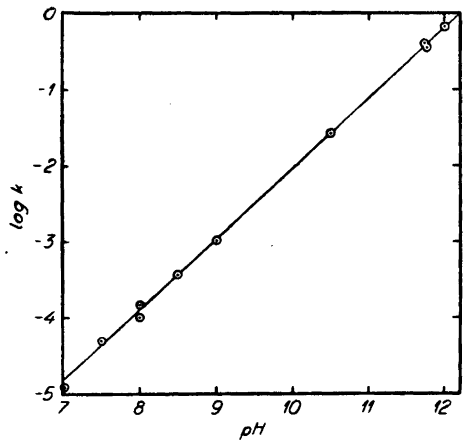


Fig. 2. The logarithm of the velocity constant $k = d \ln c/dt$ (min^{-1}) versus pH. $\mu = 0.83$; temperature 20.0° C.

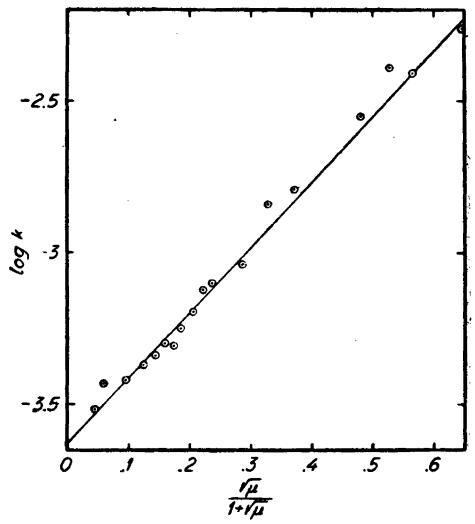


Fig. 3. log k versus $\sqrt{\mu}/(1 + \sqrt{\mu})$. pH = 10.50. Temperature 20.0° C. Except at the lowest ionic strengths the concentration of pentathionate was 0.00673 M.

This shows that the reaction is of the first order with regard to pentathionate and the last equation allows us to calculate the rate constant from the experimental results.

2. From Fig. 2 we find

$$\log k = 0.96 \text{ pH} + \text{a constant} \quad (7 \leq \text{pH} \leq 12)$$

so the reaction is almost first-order with regard to the hydroxyl ion concentration. The deviation from first order is only four per cent, which corresponds to the factor $10^{0.2} = 1.6$ over the interval investigated. This deviation is most probably due to systematic errors although we have not been able to trace their origin. In the following discussion we shall consider the reaction to be first order with respect to hydroxyl ion as well as to pentathionate ion.

Therefore the rate-determining step is a reaction between a pentathionate ion and a hydroxyl ion. As these two ions have the same sign, the rate must show a positive salt effect.

3. Fig. 3 provides a qualitative proof of this. To test it quantitatively we calculate the influence of changing the ionic strength at constant hydroxyl ion activity (*activity* because a potentiometric method was used).

Assuming, according to Brønsted [7] that a pentathionate ion A and a hydroxyl ion B form a complex M

$$A + B \rightleftharpoons M \rightarrow \ldots$$

and the velocity is proportional to the concentration of this complex, we have

$$-\frac{dc_A}{dt} = k' \; c_M$$

Using the thermodynamic equilibrium constant

$$K = \frac{a_M}{a_A \; a_B}$$

and the relation between activity, activity coefficient and concentration

$$a = f \; c$$

we derive

$$-\frac{dc_A}{dt} = k' \; K \; c_A \; a_B \frac{f_A}{f_M} = k'' \; c_A \frac{f_A}{f_M}$$

or

$$\log k = \log \left(-\frac{d \; \ln c_A}{dt} \right) = \log f_A - \log f_M + \text{a constant}$$

Introducing the Debye-Hückel equation as modified by Güntelberg [8]

$$\log f_Z = -0.50 \; z^2 \frac{\sqrt{\mu}}{1 + \sqrt{\mu}}$$

which gives a good approximation up to $\mu = 0.1$ we arrive at

$$\log k = 0.50 \; (z^2_M - z^2_A) \frac{\sqrt{\mu}}{1 + \sqrt{\mu}} + \text{a constant.}$$

With $z_A = -2$, $z_B = -1$, $z_M = z_A + z_B = -3$, this salt effect equation becomes

$$\log k = 2.5 \frac{\sqrt{\mu}}{1 + \sqrt{\mu}} + \text{a constant}$$

whilst with $z_A = z_B = -1$, $z_M = -2$ it becomes

$$\log k = 1.5 \frac{\sqrt{\mu}}{1 + \sqrt{\mu}} + \text{a constant.}$$

The experimental value 2.2 obtained from the slope of the line in Fig. 3 is close to 2.5 and seems therefore to decide for the first choice.

4. Investigation has shown that the velocity is not influenced by the presence of thiosulphate, trithionate or tetrathionate ions, except for their contribution to the ionic strength. This enables us to exclude certain mechanisms otherwise possible.

5. Finally we have examined whether the reaction is generally base-catalysed. At pH 10.50 and $\mu = 0.83$ a part of the potassium chloride was substituted by ammonium chloride to such an extent that the concentration of free ammonia was 0.067 *M* (calculated) and in other experiments by potassium acetate to give an acetate ion concentration of 0.083 *M* (calculated). In both instances we found within 10 per cent the same velocity as usually at this pH and ionic strength. This agrees with results obtained by Foss [1,p. 113] concerning the influence of acetate ions.

Therefore we conclude that the reaction is a reaction with the hydroxyl ion.

6. The numerical results of the present investigation can be expressed in the following empirical equation

$$\log k = -12.7 + 0.96\ \mathrm{pH} + 2.2\frac{\sqrt{\mu}}{1+\sqrt{\mu}} \pm 0.1 \text{ at } 20.0^\circ\ \mathrm{C}$$

$$\text{where } k = \frac{\mathrm{d}\ln c_{S_5O_6^{--}}}{\mathrm{d}t}\ \mathrm{min}^{-1},\ 7 \leq \mathrm{pH} \leq 12,\ 0 < \mu \leq 3.3.$$

CONCLUSION

All our results agree with the equations (2) and (3), when (2) is split up in (2a) and (2b)

$$S_5O_6^{--} + OH^- \rightarrow S_2O_3^{--} + HO \cdot S \cdot S_2O_3^- \qquad (2a)$$

$$HO \cdot S \cdot S_2O_3^- + OH^- \rightarrow S(OH)_2 + S_2O_3^{--} \qquad (2b)$$

(2a) is rate determining and (2b) is not.

This is seen as follows: If (2b) were rate determining (2a) must be reversible. But in that case the overall velocity would be proportional to the square of the hydroxyl ion concentration and the reaction would be inhibited by thiosulphate ions, both of which results disagree with the experiments.

According to Foss [1] (2a) is also the first step in the reaction yielding sulphur + tetrathionate (*l.c.*p.113) the next step being

$$HO \cdot S \cdot S_2O_3^- + S_2O_3^{--} \rightarrow S_4O_6^{--} + S + OH^-$$

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The Effects of Anesthetics on Blood Coagulation in Vitro

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In the discussion on the nature of cell stimulation * several authors, *e.g.* Heilbrunn [1,2], Kraus and Fuchs [3], and Runnström and coworkers [4,5], have suggested that the typical changes of protoplasm viscosity are related to the natural coagulation of blood. (*Cf.* also works on the connection between the latter phenomenon and *rigor mortis* [6,7,8], and Kühne's monograph on contractility [9].) Some of the above authors (*vide* especially Heilbrunn [1] and Runnström [5]) have claimed that these changes of protoplasm viscosity constitute a universal and fundamental feature of cell stimulation.

Another common property of all living cells is their ability to alter their responsiveness or automatic activity under the influence of a number of agents, anesthetics (or narcotics), most of which are lipophilic chemicals. When administered in medium doses **, anesthetics produce anesthesia (narcosis) in the cells, *i.e.* "a physiological condition in which the normal responsiveness or automatic activity of the living system — organism, tissue, or cell — is temporarily decreased or abolished" (Lillie [10b]). With large doses of anesthetics these effects become irreversible, *i.e.* they lead to death. Further, it is a well established fact that anesthetics, when applied in small subanesthetic doses, increase the responsiveness of cells (Winterstein [11, pp. 13–20]; Seelich [12]). This can be observed as an increased degree of contraction of a muscle or as a decreased stimulation threshold (*cf.* Graham [13], Blume [14]). The excitability increasing effect of anesthetics on nerves has been studied by Thor [15] and Blume (*l.c.*).

If there is any analogy or homology between the increase of plasma viscosity after stimulation and in blood coagulation, the influence of anesthetics on the latter must be of great theoretical interest. The present paper

* It is not easy to define generally the concept of stimulation, but at present we ascribe to the word the response elicited in a living system when some factor (natural or otherwise) in its environment is suddenly changed more than a certain amount, the stimulation threshold. This response is an activity change which is specific for the living system in question: a muscle, for instance, contracts whatever the nature of the environmental change (stimulus). (*Cf.* Heilbrunn [1], chap. 35 and Lillie [10a]).

** In this general discussion we prefer the term "dose" to "concentration", as physical agents too (*e.g.*, heat, cold) may produce a state of anesthesia.

is to be regarded as a preliminary investigation of the effects of anesthetics, typical local anesthetics as well as general narcotics, on the coagulation process. — The chief difference between general and local anesthetics lies in their mode and site of application, conditioned by physical properties (volatility, solubility, diffusivity) of the molecules. However, the fact that typical general anesthetics such as ethanol (Gallego [16]) and ether (Lorente de Nó [17, p. 356]) act through a lowering of the membrane potential of the nerve, whereas typical, local anesthetics, *e.g.* procaine (Bennett *et al.* [18]) and cocaine (Lorente de Nó [17, p. 115]) do not lower this potential, seems to be a fundamental difference in the mode of action which has to be explained in any stimulation-narcosis theory. That is the reason why we distinguish between the two types of substances.

In this introduction the main features of the process of blood coagulation, in so far as they are known, must be briefly recapitulated (*cf.* Astrup [19], Seegers [20], and handbooks in enzymology). Two phases of the process can be recognized: (1) From injured cells a thrombokinase is liberated, which in the presence of Ca ions activates the blood protein, prothrombin, to thrombin. As thrombin is formed, it autocatalyzes the formation of more thrombin from prothrombin; for this autocatalysis Ca ions and a globulin, Ac-globulin, are necessary. (2) The thrombin thus formed catalyzes the transformation of the blood protein, fibrinogen, to insoluble fibrin, which clots. For this part of the process Ca ions are not necessary.

Several factors contained in the blood regulate the rapidity of the clotting, *e.g.* the polysaccharide acid, heparin, and several proteins [20]. Of great interest from the standpoint of a possible relation between cell stimulation and blood coagulation is the possibility of extracting thrombokinases (or substances with thromboplastic activity) from all kinds of cells, many of which under normal circumstances never come in contact with blood, *e.g.* semen [7,21], yeast [7,22,23], protozoa [7]. In experiments which will be published in another context, we have found thromboplastic activity in human semen, yeast, *Vicia faba* roots, and in *Drosophila melanogaster*. All these activities have a more or less specific ability to initiate or accelerate the coagulation process *in vitro*.

I. EFFECTS OF ANESTHETICS IN MEDIUM CONCENTRATIONS

In all experiments horse blood was used, which was drawn into a sodium citrate solution to a final concentration of 0.75 per cent of $Na_3C_6H_5O_7 \cdot 2H_2O$. The blood cells were centrifuged off immediately after the drawing at 1 000 *g* for 45 min. Thromboplastic substances were extracted from different specimens according to the very simple methods of Lehmann [24, *cf.* 24a] and Quick [25]. In the experiments presented here, extracts from hare brain and from horse sciatic nerve were used. As regards the mode of preparation, see Table 1 and the legends to the figures. The coagulation times of different mixtures were determined according to the methods mentioned [24,25], at 37° C. In the first experiments (Fig. 1) a mixture of 0.25 ml each of thrombokinase solution,

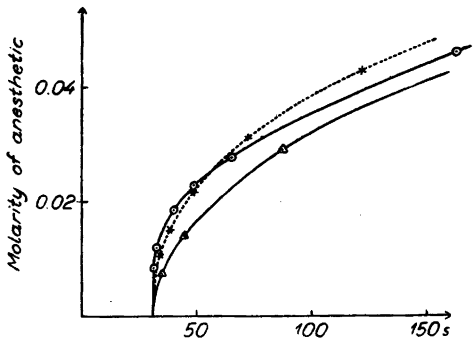


Fig. 1. The influence of xylocaine (×), N-diethylaminoacetanilide (⊙), and procaine (△) on the coagulation time of blood. Thrombokinase II (cf. Table 1).

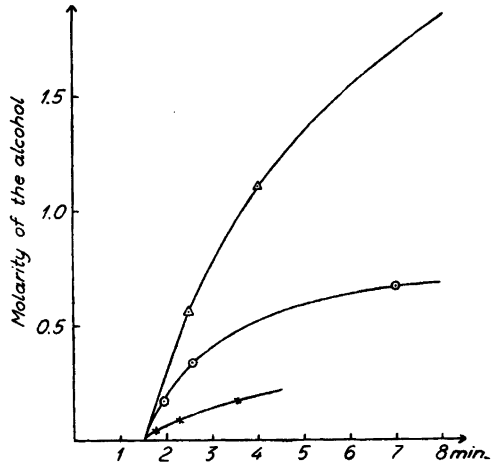


Fig. 2. The influence of n-alkanols on the coagulation time of blood. ×: 1-butanol, ⊙: 1-propanol, △: ethanol. Thrombokinase I (cf. Table 1).

anesthetic (both in saline), and citrate plasma was left to reach the temperature of the thermostat. Then 0.25 ml of 1.0 per cent $CaCl_2$ at 37° C. was added and the stop watch set. In later experimental series the amounts of component solutions were 0.15, 0.10, 0.10, and 0.10 ml respectively. The clotting times were noted in seconds. The pH of all component solutions was adjusted to 7.4.

From Figs. 1 and 2 it will be seen that when medium (*i.e.* approximately anesthetic) concentrations of anesthetics are applied — in the diagrams the concentration of the anesthetic is given in moles per liter of reaction mixture — the coagulation times are markedly prolonged: Some factor accelerating the coagulation process is inhibited by the anesthetics. Further, it can be observed that the activities of the alkanols in this respect (Fig. 2) parallel their lipid solubilities (as in narcosis; *cf.* Meyer and Hemmi [26]) or surface activities (Traube's rule; *cf.* Seelich [12]).

In order to determine the point of attack of the anesthetics, their effect was studied separately on each of the two phases of the clotting process. A fibrinogen-free prothrombin was prepared by acid precipitation of diluted plasma and a prothrombin-free fibrinogen by precipitation with three-fifths saturated NaCl, both according to Eagle and Harris [27]. If mixtures of a thromboplastic substance, prothrombin, and $CaCl_2$ are incubated for different times before the addition of fibrinogen, we get the *clotting time-incubation time* curve shown in Fig. 3 (a). In this case, of course, the clotting time has been

determined from the moment when the fibrinogen was added. If we now let the incubation, *i.e.* prothrombin activation, occur in the presence of an anesthetic (in this case, procaine) we get markedly prolonged clotting times (curve b). On the other hand, an addition of the same amount of anesthetic together with the fibrinogen is without any effect.

Although the action of the anesthetic can thus be localized to the first phase of the coagulation process, our experiments have not yet proved definitely which of the factors determining this phase is inhibited. However, the lipophilic nature of the anesthetics, and especially the fact that the inhibition by alkanols parallels the lipophilic properties of the latter, makes it most probable that the thromboplastic substances, which are typical lipo-proteins (Chargaff and coworkers [28]), are the points of attack. Considering the glyco-albumin nature of prothrombin [20] and the somewhat similar properties of Ac-globulin, it seems unlikely that these substances are inhibited. Further, it was demonstrated above that the thrombin is not inactivated; it is therefore unlikely that its chemically related precursor becomes inactivated. Compare this too with the fact that fat solvent anesthetics do not inhibit the precipitin reaction [29].

We have not been able to find any simple stoichiometric relation between thrombokinase (T) and anesthetic (A)

$$\frac{(TA)}{(T)\ (A)} = k_1,\ (k_1 = \text{const.}) \tag{1}$$

where (TA) is the concentration of inactivated thrombokinase and (T) is the concentration of free thrombokinase, both in units equivalent to the anesthetic. If we determine the relation between *clotting time* and *anesthetic concentration* at some concentrations of T so dilute that the activity of T may be considered proportional to its concentration, and determine (T) by comparison with clotting times obtained by dilution of the same thrombokinase solution, we get the $(A)_o$, (T) curves shown in Fig. 4; $(A)_o = (A) + (TA)$. If equation (1) be correct, these curves should be represented by

$$\frac{(1-x)\ [1 + k_1(T)_o x]}{k_1 x} = (A)_o \tag{2}$$

where $(T)_o$ is the total concentration of T in units equivalent to $(A)_o$, and x is the relative concentration of non-inactivated T, *i.e.*, $x = (T)/(T)_o$. Equation (2) fits any single curve, when $(T)_o$ is taken small enough; at greater thrombokinase concentrations the curves are often concave, contrary to the hypothe-

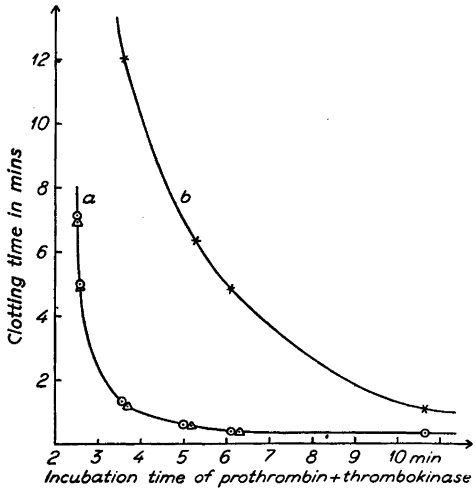


Fig. 3. Effect of procaine (0.03 mol/l of reaction mixture) on fibrin formation (△) and on prothrombin activation (×). No anesthetic: ⊙. Cf. text. Thrombokinase II (Table 1).

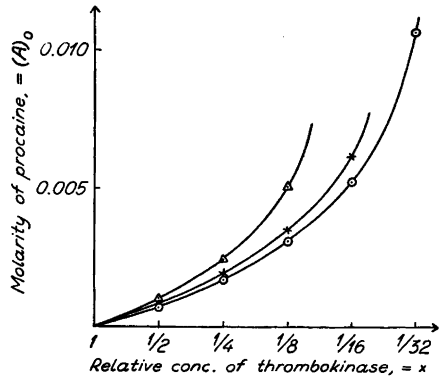


Fig. 4. Calculated decrease of thrombokinase concentration as a function of amount of procaine added (cf. text). Thrombokinase I (Table 1), dilution 1/400 (⊙), 1/800 (×), 1/1600 (△).

sis. But the k_1 value calculated from one curve is not valid at another thrombokinase concentration, $(T)_o$. As will already be seen from Fig. 4, the anesthetic is relatively more effective at higher concentrations of T, which would be impossible if the whole thing were a simple inactivation represented by $T + A \rightleftharpoons TA$, *i.e.* equation (1). Fig. 4 shows the effect of procaine; similar results are obtained with butanol.

These findings would be hard to explain if we had used pure thrombokinase preparations, because all the substances from the blood exerting an influence on the process were present in constant concentrations throughout the experiments. Differences in pH between individual experiments, due to the degree of dilution of the thrombokinase or anesthetic, could possibly have an effect in this direction, (a) if a weak base anesthetic [30] was used, or (b) if some part of the coagulation process was strongly dependent on the pH level. A pH effect can, however, be excluded because (a) *e.g.* butanol behaves similarly to the weak base anesthetic, procaine, and (b) the pH was constant to within a few hundredths in every series of experiments with one anesthetic. The most probable explanation of the complicated effect described here would be the presence in the thrombokinase solution and (or) the blood plasma of contaminating substances, one or several of which are inhibitors of the throm-

bokinase. These substances, which we will call antithrombokinase, I, can be bound or inactivated, respectively, by anesthetics, A. In point of fact, a lipid antithrombokinase can be isolated from brain tissue (Tocantins *et al.*[31]; *cf.* Hecht [32]), blood plasma, and other tissues (Overman *et al.*[33]; Chargaff [28b]). We therefore suggest hypothetically that the following reactions determine the actual concentration of thrombokinase, (T), in the presence of an anesthetic:

$$I + T \rightleftharpoons TI \quad (TI)/(I)(T) = k_2 \tag{3}$$
$$T + A \rightleftharpoons TA \tag{1}$$
$$I + A \rightleftharpoons IA \quad (IA)/(I)(A) = k_3 \tag{4}$$

II. ACTION OF ANESTHETICS IN LOW CONCENTRATIONS

If the antithrombokinase, I (*cf.* above), were inactivated more readily than the thrombokinase, it would be possible to find some (low) concentrations of anesthetics at which the amount of active thrombokinase would be greater than in the unanesthetized standard.* This is also the case with both the general anesthetics, butanol and chloroform, and procaine. Each of these substances in low concentrations gives a somewhat *shorter* coagulation time than the control. Fig. 5 shows the influence of butanol on two different diluted thrombokinase preparations. (*Cf.* the concentrations given in Fig. 2.) Chloroform produces a similar shortening of the coagulation time at about 0.1 mmoles per liter (0.8 mmoles per liter is retarding). Procaine has a slight accelerating effect at concentrations of about 0.75 mmoles per liter (*cf.* the retarding concentrations in Fig. 1).

The well-known ability of very strong thrombokinase solutions to give a longer coagulation time than the somewhat more diluted solutions [34,31] was also found to be valid for our preparations (Fig. 6). The accelerating effect of low concentration anesthetics on the coagulation process does not, however, constitute inhibition of the retardation occurring in very concentrated thrombokinase solutions. This is proved by the following facts: (a) The short coagulation time of point 2 (Fig. 6) cannot be reached if we add the anesthetic to the thrombokinase concentration of point 1; (b) the accelerating effect of low concentration anesthetics also occurs if we add the anesthetic to throm-

* A derivation of the equations, (1, 3, 4) above, preliminarily disregarding the formation of TIA, at the limit $(A)_0 = 0$ gives a positive value of $d(T)/d(A)_0$, *i.e.*, an increasing amount of T on the first addition of anesthetic, if

$$\frac{k_3}{k_1} > \frac{(I)}{(T) + 1/k_2}$$

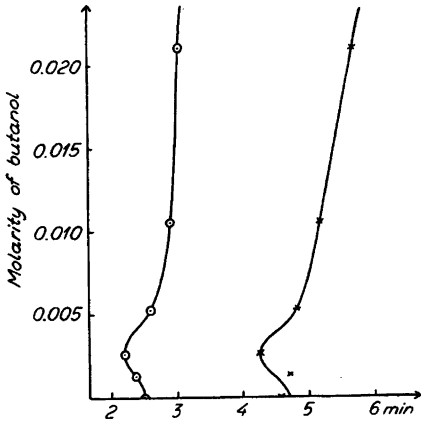


Fig. 5. Effect of low concentrations of butanol on the clotting time. Thrombokinase I (Table 1), dilution 1/1600 (⊙) and 1/3200 (×).

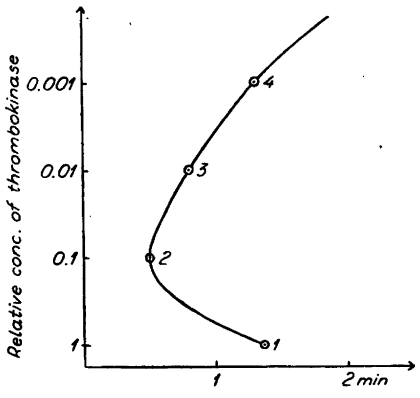


Fig. 6. Effect of dilution of a high concentration of thrombokinase (I, Table 1, not centrifuged) on the clotting time.

bokinase of concentrations 3 and 4 (Fig. 6). In such cases the accelerating effect of procaine is not always demonstrable — at all events it is only very slight — whereas that of butanol is evident (Fig. 5). If the inhibition of thrombokinase by antithrombokinase follows the simple relation (3), it can be calculated that the concentration of free thrombokinase cannot decrease with increasing concentrations of a T + I preparation (*cf.* Tocantins *et al.*[31]). (It must be pointed out, however, that these equations are only a rough working hypothesis at the present stage of the investigation. Already the fact that concentration must be used instead of activity makes them imperfect.)

In this connection it should be mentioned that the general anesthetics (butanol and chloroform were investigated here) have the ability without addition of any thromboplastic substance to initiate the coagulation process in the presence of Ca ions (*cf.* Nolf[35]; Scheuring[36]). This effect, which occurs at high concentrations of the anesthetics, is not found in the case of procaine. The latter substance only retards the slow coagulation of recalcified plasma, at about the same concentrations which inhibit the rapid clotting in the presence of thrombokinase. The coagulation initiating effect of chloroform is the most obvious, and perhaps of a special nature[35-37]; but this effect of both chloroform and butanol is very dependent on the state of the plasma (origin, time and temperature of aging). Thus it will be seen that the effects of anesthetics on blood coagulation are rather complicated.

III. DISCUSSION

Earlier knowledge of the effects of anesthetics on blood coagulation mostly consists in reports of changes in bleeding time in surgical cases under different conditions of anesthesia. The facts known are highly contradictory, which can well be understood from the complicacy demonstrated by our experiments *in vitro*. (*Cf.* the review by Adriani [38].) For instance, barbiturates prolong or shorten the coagulation time (Aykut [39]); ether narcosis shortens it, whereas procaine has no effect (Levy *et al.*[40]). Ether and chloroform increase the rate of inactivation of thrombin (Pálos [41]).

If we regard the hypothesis of an analogy or homology between blood coagulation and cell stimulation, it is of interest to compare the minimum concentrations for narcosis with the relative amount of thrombokinase inactivated at these concentrations (if our assumption that thrombokinase is inhibited by anesthetics is correct). It is noteworthy that a fairly high percentage is really inactivated at these concentrations (Table 1). Before it can be decided whether the thrombokinase extracted from different tissues is the substance inhibited in anesthesia of the same tissue, the following conditions must be fulfilled: (a) It must be proved conclusively that the thrombokinase is the substance inhibited in the case of blood coagulation; (b) the thrombokinase must be extracted from the tissue used for anesthesia investigations. (For unknown

Table 1.

Anesthetic	Minimum concentration for narcosis of frog nerve moles/l	Calculated decrease of the thrombokinase conc., in per cent, at anesthetic conc. of column 2	Source and method of thrombokinase preparation
Ethanol	1.45 [55]		
	0.6 – 1 [14]		
	1 – 2 [16]		
	Average: 1	93	Thrombokinase I. Dried hare brain. 1 g/20 ml of saline; centrifuged for 5 min at 1 000 *g*. Diluted 1 000 – 3 000 times.
1-Propanol	0.3 [55]	85	
1-Butanol	0.094 [55]	65	
Procaine	0.0125 [50]	95 – 98	
		83	Thrombokinase II. Dried ischiatic nerve from horse. 1.2 g/20 ml of saline; decanted after 12 hrs at 0° C. Not diluted.
Xylocaine	0.0038 [30]	25	
N-Diethylamino acetanilide	0.0095 [30]	25 – 40	

reasons our attempts to make a thrombokinase from frog nerve yielded only rather weak preparations, not suitable for experiments); (c) the blood coagulation and tissue anesthesia must be investigated at the same temperature. However, the possible role of the thromboplastic substances in cell stimulation and anesthesia must be taken into recount.

From a more general point of view the present investigation strongly supports the hypothetically proposed relationship between cell stimulation and blood coagulation. It is of interest to review some data on essential similarities and relations between the two processes:

1. Both involve a remarkable increase in the viscosity of plasma.

2. Both processes are checked and promoted, respectively, by similar concentrations of fat solvent anesthetics.

3. Both processes require Ca ions, and are therefore inhibited by other cat-ions [42,43] (*e.g.* the anesthetics, K^+ and Mg^{2+} [44]). Ca ions in a too high concentration [45] also inhibit both processes.

4. Heparin-like substances appear in connection with cell stimulation [46].

5. An important fact in this connection is the general occurrence in cells of thromboplastic substances (*cf.* p. 342 above).

6. All the substances necessary in the blood coagulation can be extracted from, *e.g.*, muscle [3].

7. Injury substances from animals promote both blood clotting and stimulation [*Cf.* 1, p. 561]. Histamine promotes blood clotting *in vivo* [47]; it has an antiheparin effect, demonstrated *in vitro* [48].

8. Substances formed in blood plasma during the coagulation process may stimulate muscles [9] and nerves [51] and cause vascoconstriction [49] (*cf.* the hypertensinase activity of the substances in question [51]). These substances can also promote cell division [52,53].

SUMMARY

1. Local as well as general anesthetics prolong the clotting time of blood when they are added to the plasma-thrombokinase mixture in *medium* (anesthetic) concentrations.

2. The prothrombin activation, not the fibrin formation, is inhibited.

3. The inhibition through *n*-alkanols runs parallel to the lipid solubilities of the latter. Therefore it is suggested that the lipoprotein, thrombokinase, is the factor inhibited.

4. Superimposed on the inhibition by anesthetics there seems to be an activation of some factor participating in the thrombin formation. This probably is due to the inactivation of an antithrombokinase, which contami-

nates the thrombokinase and (or) the blood. In *low* (subanesthetic, stimulating) concentrations, anesthetics shorten the coagulation time of blood.

5. *High* concentrations of anesthetics promote the coagulation of blood without any addition of thrombokinase (observed in the cases of *n*-butanol and chloroform).

6. Data are related to observations of the effects of anesthesia on bleeding time. They are also compared with known similarities between blood coagulation and the protoplasm coagulation which occurs after stimulation.

We wish to express our gratitude to Professor K. Myrbäck, the Head of this institute, for valuable discussions, and to Dr. S. Bodin of the State Veterinary Medical Institute for supplying us with horse blood. The investigation was supported by *Statens Naturvetenskapliga Forskningsråd.*

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Potentiometric and Spectrophotometric Studies on 8-Quinolinol and Its Derivatives. III. 8-Quinolinol Chelates of Barium, Strontium and Magnesium in Aqueous Solutions

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The method used for the investigation of calcium chelate [1] has also been used in this study. In deriving the equations for the calculation of the equilibrium constants, however, some approximations, which were quite permissible, but not inevitable, were made and the exact equations are now given. The stability constants of the complexes may be calculated by means of the following equations (for an explanation of the notation, see ref. 1).

$$\Sigma\ i\ k_i'([\mathrm{HOx}]/(\mathrm{H}^+))^{i-1} - c_{\mathrm{Ox}}'\ (\mathrm{H}^+)/[\mathrm{M}^{++}][\mathrm{HOx}] = 0 \qquad (1)$$

$$c_{\mathrm{Ox}}' = c_{\mathrm{Ox}} - [\mathrm{HOx}](1 + (\mathrm{H}^+)/K_1' + K_2'/(\mathrm{H}^+)) \qquad (2)$$

$$[\mathrm{HOx}] = (c_{\mathrm{Ox}} + c_{\mathrm{A}} - c_{\mathrm{B}} + [\mathrm{OH}^-] - [\mathrm{H}^+])/(1 + 2\ (\mathrm{H}^+)/K_1') \qquad (3)$$

$$[\mathrm{M}^{++}] = c_{\mathrm{M}}/(1 + \Sigma k_i'([\mathrm{HOx}]/(\mathrm{H}^+))^i) \qquad (4)$$

From equations (1) and (4) we obtain:

$$\Sigma(i - c_{\mathrm{Ox}}'/c_{\mathrm{M}})([\mathrm{HOx}]/(\mathrm{H}^+))^i k_i' - c_{\mathrm{Ox}}'/c_{\mathrm{M}} = 0 \qquad (5)$$

For the calculation of the solubility equilibria the following equation is needed:

$$[\mathrm{M}^{++}] = (2c_{\mathrm{M}} - c_{\mathrm{Ox}}')/(2 + \Sigma\ (2 - i)([\mathrm{HOx}]/(\mathrm{H}^+))^i k_i') \qquad (6)$$

The concentration [HOx] may be calculated from equation (3) and thus the constant

$$K_{\mathrm{S}} = S/K_2' = [\mathrm{M}^{++}][\mathrm{HOx}]^2/(\mathrm{H}^+)^2 \qquad (7)$$

may be calculated from the known values of the stoichiometric concentrations, the dissociation constants of 8-quinolinol, the constants k'_i, and the pH of the solution.

Of the constants k'_i, only the first, k'_1 can be calculated accurately, because the last term in equation (1) is, within the experimental error, independent of the quotient $[HOx]/(H^+)$ when the latter is varied from $0.987 \cdot 10^5$ to $7.05 \cdot 10^5$. Thus in equation (1) the term including the constants k'_i $(i > 1)$ are negligible. Under the conditions prevailing in the cases investigated here the first complex is the only one of importance. The higher complexes would be expected to play a more important role at higher ligand concentrations, which are possible only at a high pH. Under such conditions, however, the potentiometric method is useless, and the spectrophotometric method is unsuitable because of the very low solubilities of the 8-quinolinolates in question.

The values obtained for the constant k'_1 are recorded in Table 1.

Table 1. Potentiometric determination of the constants k'_1 in barium, strontium and magnesium chloride solutions at 20° C.

		Barium	complex	Strontium	complex	Magnesium	complex
$\sqrt{I}$	log F	pk'_1	$pk'_{1,0}$	pk'_1	$pk'_{1,0}$	pk'_1	$pk'_{1,0}$
0.115	0.148	7.98	7.83	7.48	7.33	5.31	5.16
0.196	0.228	8.06	7.83	7.58	7.34	5.38	5.17
0.320	0.324	8.16	7.84	7.67	7.35	5.47	5.15
0.720	0.509	8.33	7.82	7.85	7.34	5.65	5.14
1.000	0.596	8.41	7.81	7.92	7.32	—	—
		mean value	7.83		7.34		5.16

These can be represented satisfactorily by the equation

$$pk'_1 = pk'_{1,0} + \frac{1.514\sqrt{I}}{1 + 1.54\sqrt{I}} \tag{8}$$

obtained previously for calcium chelate [1]. The constants

$$k_{1,0} = \frac{(MOx^+)}{(M^{++})(Ox^-)} \tag{9}$$

may be calculated from the relation

$$\log k_{1,0} = pK_{2,0} - pk'_{1,0} \tag{10}$$

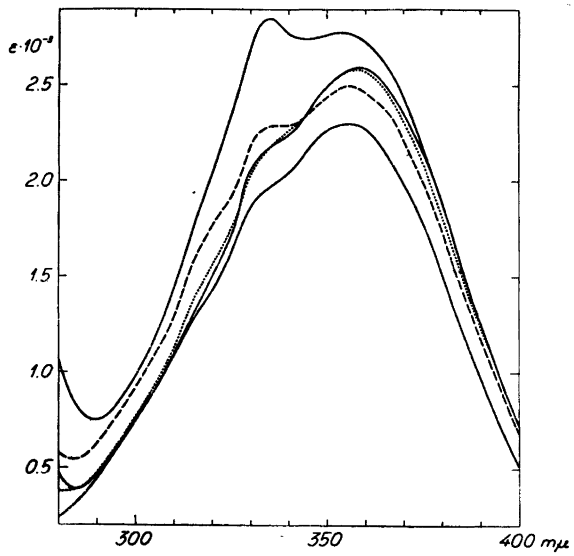


Fig. 1. Ultra-violet absorption spectra of the 8-quinolinol complexes of alkaline earth metals. Upper full line Ox^-; middle full line $CaOx^+$; lower full line $MgOx^+$; broken line $SrOx^+$; dotted line $BaOx^+$.

Using the value $pK_{2,0} = 9.90$ of Näsänen, Lumme Mukula [2] we obtain

	$MgOx^+$	$CaOx^{+1}$	$SrOx^+$	$BaOx^+$	
$\log k_{1,0}$	4.74	3.27	2.56	2.07	(20° C)

The results obtained spectrophotometrically using the method used in the study of calcium chelate were consistent with the above. In Fig. 1 the molar extinction coefficients of the complex ion MOx^+, as well as that of the ligand Ox^-, are represented over the range 280—400 mμ. The spectra of the complexes seem to resemble each other and the spectrum of Ox^- in main features. The relatively small differences are irregular and may in part be due to experimental error. The effect of the latter are relatively great because of the indirect method.

The results relating to the solubility equilibria of magnesium chelate are recorded in Table 2. The constant K_S may be represented by the equation

$$\log K_S = 4.248 + \frac{2.018\ \sqrt{I}}{1 + 1.90\ \sqrt{I}} - 0.402\ I \quad (20° \text{ C}) \qquad (11)$$

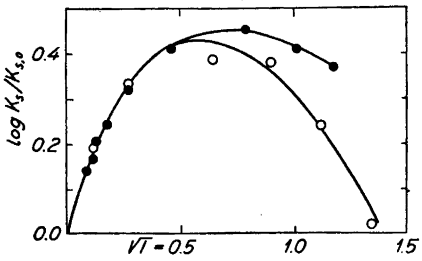


Fig. 2. Solubility products of 8-quinolinol chelates of magnesium and calcium in aqueous solutions of potassium chloride as a function of the ionic strength at 20° C.

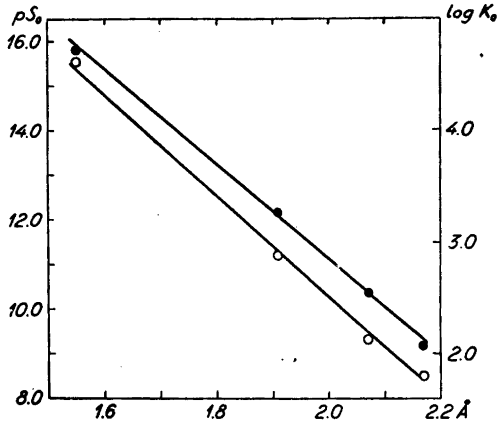


Fig. 3. The thermodynamic solubility product and the complex constant of the chelates of alkaline earth metals as a function of the metallic (octahedral) radius.

Table 2. Solubility equilibria of magnesium chelate in potassium chloride solutions at 20° C.

$c_{Ox} = 3.41 \cdot 10^{3}$. $c_{Mg} = 2.81 \cdot 10^{3}$. $c_{A} = 3.41 \cdot 10^{3}$. $c_{B} = 5.55 \cdot 10^{3}$.

$\sqrt{I}$	pH	log K_S
0.114	6.53	4.44
0.270	6.60	4.58
0.640	6.63	4.63
0.900	6.63	4.62
1.180	6.57	4.49
1.340	6.48	4.27

In Fig. 2, log K_S for the magnesium and calcium chelates are plotted against ionic strength. The pH is sensitive to the value of K_S only when $c_B < c_A - c_{Ox}$. Under these conditions the barium and strontium chelates precipitate only if the metallic ion concentration is considerable. The extrapolation to zero ionic strength in these cases was done by means of the equation

$$\log K_S = \log K_{S,O} + \frac{2.018\sqrt{I}}{1 + 2.24\sqrt{I}} - 0.203\, I \quad (20^\circ\,C) \qquad (12)$$

obtained previously for calcium chelate [1]. This calculation gave the values

	$SrOx_2$	$BaOx_2$	
log $K_{S,O}$	10.47	11.31	(20° C)

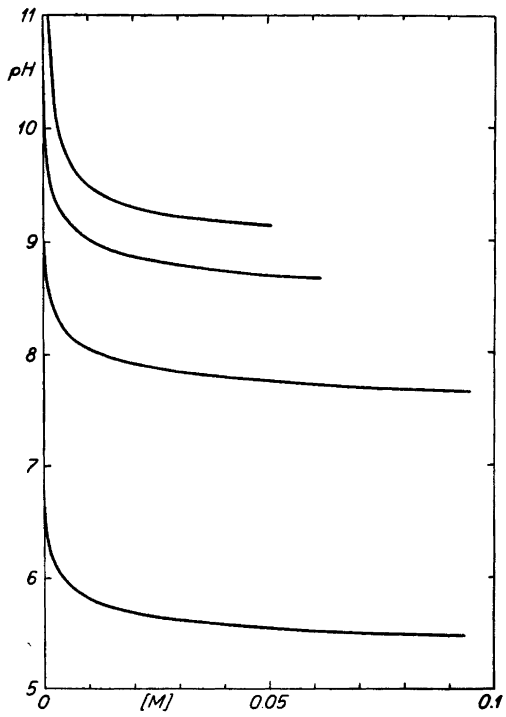


Fig. 4. Precipitation of alkaline earth metals with 8-quinolinol. $[Ox] = 3.78 \cdot 10^{-3}$.

From the values of the thermodynamic constants:

	$MgOx_2$	$CaOx_2$	$SrOx_2$	$BaOx_2$	
$\log K_{s,o}$	4.25	8.76	10.47	11.31	(20° C)

the thermodynamic solubility products

$$S_0 = (M^{++})(Ox^-)^2 \tag{13}$$

may be calculated. Using the relation [1]

$$pS_0 = 2\ pK_{2,0} - \log K_{s,o} \tag{14}$$

we obtain

	$MgOx_2$	$CaOx_2$	$SrOx_2$	$BaOx_2$	
pS_0	15.55	11.22	9.33	8.49	(20° C)

The quantities pS_0 and $\log k_{1,0}$ seem to vary approximately linearly with the metallic radius [3] as may be seen in Fig. 3.

For the calculation of the total concentration of metal in relation to the pH, the following equations were used:

$$[\mathrm{HOx}] = \frac{[\mathrm{Ox}]}{1 + K_2'/(\mathrm{H}^+) + (\mathrm{H}^+)/K_1' + k_1'[\mathrm{M}^{++}]/(\mathrm{H}^+)} \tag{15}$$

$$[\mathrm{M}^{++}] = K_s\ (\mathrm{H}^+)^2/[\mathrm{HOx}]^2 \tag{16}$$

$$[\mathrm{M}] = [\mathrm{M}^{++}](1 + k_1'[\mathrm{HOx}]/(\mathrm{H}^+)) \tag{17}$$

where [Ox] and [M] are the total cocentrations of 8-quinolinol and of the metal in the solutions respectively. The results in the case $[\mathrm{Ox}] = 3.78 \cdot 10^{-3}$ are presented in Fig. 4, where [M] is plotted against pH. From this figure the pH at which precipitation begins can be seen. The figure also indicates the completeness of precipitation at different pH's. It must be emphasised, however, that here $[\mathrm{Ox}] = 3.78 \cdot 10^{-3}$ and that [M] is relatively highly dependent on [Ox] *i.e.* on the excess of reagent, as is evident from equations (15), (16) and (17).

SUMMARY

The equilibria of the 8-quinolinol chelates of the alkaline earth metals in aqueous solution have been investigated. The equilibrium constants of the first complexes have been determined potentiometrically and spectrophotometrically. When the pH is not very high, only the first complexes have any significance. The potentiometric and spectrophotometric results were consistent. The absorption spectra of the first complexes were determined over the range 280—400 mμ. In main features the spectra are similar to the spectrum of the ligand Ox^-. The solubility products of the 8-quinolinolates of alkaline earth metals have been determined.

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The Isolation of Chondrosamine from Gangliosides and from Submaxillary Mucin *

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According to Klenk [1], the gangliosides are composed of fatty acids, sphingosine, hexoses (in the main galactose), and a hydroxyamino acid, *i.e.* neuraminic acid ("Neuraminsäure"), with the probable composition $C_{10}H_{19}NO_9$. The structure of the latter is unknown. In 1938 one of us [2] noted an agreement in certain qualitative reactions between gangliosides and a mucopolysaccharide present in submaxillary mucin, and therefore submitted hydrolysate of gangliosides to hexosamine determination according to the colorimetric method of Elson and Morgan. Such analyses showed a hexosamine content of about 9 % in pure gangliosides. In view of this result, Klenk [3] tested neuraminic acid for hexosamine, but found no reducing substance in the hydrolysate of this substance. In 1948 Brante [4] applied paper partition chromatography in studies on water-soluble split products of brain lipids. He obtained from ganglioside hydrolysates a very distinct spot, which reduced *m*-phenylendiamine and gave a positive reaction with the Elson-Morgan reagent and with ninhydrin. With different solvents, this spot moved at practically the same rate as chondrosamine in chondroitin sulphuric acid hydrolysates, a rate which differed slightly from that of glucosamine in identical conditions. These findings thus strongly supported the supposition that the ganglioside contains hexosamine, and also suggested that the hexosamine present was chondrosamine. We felt, however, that the question could only be definitely answered by isolation of the substance.

Owing to its greater solubility, chondrosamine hydrochloride does not crystallize from water or water methanol mixtures as readily as glucosamine hydrochloride. When preparing chondrosamine hydrochloride from chondroi-

* A preliminary report on this investigation has been published in this journal, 4 (1950) 717.

tin sulphuric acid by Levene's [5] method we at first had some difficulty in obtaining a crystalline product. If, however, the hydrolysate is evaporated *in vacuo* to a sticky syrup and then dissolved in methanol, and this process repeated a few times, there appears on resolution a crystalline residue which increases on standing. From the mother liquors more crystalline material can obtained by the same treatment. Probably, the removing of the last traces of water by the repeated evaporations with methanol favoured the crystallization. This experience was acted on in the subsequent work. As will be described in detail in the experimental part, we succeeded in isolating from the ganglioside a hexosamine which, by its optical rotation and X-ray diffraction pattern, was identified as chondrosamine. Nothing indicated the coexistence of glucosamine in the analysed material.

The finding that the ganglioside contains chondrosamine prompted us to investigate whether the main carbohydrate of the submaxillary mucin does so, too. — In 1936 one of us [6] showed this mucin to contain two different carbohydrate groups. The main carbohydrate was isolated in small amounts in crystalline form. It was thought to be of disaccharide nature, and to consist of an acetylated hexosamine and a polyhydroxy acid, which was not a hexuronic acid. This view was based on the results of elementary analyses and acetyl determinations, as also on the reducing power and acidity of the substance, and on certain colour reactions, in the first place the Ehrlich reaction with *p*-dimethyl-aminobenzaldehyde. Hexosamine was not isolated from the substance, however. Unlike ordinary N-acetylhexosamine compounds, the main carbohydrate of the submaxillary mucin gives a strong purple reaction on heating with the Ehrlich reagent, even when not previously boiled in alkaline solution. It is further characterized by a very marked humin formation on treatment with acids in the heat and, as has been found later on, by giving a purple colour when tested with Bial's reaction, and by giving off CO_2 on heating with 12 % HCl in the same way as hexuronic acids. Besides this substance, for which we propose the provisional name *sialic acid*, the mucin contains small amounts of carbohydrate of the dihexose-hexosamine type, as well as of the bloodgroup antigen type. If the mucin is purified by frequent reprecipitation from acid solution by diluting with water, the content of the two neutral carbohydrates is reduced to traces, whereas the sialic acid content increases to about 25—30 % of the mucin. A good yield of hexosamine isolated from such a mucin preparation could not be derived from the neutral carbohydrate components. In fact, from 2 g of such a mucin preparation we succeeded in isolating somewhat more than 0.2 g almost pure chondrosamine hydrochloride. — However, this chondrosamine did not derive from the sialic acid. This is shown in the following observations. Boiling the mucin in water

for 1—2 hrs partially decomposes it. If the material thus obtained is evaporated *in vacuo* to dryness and the residue extracted with methanol, part of it dissolves. Fractionation of the methanol solution with increasing amounts of ether yield products which contain increasing amounts of sialic acid (as determined quantitatively with the help of the Bial reaction or of the direct Ehrlich reaction) and decreasing quantities of hexosamine (as determined according to Elson and Morgan after hydrolysis with 2 *N* HCl for 2—12 hrs).

The gangliosides give the reactions characteristic of sialic acid, *i.e.* direct Ehrlich reaction, purple Bial reaction, and strong humin formation and liberation of CO_2 on heating with mineral acid. It may therefore be assumed that the gangliosides contains this (or some closely related) substance. As to the relation between the sialic acid and the neuraminic acid, it should only be pointed out that the isolation procedure for the neuraminic acid includes heating with methanolic HCl and a prolonged heating with baryta, by which certain groups, *e.g.* acetyl groups, might have been split off. The neuraminic acid has so far been isolated only in the form of its non-reducing methoxyl compound. Also this substance gives a strong purple Bial reaction and the direct reaction with the Ehrlich reagent.

Taken altogether, the above observations indicate that the sialic acid both in the submaxillary mucin and in the gangliosides is accompanied by, and probably bound with an easily split linkage to the chondrosamine. As regards the composition of the acid, it is clear that if it contains hexosamine, of which there is at present no direct evidence, this must be in such a combination that it is not liberated on treatment with mineral acids in the heat.

As sialic acid occurs not only in submaxillary mucin and in the gangliosides but also in several other glycoproteins, *e.g.* those of the blood serum, the elucidation of the structure of the sialic acid should be an important task. Attempts to this end are at present in progress in this Institute, as is work on the questions of the relation of the sialic acid to the acid glycoprotein in serum demonstrated by Winzler *et al.*[7], and to the influenza-virus haemagglutination inhibitors.

EXPERIMENTAL

Preparation of ganglioside. In the main the directions given by Klenk [1] were followed. However, the substance was in addition dissolved in water and thoroughly dialysed, in order to remove low-molecular organic and inorganic impurities. The preparation used contained only 0.04 % P. The hexosamine content (determined according to the Elson-Morgan method as modified by Blix [8]) was about 8 %, and the content of neuraminic acid, as determined according to Klenk with the aid of the Bial reaction, about 20 %. The substance gave a clear solution in water.

Isolation of chondrosamine hydrochloride from ganglioside. 1.5 g was heated under reflux with 30 ml 5 *N* HCl for 12 hrs. The humin formed was filtered off and the filtrate

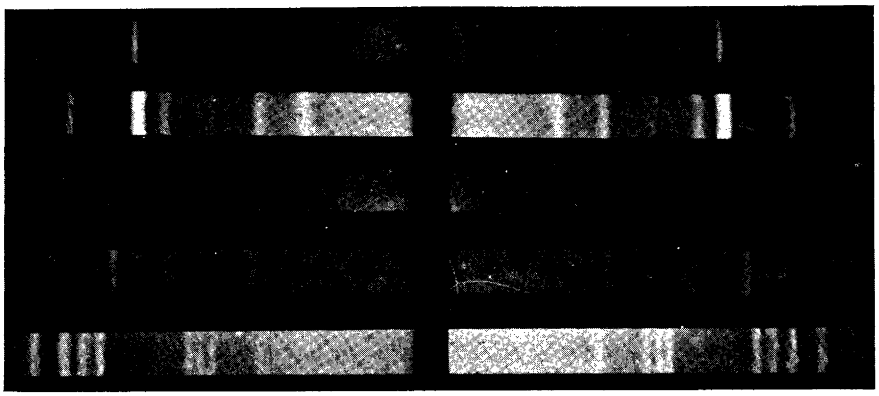


Fig. 1. X-ray powder diagrams of α-chondrosamine HCl (1), hexosamine HCl from ganglioside (2), β-chondrosamine HCl (3), hexosamine HCl from submaxillary mucin (4), α-glucosamine HCl (5).

was shaken twice with two vol. petroleum ether. 1.5 g blood coal was then added to the aqueous phase. The mixture was boiled for a few minutes, filtered and evaporated to a syrup. The syrup was redissolved in warm methanol, and insoluble residues (which contained very little hexosamine) filtered off and discarded. As repeated evaporations from methanol solution yielded no crystalline deposit, the material was heated to 100° with 1 *N* HCl for 24 hrs. The hydrolysate was then treated exactly as described above. After the treatment with blood coal, the solution was evaporated to a syrup. This was taken up in 5 ml methanol, and the solution once more evaporated. After standing for some days, a crystalline deposit appeared, which did not dissolve on addition of methanol. The crystals were washed repeatedly with methanol. On evaporation of the mother liquor, more crystalline material was formed and treated as above. Further repeated evaporations of methanol solution of the material gave more crystalline substance. All crystalline material was collected, and dissolved in about ½ ml distilled water. Methanol was added until crystallization started, and the mixture kept for some days at + 4°. The crystalline deposit was then filtered off, washed and dried. Hexosamine determination according to Elson and Morgan give 84.0 % (calc. 83.1 %). N = 6.32 % (calc. 6.50 %). Optical rotation $[\alpha]_D^{20}$: + 88° (end value). To judge from the N value and the rotation value, the substance still contained some impurities. The X-ray diffraction picture of a once more recrystallized specimen (Fig. 1) showed complete agreement with that of an authentic specimen of α-chondrosamine hydrochloride.

Preparation of submaxillary mucin. The substance was prepared according to the old method of O. Hammarsten, as given by one of us [6]. Some details which are of practical importance in the preparation but which have not been pointed out earlier, should, however, be given here. In order to obtain a coherent mucin clot on dilution of the acid mucin solution with water, it is essential that the submaxillary glands should not be finely minced before the extraction. The gland should only be divided into pieces of about the size of a hazel nut, or somewhat larger. When the glands are minced, the coextracted globulins will prevent the mucin precipitating as a clot, and thereby render it difficult or impossible to isolate. A closer observation of the extraction procedure has shown that

the mucin is in fact, not extracted in the true sense, but flows slowly out into the water from all greater and smaller ducts. If the "extraction" is carried out at + 2°— + 4°, mucin clots may be obtained from the water "extracts" on 5—6 subsequent days. The preparation here investigated was reprecipitated 6 times from acid solution by diluting with water. The dry substance contained 16 % hexosamine and 9.9 % N.

Isolation of chondrosamine hydrochloride from submaxillary mucin. 2 g of the mucin was heated under reflux on the boiling water bath with 30 ml 5 *N* HCl for 14 hrs. To the solution were then added 100 ml methanol and 1.5 g blood coal. The mixture was boiled for some minutes, and filtered. The filtrate was evaporated to a syrup, the latter warmed with 10 ml methanol, and undissolved material filtered off. The filtrate was again evaporated, taken up in 5 ml methanol, and filtered. After a new evaporation, the residue was heated for 18 hrs to + 100° under reflux with 1 *N* HCl. The treatment of the hydrolysate described above was then repeated, the residue taken up in methanol, and the solution thereafter evaporated three times more. Finally, the residue was taken up in 50 ml methanol, and ether was added until a precipitate began to appear. On standing in an open dish for four days, a crystalline deposit was formed. This was filtered off and washed. Repeated evaporations and dissolvings in methanol enabled us to obtain additional crystalline material from the mother liquor. The crystals were collected, dissolved in very little water, and crystallized by adding methanol. Of the fairly pure substance somewhat more than 0.2 g was obtained, which is about 2/3 of the hexosamine present in the mucin taken. Hexosamine determination according to Elson and Morgan gave 84.3 %. N = 6.28 (Dumas). $[\alpha]_D^{20}$: + 93° (end value). The X-ray diffraction pattern was that typical for β-chondrosamine hydrochloride (Fig. 1).

SUMMARY

The isolation and identification of chondrosamine from submaxillary mucin and from gangliosides is described. In these substances, the chondrosamine accompanies and seems to be loosely bound to a probably disaccharidic, structurally still unknown, substance, which is provisionally called *sialic acid*. Some characteristics of the latter are given, and its relation to the so-called neuraminic acid, isolated by Klenk from gangliosides, is shortly discussed.

Our thanks are due to Mr. E. Lindberg for most valuable assistance in the preparative work.

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Interaction of Amino Compounds with Monosaccharides

A Spectrophotometric Study of the Colour with Ehrlich's Reagent

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The differing results on the glucosamine content of the jelly coat of the sea-urchin egg obtained by Krauss [1,2], Vasseur [3], and Vasseur and Immers [4] prompted us to undertake a more careful study of this matter.

Among the hydrolysis products of the jelly coat substance are about 45 per cent of reducing sugars, 1.6 per cent of lysine, and glycine, but no hexosamine [3,4]. As has been noted previously [5,6] a mixture of a reducing sugar with lysine or glycine gives a positive colour reaction for hexosamine when treated according to the procedure described by Elson and Morgan [7]. In contradistinction to glucosamine, a mixture of reducing sugar and, for example, lysine gives a red colour with Ehrlich's reagent after heating with sodium carbonate buffer alone [4], *i.e.* in the absence of acetylacetone. The optimum pH values for glucosamine and for the sugar — amine mixture differ also. This enabled us to give a possible method for the elimination of this error [8]. A more detailed account on these investigations is given in this paper.

METHOD

In a "Hysil" test tube (150 × 15 mm), marked at 5 ml, 0.5 ml of the sample was mixed with 0.5 ml of the buffer solution (usually *M* sodium carbonate — sodium bicarbonate), containing acetylacetone if desired. A larger volume of this mixture was prepared when the pH of the solution was to be measured. The pH was determined with a glass electrode (Radiometer PHM 11a, Copenhagen), the pH values between 9.0 and 11.5 being corrected for the sodium ion content of the solution.

The mixture (1.0 ml) was heated in the stoppered tube in a vigorously boiling water bath for an appropriate time, then cooled and 2 ml of 95 per cent ethanol was added, and the contents were mixed. To this mixture was added 0.5 ml of Ehrlich's reagent (0.8 g *p*-dimethylaminobenzaldehyde — Eastman Kodak, three times recrystallised from 95 per cent ethanol — in 30 ml of 95 per cent ethanol plus 30 ml of concentrated hydrochloric

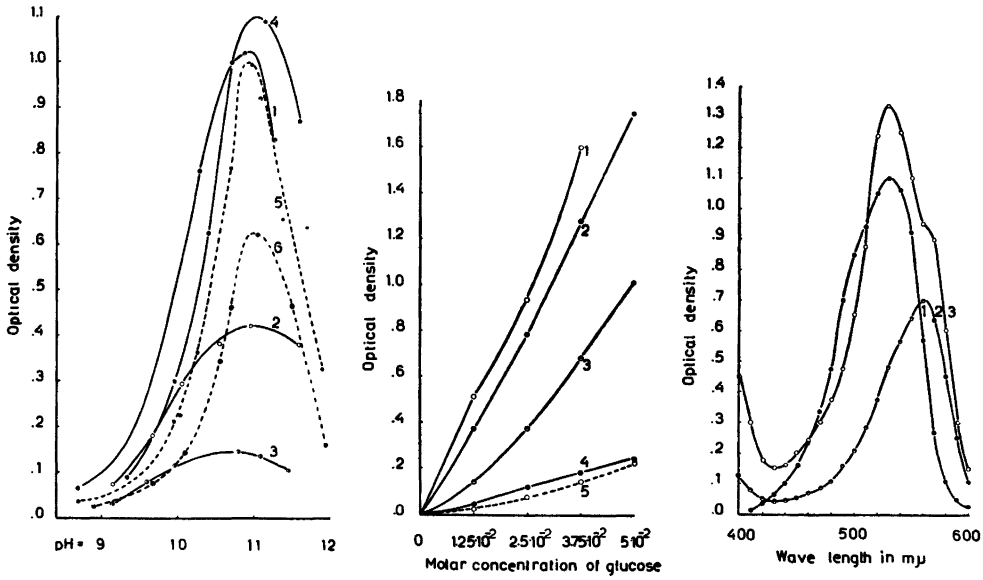


Fig. 1. Influence of pH during the heating on the colour formation with Ehrlich's reagent. Mixture of 0.50 ml of M *carbonate buffer plus 0.25 ml of 0.05* M *glucose solution and 0.25 ml of 0.025* M *solution of: lysine · 2 HCl (curve 1), β-alanine (curve 2), glycine (curve 3), ethylamine · HCl (curve 4). Mixture of 0.50 ml of* M *phosphate buffer plus 0.25 ml of 0.05* M *glucose solution and 0.25 ml of 0.025* M *solution of: lysine · 2 HCl (curve 5), ornithine · 2 HCl (curve 6). Optical density at 570 mμ, after 30 minutes.*

Fig. 2. Effect of carbon chain length of amino acids on the reaction with glucose. 0.5 ml of M *carbonate buffer of pH 10.90 plus 0.5 ml of mixture of amino acid (0.0125* M*) and glucose (from 0 to 0.05* M*). Curves: 1 — lysine, 2 — ornithine, 3 — β-alanine, 4 — α,γ-diaminobutyric acid, 5 — glycine. Optical density at 570 mμ, after 30 minutes.*

Fig. 3. Absorption spectra: curve 1 — a 0.00125 M *glucosamine · HCl solution treated with 1.96 per cent (v/v) acetylacetone in* M *carbonate buffer, at pH 9.55; curve 2 — a mixture of 0.025* M *glucose and 0.0025* M *lysine · 2 HCl treated with* M *carbonate buffer at pH 10.90; curve 3 — a mixture of 0.025* M *glucose and 0.0025* M *lysine · 2 HCl treated with 1.96 per cent (v/v) acetylacetone in* M *carbonate buffer at pH 10.50. Readings 30 minutes after the addition of Ehrlich's reagent.*

acid, sp.gr. 1.19) and then ethanol to the 5 ml mark. The solution was thoroughly mixed and the colour was measured after certain time intervals with a Beckman spectrophotometer model DU, wavelengths 400 to 600 mμ, slit width 0.015 to 0.04 mm, using a 10 mm Corex cell.

EXPERIMENTAL

1. *Influence of pH and buffer during heating on the formation of coloured products.* Glucose was heated with different amino compounds at various pH values in the presence or in the absence of acetylacetone. The buffer used in

most experiments was *M* sodium carbonate — bicarbonate, but 0.5 *M* sodium phosphate (tribasic-dibasic) also was tried, giving essentially the same results (see Fig. 1). The optimum pH is about 10.8 to 11.2 for the glucose — amine mixtures either with or without acetylacetone. On the other hand, for glucosamine the optimum pH is about 9.5 to 9.6 [9].

2. *Influence of the structures of the amino compound and carbohydrate on the ability to give coloured products.* Of the α-amino acids lysine and ornithine give by far the most intense colours [4]; glycine, arginine, and asparagine give a much weaker coloration, and all the other α-amino acids tested, including alanine, give no colour when tested separately with carbohydrate (without acetylacetone). A weak colour is given also by α, γ-diaminobutyric acid (Fig. 2, curve 4), and β-alanine gives a colour intermediate in intensity between the former and ornithine (Fig. 2, curve 3). Acetamide and hydroxylamine do not give any colour under these conditions.

Only primary amines give a positive reaction; secondary and tertiary amines do not react under these conditions (see Table 1).

Table 1. Optical density at 570 mμ of the coloured product of glucose with primary, secondary, and tertiary methylamine in M *carbonate buffer at pH 10.95.*

0.25 ml 0.025 *M*	0.25 ml glucose			
	0.05000 *M*	0.02500 *M*	0.01250 *M*	0.00625 *M*
$CH_3NH_2 \cdot HCl$	1.600	.880	.317	.085
$(CH_3)_2NH \cdot HCl$	.067	.045	.050	.000
$(CH_3)_3N \cdot HCl$	.075	.025	.015	.000

These results demonstrate that (1) the amino group must be unsubstituted, *i.e.* only primary amino groups react; (2) the colour is more intense when the amino group is remote from carboxyl, hydroxyl, and other electron-attracting groups, *i.e.*, a stronger coloration develops when the carbon chain separating the carboxyl and the amino groups of the amino acid is lengthened (Fig. 2).

In the presence of acetylacetone, ornithine — glucose give a more intense colour than lysine — glucose (*cf.* Immers and Vasseur [8]).

On the other hand, the structure of the monosaccharide does not seem to be equally essential. Glucose, galactose, mannose, fructose, arabinose, xylose, fucose, and rhamnose have all the same action. The behaviour of lactic aldehyde and other similar compounds would be of greatest interest for studying the mechanism of this reaction.

3. *Character and stability of the colour.* a) *Under ordinary conditions*, i.e. *in strongly acid medium.* The absorption curves (see Fig. 3) for glucosamine (curve 1), glucose — lysine without acetylacetone (curve 2), and glucose — lysine with acetylacetone (curve 3) are all different. The glucosamine curve has its maximum at 530 mμ. The glucose — lysine curve without acetylacetone has one maximum, at 560 mμ, but in the presence of acetylacetone two maxima, one at 530 mμ and a smaller one at about 570 mμ. These curves were obtained by measuring the optical density 30 minutes after the addition of Ehrlich's reagent.

When the solutions are kept at room temperature, the character of the colour changes (see Figs. 4 to 6) and the intensity decreases. The maximum of the glucosamine curve (Fig. 4) shifts from 530 mμ initially to 510 mμ after 21 hours *. The presence of the compound giving the absorption at 510 mμ is evident even after 20 minutes, just as the 530 mμ compound is still present after 21 hours. In each case the presence of the minor component is indicated by a bulge on the curve. The colour intensity decreases slowly with time.

In the absorption curve of the glucose — lysine mixture without acetylacetone two maxima, one at 570 mμ and the other at 510 mμ, gradually displace the maximum at 560 mμ (Fig. 5). The intensity of the 570 mμ peak is lowered on keeping at room temperature, whereas the intensity of the 510 mμ peak seems to increase slightly.

The absorption of the glucose — lysine solution with acetylacetone does not show any of these pronounced changes with time (Fig. 6). The main effect seems to be a diminution of the colour intensity. However, the peak at 530 mμ fades more rapidly than that at 570 mμ.

b) *At different pH.* The colour intensity of the products formed is sensitive to differences in pH. The colour is most stable in strongly acid media, below pH 1.3. When the solution is made less acid, the colour gradually fades and disappears completely at a pH of approximately 8.5 (Table 2).

Table 2. Colour intensity of glucose — lysine chromophore without acetylacetone at various pH values.

pH	1.00	2.32	2.85	3.60	5.40	6.35	8.70
Optical density at 570 mμ	.867	.540	.485	.393	.269	.152	.030

* After the experimental part of this work was completed, the paper by Schloss [10] appeared, containing *inter alia* a similar statement.

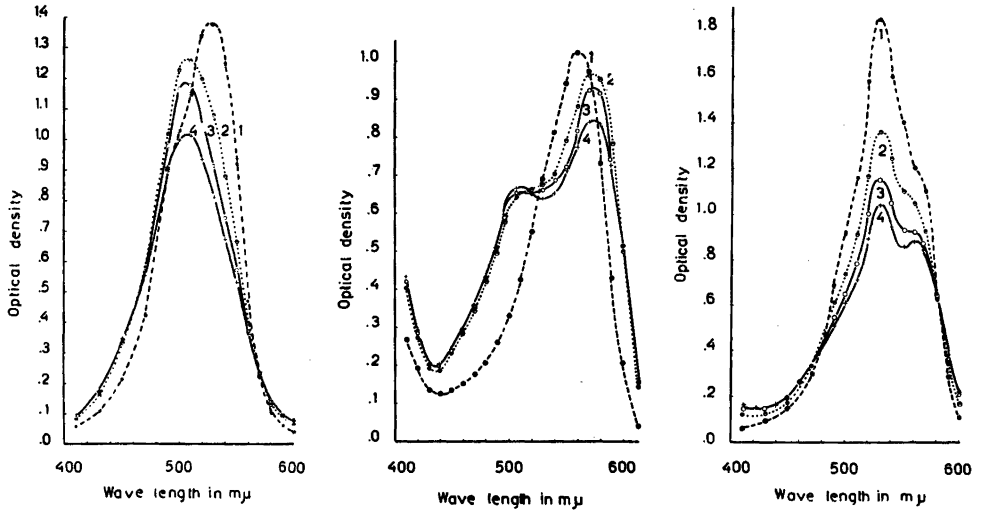


Fig. 4. Change of absorption spectrum of the chromophore of 0.0015 M *glucosamine · HCl treated with 1.96 per cent acetylacetone in* M *carbonate buffer at pH 9.55 and developed with Ehrlich's reagent, on keeping at room temperature. Curves: 1 — after 20 minutes, 2 — 21 hours, 3 — 45 hours, 4 — 68 hours.*

Fig. 5. Change of absorption spectrum of the chromophore of 0.025 M *glucose and 0.0025* M *lysine · 2 HCl treated with* M *carbonate buffer at pH 11.00 and developed with Ehrlich's reagent, on keeping at room temperature. Curves: 1 — after 20 minutes, 2 — 18 hours, 3 — 25 hours, 4 — 42 hours.*

Fig. 6. Change of absorption spectrum of the chromophore of 0.025 M *glucose and 0.007* M *lysine · 2 HCl treated with 1.96 per cent acetylacetone in* M *carbonate buffer at pH 10.80 and developed with Ehrlich's reagent, on keeping at room temperature. Curves: 1 — after 20 minutes, 2 — 21 hours, 3 — 45 hours, 4 — 68 hours.*

When this colourless solution is re-acidified, the colour reappears, the intensity being higher in more acid solution, just as before. The solution may be kept alkaline for many hours without losing the capacity to develop the red colour when re-acidified.

However, there is a difference in reactivity of the coloured products towards alkali. The colour derived from glucosamine or from glucose — lysine with acetylacetone disappears immediately upon addition of excess sodium hydroxide, even when the coloured sample has been standing for many hours. The colour from a glucose — lysine mixture without acetylacetone, on the other hand, does not disappear completely on the addition of alkali, provided that the colour has been developed some 18 hours or more before, and the solution thereafter has been kept at room temperature. There is, however, a decrease in intensity. Only the absorption maximum at 570 mμ is evident after this treatment.

4. *Behaviour of the glucose — lysine chromogen without acetylacetone when kept at different pH values before the addition of Ehrlich's reagent.* Samples of glucose (0.05 *M*, 1 part) and lysine (0.025 *M*, 1 part) were heated with carbonate buffer (*M*, pH 10.95, 2 parts) in the usual manner. To 1.0 ml aliquots were added (1) 0.50 ml of distilled water; (2) 0.20 ml of 6 *N* HCl and 0.30 ml of distilled water; (3) 0.30 ml of 6 *N* HCl and 0.20 ml of distilled water; (4) 0.50 ml of 6 *N* HCl. The pH values after these additions were 10.9, 6.7, 3.35, and < 1.2, respectively.

The samples were allowed to stand for different lengths of time, 0, 4, 21, 45, and 71 hours, before the colour was developed with Ehrlich's reagent. Thereafter the absorption curve between 400 and 600 mμ was measured for each sample after 40 minutes, 22 hours, 46 hours, and 71 hours. Lack of space does not allow the publication of all these curves, but Table 3 gives some data

Table 3. Change in optical density at 510 and 570 mμ of the glucose-lysine chromophore without acetylacetone when the chromogen was kept at different pH values before the addition of Ehrlich's reagent or p-dimethylaminobenzaldehyde (p-DAB).

Time in hours before the addition of Ehrlich's reagent or p-DAB	Measurement 40 minutes after the addition of							
	Ehrlich's reagent						p-DAB	
at pH	10.90		3.35		<1.2		<1.2	
wavelength, mμ	510	570	510	570	510	570	510	570
0	.685	2.00	.630	1.90	.580	1.05	.650	1.38
4	.652	1.90	.560	1.56	.420	.660	.430	.710
21	.710	2.00	.452	1.07	.317	.320	.340	.330
45	.627	1.80	.384	.740	.273	.205	.297	.210
71	.665	2.00	.318	.488		.185		.183
Time in hours before the addition of Ehrlich's reagent or p-DAB	Measurement 46 hours after the addition of							
	Ehrlich's reagent						p-DAB	
at pH	10.90		3.35		<1.2		<1.2	
wavelength, mμ	510	570	510	570	510	570	510	570
0	.958	1.50	.880	1.25	.730	.583	.940	.785
4	.945	1.54	.795	1.08	.625	.390	.790	.510
21	.995	1.58	.660	.720	.525	.270	.685	.305
45	.915	1.38	.620	.530	.500	.232	.670	.255

for the changes in intensity at the essential wavelengths, 510 and 570 mμ (*cf.* Fig. 5). The variations in the individual data are rather high in this particular experiment owing to the large number of operations which had to be performed simultaneously or in quick succession.

On keeping, the samples of pH 10.9 showed very little if any change in the intensity of the colour and the character of the curves, provided that the measurements were carried out at the same time after the addition of Ehrlich's reagent. The curves obtained after 40 minutes were all of the kind shown in Fig. 5, curve 1 (with but one maximum at ca. 560 mμ), and the curves obtained 46 hours after the addition of Ehrlich's reagent were similar to curve 4 in Fig. 5 (with one maximum at 570 mμ and another at 510 mμ). Thus, keeping at room temperature at the same pH as that at which the heating of the sample had been carried out, does not affect the curve.

On the other hand, when the pH of the sample is lowered after the heating at pH 10.9 and the sample is allowed to stand at this lower pH before Ehrlich's reagent is added, the character of the curves changes. This change is the more pronounced the more the sample has been acidified. The intensity of the colour is decreased considerably. The most interesting feature is, however, the appearance of the 510 mμ peak in the 40 minutes curves and the dominance of this peak in the 46 hours curves. By changing the pH of the sample to a very low value (< 1.2), after the heating has been carried out at pH 10.9, and allowing the sample to remain at this pH one or two days at room temperature, the 570 mμ component disappears almost completely, whereas the 510 mμ component becomes the dominating chromogen. These two components may thus be obtained almost free from each other and this should facilitate a closer study of their structures (*cf.* Schloss [10]).

These experiments show that the 570 mμ component is stable in alkaline solution but unstable in acid solution. It seems very probable that the 570 mμ component is transformed partly to the 510 mμ component in acid media. On the other hand, in alkaline media the 510 mμ component is less stable, and seems to be transformed partly to the 570 mμ component (see section 3b).

5. *Effect of concentration of the two components (sugar and amine) on the colour intensity.* Different mixtures of glucose and lysine in carbonate buffer of pH 10.9 without acetylacetone were treated in the usual way. The concentration of one of the components was kept constant and the concentration of the other was varied (Table 4). It thus becomes evident (1) that glucose is degraded to a greater extent than is lysine, and (2) that the degradation of glucose is less pronounced when higher concentrations of lysine are present simultaneously. Thus, the reaction between lysine and glucose will compete with the degradation of glucose, so that when lysine has reacted with glucose, the

Table 4. Effect of concentration of glucose and lysine on the colour intensity, optical density at 570 mμ.

Concentration of lysine, molarity	Concentration of glucose, molarity				
	$3.125 \cdot 10^{-3}$	$6.25 \cdot 10^{-3}$	$1.25 \cdot 10^{-2}$	$2.5 \cdot 10^{-2}$	$5.0 \cdot 10^{-2}$
$3.125 \cdot 10^{-3}$	.000	.050	.225	.620	1.105
$6.25 \cdot 10^{-3}$	.020	.080	.280	.770	1.57
$1.25 \cdot 10^{-2}$	.030	.110	.385	.860	1.90
$2.5 \cdot 10^{-2}$	.045	.180	.365	.880	1.90
$5.0 \cdot 10^{-2}$	.055	.165	.450	.920	2.00

latter will not be liable to be degraded to the same extent as when present alone or together with a lower concentration of lysine.

6. *Effect of pretreatment of the two components (sugar and amine) on the colour intensity.* Table 5 shows some of the results of two series of experiments, in which lysine and glucose were treated separately or together (0 or 20 minutes heating at pH 9.60) and then treated in the normal way with or without acetylacetone.

The results are very complex, but some conclusions may be drawn from the experiments. (1) The 570 mμ peak is the dominating one in the absence of acetylacetone (columns with even numbers) and the 530 mμ peak in the presence of acetylacetone (columns with uneven numbers). (2) The very low value for the non-heated glucose — lysine mixture with acetylacetone (columns 3 and 7) is increased very considerably when the mixture is heated before the acetylacetone is added (column 1). (3) The optical density without acetylacetone is nearly doubled when the mixture is heated for 20 minutes before the ultimate heat-treatment (columns 2, 4, 8). (4) When glucose and lysine have been heated separately, the optical density in the absence of acetylacetone is decreased (columns 6, 8), but in the presence of acetylacetone it is increased (columns 5, 7). (5) The optical density both with and without acetylacetone is barely influenced if lysine alone is heated before (columns 7, 9, and 8, 10, respectively), but there is a considerable decrease in optical density without acetylacetone (columns 8, 12) and an increase with acetylacetone (columns 7, 11) if glucose is heated separately before the ultimate heat-treatment.

There seems, therefore, to exist in the heated glucose solution a degradation product of glucose which in the absence of acetylacetone does not contribute to the formation of the chromogen, but which in the presence of acetylacetone has this property.

Table 5. Effect of pretreatment of glucose and lysine on the colour intensity.

Column no.	1	2	3	4	5	6	7	8	9	10	11	12
0.005 *M* lysine in *M* carbonate buffer, pH 9.60; no heating; ml (A)	—	—	—	—	—	—	.25	.25	—	—	.25	.25
0.05 *M* glucose in *M* carbonate buffer, pH 9.60; no heating; ml(B)	—	—	—	—	—	—	.25	.25	.25	.25	—	—
0.005 *M* lysine + 0.05 *M* glucose in *M* carbonate buffer, pH 9.60; no heating; ml (C)	—	—	.25	.25	—	—	—	—	—	—	—	—
As A but 20 min. heating; ml	—	—	—	—	.25	.25	—	—	.25	.25	—	—
As B but 20 min. heating; ml	—	—	—	—	.25	.25	—	—	—	—	.25	.25
As C but 20 min. heating; ml	.25	.25	—	—	—	—	—	—	—	—	—	—
M carbonate buffer, pH 9.60; ml	.25	.75	.25	.75	—	.50	—	.50	—	.50	—	.50
6 per cent acetylacetone reagent (in *M* carbonate buffer), pH 9.60; ml	.50	—	.50	—	.50	—	.50	—	.50	—	.50	—
20 minutes heating of final mixture	+	+	+	+	+	+	+	+	+	+	+	+
Optical density at 530 mμ Exp.1	.375	.310	.075	.192	.175	.110	.080	.185	.045	.205	.225	.085
» 2	.460	.380	.050	.195	.225	.108	.096					
Optical density at 570 mμ Exp.1	.470	.465	.060	.295	.145	.153	.065	.292	.043	.320	.185	.115
» 2	.620	.590	.045	.295	.205	.135	.085					

7. *Change of ultraviolet spectrum of glucose in sodium carbonate — bicarbonate buffer of pH 9.6, and of this buffer alone upon heating.* The ultraviolet spectrum of a glucose solution in carbonate buffer (pH 9.6) undergoes very complicated variations when the solution is heated for different lengths of time. In addition to the peak at ca. 265 mμ [11], a peak at 290 mμ appears after 20 minutes heating. This peak fades shortly afterwards. By analysing the ultraviolet spectrum of the carbonate buffer alone it could be demonstrated that this peak at 290 mμ must be attributed entirely to the buffer and not to any degradation product of glucose.

When the heated samples were acidified, only one maximum (at 250 mμ) remained in the 220 to 320 mμ region. The increase of the optical density at 250 mμ indicates that during the first 20 to 30 minutes of heating (at pH 9.6) a compound is formed, which exhibits a maximum at 250 mμ in acid media and at 265 mμ in alkaline media. Thereafter another compound with maximal absorption at a lower wavelength than 220 mμ is formed.

GENERAL DISCUSSION

The colorimetric method for the determination of glucosamine as described by Elson and Morgan [7] may give erroneous results when simple carbohydrates and amino compounds are present simultaneously. This may often be the case when hydrolysates from biological material are analysed. In the present investigation it has been shown that when monosaccharides are heated in alkaline media (with or without the addition of acetylacetone) with certain amino acids or primary amines, the resulting solutions give red colorations with Ehrlich's reagent. The coloured products exhibit maximal absorption in the same part of the spectrum as the glucosamine reaction product. The intensity of the colour depends on the following: (1) the pH during the heating in alkaline medium; (2) the acetylacetone concentration; (3) the nature of the carbohydrate and amino compounds; (4) the pH after the addition of Ehrlich's reagent; (5) the concentration of the carbohydrate and the amine.

The optimal pH during the heating is 9.5 to 9.6 for the glucosamine reaction [9], but pH 10.8 to 11.2 (Fig. 1) for the sugar — amine mixtures. The optimal acetylacetone concentration for the sugar — amine mixtures is about 0.8 per cent (v/v) in the acetylacetone reagent (Fig. 7), *i.e.* about 0.4 per cent in the reaction mixture. For glucosamine, on the other hand, the colour intensity increases with the concentration of acetylacetone up to at least 3 per cent in the acetylacetone reagent (Fig. 7, curve 1). Elson and Morgan [7] use 1.96 per cent acetylacetone at pH 9.5. The same concentration and pH have been used in many of the modifications of this method [12,13,14]. Sørensen [9] and Blix [15], however, propose the use of higher concentrations of acetylacetone.

Our own investigations show that usually the sugar — amine error is reduced by using a higher acetylacetone concentration. Therefore, 4 to 5 per cent of acetylacetone at pH 9.5 to 9.6 is recommended. On the other hand, sugar — amine interaction can be detected most easily by carrying out analyses at other acetylacetone concentrations as well, and at another pH. It is advisable to test for the sugar — amine error by analysing a sample at pH 10.8 to 11.2 with 0.8 per cent acetylcetone in the acetylacetone reagent or even without acetylacetone and to read the sample both at 530 mμ, which is

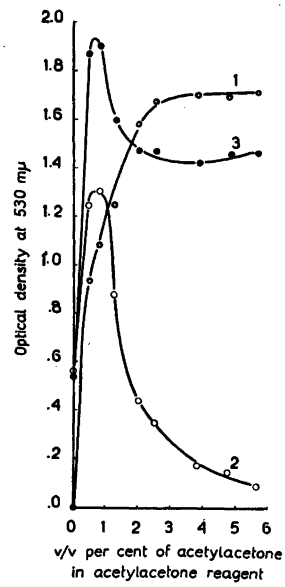


Fig. 7. Influence of acetylacetone concentration on the colour formation with glucosamine (curve 1; 0.0015 M*), glucose plus lysine (curve 2; 0.025* M *and 0.0025* M*, respectively), and a mixture of these (curve 3; 0.0015* M *glucosamine · HCl, 0.025* M *glucose, 0.0025* M *lysine · 2 HCl). Measurement of optical density at 530 mμ, 30 minutes after the addition of Ehrlich's reagent.*

the optimum for glucosamine and for the sugar — amine mixtures with acetylacetone, and at 570 mμ, the optimum for the sugar — amine mixtures without acetylacetone.

Curve 3 (Fig. 7) which relates to the colours produced by mixtures of glucosamine, glucose and lysine at different acetylacetone concentrations, indicates that the two reactions counteract each other to some extent. This becomes particularly evident at an acetylacetone concentration higher than 2 per cent, where the colour intensity is lower than that for glucosamine alone, thus giving rise to a negative error. The region where the glucosamine curve and the glucosamine — glucose — lysine curve intersect, depends on the concentrations of the interfering substances [8].

As to the chemical nature of the different chromogens, Gottschalk and Partridge [16,17] have recently suggested that on alkaline treatment a glucose — lysine mixture gives a Schiff's base, the enol form of which gives a dihydropyrazine condensation product. This in turn is assumed to form with *p*-dimethylaminobenzaldehyde the chromophore in the Ehrlich reaction.

Our results indicate, however, that the reactions may be still more complex. A Schiff's base is formed, when simple sugars react with almost all amino acids under alkaline conditions (*cf.* 11, 18 for literature). However, a coloured product with Ehrlich's reagent is formed only with a few of these amino acids and the degree of colour formation depends on the distance between the reacting amino group and electron-attracting groups which may be present.

Under the conditions of the glucosamine reaction, the 1 : 1 ratio calculated for the Schiff's base reaction cannot be expected to give the maximum colour, since some of the glucose is degraded, as shown in sections 5 and 7 (see also Frankel and Katchalsky [19]). A higher monosaccharide concentration, as compared with the amino compound, must therefore be present in order to give maximal colour. This is the case with hydrolysates of many mucoid and mucopolysaccharide preparations, the observed values of the hexosamine content of which will be in error for this reason.

Furthermore, the experiments reported in section 6 and those illustrated in Fig. 7 demonstrate that the presence or absence of acetylacetone is of very great significance. The maxima for the reaction products occur at different wavelengths in the different cases. When the coloured samples are allowed to stand at room temperature, these maxima shift, but the displacement is different for the different types of products.

Finally, the different behaviour towards alkali, reported in section 3b, indicates basic differences in these reactions.

SUMMARY

When monosaccharides are heated in alkaline media (pH 9 to 12) with certain amino acids (*e.g.* lysine) or primary amines, in the presence or in the absence of acetylacetone, red coloured products are obtained with Ehrlich's reagent. These interfere with the colorimetric determination of glucosamine by the method of Elson and Morgan [7].

This interference can be controlled by carrying out the colorimetric determination on the unknown sample parallel with the glucosamine standard (1) at pH 9.5 to 9.6 and at pH 10.8 to 11.2; (2) with different acetylacetone concentrations, *e.g.* 0, 0.8, and 4 to 5 per cent in the reagent; (3) by determining the absorption at 530 and 570 mμ.

Furthermore, the main results may be summarised as follows. The optimum pH for glucosamine is 9.5 to 9.6, for the sugar — amine mixtures 10.8 to 11.2.

The amino compound must have the amino group unsubstituted. The intensity of the colour is increased when the distance between the reacting amino group and electron-attracting groups (-COOH, >CO, $-\overset{|}{\underset{|}{C}}OH$, etc.), which might be present, is increased.

The absorption curve for glucosamine has its maximum at 530 mμ, but this shifts to 510 mμ on keeping. The glucose — lysine mixture without acetylacetone has its maximum at 560 mμ, but on keeping two maxima appear,

one at 570 and another at 510 mμ. The glucose — lysine mixture with acetylacetone has two maxima, at 530 and 570 mμ, respectively.

When the coloured, strongly acid solutions are made alkaline, the colour disappears completely, except for the glucose — lysine mixture without acetylacetone which has been standing for about a day or more at room temperature.

The 570 mμ component of the glucose — lysine mixture without acetylacetone is stable in alkaline but unstable in acid media, whereas the reverse is true for the 510 mμ component.

The degradation of glucose in alkaline solution is counteracted by its reaction with lysine to form the chromogen.

A degradation product of glucose is formed in alkaline solution which upon further heating with lysine and in the presence but not in the absence of acetylacetone contributes to the formation of the chromogen.

In a pure sodium carbonate — bicarbonate buffer of pH 9.6 a compound is formed upon heating, characterized by an absorption maximum at 290 mμ.

We express our thanks to Professor John Runnström, head of the Wenner-Gren Institute, for his interest during the work. We are indebted to Professor Gunnar Blix and Professor Erik Jorpes for samples of acetylacetone, glucosamine, *etc.*, when these chemicals were not available commercially.

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Periodate Oxidation of the Jelly Coat Substance of *Echinocardium cordatum*

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The jelly coat which surrounds sea-urchin eggs is composed of a polysaccharide ethereal sulphate in combination with protein, the former being the main component [1]. Analyses carried out with the aid of paper partition chromatography [2] revealed the presence of but one sugar, fucose, in the hydrolysate from *Echinocardium cordatum* jelly. Chemical studies on the egg jellies of other species of sea-urchin [1–4] suggest that the comparatively high degree of specificity which the jellies show in the sperm-agglutination test [5–7] may depend on the carbohydrate component [8] of the jelly substance. Furthermore, these studies showed that polyfucose sulphate is the major carbohydrate component, not only in *E. cordatum* jelly (where no other sugar was detected), but also in *Strongylocentrotus droebachiensis* and *Paracentrotus lividus* jellies. In the latter two species the minor carbohydrate component is built up from galactose and glucose residues respectively [2]. It is therefore of interest to investigate the structure of the polyfucose sulphates in order to see if they differ, and the present paper presents the results of some studies on the structure of the *E. cordatum* jelly substance.

EXPERIMENTAL

Material. The material used was collected in the summer of 1949 and prepared in the usual manner [1] by dissolution in acidified sea-water (pH approximately 5.0), cleaning by repeated centrifugation, and precipitation with ammonium sulphate. The resulting precipitate was stored in the cold.

Very concentrated solutions were obtained from this precipitate by dialysis, after the addition of some water, against running tap water until free from sulphate ions and then against cold distilled water until free from chloride ions.

Methods. The solid content of these solutions was determined by evaporating and drying at 105°. Total nitrogen was determined both on dry samples and on aliquots of

solution by the procedure described by Parnas [9], using the combustion fluid of Arnold and Wedemeyer (*cf.* Parnas [9]).

Total sulphur was determined by the Grote – Krekeler method [10]. The absorption liquid and the ash were treated separately, and the sulphate was weighed as $BaSO_4$. As the ash was more or less insoluble, most probably owing to the presence of dead-burned $CaSO_4$, the apparent sulphate figure for the ash was corrected on this assumption. In most cases the entire ash could be considered to be composed of anhydrous $CaSO_4$. The excellent agreement between the values for the total sulphur obtained in this way and the values for the acid-hydrolysable sulphate (Table 1) suggests that this assumption is

Table 1. Analysis of total sulphur (Grote and Krekeler [10]) and acid-hydrolysable sulphate of the jelly substance of Echinocardium cordatum.

Preparation	Total sulphur as sulphate (SO_4)				Acid-hydrolysable sulphate (SO_4)		
	weight of substance mg	in abs. liquid per cent	in ash ($CaSO_4$) per cent	total per cent	weight of substance mg	time at 127° hours	per cent
Ec 49 : 1					27.97	0 *	17.64
					27.93	3	18.41
Ec 49 : 2	27.5	10.92	9.74	20.66			
	69.1	10.91	9.69	20.60			
Ec 49 : 3	29.0	12.76	9.96	22.72	29.72	1	22.44
	34.1	11.82	10.56	22.38			

* *i. e.* the gas was turned off immediately after the temperature had reached 127°. Fifteen minutes was allowed for the steam to displace the air in all autoclaving experiments.

justified. Acid-hydrolysable sulphate was obtained as $BaSO_4$ after hydrolysing weighed samples of the jelly solution with 0.5 *N* HCl in an autoclave at 127° *Cf.* [1]. The total fucose in the jelly solution was determined by the procedure described by Dische and Shettles [11].

Quantitative analyses of the monosaccharide content of the *E. cordatum* jelly hydrolysate were carried out following the procedure described by Flood, Hirst, and Jones [12], and the colorimetric determination described by Blass, Macheboeuf, and Nunez [13].

The jelly was hydrolysed with 0.5 *N* H_2SO_4 for 5 hours in a flask equipped with a reflux condenser and immersed in a boiling water bath. The fucose content [11] after hydrolysis was 33.6 per cent, compared with 34.4 per cent before hydrolysis. The optical rotation of the hydrolysate was strongly negative, indicating that the fucose is in the L-form. No reference sugar [12] was added to the hydrolysate. Instead, the concentrations of the various sugars were related to the concentration of fucose in the jelly before hydrolysis, *i. e.* 34.4 per cent of the dry weight. The hydrolysate was neutralised with $BaCO_3$ and freed from amino acids on columns of ion exchange resins (Amberlites IR 100 and IR 4 B). n-Butanol: water (6 : 1) was used for developing the chromatograms which were run on Munktell OB filter paper sheets. The techniques described by Trevelyan, Procter, and Harrison [14], and by Hough, Jones, and Wadman [15] were employed for the detection and

identification of the sugars. The sugars from the sections of the chromatograms corresponding to galactose and glucose were estimated as galactose, since the amount of glucose was very small, probably less than one third of the galactose content. The values obtained for galactose (+ glucose) were 1.55, 1.53, 1.58, mean 1.56 per cent, and for mannose 0.54, 0.21, 0.65, mean 0.50 per cent, referred to fucose as 34.4 per cent.

Periodate oxidation: Periodate oxidation of the jelly substance was carried out at 50° in a solution prepared as above which was 0.533 millimolar in $NaIO_4$ and buffered to pH 3.5 with a phthalate buffer [16]. About two thirds of the periodate had reacted after twelve hours.

The pH 3.5 was chosen since model studies on the periodate oxidation of maltose, melibiose, a dextrin containing 1,3-linkages, dextran, and other sugars [17] have shown that at this pH the amount of periodate consumed during the initial rapid phase of the oxidation is proportional to the number of α-glycol groupings in the carbohydrate. The rate of "overoxidation" [17–22] is much less at this pH than in more acidic solutions [18] or, in particular, at pH 7 [19]. At known times five-ml samples, equivalent to 10.0 mg of untreated jelly substance, were withdrawn with a pipet and run into 10 ml of ice-cold phosphate buffer of pH 7 [19, 23]. Solid potassium iodide was added and after a few minutes the mixture was titrated with 0.01 *N* thiosulphate. A blank, without jelly substance, was run simultaneously. Fucose was determined in these titrated samples after dialysis, and in the untreated sample, by the method of Dische and Shettles [11], and nitrogen according to Parnas [9]. The effect of dialysis alone on the fucose value was determined on an aliquot to which periodate was not added. This sample was treated with the same amount of phosphate buffer, potassium iodide, and starch as was added to the periodate-treated samples, and a little thiosulphate was added before the dialysis. The results of the oxidations carried out at pH 3.5 are summarised in Table 2. The values for the nitrogen content of the supernatants are not reliable, because it was found out that the cellophane of the dialysis bags under these conditions slowly went into solution, thus contaminating the samples and increasing their nitrogen content. It seems fairly certain that the entire nitrogen content ot the supernatant of all the periodate-treated samples, possibly except of the very first, comes from this source. After the last sample for periodate, etc. determinations had been withdrawn, the remainder of the reaction mixture, equivalent to 50.0 mg of untreated jelly substance, was dialysed. The precipitate and the supernatant were thereafter treated separately. They were hydrolysed with 0.5 *N* HCl in an autoclave for 3 hours at 127°, and the sulphate was precipitated and weighed as $BaSO_4$. The supernatant contained 17.45 per cent of sulphate, and the precipitate gave 1.8 mg (= 1.48 per cent as SO_4) of a brown precipitate, which probably contained some $BaSO_4$ but which was not analysed further.

In another experiment, at pH 1.7, a much stronger periodate solution was used so that there was more than a fivefold excess of periodate even after ten hours. The fucose content was affected in the same way as in the before-mentioned experiment, but the periodate consumption increased rapidly even after the polysaccharide was broken down.

DISCUSSION

The carbohydrate analyses by the quantitative micromethod of Flood, Hirst, and Jones [12] as modified by Blass, Macheboeuf, and Nunez [13] showed the presence of very small amounts of galactose, mannose, and glucose (total

about 2 per cent) as well as fucose (34.4 per cent). The former three sugars were present in this jelly preparation in such low amounts that it appears very doubtful if they really form part of the jelly molecule. It seems more probable that they represent a slight impurity derived from the eggs. This is all the more likely in view of the fact that *E. cordatum* eggs are known to be very fragile.

The analyses of total sulphur and acid-hydrolysable sulphate (Table 1) confirm the results obtained previously with *S. droebachiensis* jelly [24] and *Echinus esculentus* jelly [1] that practically all of the sulphur is present as easily acid-hydrolysable sulphate. The figures for the fucose content and for the content of acid-hydrolysable sulphate (and total sulphur) agree very well with the concept [1] that the polysaccharide part of the jelly is built up of fucose sulphate units (Table 3).

The periodate oxidation gives some very interesting information on the structure of the polysaccharide part of the *E. cordatum* jelly. During the first half hour of reaction at pH 3.5, periodate is consumed very rapidly; thereafter the rate decreases. During the initial rapid reaction half of the fucose residues are destroyed (Table 2), but the remainder are intact even after twelve hours of periodate treatment.

Table 2. Periodate oxidation of the jelly substance of Echinocardium cordatum at pH 3.5 and 50°.

Time of periodate treatment, min	Untreated		1	10	30	60	120	240	360	540	730
	non-dialysed	dialysed									
Periodate consumed, μmol IO_4^-/mg dry subst.			0.289	1.024	1.32	1.50	1.73	2.08	2.43	2.83	3.24
Total fucose, per cent	30.7	25.3	21.5	17.9	13.9	13.9	12.4	14.9	14.9	14.0	12.8
Fucose in supernatant, per cent	29.3	23.4	20.2	15.6	12.2	13.0	10.0	13.0	14.3	13.2	12.5
Nitrogen in precipitate, per cent	4.8	0.7	3.1	4.0	3.5		3.0	2.7		2.1	
Nitrogen in supernatant, per cent		4.5	2.1	1.6	1.7		1.0	1.3		1.3	
Nitrogen in supernatant corrected for contamination from dialysis bag (see text), per cent		ca. 3.3	<1.0	<0.3	<0.4		0	0		0	

Further at pH 1.7 half of the fucose residues are destroyed during the first half hour, but no more are oxidised in spite of the large excess of periodate present. However, the plot of periodate consumed against time shows no break after 30 minutes so that in this case the periodate consumption cannot be used to calculate the number of α-glycol groupings in the jelly substance.

On the other hand, the experiment carried out at pH 3.5 lends itself better to such calculation as there is a pronounced break in the rate of periodate consumption, which occurs simultaneously with the termination of the breakdown of the fucose residues in the jelly substance. If the reacting fucose residues each consume one molecule of periodate (see below), then the periodate consumption should be 0.934 μmol per mg of dry substance. The actual periodate consumption at this point (30 minutes) is however 1.32 μmol per mg and this fact indicates that structures other than the polyfucose sulphate consume periodate as well. The galactose, glucose, and mannose, present as impurities (see above), would consume not more than 0.23 μmol of periodate per mg of dry substance, probably considerably less, if they are in the form of neutral polysaccharides. Therefore, other components of the jelly must be considered to be oxidised by the periodate.

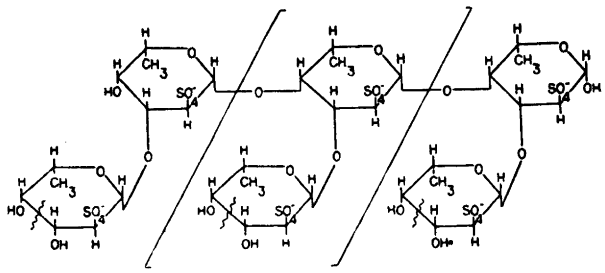
This assumption is substantiated by the observations that a precipitate is formed in the jelly solution during the course of periodate oxidation. This precipitate first becomes evident after one hour, and its formation seems to be essentially complete after four hours. When these samples were dialysed (for the fucose determination), a flocculent precipitate was formed in all the samples except the non-oxidised sample in which comparatively little precipitate was formed. Part of this precipitate seemed to consist of retrograded starch (from the periodate titration). However, the analyses of the clear supernatants and of the precipitates (Table 2) show that, whereas practically all of the fucose is in the clear solution, the major part of the nitrogen is contained in the precipitate. Moreover, the nitrogen values indicate a slow decrease in the nitrogen content of the precipitate during the periodate treatment. This indicates that the protein component is attacked by the periodate and precipitated in denatured form. Desnuelle, Antonin, and Casal [25] found that egg albumin, when treated with periodate at pH 5.5 and 27°, was partly oxidised and precipitated. The precipitated albumin did not contain any cysteine and cystine and also the tryptophane and tyrosine contents were reduced. On the other hand, the methionine, threonine, and serine contents remained practically constant.

Acid-hydrolysable sulphate analyses showed that about 95 per cent of the original sulphate was in the supernatant when the periodate oxidation was complete. This together with the fucose analyses again demonstrates that each

fucose residue carries one sulphate group. Furthermore, it demonstrates that the sulphate linkage is not split during periodate oxidation, even in the oxidised fucose residues.

The fucose content before and after oxidation with periodate indicates that the fucose sulphate residues in the polysaccharide cannot be linked in an unbranched chain since in such a structure only one or both (or possibly none) of the terminal residues have the unsubstituted α-glycol grouping which is a prerequisite for attack by the periodate ion [26].

This leaves as the most probable structure a branched polysaccharide chain. If it is assumed that the fucose residues are in the pyranose form, and that the sulphate groups are attached to the same carbon atoms of the fucose residues in both the main chain and the branches, only a few structures will account for the analytical results.



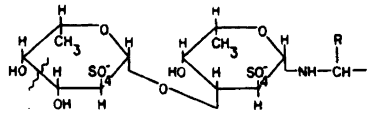
I

One of the possible structures is shown in I, in which the points of attack by the periodate ion are indicated with the wavy lines. The configuration of the glycosidic linkage is not known. Two other possibilities are (1) a main chain linked by 1 : 3-linkages with the branching point at C_2 and with the sulphate at C_4 both in the main chain and in the branches; (2) the same main chain but with the branching point at C_4 and the sulphate at C_2.

The structure of the polyfucose sulphate, fucoidin, extracted from the brown seaweed, *Fucus vesiculosus*, appears to be quite dissimilar [27]. Hydrolysis of methylated fucoidin gave L-fucose (ca. 1 part), 3-methyl L-fucose (ca. 3 parts), and 2 : 3-dimethyl L-fucose (ca. 1 part). The sulphate was considered to be linked to C_4. Only one fucose residue of five would thus be oxidised by periodate. The periodate consumption by the fucoidin prepared from *Himanthalia lorea* [28] corresponds to the breakdown of one fucose residue of between three and four monosaccharide units [27].

However, the results on the *E. cordatum* jelly may be interpreted in still another way. The fucose sulphate residues might be attached to the protein in

a similar fashion to the way in which, according to Aminoff and Morgan [29], the fucose residues of the blood group A substance are linked to hexosamine: (L-fucose)-(L-fucose)-(N-acetylhexosamine) —. In such a structure there would be one terminal fucose sulphate residue which is destroyed by periodate and another residue which cannot be attacked by periodate since it has no free α-glycol grouping (II).



II

This structure would be in line with the interpretation by Tyler [7] that the "protein and polysaccharide constituents do not exist as such in the molecule but rather that the various sugars and amino acids are interlinked". However, the formation upon periodate oxidation of a precipitate, which most probably consists of denatured protein, and of a clear supernatant, which contains the polysaccharide, confirms the hypothesis that the jelly substance is composed of a protein and a polysaccharide component.

It seems rather likely that the fucose sulphate residues form a polysaccharide with a structure similar to that depicted in I. This polysaccharide is then attached to the protein by comparatively strong linkages [1, 7]. One of these linkages may be of the kind shown in II. Isbell and Frush [30] have shown that the hydrolysis of arabinosylamine, which contains such a linkage, proceeds at a maximum rate at pH 5. It is a remarkable fact that the jelly coat of most sea-urchin species can be dissolved at about this pH.

Table 3. Fucose, sulphate, and nitrogen contents of the Echinocardium cordatum jelly preparations used in this study.

Preparation	Collected	Fucose		Sulphate		Poly-fucose sulphate per cent	Nitrogen per cent	Protein N × 6.25 per cent
		per cent	mmoles/g	per cent	mmoles/g			
Ec 49 : 1	July 5 to 20	30.7	1.87	18.4	1.92	46.5	4.8	30
Ec 49 : 2	July 19 to 28	34.4	2.10	20.6	2.15	52	3.4	21
Ec 49 : 3	July 29 to August 9	43.0	2.62	22.5	2.34	57—64	4.0	25

The data in Table 3 indicate the possibility of a seasonal variation in the composition of the jelly coat. In the beginning and middle of July, when the eggs are underripe, the content of polyfucose sulphate is low (Ec 49 : 1); it increases slightly during the latter part of the month (Ec 49 : 2) and attains its maximum value during the height of the spawning season (Ec 49 : 3). However, the data indicate also that about 23, 27, and 15 per cent, respectively, of the jelly substance are not accounted for in these preparations. Therefore, further investigations will be needed to prove the seasonal variation. On analogy with similar substances it seems probable that most of the unidentified material is bound water [cf. 28].

SUMMARY

The jelly coat substance of the *Echinocardium cordatum* egg consists of polyfucose sulphate in combination with protein. On periodate oxidation (at pH 3.5 and 1.7) half of the fucose residues are destroyed rapidly, but the remaining residues are not attacked even after prolonged periodate treatment.

This result suggests that the polysaccharide is built up in a comb-like fashion with one main chain of fucose sulphate residues each of which carries another fucose sulphate residue as a side chain.

The protein component is oxidised by the periodate as well and is precipitated during the reaction. The oxidised polysaccharide, on the other hand, remains in solution.

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Studies on Carbamates

V. The Carbamates of α-Alanine and β-Alanine

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1. In previous investigations has been studied the equilibrium conditions and reaction mechanism of the formation and decomposition in aqueous medium of ammonium carbamate [1,2], and of the carbamates formed by dimethylamine [2], ethylamine and diethylamine [3], and hydrazine [4].

The present investigation deals with the corresponding conditions with regard to the carbamates formed by α-alanine and β-alanine. The conditions have been shown to be analogous to those of the carbamates previously investigated, only differing somewhat quantitatively.

On the following pages α-alanine, $CH_3 \cdot CHNH_3^+ \cdot COO^-$, as well as β-alanine, $CH_2NH_3^+ \cdot CH_2 \cdot COO^-$, both of which should be regarded as amminium ions, will frequently be referred to as "AmH^+". Accordingly the corresponding bases $CH_3 \cdot CHNH_2 \cdot COO^-$ and $CH_2NH_2 \cdot CH_2 \cdot COO^-$ will be referred to as "Am". "carbamates" signify the carbamates of the alanines, *i. e.* $CH_3 \cdot CHNHCOO^- \cdot COO^-$ and $CH_2NHCOO^- \cdot CH_2 \cdot COO^-$.

2. The following substances have been used in the present experiments:

dl-α-Alanine, L. Light & Co. Ltd., consisting of small needle-shaped crystals, the melting point of which was found to be 260 to 262° C, and the molecular weight, determined by means of the Kjeldahl method, was 89.25 (theoretically 89.09). β-Alanine was specially prepared and furnished us through the generosity of Løvens kemiske Fabrik (Leo Pharmaceutical Products), Copenhagen. The analysis was stated to yield the following results: melting point 198° C, 99.5 % of alanine by formol titration, 98.3 % of alanine by the Kjeldahl method, less than 0.1 % loss by drying, less than 0.1 % of ash, and a slight trace of halogen. We found the melting point to be 196—199° C, and 98.4 % of alanine by the Kjeldahl method.

3. No attempt has been made to isolate the carbamates and carbonates as substances. Solutions of carbamate were prepared by neutralizing the amminium ion with the equivalent amount of sodium hydroxide, *e.g.* in the case of *α*-alanine "$CH_3 \cdot CHNH_3^+ \cdot COO^- + Na^+ + OH^- = CH_3 \cdot CHNH_2 \cdot COO^- + Na^+ + H_2O$", whereupon a deficient amount of carbon dioxide was added to the solution; practically all of the carbon dioxide is thus converted to carbamate "$CH_3 \cdot CHNH_2 \cdot COO^- + CO_2 = CH_3 \cdot CHNHCOO^- \cdot COO^- + H^+$", the latter of course being neutralized by excess amine. Solutions of carbonate were made from equivalent amounts of amminium ion and sodium carbonate, "$2CH_3 \cdot CHNH_3^+ \cdot COO^- + 2\,Na^+ + CO_3^{--} = (CH_3 \cdot CHNH_3 \cdot COO^-)_2CO_3 + 2Na^+$". Solutions of carbamate as well as solutions of carbonate thus contain sodium ions; this, however, is of no importance in the present investigation.

4. The method of analysis was, as in previous investigations, precipitation with barium chloride, *i.e.* barium chloride is added to 100 ml of the solution, if necessary previously made alkaline by the addition of sodium hydroxide. In this way the carbonate is precipitated, whilst the carbamate remains dissolved. By centrifugation the precipitate and the supernate are separated as quickly as possible. If the supernate is left standing or is heated, the carbamate decomposes forming a precipitate of barium carbonate, the amount of which can be determined by titration with 0.1 *N* hydrochloric acid.

Since solutions containing only carbonate, and no carbamate, upon precipitation with barium chloride yield a supernate precipitating a small amount of barium carbonate, it was found necessary to carry out blank experiments. The value of the blanks amounts to about 3 units of the percentage. All of the experimental results in the later tables are corrected with regard to the value of the blank experiments.

5. All of the experiments were carried out at 18° C, and the velocity constants were calculated by means of Brigg's logarithms, the time unit being the minute.

As in earlier investigations the activity coefficient f of monovalent ions was calculated from the expression of Bjerrum [5]: $-\log f = 0.3 \sqrt[3]{c_{ion}}$.

6. The calculation of certain experiments was carried out on the basis of the acidic dissociation constant, K'_{AmH^+}, for

$$\begin{array}{lcl} CH_3 & & CH_3 \\ | & & | \\ CHNH_3^+ & \rightleftharpoons & CHNH_2 + H^+ \\ | & & | \\ COO^- & & COO^- \\ \text{acid} & & \text{base} \end{array}$$

and the corresponding value of β-alanine. These constants were calculated by us (Table 1) on the basis of determinations of the hydrogen ion activity in solutions containing equal moles of corresponding acid and base, prepared by the mixing of alanine with half the number of moles of sodium hydroxide. The determinations were carried out by means of a hydrogen electrode against a 0.1 N calomel electrode, and were calculated from the expression $pa_H = -\log a_H{}^+ = (E-E_0)\frac{F \log e}{R\,T}$, E_0 being fixed at 0.3360 [6]. No corrections were made for the diffusion potentials, these being insignificant as in the glycine measurements in the investigation just cited.

Table 1. Determination of the acidic dissociation constants of the alanines.

c_{acid}	c_{base}	pa_H	
		α	β
0.1	0.1	10.00	10.42
0.05	0.05	10.01	10.42
0.02	0.02	10.02	10.41
0.01	0.01	9.99	10.40
	i.e. pK':	10.00	10.41

accordingly the expression

$$\frac{a_H{}^+ \cdot c_{CH_3 \cdot CHNH_2 \cdot COO^-}}{c_{CH_3 \cdot CHNH_3{}^+ \cdot COO^-}} = K'_{AmH^+}$$

and the corresponding one for β-alanine has a practically constant value independant of the ion concentration. From this it may be further concluded that the activity coefficients of the divalent ampho-ion $CH_3 \cdot CHNH_3^+ \cdot COO^-$ ($CH_2NH_3^+ \cdot CH_2 \cdot COO^-$, respectively) are of practically the same magnitude as are those of the monovalent ion $CH_3 \cdot CHNH_2 \cdot COO^-$ ($CH_2NH_2 \cdot CH_2 \cdot COO^-$, respectively). This fact is used later on.

On the reaction "amine + carbon dioxide ⇌ carbamic acid"

1. *The reaction from left to right.* 500 ml of an aqueous solution of alanine, containing excess sodium hydroxide, were placed in a 2 liter flask; if, for instance, 250 ml of 0.2 M alanine are added to 250 ml of 0.4 M NaOH, the

resulting solution will have $c_{NaOH} = 0.10$ and $c_{Am} = 0.10$. In the gas phase above this solution approximately 250 ml of carbon dioxide were substituted for an equal amount of atmospheric air, and the flask was shaken vigorously for about 2 minutes to obtain absorption of the carbon dioxide. In 100 ml of the reaction mixture the carbamate content was determined immediately, and in another 100 ml of the reaction mixture carbamate + carbonate were determined. Per cent carbamate in table 2 indicates how many per cent of the carbon dioxide absorbed have been converted to carbamate.

Table 2. Carbon dioxide in alanine + NaOH. 18°.

	Initial solution		Absorbed CO_2 Mol./liter	% carba-mate	Final solution		Mean		$k_{CO_2 \cdot Am}$	
	c_{NaOH}	c_{Am}			c_{NaOH}	c_{Am}	c_{NaOH}	c_{Am}		Mean
α	0.10	0.10	0.0193	41	0.07	0.09	0.09	0.10	$10^{4.81}$	
	0.10	0.15	0.0188	55	0.07	0.14	0.09	0.15	$10^{4.87}$	$10^{4.82}$
	0.06	0.09	0.0116	49	0.04	0.08	0.05	0.09	$10^{4.77}$	
β	0.10	0.10	0.0175	54	0.07	0.09	0.09	0.10	$10^{5.05}$	
	0.20	0.10	0.0154	34	0.17	0.10	0.19	0.10	$10^{5.02}$	$10^{5.04}$
	0.20	0.20	0.0129	53	0.18	0.19	0.19	0.20	$10^{5.05}$	

The results of the experiments can be interpreted in that carbon dioxide is reacting partly with the amine, partly with the hydroxyl ion:

$$\begin{array}{l} CH_3 \\ | \\ CHNH_2 + CO_2 \\ | \\ COO^- \end{array} \rightleftharpoons \begin{array}{l} CH_3 \\ | \\ CHNHCOOH \\ | \\ COO^- \end{array}$$

$$OH^- + CO_2 \rightleftharpoons HCO_3^-$$

according to the following equation:

$$\frac{dc_{CO_2}}{dt} = -\left(k_{CO_2 \cdot Am} \cdot c_{Am} + k_{CO_2 \cdot OH^-} \cdot c_{OH^-}\right) c_{CO_2}$$

For the interrelation between the percentages of carbamate formed and carbonate formed we have the expression

$$\frac{k_{CO_2 \cdot Am} \cdot c_{Am}}{k_{CO_2 \cdot OH^-} \cdot c_{OH^-}} = \frac{\text{per cent carbamate}}{\text{per cent carbonate}}$$

Upon introduction into this expression of the mean values of the concentrations at the beginning and at the end of the experiment for c_{OH^-} and c_{Am}, and of the value $10^{5,02}$ for $k_{CO_2 \cdot OH^-}$[7], the values $10^{4,82}$ and $10^{5,04}$ are obtained for $k_{CO_2 \cdot Am}$ for α-alanine and β-alanine, respectively, *i.e.* carbon dioxide reacts with α-alanine approximately 1.5 times slower than it does with β-alanine.

2. *The reaction from right to left.* Previously it has been shown that ordinary carbamate, NH_2COO^-, by acidification is extremely quickly and completely decomposed into carbon dioxide and ammonia, or rather ammonium ion. The decomposition of the alanine carbamates in acid solution has not been investigated directly. As shown in a previous investigation it is possible, however, by means of the velocity constant, $k_{CO_2 \cdot Am}$, to estimate that these carbamates, too, are decomposed practically instantaneously and completely in acid solution.

3. *The equilibrium "carbamate ⇌ carbonate".* In acid and in strongly basic solutions the carbamates are practically completely decomposed into carbon dioxide and carbonate, respectively, but in a weakly basic solution an equilibrium, which can be easily determined, is established,

$$\text{carbamate} \rightleftharpoons \text{carbonate}$$

For the determination of the equilibrium value 0.02 *M* solutions of $(AmH)_2CO_3$ containing Am and AmH^+ as well, were prepared, see Table 3. Accordingly the initial composition of the solution in *e. g.* the experiment "0.02 *M* $(AmH)_2CO_3$, 0.05 *M* AmH^+, 0.05 *M* Am" was 0.14 *M* alanine, 0.05 *M* NaOH and 0.02 *M* Na_2CO_3. The solutions were left until an equilibrium was established. In the table "% carbamate" indicates the percentage of carbonate converted to carbamate. The constitution of the equilibrium solutions was calculated. pK of the carbamic acids supposedly being approximately 5 the amount of free carbamic acid present in these solutions is insignificant, *i. e.* $c_{\text{carbamate ion}}$ can be fixed at that of the carbamate found analytically. The concentrations of AmH^+, Am and HCO_3^- were calculated from the following expressions:

$$\frac{c_{Am} \cdot c_{HCO_3^-}}{c_{AmH^+} \cdot c_{CO_3^{--}} \cdot f} = \frac{K'_{AmH^+}}{K_{HCO_3^-}} = \frac{K'_{AmH^+}}{10^{-10.3}}$$

$c_{\text{carbamate}} + c_{HCO_3^-} + c_{CO_3^{--}} =$ total concentration of CO_2

$c_{\text{carbamate}} + c_{AmH^+} + c_{Am} =$ total concentration of alanine,

$c_{Am} = c_{Am}$ (column 4 in the table) $+ c_{HCO_3^-}$,

where $c_{AmH^+} = c_{CH_3 \cdot CHNH_3^+ \cdot COO^-}$ ($c_{CH_3NH_3^+ \cdot CH_2 \cdot COO^-}$, respectively), and $c_{Am} = c_{CH_3 \cdot CHNH_2 \cdot COO^-}$ ($c_{CH_3NH_2 \cdot CH_2 \cdot COO^-}$, respectively).

Table 3. The solutions of carbonate-carbamate in equilibrium. 18°.

	Initial solution			% carbamate	Equilibrium				K_{Eq}	
	$c_{(AmH)_2CO_3}$	c_{AmH^+}	c_{Am}		c_{AmH^+}	c_{Am}	$c_{carbamate}$	$c_{HCO_3^-}$		Mean
α	0.02	0.05	0.05	26 [1]	0.075	0.060	0.0052	0.0097	$10^{-0.96}$	$10^{-0.97}$
	0.02	0.10	0.10	39 [2]	0.125	0.108	0.0078	0.0075	$10^{-0.99}$	
β	0.02	0.05	0.05	44 [3]	0.076	0.055	0.0089	0.0049	$10^{-1.52}$	$10^{-1.51}$
	0.02	0.10	0.10	59 [4]	0.125	0.104	0.0117	0.0035	$10^{-1.51}$	

[1] Mean of 4 determinations: 25.3, 27.4, 25.4, 25.7.
[2] » » 4 » 39.1, 39.1, 39.1, 38.5.
[3] » » 4 » 44.8, 45.0, 44.1, 44.0.
[4] » » 3 » 57.5, 60.3, 58.1.

Further the equilibrium constants of the reactions "carbamate ion + H_2O = HCO_3^- + Am", *i. e.*

$$\frac{a_{Am} \cdot a_{HCO_3^-}}{a_{\text{carbamate ion}}} = \frac{c_{CH_3 \cdot CHNH_2 \cdot COO^-} \cdot f \cdot c_{HCO_3^-} \cdot f}{c_{CH_3 \cdot CHNHCOO^- \cdot COO^-} \cdot f^2} = K_{Eq}$$

and the analogous one for β-alanine, in both of which the activity coefficients neutralize each other, were calculated. The constants are listed in the last column of Table 3.

The velocity of the conversion "carbamate ⇌ carbonate"

In Table 4 are listed data on the velocity when equilibrium is ensuing in buffer solutions of pa_H = about 10. All experiments are from the carbonate side.

In addition to the experimental data are listed in Table 4 in the column $k_{amate} + k_{onate}$ the values of $\frac{1}{t} \log \frac{K \cdot a}{K \cdot a - (1 + K)x}$, the expression for a process which is monomolecular from both sides and which does not run to completion. The constant K signifies the relation between the equilibrium values of the concentrations of carbamate and carbonate. The sum "$k_{amate} + k_{onate}$" is approximately constant within each experiment. k_{amate} is the

Table 4. Velocity constants for the process: carbamate ⇌ carbonate; pa_H = ca. 10. 18°.

	Initial solution			Min.	% carbamate	$k_{amate} + k_{onate}$
		c_{AmH^+}	c_{Am}			
α	0.02 *M* $(AmH)_2CO_3$	0.05	0.05	40	9.7	0.00506
				160	22.1	0.00515
				1494	25.3	Mean: 0.0051
				4202	25.4	k_{amate}: 0.0038
						k_{onate}: 0.0013
	0.02 *M* $(AmH)_2CO_3$	0.10	0.10	40	10.5	0.00340
				80	17.7	0.00330
				120	22.4	0.00309
				155	26.3	0.00316
				1080	39.1	Mean: 0.0033
				2520	39.1	k_{amate}: 0.0020
						k_{onate}: 0.0013
β	0.02 *M* $(AmH)_2CO_3$	0.05	0.05	43	7.8	0.00193
				81	13.6	0.00196
				161	22.5	0.00190
				241	28.9	0.00189
				1320	44.8	Mean: 0.00192
				2910	44.1	k_{amate}: 0.00107
						k_{onate}: 0.00085
	0.02 *M* $(AmH)_2CO_3$	0.10	0.10	40	6.3	0.00123
				80	11.7	0.00121
				155	19.9	0.00116
				240	27.7	0.00116
				1269	56.1	0.00107
				2655	57.5	Mean: 0.00117
				4486	58.1	k_{amate}: 0.00048
						k_{onate}: 0.00069

velocity constant for the conversion of carbamate to carbonate, and k_{onate} the one for the reversed reaction.

By means of $\frac{k_{onate}}{k_{amate}} = \frac{\text{equilibrium value of } c_{carbamate}}{\text{equilibrium value of } c_{carbonate}} = K$, the values of k_{amate} and k_{onate} are obtained.

In Table 5 are presented data on the decomposition of the carbamates in strong basic solution, pa_H = about 13. The solutions of the carbamates were prepared by dissolving carbon dioxide in a mixture of practically equal moles alanine and sodium hydroxide and subsequently add the desired excess sodium hydroxide. The initial composition of *e. g.* the solution "0.018 *M* carbamate, 0.18 *M* OH^- and 0.08 *M* Am" was accordingly 0.10 *M* alanine, 0.30 *M* NaOH, and 0.018 *M* CO_2. k_{amate} is the value of $k = \frac{1}{t} \log \frac{a}{a-x}$.

The experiments may be interpreted in a way similar to the one used by the carbamates previously examined. The conversion is a two-stage reaction

$$\text{Carbonate} \rightleftharpoons \text{carbon dioxide}$$
$$\text{carbon dioxide} + \text{amine} \rightleftharpoons \text{carbamate}$$

and it is possible to calculate in advance the velocity constants.

The conversion "carbon dioxide ⇌ carbonate" is known to take place through two reactions: "$CO_2 + H_2O \rightleftharpoons H_2CO_3$" and "$CO_2 + OH^- \rightleftharpoons HCO_3^-$"; as the pa_H of the solution is about 10 or more, only the latter reaction need, as previously shown, to be taken into consideration.

k_{amate} is calculated from the expression previously deduced [3]

$$k_{amate} = \frac{k_{CO_2 \cdot Am} \cdot K_{Eq} \cdot K_{H_2O} \cdot 1/K_{CO_2}}{c_{OH^-} + \frac{k_{CO_2 \cdot Am}}{k_{CO_2 \cdot OH^-}} \cdot c_{Am}}$$

i.e. in order to calculate the velocity at which a carbamate, following the above mentioned sequence of reactions, is decomposed, only two experiments need to be carried out, *viz.* 1) a determination of the distribution of carbon dioxide on carbamate and carbonate upon introducing the gas into a solution of amine and sodium hydroxide, thus attaining the value of $k_{CO_2 \cdot Am}$, and 2) a determination of the carbamate-carbonate equilibrium value *i. e.* K_{Eq}.

From the expression for k_{amate} it is seen that for a weakly basic solution the c_{OH^-} of the denominator is but slight compared to the other term of the denominator, *i. e.* in such solutions the velocity of the decomposition of carbamate is inversely proportional to the concentration of amine.

k_{onate} is calculated from the expression previously deduced [2]

$$k_{onate} = \frac{k_{HCO_3^-}}{1 + \frac{a_{H^+}}{K_{H_2CO_3}} \cdot f + \frac{K_{HCO_3^-}}{a_{H^+}} \cdot \frac{1}{f}}$$

The middle term of the denominator being so slight that it is discarded.

Table 5. Velocity constants for the process: carbamate → carbonate; pa_H = ca. 13. 18°.

	Initial solution			Min.	% carbamate left	k_{amate}
	$c_{carbamate}$	c_{NaOH}	c_{Am}			
α	0.018	0.18	0.08	0	100	
				384	70.3	0.000398
				1049	36.0	0.000423
				1568	21.7	0.000424
				2538	8.2	0.000428
				2988	5.0	0.000437
				3921	1.8	0.000443
				ca. 1 month	0	Mean: 0.00043
β	0.019	0.18	0.08	0	100	
				362	89.2	0.000137
				1382	60.8	0.000156
				1744	53.3	0.000157
				2906	37.2	0.000148
				3381	30.2	0.000154
				4210	22.3	0.000155
				ca. 2 months	0	Mean: 0.00015
	0.020	0.18	0.18	0	100	
				402	90.8	0.000104
				1350	70.4	0.000113
				1855	62.7	0.000109
				2715	50.4	0.000110
				3182	44.6	0.000110
				ca. 1 month	0	Mean: 0.00011

In calculating the values of k_{amate} and k_{onate} were used $K_{H_2O} = 10^{-14.22}$, $K_{CO_2} = 10^{-6.52}$, $K_{HCO_3^-} = 10^{-10.3}$, $k_{HCO_3^-} = 10^{-2.74}$, $k_{CO_2 \cdot OH^-} = 10^{-5.02}$ and for K_{Eq} the values obtained in the preceding paragraph, these being $10^{-0.97}$ for α-alanine and $10^{-1.51}$ for β-alanine.

Table 6 contains a survey of the experimental and calculated values of k_{amate} and k_{onate}. Considering the nature of the conditions the agreement may be described as satisfactory.

Table 6. Velocity constants, experimental and calculated.

	Initial solution					k_{amate}		k_{onate}	
	$c_{(AmH)_2CO_3}$	$c_{carbamate}$	c_{AmH^+}	c_{Am}	c_{NaOH}	exp.	calc.	exp.	calc.
α	0.02		0.05	0.05		0.0038	0.0041	0.0013	0.0013
	0.02		0.10	0.10		0.0020	0.0021	0.0013	0.0012
		0.018		0.08	0.18	0.00043	0.00062		
β	0.02		0.05	0.05		0.0011	0.0012	0.00085	0.00087
	0.02		0.10	0.10		0.00048	0.00063	0.00069	0.00074
		0.019		0.08	0.18	0.00015	0.00025		
		0.020		0.18	0.18	0.00011	0.00018		

SUMMARY

The velocity constant of the reaction "$CH_3 \cdot CHNH_2 \cdot COO^- + CO_2 = CH_3 \cdot CHNHCOOH \cdot COO^-$" and the equilibrium constant for the reaction "$CH_3 \cdot CHNHCOO^- \cdot COO^- + H_2O = HCO_3^- + CH_3 \cdot CHNH_2 \cdot COO^-$" have been determined. The velocity of the decomposition of $CH_3 \cdot CHNHCOO^- \cdot COO^-$ in basic medium was investigated and may be explained in assuming that the decomposition is a two-stage reaction, *viz.*

carbamate = alanine + carbon dioxide
carbon dioxide = carbonate

The carbamate of β-alanine was studied in a similar way.

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Studies on Carbamates

VI. The Carbamate of Glycine

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In a previous investigation [1], during which was studied the equilibrium conditions and the reaction mechanism of the formation and decomposition in aqueous medium of the carbamates formed by ammonia, methylamine and dimethylamine, a few experiments were done concerning the carbamate of glycine. The present investigation is supplementary to those previous experiments. The equilibrium conditions and reaction mechanism of glycine being analogous to those of the alanines, and the experimental method being similar, we find that on the whole it is sufficient here to state the experimental data and the calculated constants, referring for further information to the investigation of the alanines [2].

The preparation of glycine used in the present investigation was one which fulfilled the demands for purity stated by S. P. L. Sørensen [3], except for a content of 0.05 per cent ammonia, an amount being, however, of no significance in the present investigation.

The following expression was used for the calculations

$$\frac{a_{H^+} \cdot c_{CH_2NH_2 \cdot COO^-}}{c^+_{CH_2NH_3 \cdot COO^-}} = K'_{AmH^+} = 10^{-9.88}\ ^4$$

Table 1. Carbon dioxide in glycine + NaOH . 18°.

Initial solution		Absorbed CO_2 Mol/liter	% carbamate	Final solution		Mean		$k_{CO_2 \cdot Am}$	
c_{NaOH}	c_{Am}			c_{NaOH}	c_{Am}	c_{NaOH}	c_{Am}		Mean
0.20	0.20	0.0191	52.5	0.17	0.19	0.19	0.20	$10^{5,04}$	
0.20	0.10	0.0188	35.6	0.17	0.09	0.18	0.10	$10^{5,04}$	$10^{5,05}$
0.10	0.10	0.0203	55.4	0.07	0.09	0.09	0.09	$10^{5,07}$	

Table 2. The solutions of carbonate-carbamate in equilibrium. 18°.

Initial solution			% carbamate	Equilibrium				K_{Eq}	
$c_{(AmH)_2CO_3}$	c_{AmH^+}	c_{Am}		c_{AmH^+}	c_{Am}	$c_{carbamate}$	$c_{HCO_3^-}$		Mean
0.02	0.05	0.05	55 [1]	0.073	0.056	0.011	0.0064	$10^{-1,48}$	$10^{-1,48}$
0.02	0.05	0.10	64 [2]	0.073	0.104	0.013	0.0041	$10^{-1,47}$	

[1] Mean of 7 determinations: 53.5, 53.5, 53.8, 56.6, 55.6, 55.7, 55.5.
[2] » » 4 » 62.9, 64.6, 63.7, 63.7.

Table 3. Velocity constants for the process: carbamate ⇌ carbonate; pa_H = ca. 10. 18°.

Initial solution			Min.	% carbamate	$k_{amate} + k_{onate}$
	c_{AmH^+}	c_{Am}			
0.02 *M* $(AmH)_2CO_3$	0.05	0.05	41	13.2	0.00287
			80	21.4	0.00263
			160	35.1	0.00269
			261	44.6	0.00267
			1425	56.6	Mean: 0.0027
			1440	55.6	k_{amate}: 0.0012
					k_{onate}: 0.0015

Table 4. Velocity constants for the process: carbamate → carbonate; pa_H = ca. 13. 18°.

Initial solution			Min.	% carbamate left	k_{amate}
$c_{carbamate}$	c_{NaOH}	c_{Am}			
0.011	0.19	0.19	0	100	
			242	92.5	0.000140
			1211	67.8	0.000139
			1680	60.1	0.000131
			2640	43.4	0.000137
			ca. 1 month	0	
					Mean: 0.00014

Table 5. Velocity constants, experimental and calculated.

Initial solution					k_{amate}		k_{onate}	
$c_{(AmH)_2CO_3}$	$c_{carbamate}$	c_{AmH^+}	c_{Am}	c_{NaOH}	exp.	calc.	exp.	calc.
0.02		0.05	0.05		0.0012	0.0013	0.0015	0.0014
	0.011		0.19	0.19	0.00014	0.00019		

SUMMARY

The velocity constant of the reaction "$CH_2NH_2 \cdot COO^- + CO_2 = CH_2NHCOOH \cdot COO^-$" and the equilibrium constant for the reaction "$CH_2NHCOO^- \cdot COO^- + H_2O = HCO_3^- + CH_2NH_2 \cdot COO^-$" have been determined. The velocity of the decomposition of $CH_2NHCOO^- \cdot COO^-$ in basic medium was investigated and may be explained in assuming that the decomposition is a two-stage reaction, *viz.*

carbamate = glycine + carbon dioxide
carbon dioxide = carbonate

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Studies on Carbamates

VII. The Carbamates of *n*-Propylamine and *iso*-Propylamine

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1. In previous investigations were studied the equilibrium conditions and the reaction mechanism of the formation and decomposition in aqueous medium of ammonium carbamate as well as of the carbamates formed by amines and other amine compounds, *e.g.* the alanines. The present investigation deals with the corresponding conditions for the carbamates formed by n-propylamine and iso-propylamine. The conditions have been shown to be analogous to those found for the carbamates previously investigated, only differing somewhat quantitatively. The experimental method likewise being similar, we refer to previous investigations [1] for information on method, theory, significance of the constants *etc.* It should be noted, though, that "Am" means propylamine and "AmH^+" propylammonium ion.

2. The following preparations were used in the experiments:

n-propylamine was prepared by catalytic reduction of 1-nitropropan by means of hydrogen of a pressure of 2 to 3 atm. at room temperature, and with Raney-nickel as a catalytic agent. It was purified through the acid oxalate by recrystallizations, until the constant melting point, 135—136°, was attained. The molecular weight of the acid oxalate, crystallizing with half a molecule crystal water [2], is theoretically 158.16. The preparation obtained showed the following molecular weight: by distilling off the amine and titrating it was found 157.5, by titration with permanganate was found 157.5, and by titration with base was found 159.1. From the acid oxalate was prepared an aqueous solution of *n*-propylamine by distilling it off with sodium hydroxide.

The *iso*-propylamine was obtained from the Rubber Industries and Sherman Chemicals Ltd. It was likewise purified through the acid oxalate, also crystallizing with half a molecule crystal water, and having a melting point of 164—165°; by titrating with base the molecular weight was found to be 159.5.

3. No attempt was made to obtain the carbamates or the carbonates as substances. The solutions of carbamates were prepared by dissolving carbon dioxide in solutions of the pure amine, practically all of the carbon dioxide thus being converted to carbamate "$CO_2 + 2C_3H_7NH_2 = C_3H_7NHCOONH_3C_3H_7$". The solutions of carbonate were prepared by mixing equivalent amounts of solutions of propylammonium chloride and sodium carbonate "$2C_3H_7NH_3Cl + Na_2CO_3 = (C_3H_7NH_3)_2CO_3 + 2NaCl$", *i. e.* the resulting solutions contain some sodium chloride too, but this is insignificant in the present investigation.

4. The method of analysis was as in previous investigations precipitation with barium chloride, causing the precipitation of carbonate, but not of carbamate. All of the data presented in the later tables are corrected for the values of blank experiments, *viz.* about 3 units of the percentage.

5. All of the experiments were done at 18° C, and the velocity constants were calculated by means of Brigg's logarithms, the unit of time being the minute. As in previous investigations the activity coefficient f for a monovalent ion was calculated from the expression of Bjerrum $-\log f = 0.3\sqrt[3]{c_{ion}}$.

6. For the acidic dissociation constants K_{AmH^+} were used the values $10^{-10,81}$ and $10^{-10,86}$ for *n*-propylammonium ion and *iso*-propylammonium ion, respectively. The corrected values of Bredigs [3] for the basic dissociation constants at 25° C are $3{,}9 \cdot 10^{-4}$ and $4{,}3 \cdot 10^{-4}$ for *n*-propylamine and *iso*-propylamine, respectively. The heat effect at the reactions being very slight, these values may be used for 18° C as well. By conversion to the acidic dissociation constants K_{H_2O} was fixed at $10^{-14,22}$.

On the reaction "amine + carbon dioxide ⇌ carbamic acid"

About 240 ml of carbon dioxide were dissolved in 500 ml of solution containing both propylamine and sodium hydroxide and placed in a 2 liter flask.

Table 1. Carbon dioxide in propylamine + NaOH. 18°.

	Initial solution		Absorb. CO_2 mol./liter	% carbamate	Final solution		Mean		$k_{CO_2 \cdot Am}$	
	c_{NaOH}	c_{Am}			c_{NaOH}	c_{Am}	c_{NaOH}	c_{Am}		Mean
n-	0.20	0.10	0.0216	45	0.167	0.090	0.183	0.095	$10^{5.21}$	$10^{5.20}$
	0.20	0.10	0.0185	43	0.171	0.092	0.185	0.096	$10^{5.19}$	
iso-	0.20	0.148	0.0207	36	0.166	0.141	0.183	0.144	$10^{4.86}$	$10^{4.83}$
	0.20	0.10	0.0182	25	0.160	0.096	0.180	0.098	$10^{4.81}$	
	0.20	0.10	0.0097	25	0.183	0.098	0.192	0.099	$10^{4.82}$	

The carbon dioxide is reacting partly with the amine, partly with the hydroxyl ion. The mixture was immediately analyzed, see Table 1, where "% carbamate" indicates how many per cent of the carbon dioxide absorbed have been converted to carbamate.

From the values of $k_{CO_2 \cdot Am}$, the velocity constant of the reaction "$C_3H_7NH_2 + CO_2 \rightarrow C_3H_7NHCOOH$", it is seen that carbon dioxide is added to *n*-propylamine about twice as fast as it is to *iso*-propylamine.

The equilibrium "carbamate ⇌ carbonate"

Table 2. The solutions of carbonate-carbamate in equilibrium. 18°.

	Initial solution				% carbamate	Equilibrium				K_{Eq}	
	$c_{(AmH)_2CO_3}$	$c_{carbamate}$	c_{AmH^+}	c_{Am}		c_{AmH^+}	c_{Am}	$c_{carbamate}$	$c_{HCO_3^-}$		Mean
n-	0.02		0.10	0.10	54 [1]	0.128	0.101	0.0108	0.0014	$10^{-1.88}$	$10^{-1.88}$
		0.021	0.10	0.099	55 [2]	0.128	0.101	0.0118	0.0015	$10^{-1.88}$	
iso-	0.02		0.10	0.096	18 [3]	0.134	0.099	0.0035	0.0023	$10^{-1.18}$	$10^{-1.20}$
		0.018	0.10	0.10	19 [4]	0.130	0.103	0.0038	0.0023	$10^{-1.21}$	

[1] Mean of 3 determinations: 53.6, 53.6, 53.7.
[2] » » 3 » 55.1, 54.6, 54.8.
[3] » » 3 » 17.4, 18.0, 17.4.
[4] » » 3 » 19.5, 18.6, 20.2.

Experiments have been done from the carbonate side as well as from the carbamate side, see Table 2, and the pa_H of the solutions is fixed about 10.8 by means of propylammoniumion-propylamine-buffers. The initial composition of the solution *e. g.* in the experiment "0.02 *M* $(AmH)_2CO_3$, 0.10 *M* AmH^+, 0.10 *M* Am" was 0.14 *M* AmHCl, 0.10 *M* Am, and 0.02 *M* Na_2CO_3. The initial composition of the solution *e. g.* in the experiment "0.021 *M* carbamate, 0.10 *M* AmH^+, 0.10 *M* Am" was 0.10 *M* AmHCl, 0.14 *M* Am, and 0.0205 (*i. e.* 0.021) *M* CO_2. The constitution of the equilibrium solutions was calculated from the equations:

$$\frac{c_{Am} \cdot c_{HCO_3^-}}{c_{AmH^+} \cdot c_{CO_3^{--}} \cdot f^2} = \frac{K_{AmH^+}}{K_{HCO_3^-}}$$

$c_{Am} = c_{Am}$ (column 5 of the table) $+ c_{HCO_3^-}$
$c_{carbamate} + c_{HCO_3^-} + c_{CO_3^{--}}$ = total concentration of CO_2
$c_{carbamate} + c_{AmH^+} + c_{Am}$ = total concentration of amine.

Furthermore the equilibrium constant K_{Eq} for the reaction "carbamate$^-$ + $H_2O \rightleftharpoons HCO_3^- + Am$" or in other words "$C_3H_7NHCOO^- + H_2O \rightleftharpoons HCO_3^- + C_3H_7NH_2$", was calculated.

The velocity of the conversion "carbamate ⇌ carbonate"

In Table 3 are presented the experiments on velocity, which have been carried out in a propylammoniumion-propylamine buffer, where an easily measurable

Table 3. Velocity constants for the process "carbamate ⇌ carbonate", pa_H = ca. 10.8. 18°.

	Initial solution			Min.	% carbamate	$k_{amate} + k_{onate}$	
		c_{Am}	c_{AmH^+}				
n-	0.02 *M* $(AmH)_2CO_3$	0.10	0.10	186	8.9		0.00043
				310	13.7		0.00041
				434	18.3		0.00042
				771	28.8		0.00043
				1467	42.3		0.00046
						Mean:	0.00043
						k_{amate}:	0.00020
						k_{onate}:	0.00023
iso-	0.02 *M* $(AmH)_2CO_3$	0.096	0.10	57	2.7		0.00130
				92	4.4		0.00138
				190	7.6		0.00129
				330	11.3		0.00135
				784	15.8		0.00126
						Mean:	0.00131
						k_{amate}:	0.00108
						k_{onate}:	0.00023
	0.018*M* solution of carbamate	0.10	0.10	0	100		
				47	89.5		0.00132
				82	82.5		0.00133
				212	62.4		0.00132
				431	41.8		0.00134
				622	32.0		0.00136
						Mean:	0.00133
						k_{amate}:	0.00107
						k_{onate}:	0.00026

Table 4. Velocity constants for the process "carbamate → carbonate". pa_H = ca. 13. 18°.

	Initial solution			Min.	% carbamate left	k_{amate}
	$c_{carbamate}$	c_{NaOH}	c_{Am}			
n-	0.019	0.181	0.081	0	100	
				240	95.3	0.000087
				464	91.5	0.000083
				696	86.8	0.000089
				1339	76.8	0.000086
				1676	71.8	0.000086
				3600	49.1	0.000086
				∞	(1)	
						Mean: 0.000086
iso-	0.009	0.191	0.090	0	100	
				172	90.3	0.000256
				352	82.5	0.000237
				712	67.9	0.000237
				1455	45.7	0.000234
				1834	38.3	0.000227
				2320	29.2	0.000230
				∞	(0)	
						Mean: 0.000237

equilibrium is established between carbamate and carbonate. In Table 4 are presented those experiments, which have been carried out in a medium containing sodium hydroxide, where carbamate is converted practically completely to carbonate, *e. g.* in the experiment "0.019 *M* carbamate, 0.081 *M* Am, 0.181 *M* NaOH", the initial composition of the solution was 0.10 *M* Am, 0.20 *M* NaOH, and 0.019 *M* CO_2; it was prepared by dissolving the carbon dioxide in a pure solution of amine, and subsequently add sodium hydroxide.

k_{amate} and k_{onate} are the velocity constants for the decomposition of the carbamate and the carbonate, respectively, and may, provided the decomposition takes place through the reactions

$$\text{carbamate} \rightleftharpoons \text{amine} + CO_2$$

$$CO_2 \rightleftharpoons \text{carbonate}$$

be calculated from the expressions:

$$k_{amate} = \frac{k_{CO_2 \cdot Am} \cdot K_{Eq} \cdot K_{H_2O} \cdot 1/K_{CO_2}}{c_{OH^-} + \frac{k_{CO_2 \cdot Am}}{k_{CO_2 \cdot OH^-}} \cdot c_{Am}} \quad , \quad k_{onate} = \frac{k_{HCO_3^-}}{1 + \frac{a_{H^+}}{K_{H_2CO_3}} \cdot f + \frac{K_{HCO_3^-}}{a_{H^+}} \cdot \frac{1}{f}}$$

In Table 5 is given a survey of the experimental and calculated values of the velocity constants. Considering the nature of the conditions the agreement may be described as satisfactory.

Table 5. Velocity constants, experimental and calculated.

	Initial solution					k_{amate}		k_{onate}	
	$c_{(AmH)_2CO_3}$	$c_{carbamate}$	c_{AmH^+}	c_{Am}	c_{NaOH}	exp.	calc.	exp.	calc.
n-	0.02		0.10	0.10		0.00020	0.00027	0.00023	0.00028
		0.019		0.081	0.181	0.000086	0.00013		
iso-	0.02		0.10	0.096		0.0011	0.0014	0.00023	0.00028
		0.018	0.10	0.10		0.0011	0.0013	0.00026	0.00026
		0.009		0.090	0.191	0.00024	0.00034		

SUMMARY

The velocity constant of the reaction "$CH_3 \cdot CH_2 \cdot CH_2NH_2 + CO_2 = CH_3 \cdot CH_2 \cdot CH_2NHCOOH$" and the equilibrium constant for the reaction "$CH_3 \cdot CH_2 \cdot CH_2NHCOO^- + H_2O = HCO_3^- + CH_3 \cdot CH_2 \cdot CH_2NH_2$" have been determined. The velocity of the decomposition of $CH_3 \cdot CH_2 \cdot CH_2NHCOO^-$ in basic medium was investigated and may be explained in assuming that the decomposition is a two-stage reaction, *viz.*

carbamate = propylamine + carbon dioxide
carbon dioxide = carbonate

The carbamate of *iso*-propylamine was studied in a similar way.

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Studies on Monoalkyl Carbonates

XII. The Monoalkyl Carbonates of Ethylene Glycol and Ethylene Chlorohydrin

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1. In previous investigations [1] were studied the equilibrium conditions and the reaction mechanism in aqueous medium of different monoalkyl carbonates, *e.g.* $CH_3CO_3^-$ and $C_2H_5CO_3^-$. The present investigation deals with the monoalkyl carbonates of ethylene glycol and ethylene chlorohydrin.

2. The ethylene glycol used was purified by distillation through a wire-gauze column [2] with 51 sets of platinum plates; the distillation took place *in vacuo*. One fraction was taken at 113° C and 32 mm Hg pressure, another at 103° C and 20 mm Hg pressure. At 760 mm Hg pressure the boiling point of both fractions was 197.3° C; $n_D^{24.9°}$ was 1.4306 and 1.4305, respectively.

An attempt was made to purify the ethylene chlorohydrin used by the same method as that applied to the ethylene glycol. The attempt failed, however, due to a splitting off of HCl during distillation, whereas an ordinary distillation at a Hg pressure of a few mm proved succesful. The fractions used had a $n_D^{20.1°} = 1.4421$, and at 760 mm Hg a boiling point of 128.6—128.9° C; a determination of halogenide content after Stepanow-Bacon gave the equivalent number 80.71 (theoretically 80.52).

3. No attempt was made to isolate the monoalkyl carbonates as substances. We were content to prepare the solutions in the following way: About 0.02 moles carbon dioxide was dissolved in one liter of a solution, which was 0.5 molar with regard to alcohol and 0.15 molar with regard to NaOH, thus converting about 50 per cent of the carbon dioxide to monoalkyl carbonate, and 50 per cent to ordinary carbonate.

4. The method of analysis was as in previous investigations precipitation with bariumchloride *i.e.* 100 ml sample solution is — provided the hydroxyl ion

concentration of the solution is less than about 0.1 molar — made 0.1 molar with regard to hydroxyl ion by means of sodium hydroxide. By the addition of barium chloride the carbonate is precipitated, whilst the monoalkyl carbonate remains dissolved. By centrifugation the precipitate and the supernate are separated as quickly as possible. By the heating of the supernate the monoalkyl carbonate is decomposed, forming a precipitate of barium carbonate, which is titrated with 0.1 *N* HCl.

It should be noted that in the case of ethylene chlorohydrin large amounts of NaOH are consumed by ethylene chlorohydrin during the heating of the supernate, and hence sufficient sodium hydroxide was added so that the supernate after the heating was about 0.1 *N* with regard to hydroxyl ion.

Since solutions containing only carbonate, and no monoalkylcarbonate, after precipitation with barium chloride will yield a slight precipitate of barium carbonate upon heating, it was necessary to carry out blank determinations along with the determinations proper. A blank determination was carried out with 100 ml of sample solution of approximately the same composition as that of the corresponding determination proper, and was precipitated, centrifuged, heated, and so on, in identical ways. The blank values amount to about 1 to 2 units of the percentage of monoalkyl carbonate. All of the data presented are corrected with regard to blank values, unless otherwise stated.

5. All of the experiments were carried out at 0° C, the centrifugations, however, in the cold room at 1° C. The velocity constants were calculated by means of Brigg's logarithms, the unit of time being the minute.

On the formation of monoalkyl carbonate from carbon dioxide and alcohol in basic solution, and on the reactions:

$$\text{alkylate}^- + CO_2 = \text{alkyl} \cdot CO_3^-$$

The experiments were carried out in the following way: 500 ml of an aqueous solution containing alcohol as well as sodium hydroxide were placed in a 2 liter flask. Approximately 250 ml of carbon dioxide were substituted for approximately 250 ml of atmospheric air in the gas phase above the solution, and the flask was shaken vigorously for half a minute. It should be explicitly noted that the ethylene chlorohydrin-solution was prepared immediately before adding the carbon dioxide, since ethylene chlorohydrin and NaOH will otherwise react with each other. The solution was immediately analyzed to determine how many per cent of the carbon dioxide absorbed had been converted to monoalkyl carbonate and carbonate, respectively. The results are presented in Table 1.

Since the monoalkyl carbonates are very rapidly decomposed, it was, especially in the case of ethylene chlorohydrin, necessary — by means of the velocity constants determined in section IV — to correct the analytically determined percentage of monoalkyl carbonate for the decomposition taking place during the 5 to 7 minutes passing between the start of the shaking with carbon dioxide, and the separation of precipitate and supernate by centrifugation. In the case of ethylene glycol the correction amounts to 2.5 units of the percentage of monoalkyl carbonate, whereas in the case of ethylene chlorohydrin it amounts to as much as about 10 units. The values listed in the table are corrected to zero time.

Table 1. Carbon dioxide in alcohol + NaOH, 0°.

	Initial solution		Absorbed CO_2 mol/liter	% alkyl-carbonate	log k'	Mean of log k'
	c_{NaOH}	$c_{alcohol}$				
CH_2OH | CH_2OH	0.15	0.50	0.0225	46.6 47.1	4.64 4.64	4.64
CH_2Cl | CH_2OH	0.15	0.50	0.0194	53.6 53.1	4.76 4.75	4.74
	0.15	0.50	0.0230	51.1	4.72	

Corresponding experiments on the alcohols previously investigated might be explained by assuming that carbon dioxide reacts partly with the alkylate on, partly with the hydroxyl ion, but not with the non-ionized alcohol:

$$CO_2 + \text{alkylate}^- = \text{alkyl} \cdot CO_3^- \qquad \text{I}$$

$$CO_2 + OH^- = HCO_3^- \qquad \text{II}$$

As shown previously this leads to the following expression:

$$k' = \frac{k_{CO_2 \cdot \text{alkylate}^-}}{K_{\text{alkylate}^-}} = \frac{\text{per cent monoalkyl carbonate}}{\text{per cent carbonate}} \cdot \frac{k_{CO_2 \cdot OH^-}}{c_{\text{alcohol}}};$$ upon

introduction in this expression of the experimental results with ethylene chlorohydrin and ethylene glycol, the k' values presented in Table 1 are obtained. Here $k_{CO_2 \cdot \text{alkylate}^-}$ and $k_{CO_2 \cdot OH^-}$ ($= 10^{4.39}$ at 0° C) signify the velocity con-

stants of reactions I and II, respectively, whilst K_{alkylate^-} is the equilibrium constant for the reaction "alcoholate ion + water = alcohol + hydroxyl ion"; k' is the velocity constant (from left to right) of the gross reaction:

$$CO_2 + \text{alcohol} + OH^- = \text{alkyl} \cdot CO_3^- + H_2O \qquad \text{III}$$

From:

$$\begin{aligned} \text{alcohol} + HCO_3^- &= \text{alkyl} \cdot CO_3^- + H_2O & &\frac{1}{K_{Eq}} \\ CO_2 + H_2O &= H^+ + HCO_3^- & &K_{CO_2}\ (10^{-6.65}) \\ H^+ + OH^- &= H_2O & &\frac{1}{K_{H_2O}}\ (10^{14.93}) \end{aligned}$$

the equilibrium constant, K', for reaction III may be calculated, and hence may also be calculated the velocity constant, $k_{\text{alkyl} \cdot CO_3^-}$, for reaction III from right to left:

$$K' = \frac{k'}{k_{\text{alkyl} \cdot CO_3^-}} = \frac{K_{CO_2}}{K_{H_2O} \cdot K_{Eq}} = \frac{10^{-6.65}}{10^{-14.93} \cdot K_{Eq}}$$

where K_{Eq} is the equilibrium constant found in section III. In this way is found for ethylene glycol $k_{\text{alkyl} \cdot CO_3^-} = 0.0063$, and for ethylene chlorohydrin $k_{\text{alkyl} \cdot CO_3^-} = 0.032$.

The equlibrium of the reactions:

$$\text{alkyl} \cdot CO_3^- + H_2O \rightleftharpoons HCO_3^- + \text{alcohol}$$

The above equilibrium was established in aqueous solutions of the alcohol, hydrogen carbonate, and carbonate. By the alcohols studied in the present investigation the equilibrium is shifted strongly to the right, and thus the determinations of per cent alkyl carbonate may be encumbered with rather large errors.

Equilibrium was established at 0° C in solutions which were 1.0 M with regard to alcohol, 0.1 M with regard to $KHCO_3$, and 0.05 M with regard to Na_2CO_3. The content of monoalkyl carbonate was determined and was calculated as per cent of HCO_3^- initially present; thus no attention was payed to carbonate. For ethylene glycol 7 determinations in 2 series (4 + 3) were carried out, and for ethylene chlorohydrin 8 determinations in 2 series (4 + 4) were carried out; the results are presented in Table 2; to illustrate, how much importance may be attached to the accuracy of the values found, are presented in the table not only the "% alkyl carbonate" corrected for blank

values, but the uncorrected ones as well. From the experimental results the equilibrium constant of the reaction,

$$K_{Eq} = \frac{c_{\text{alcohol}} \cdot c_{HCO_3^-} \cdot f}{c_{\text{alkyl} \cdot CO_3^-} \cdot f}$$

may be calculated, the activity coefficients neutralizing each other. It should be noted that the above weakly basic solutions were left for about 30 hours before being analyzed. During this time some ethylene chlorohydrin must have been decomposed. An analysis showed, however, that the amount decomposed was so slight as to be insignificant. After 30 hours the molarity of ionogen chlorine in the 1 *M* ethylene chlorohydrin was no more than 0.0008.

Table 2. The solution of carbonate-alkylcarbonate in equilibrium. 0°.

	Initial solution			% alkylcarbonate		K_{Eq}
	c_{alcohol}	c_{KHCO_3}	$c_{Na_2CO_3}$	incorr.	corr.	
CH_2OH | CH_2OH	1.0	0.1	0.05	4.18	3.50 [1]	$10^{1,44}$
CH_2Cl | CH_2OH	1.0	0.1	0.05	1.70	0.88 [2]	$10^{2,05}$

[1] Mean of 7 determinations: 3.43, 3.48, 3.67, 3.43, 3.48, 3.58, 3.45.
[2] » » 8 » 0.96, 0.78, 0.93, 0.86, 0.91, 0.89, 0.83, 0.87.

On the velocity of the reactions:

$$\text{alkyl} \cdot CO_3^- + H_2O = HCO_3^- + \text{alcohol}$$

Concerning the preparation of the solutions used we refer to the introduction. The initial concentration of monoalkyl carbonate was in the case of ethylene glycol approximately 0.010 *M*, and in the case of ethylene chlorohydrin approximately 0.006 *M*. The concentration of alcohol was in both experiments 0.49 *M*, and the molarity of sodium hydroxide was 0.12 and 0.11, respectively.

In Table 3 "% alkyl carbonate left" indicates how many per cent of the monoalkyl carbonate have not yet been decomposed. In both cases the reaction may, after a sufficiently long time, be considered as having run to completion, and as k_{mono} is found the calculated value of $\frac{1}{t} \log \frac{a}{a-x}$.

The experiments are interpreted in a way similar to the one applied to the monoalkyl carbonates previously investigated. The decomposition takes place through the reactions:

$$1.\quad \text{alkyl} \cdot CO_3^- = \text{alkylate}^- + CO_2$$
$$2.\quad CO_2 + OH^- = HCO_3^-$$

and, as shown in a previous investigation [1b], the velocity constant k_{mono} may at pH > about 10 be calculated by means of the following expression:

$$k_{mono} = \frac{k_{\text{alkyl} \cdot CO_3^-}}{1 + \frac{k_{CO_3 \cdot \text{alkylate}^-}}{K_{\text{alkylate}^-}} \cdot \frac{c_{\text{alcohol}}}{k_{CO_3 \cdot OH^-}}} = \frac{k' \cdot K_{Eq} \cdot \frac{K_{H_2O}}{K_{CO_3}}}{1 + k' \cdot \frac{c_{\text{alcohol}}}{k_{CO_3 \cdot OH^-}}}$$

which, upon introduction of the constants, gives for ethylene glycol:

$$k_{mono} = \frac{0.0063}{1 + 1.78 \cdot c_{\text{alcohol}}} = 0.0034 \text{ (calc.)}$$

Table 3. Monoalkyl carbonate in NaOH + alcohol, 0°.

	Initial solution			Min.	% alkyl-carbonate left	k_{mono}
	$c_{\text{alkylcarbonate}}$	c_{NaOH}	c_{alcohol}			
CH_2OH | CH_2OH	0.010	0.12	0.49	0	100	—
				20	84.6	0.00363
				44	70.3	0.00348
				88	46.6	0.00376
				148	28.9	0.00365
				195	20.0	0.00358
				256	11.2	0.00371
					Mean:	0.0036
CH_2Cl | CH_2OH	0.006	0.11	0.49	0	100	—
				8.5	72.0	0.0168
				17	51.6	0.0172
				25	35.9	0.0180
				33	25.9	0.0180
				40	18.7	0.0181
				49	15.0	0.0170
					Mean:	0.018

and for ethylene chlorohydrin:

$$k_{\text{mono}} = \frac{0.032}{1 + 2.24 \cdot c_{\text{alcohol}}} = 0.015 \text{ (calc.)}$$

0.49 being introduced for c_{alcohol}.
The experimental and the calculated values agree surprisingly well; especially when the errors of determination of K_{Eq} and k' are taken into consideration.

SUMMARY

The velocity constant of the reaction "$CH_2Cl \cdot CH_2O^- + CO_2 = CH_2Cl \cdot CH_2CO_3^-$" and the equilibrium constant for the reaction "$CH_2Cl \cdot CH_2\,CO_3^- + H_2O \rightleftharpoons CH_2Cl \cdot CH_2OH + HCO_3^-$" have been determined. The velocity of the decomposition of $CH_2Cl \cdot CH_2CO_3^-$ in strongly basic medium was investigated and may be explained in assuming that the decomposition is a two-stage reaction, *viz.*

$$\begin{aligned} CH_2Cl \cdot CH_2CO_3^- &= CH_2Cl \cdot CH_2O^- + CO_2 \\ CO_2 + OH^- &= HCO_3^- \end{aligned}$$

The monoalkyl carbonate of ethylene glycol was studied in a similar way.

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The Molecular Weight of Leghemoglobin

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Hemoglobin present in the root nodules of leguminous plants, called in brief leghemoglobin *, oxidizes so rapidly when isolated from the nodules that unless oxidation-preventing substances are added the hemoprotein appears in the form of hemiglobin. We have, therefore, made determinations of the molecular weight of leghemiglobin.

During electrophoresis leghemiglobin purified by ammonium sulphate precipitations separates into two components [1]. In the present paper an attempt is made to characterize these components more closely and to determine the molecular weight of the one which probably represents pure leghemiglobin.

Leghemiglobin preparations

Leghemiglobin was prepared in a similar way as before [2]. The nodules of soya bean were crushed in an equal weight of saturated ammonium sulphate solution (58 % saturation) centrifuged and the ammonium sulphate saturation adjusted to 58 %. After standing overnight at 0° C the precipitate was separated and the ammonium sulphate saturation was raised to 75 %. After again setting aside overnight at 0° C, the precipitate was separated and dissolved in a small quantity of distilled water, dialyzed against running tap water, and finally against distilled water. Precipitation and dialysis were repeated several times.

The preparation was then subjected to lyophilization. This gives leghemiglobin in the form of a pink amorphous powder, which dissolves immediately and completely in distilled water. In the dry state the leghemiglobin is quite stable. The material obtained was used for preparing concentrated solutions for osmotic investigations and preparative electrophoretic work.

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* Keilin and Hartree (*Nature* **168** (1951) 266) have expressed the opinion that the prefix "leg" is inadequate, and furthermore that it is ambiguous in English. However, it would appear to be a logical abbreviation of the Latin word "legumen" from the verb "legere", and we shall continue to use the term "leghemoglobin" rather than the unwieldy "hemoglobin of leguminous root nodules". A. I. Virtanen.

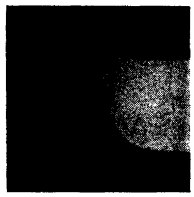


Fig. 1. Ascending limb, pH 6.40, u = 0.1, t = 120 min. Phosphate buffer.

The material had the following characteristics.

Spectrum. The spectrophotometric (Beckman) investigation showed the trivalent form of hemin iron, *i.e.*, leghemiglobin.

Electrophoresis. This showed the existence of two components as shown in Fig. 1. The apparatus used was Perkin-Elmer.

Iron content. Iron was determined photometrically by the *o*-phenanthroline method [3]. Combustion of the material was performed with sulphuric acid with addition of hydrogen peroxide, and 1 % hydroxylamine was used as reducing agent. The intensity of the colour of the ferrous *o*-phenanthroline complex was estimated using a Klett-Summerson photometer with filter 50, and the amount of iron was read from a calibration curve constructed with known concentrations. The iron content was 0.26—0.27 % calculated on the dry protein basis.

Separation of the two electrophoretically separable components was carried out by Klett electrophoresis apparatus during 7 hours in a 15 ml cell with 1 % leghemiglobin solution in a phosphate buffer (pH 7.10, $\mu = 0.1$). The potential gradient F was 8.9 volt/cm.

Two determinations of the iron content of the faster component gave the values 0.345 and 0.360 % of the dry matter. The former determination was made with a larger quantity of material and is probably the more accurate. The slower component was used for a diffusion measurement.

Diffusion measurements

Diffusion measurements were carried out at 20° C in an electrophoresis apparatus (Klett), a 10 ml preparative electrophoresis cell serving as the diffusion cell. To get evenly exposed photographs, we used a red filter, and a tungsten lamp as light source. The diffusion was followed by the Philpot-Svensson slit method.

The leghemiglobin preparation used in the diffusion measurements contained the two electrophoretically separable components (0.26—0.27 % iron). The solution was 0.8—0.9 %, and had been dialyzed against 1 molal NaCl ($\eta/\eta_0 = 1.087$) which was used to fill the upper compartments of the cell into which leghemiglobin was diffusing. The pH of the protein solution was $\sim$ 5.6.

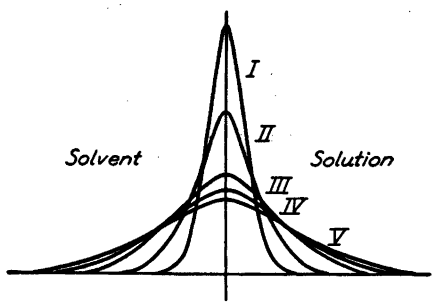


Fig. 2. Diffusion diagrams I, II, III, IV, and V refer to exposures taken 171, 388, 1 200, 1 586, and 1 836 minutes after the beginning of the diffusion process, and show the concentration gradient as a function of the distance from the initially sharp boundary between solvent and solution.

The curves in Fig. 3 characterize the course and momentary state of diffusion at five different times. The diffusion constant D has been calculated from these curves using the "area method" and is defined in the following way

$$D_A = \frac{A^2}{4 \cdot \pi \cdot t \cdot H_{max}^2}$$

where A denotes the area between the curve and x-axis, H_{max} is the maximum height of the curve, and t is the time after the formation of the boundary.

In our experiment the angle of inclination of the diagonal slit $\Theta = 30°$. The magnification factor of the camera was 0.95. From the curves in Fig. 2 we obtained the following values of D_A given in units of 10^{-7} c.g.s.

Diffusion time, mins.	171	388	1 200	1 586	1 836
D_A	8.50	8.92	7.90	8.40	8.84
D_A, corrected for water	9.23	9.70	8.58	9.12	9.60

After graphical levelling of the corrected values

$$D_A = 9.08$$

Another diffusion measurement was carried out with a more dilute solution of leghemiglobin (ca. 0.3 %) in phosphate buffer (0.01 M Na_2HPO_4, 0.023 M NaH_2PO_4 and 0.047 M NaCl, pH 6.4). The conditions were the same as before. $\Theta = 75°$. We obtained the following values of D_A in units of 10^{-7} c.g.s.

Diffusion time, mins.	1 705	2 650	3 810
D_A (in phosphate buffer)	8.88	8.65	9.15

after graphical levelling of the values

$$D_A = 8.25 \text{ (in phosphate buffer)}$$

A diffusion measurement was also carried out with the electrophoretically slower component [1], using the same method. The diffusion cell was a 2 ml

analytical electrophoresis cell. $\Theta = 81°$. Protein concentration: 0.1—0.2 %. Medium: phosphate buffer (0.02 *M* NaH_2PO_4, 0.046 *M* Na_2HPO_4, 0.094 *M* NaCl, pH 6.6). The values of D_A in units of 10^{-7} c.g.s. obtained were as follows:

Diffusion time, mins.	492	1 140	1 557
D_A (in phosphate buffer)	4.8	3.7	3.6

after graphical levelling of the values

$$D_A = 4.1 \text{ (in phosphate buffer)}$$

Unfortunately we could not make any diffusion measurements in lack of material.

Ultracentrifuge studies

The apparatus employed was an air-driven ultracentrifuge (Phywe) and the "schlieren" method was used to follow the course of the sedimentation. The speed used was 50.000 r.p.m, and the distance from the axis of rotation to the meniscus of the liquid was 5.75 cm. Because of the relatively low molecular weight of this protein, a long time was required to obtain an adequate displacement of the boundary and considerable diffusion took place thus making it difficult to determine accurately the maxima of the displacement curves obtained by the schlieren method.

Solutions of 0.3—0.4 % leghemiglobin in phosphate buffer (0.01 *M* Na_2HPO_4, 0.023 *M* NaH_2PO_4 and 0.047 *M* NaCl, pH 6.40) were used. Our preparations indicate a considerable homogeneity in the ultracentrifuge field, although some separation into components was detectable. However, in contrast to the electrophoretic behaviour, two components of equal concentration could not be distinguished. The predominating component showed a sedimentation constant *s* in Svedberg units (1 S = 10^{-13} c.g.s. units)

$$s_{20} = \sim 2 \text{ S (in phosphate buffer)}$$

The other component had a greater molecular weight than the predominating one but its concentration was very much less. It could only be observed in the early stages of ultracentrifugation, and disappeared during the run. The sedimentation constant of this component seemed to be between 5 S and 6 S (the actual value obtained was 5.3 S).

Molecular weight from the sedimentation rate

The molecular weight *(M)* was calculated by means of the equation

$$M = \frac{RT \cdot s_{20}}{D\ (1 - V\varrho)}$$

where R = gas constant, T = absolute temperature, ϱ = density of the solvent and V = partial specific volume of the protein.

Taking $s_{20} = 1.90 \cdot 10^{-13}$ (the value of Pedersen, see below)

$D_{20} = 9.08 \cdot 10^{-7}$

$V = 0.749$ (the partial specific volume of blood hemoglobin) we obtain the molecular weight 20 300.

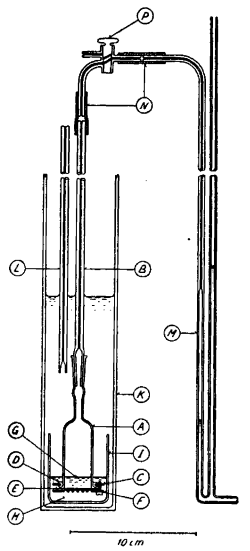


Fig. 3. The osmometer.

OSMOTIC MEASUREMENTS

The osmotic measurements were made because the ultracentrifuge studies indicated that our preparations precipitated with ammonium sulphate consisted of a molecular mixture the chief part of which contained homogeneous protein of low molecular weight.

Apparatus. The osmometer used for these experiments described by Güntelberg and Linderstrøm-Lang [4] and illustrated by Fig. 3 was placed in a thermostat at a temperature of 20 ± 0.002° C.

Experimental procedure.

The membranes used in our investigations were prepared according to the principle of Bjerrum and Manegold [5]. An iron ring was placed in a large Petri dish containing mercury, and collodion was poured on to the surface of the mercury inside the ring. The dish was covered with a glass plate until the collodion had spread evenly, then the plate was raised enough to allow the collodion to dry under a free access of air. After 3 hours most of alcohol and ether had evaporated and the iron ring with the wet membrane was removed from the mercury and allowed to dry in the air. To get the right permeability the iron ring with the membrane was put into water. If this was done too early or too late the permeability became accordingly too great or too small. This is naturally a question of experience. The permeability of our membranes was, expressed in terms of Bjerrum and Manegold [6]. $D \sim 10^{-7}$. It appeared that the permeability decreased during the osmosis.

Small membranes cut off from the large one were stored in 1 molal NaCl solution for half an hour, dried between filter papers, and placed on the bottom of a steel plate F. The glass vessel A, the flange of which was greased with vaseline, was pressed against F and fixed to the bottom plate with 3 screws.

4.5 ml of the test solution, freed from air, were put into the chamber which was then filled with toluene. The capillary tube B was greased with a special grease [7] insoluble in toluene, filled with toluene and put in position. The chamber was then placed in a beaker containing 25 ml of the outer solution (1 molal NaCl) care being taken to ensure that there were no air bubbles in the holes of the steel plate. The beaker was then filled

with toluene and lowered into the glass cylinder K containing toluene. Since the room temperature was higher than the temperature of the thermostat, the toluene in B had to retract to the middle part of the tube so that the toluene column in B was often 2—5 cm higher or lower than in L. The two columns were approximately levelled by the application of a pressure higher or lower than the osmotic pressure assumed. In this way it is possible to find the protein concentration without analysing the test solution, provided the exact concentration of the stock solution and the capillary volume per cm are known so that the dilution or concentration of the solution can be determined.

The outer solutions were normally 1 molal with respect to NaCl. The stock solution for the inner solution — the test solution — was prepared by dissolving the freeze-dried preparation in a small quantity of distilled water. The pH of this solution was $\sim$ 5.7, and several more dilute solutions were prepared so that the concentration of NaCl became 1 molal.

To determine the concentration of the inner solutions we used the procedure described above and did not analyze the inner solutions at equilibrium. The quantities were calculated on the dry protein basis.

The osmometer was left overnight at 20° C to reach equilibrium. The osmotic pressure was measured as the pressure externally applied by means of the manometer M, at which the meniscus in B is stationary, corrected for the difference in height of the columns in B and L. This was done in the following way.

The rate at which the meniscus moved in periods of 5 minutes was measured with a cathetometer at four different pressures, *i. e.* about 2 and 4 cm above and below the pressure at which the meniscus was expected to be stationary, and the true value was then found by graphical interpolation. The same experiment was continued for several days to test for possible leakage of the cell.

Molecular weight from the osmotic measurements

The apparent molecular weight (M_{app}) was calculated from each measurement by the equation [4]

$$M_{app} = \frac{RT \cdot \gamma}{\omega' \cdot p}$$

where γ = protein g per 1 000 g water, p = osmotic pressure measured in cm H_2O, ω' = specific volume of water plus specific volume of NaCl in solution = 1.02 l, RT (20°) = 24 870 l, cm H_2O/Mol.

Table 1 shows the experimental results at different concentrations of protein.

To obtain the correct value for M from the above equation, the usual procedure is to determine the osmotic pressure over a series of protein concentrations and to plot M_{app} against γ. The method of the least squares can also be used by applying the equation: $M_{app} = a + b\,\gamma$ where

$$a = \frac{\Sigma\gamma\,\Sigma M_{app}\gamma - \Sigma\gamma^2\,\Sigma M_{app}}{\Sigma\gamma\,\Sigma\gamma - n\Sigma\gamma^2};\quad b = \frac{\Sigma\gamma\,\Sigma M_{app} - n\Sigma\gamma\,\Sigma M_{app}}{\Sigma\gamma\,\Sigma\gamma - n\Sigma\gamma^2}$$

(n = number of experiment)

Table 1. Osmotic pressures and apparent molecular weights of leghemiglobin preparations at 20° C in 1 molal NaCl, pH 5.7.

No.	γ (protein, g/1 000 g H_2O)	p pressure in cm H_2O	M_{app}
1	4.6	5.7	19 700
2	5.6	6.6	21 000
3	7.1	8.7	19 950
4	8.8	10.4	20 650
5	11.3	13.1	21 000
6	12.7	13.3	23 600
7	19.6	22.3	21 400
8	32.0	33.7	23 200

Calculating a and b from the values in Table 1, excluding experiment 6, we get

$$a = (M) = 19\,617;\ b = 107.7$$

If, however, we determine the values a and b, neglecting experiments 1, 6, and 8, we obtain

$$a = (M) = 20\,132;\ b = 63.8$$

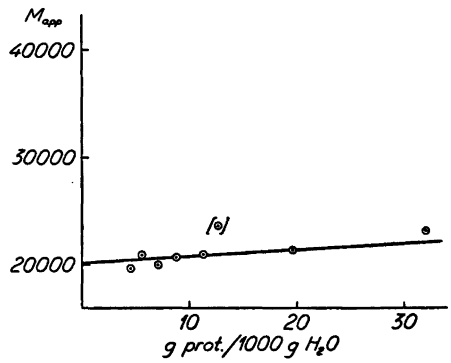


Fig. 4. Correlation between M_{app} and γ. The straight line has been constructed on the basis of the last values of a and b.

Earlier studies

In 1945—1947, Dr. K. O. Pedersen kindly carried out ultracentrifuge studies on three hemiglobin preparations sent by one of us (V.). The preparations had not been examined electrophoretically and hence their homogeneity was not established. These three preparations gave different values in diffusion measurements but approximately the same s_{20} (= 1.90 S) for the red-brown chief component. In addition, the preparations contained components with s_{20} within the region 5—12 S.

In regard to the first preparation, Pedersen found that "the great difference between D_A and D_m indicates that the preparation is non-homogeneous". He was very uncertain about the diffusion values but if they were used the most likely value for M was $\sim$ 34 000[2].

Pedersen made a number of diffusion experiments using the third sample and obtained D_A values between 8.2×10^{-7} and 10.8×10^{-7}. After a critical examination of the values he came to the conclusion that the molecular weight of leghemiglobin probably does not exceed 19 000 [8].

Because of the heterogeneity and differences between the samples no definite conclusions could be made of the mol.wt. of the pure leghemiglobin.

DISCUSSION

On the basis of the results recorded in the previous paper [1] and in the present one, certain conclusions can be drawn about the composition of the leghemiglobin preparations isolated from the root nodules, and the molecular weight of pure leghemiglobin. The molecular weights obtained for our leghemiglobin preparations, by the osmotic method, have been about 20 000.

Svedberg and Eriksson-Quensel [9] have found in some lower animals erythrocruorins with molecular weights of the same order, *i.e.* between 17 000 and 34 000.

	M	s_{20}
Erythrocruorin, *Thyone briareus*	23 600	2.6 S
» *Myxine glutinosa*	23 100	2.3 S

As the simplest explanation of the behaviour of these erythrocruorins, the authors advanced the idea "that they represent mixtures of the molecules 34 500 and 17 250, that is, mixtures of molecules with two and one hemin group per molecule, respectively".

The applicability of this explanation to our leghemiglobin preparations with molecular weights of $\sim$ 20 000 seems unlikely. The electrophoresis studies have revealed two components of equal concentration. The hemin content of the faster one is twice that of the slower one. If the faster component is hemin protein with a molecular weight of about 17 000, then the slower one must have a molecular weight considerably higher than 34 000 since it gave a diffusion constant $D_A = 4.1 \cdot 10^{-7}$. True, this value must be taken with some reserve, because the determination was made at a very low concentration, but it justifies the assumption of a large molecule or of a highly asymmetric molecule (unless hydration is considered). Calculation of the molecular weight of the slower component gives a value of $\sim$ 45 000, when $s_{20} = 1.9$ S and $D_A = 4.1 \cdot 10^{-7}$. The suggestion that the leghemiglobin preparations are a mixture of hemin proteins with mol. weights of $\sim$ 17 000 and $\sim$ 34 000 is thus unjustified.

The molecular weight ~ 20 000 found by osmotic measurements for the leghem*i*globin preparation containing 0.26 % Fe, the iron content of the electrophoretically faster component, 0.35 % (corresponding to 1 atom of iron per mol. weight ~ 17 000), and the occurrence of a slower component with a higher mol. weight, can be explained by assuming that the faster component is pure leghem*i*globin with mol. weight ~ 17 000 and the slower one a heavier protein.

The ultracentrifuge studies have shown that our preparations of leghem*i*globin containing 0.26 % Fe contain a predominating component with a sedimentation constant of about 1.9 S and a heavier one, which, in our investigations, gave a sedimentation constant of 5—6 S. This component seems to form a part of the electrophoretically slower component with low iron content. We assume it to be some kind of specific, relatively stable, adsorption compound of a heavy iron-free protein and leghem*i*globin, possibly a globulin surrounded by leghem*i*globin molecules. However, it must be admitted that in this case it is somewhat surprising that the ratio of the low-hemin and high-hemin (leghem*i*globin) components has been the same in our electrophoresis experiments with different preparations.

On the basis of the above, leghem*i*globin should be analogous to myoglobin and erythrocruorin from the species *Petromyzon* with the following properties:

	M	s_{20}	I. P.
Myoglobin	17 600 [10]	2.04 S [10]	6.99 [11]
Erythrocruorin, *Petromyzon*	19 100 [9]	1.9 S [9]	5.6 [9]
Leghem*i*globin	~17 000	1.9 S	4.4

Leghem*i*globin has the lowest I.P. of all the known hemoglobins and similar oxygen carriers. Its histidine content is very low hardly 1/4 of that of myoglobin. In this respect as well as in respect of the I. P., the erythrocruorins are between leghem*i*globin and myoglobin.

SUMMARY

Ultracentrifuge studies of our leghem*i*globin preparations containing 0.26—0.27 % Fe indicate a predominating component with a sedimentation constant 1.9 S, and a smaller heavier component.

The molecular weight of our preparations, estimated by the osmotic method, were found to be about 20 000. This is in good agreement with the values obtained from the rate of sedimentation ($s_{20} = 1.9$ S) and from diffusion measurements ($D_{20} = 9.08 \cdot 10^{-7}$). The iron content of the electrophor-

etically faster component was ~ 0.35 % indicating one hemin group per mol. weight ~ 17 000.

The molecular weights of the two components, which can be separated elctrophoretically have been discussed. The results suggest that the faster component with I. P. 4.4 has a molecular weight of ~ 17 000, and this component is assumed to be pure leghemiglobin.

We are deeply indebted to Dr. E. Uroma, Director of the State Serum Institute, for allowing us to use the equipment for lyophilization, diffusion, electrophoretic separation, and ultracentrifugation. We wish to express our gratitude to Phil. Mag. Antti Louhivuori, Head of the Chemical Department of the same Institute, for his valuable assistance in the course of the work, and to Dr. Korsgaard-Christensen of the Carlsberg Laboratory, Copenhagen, for his valuable advice on the preparation of the osmosis membrane.

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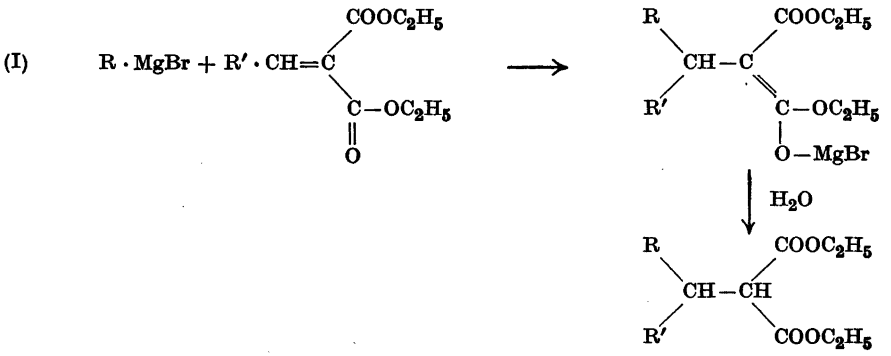
The Action of Arylmagnesium Bromides on Benzylidenemalonic Esters

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Kohler [1] has shown that ethyl diphenylmethylmalonate is formed when phenylmagnesium bromide is allowed to react with ethyl benzylidenemalonate (Equation I; R = R′ = phenyl). Although the reaction formally resembles the reaction between arylmagnesium halides and cinnamic esters, which reaction has recently been investigated by Fuson and Jackson [2] and by Hauser, Yost, and Ringler [3], very little seems to have been done to ascertain its limitations. The author of this paper has previously prepared ethyl phenyl-*p*-tolylmethylmalonate (R=*p*-tolyl, R′=phenyl), ethyl phenyl-*p*-anisylmethylmalonate (R = *p*-anisyl, R′ = phenyl), and ethyl phenyl-*α*-naphthylmethylmalonate (R = *α*-naphtyl, R′ = phenyl) from the corresponding Grignard reagents and ethyl benzylidenemalonate [4]. However, when an attempt was made to prepare ethyl di-*o*-anisylmethylmalonate from *o*-anisylmagnesium bromide and ethyl *o*-methoxybenzylidenemalonate, an oil was obtained from which different substances were isolated, but not the desired ester.

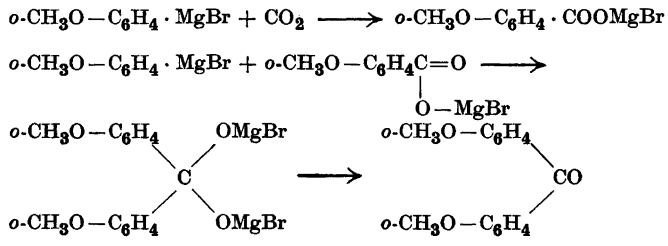
(I)



As it was of some interest to establish if ethyl phenyl-o-anisylmethylmalonate can be prepared either from ethyl benzylidenemalonate and *o*-anisylmagnesium bromide or from ethyl *o*-methoxybenzylidenemalonate and phenylmagnesium bromide, some experiments were performed. The latter substances gave ethyl phenyl-*o*-anisylmethylmalonate, which by saponification and decarboxylation was transformed into *β*-phenyl-*β*-*o*-anisylpropionic acid. The product of the reaction between *o*-anisylmagnesium bromide and ethyl benzylidenemalonate was, however, an oil from which no pure compound has hitherto been isolated. In order to determine whether ethyl phenyl-*o*-anisylmethylmalonate had formed, the oil was saponified and the oily acids were heated to 200—210° in order to obtain the more easily crystallizing *β*-phenyl-*β*-*o*-anisylpropionic acid, but only oils were formed. Since phenylmagnesium bromide reacts in the normal way with ethyl *o*-methoxybenzylidenemalonate, but *o*-anisyl-magnesium bromide and ethyl benzylidenemalonate react abnormally, experiments were made to determine how *o*-tolylmagnesium bromide reacts with ethyl benzylidenemalonate. An oil was obtained and pure diarylmalonate could not be isolated. After saponification and decarboxylation of the oil was, however, *β*-phenyl-*β*-*o*-tolylpropionic acid isolated.

From the fact that ethyl diarylmethylmalonates are not formed when *o*-anisylmagnesium bromide reacts with ethyl benzylidenemalonate and its *o*-methoxy derivative and that the corresponding ethyl diarylmethylmalonates are formed when o-tolylmagnesium bromide and phenylmagnesium bromide are allowed to react with ethyl benzylidenemalonate and ethyl *o*-methoxybenzylidenemalonate, respectively, it was concluded that *o*-anisylmagnesium bromide reacts abnormally. In order to prove this, carbon dioxide was introduced into an ether solution of *o*-anisylmagnesium bromide. A small amount of impure *o*-methoxybenzoic acid was isolated from the reaction mixture, the principal product being di-*o*-methoxybenzophenone, which substance is probably formed according to Equations II.

(II)



In connection with the syntheses of different *β*-aroyl-propionic acids from arylmagnesium bromides and succinic anhydride Baddar and El-Assal [5]

observed a similar abnormal reaction of *o*-anisylmagnesium bromide, although they did not attribute it to this Grignard reagent, but to the first product of the reaction, *β*-*o*-anisoylpropionic acid.

EXPERIMENTAL

Action of *o*-anisylmagnesium bromide on ethyl benzylidenemalonate

A Grignard reagent was prepared from 2.4 g of magnesium turnings and 18.7 g of *o*-bromoanisol in 50 ml of dry ether. The solution was filtered in an atmosphere of hydrogen and the filter was washed with about 30 ml of dry ether. The combined ether solutions were then gradually added to a solution of 18.6 g of ethyl benzylidenemalonate in 500 ml of dry ether. Every drop caused the formation of a yellow precipitate. When the addition was complete, the mixture was warmed for one hour on the water bath. The precipitate was filtered off and treated with a mixture containing 80 ml of water, 20 ml of conc. hydrochloric acid, and 80 ml of ether. The solutions were allowed to separate and the aqueous layer was discarded. The ether solution was shaken with a dilute solution of sodium hydroxide in order to remove the small amounts of acids remaining, and finally with water. After evaporation of the ether, there remained 15.87 g of an oil which did not crystallize. Attempts were made to purify it by chromatography, but only oily fractions were obtained, and when these fractions were saponified and heated to 200—210°, tarry oils remained which did not crystallize.

The ether filtrate from the yellow precipitate was teated in a similar way and an additional steam distillation was performed in order to remove anisol and unchanged *o*-bromoanisol, but also in this case an oil (7.79 g) remained that did not crystallize. Chromatography gave no purification. When the chromatographical fractions were saponified and heated to 200—210°, tarry oils formed again.

Action of phenylmagnesium bromide on ethyl *o*-methoxybenzylidenemalonate

A Grignard reagent was prepared from 2.4 g of magnesium turnings and 15.7 g of bromobenzene in 50 ml of dry ether. The solution was filtered in an atmosphere of hydrogen and the filter was washed with about 30 ml of dry ether. The combined ether solutions were then gradually added to a solution of 20 g of ethyl *o*-methoxybenzylidenemalonate in 500 ml of dry ether. Also in this case a yellow precipitate formed, and when the addition was completed, a lump had formed that stopped the stirrer. After the mixture had been warmed for one hour on the water bath, the ether solution was decanted into a mixture of 40 ml conc. hydrochloric acid and 200 ml of water. The lump was washed with dry ether and the washings were combined with the main portion. The mixture was shaken and the ether layer was isolated. It was washed with water, and after it had been dried with anhydrous sodium sulfate, the ether was evaporated. The residue, a yellow oil, was treated with ligroin and after some time 5.86 g of crystals separated. The substance, ethyl phenyl-*o*-anisylmethylmalonate, was recrystallized from ethyl alcohol, after which it melted at 86—87°.

$C_{21}H_{24}O_5$	Calc.	C	70.76	H	6.79
	Found	»	70.67	»	6.71

Two grams of the recrystallized substance were dissolved in 100 ml of alcohol and a mixture containing 2 g of potassium hydroxide, 2 ml of water, and 25 ml of alcohol was added. The solution was heated for two hours on a boiling water bath. The alcohol was then evaporated and the residue was dissolved by mixing with 100 ml of ether and 100 ml of water. After separation, the ether solution was dried with anhydrous sodium sulfate. After the ether had been evaporated, there remained only a very small amount of an oil (0.01 g). The aqueous solution was acidified and after some time 1.77 g of crystals had formed.

Nine tenths of a gram of this substance were heated in the oil bath to 200—210°, during which carbon dioxide was evolved. When the melted substance had cooled, it was crystallized from benzene. The melting point of the substance, β-phenyl-β-*o*-anisylpropionic acid, was 133—134°.

$C_{16}H_{16}O_3$	Calc.	C	74.98	H	6.29
	Found	»	74.79	»	6.29

The lump that had formed when the phenylmagnesium bromide was added to the solution of ethyl *o*-methoxybenzylidenemalonate was treated with a mixture of ether, water, and conc. hydrochloric acid, but no pure substance could be isolated.

Action of *o*-tolylmagnesium bromide on ethyl benzylidenemalonate

A Grignard reagent was prepared from 2.8 g of magnesium turnings and 20.5 g of *o*-bromotoluene in 50 ml of dry ether. To the solution 500 ml of dry ether were added and to this dilute mixture 24.8 g of ethyl benzylidenemalonate dissolved in 100 ml of dry ether were slowly added. During this addition two layers formed. The upper one was an ether solution, but the lower one was a rather thick oil. After the mixture had been warmed for one hour on the water bath, the two layers were treated separately by pouring into mixtures of ether and dilute hydrochloric acid.

No pure substance could be isolated from the ether layer, but when 2.01 g of the oil that remained when the ether had been distilled off were saponified with an alcoholic solution containing 4 g of potassium hydroxide in the way described in connection with the saponification of ethyl phenyl-*o*-anisylmethylmalonate, 1.46 g of an acid were obtained. This acid was heated without further purification in the oil bath to 200—210°, during which carbon dioxide was evolved. When the melted substance had cooled, it was dissolved in a small quantity of benzene, and after some time β-phenyl-β-*o*-tolylpropionic acid, m.p. 131—132°, precipitated.

$C_{16}H_{16}O_2$	Calc.	C	79.97	H	6.71
	Found	»	79.92	»	6.68

When the oily layer was treated in the same manner a small quantity of the same acid was isolated, but only after the oil had been chromatographically purified.

Another experiment was performed without the addition of 500 ml of dry ether. The results were similar, but the yield of β-phenyl-β-*o*-tolylpropionic acid from the oily layer was somewhat larger.

The reaction between *o*-anisylmagnesium bromide and carbon dioxide

A Grignard reagent was prepared from 0.96 g of magnesium turnings and 5 g *o*-bromoanisol in 20 ml of dry ether. Carbon dioxide was then conducted into the solution during two hours. The reaction mixture was poured into dilute hydrochloric acid to which ice

had been added. More ether was added, the mixture was shaken and the ether solution was separated. Acids and phenols were removed by extraction with a dilute solution of sodium hydroxide. The ether layer was removed, and after drying with anhydrous sodium sulfate, the ether was evaporated. The residue (1.80 g) crystallized after a few hours and was then treated with a small quantity of ether. Crystals (1.04 g) of di-*o*-methoxybenzophenone (m.p. 98—100°) were filtered off. From the filtrate a small amount (0.09 g) of the same substance was isolated.

The alkaline water solution was acidified and the oil that separated was dissolved in ether. Organic acids were removed from the ether solution by shaking with a solution of sodium carbonate, and from this solution, a small quantity (0.20 g) of impure *o*-methoxybenzoic acid was isolated. The ether solution was shaken with a dilute solution of sodium hydroxide, from which 0.31 g of an oil separated after addition of hydrochloric acid. The oil smelled of guaiacol, but this substance could not be isolated.

This experiment was performed with *o*-bromoanisol prepared from *o*-anisidine [6]. The results were exactly similar when the experiment was repeated with *o*-bromoanisol prepared from *o*-bromoanisol [7].

SUMMARY

1. Ethyl phenyl-*o*-anisylmethylmalonate is formed when phenylmagnesium bromide is allowed to react with ethyl *o*-methoxybenzylidenemalonate, but not when *o*-anisylmagnesium bromide reacts with ethyl benzylidenemalonate.

2. It was established that ethyl phenyl-*o*-tolylmethylmalonate is formed in the reaction between *o*-tolylmagnesium bromide and ethyl benzylidenemalonate by isolating β-phenyl-β-*o*-tolylpropionic acid from the reaction product after saponification and decarboxylation.

3. When carbon dioxide is introduced into a solution of *o*-anisylmagnesium bromide, di-*o*-methoxybenzophenone is formed as the main product. Only a small amount of impure *o*-methoxybenzoic acid was isolated.

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1,2,4,5-Tetrahalogeno-Cyclohexanes and Structural Problems Related to Them

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Three members of the series of 1,2,4,5 halogen substituted cyclohexanes have been known for some time and the configurations of their molecules determined. These are the tetrachloro-compound melting at 176°C and the tetrabromo-compound of m.p. 187° C, both having their halogen atoms in the positions 1ε, 2ε, $4\varkappa$, $5\varkappa$[1,2], and the tetrabromo-compound melting at 218° in which all bromine atoms occupy $\varkappa$ positions[3].

In the present communication a series of new substances will be described, some of which exhibit structural properties which seem to justify a more detailed description of the compounds.

All the substances were prepared from 1,4-cyclohexadiene by adding solutions of the appropriate halogens in an inert solvent to solutions of the diene, or in some cases of 1,2-dichlorocyclohexene(4) of m.p. 42° C which is formed in the first step of the addition reaction when a chlorine solution is added to the diene. In such cases the dichlorocyclohexene was first isolated and purified.

In the case of chlorine addition *two* different tetrachlorocyclohexanes were obtained, one of which is identical with the substance already mentioned of m.p. 176° C. The second substance crystallizes from hot solutions in carbon tetrachloride in fine needles melting at 226° C. When the solvent (ethyl acetate) was evaporated from solutions of the latter substance at room temperature, more compact crystals of a different habit were occasionally obtained. This new modification (the α modification) is stable at lower temperatures, the other modification (β modification) above 60—65° C.

An exact determination of the transition point is difficult because of the low rate of transition at temperatures near this point. X-ray photographs show that the β modification is isomorphous with the tetrabromocyclohexane

prepared by Wibaut and Haak [3]. However, the crystals so far examined are all twinned and built up of submicroscopical lamellae. The isomorphism just mentioned proves that in the molecules of the new 1,2,4,5-tetrachlorocyclohexane all chlorine atoms are in $\varkappa$ positions. It has subsequently been possible to prove that this holds true also for the molecules in the α modification (see below).

When *bromine* is added to the 1,2-dichlorocyclohexene(4) *two* different 1,2-dichloro-4,5-dibromocyclohexanes are also formed, one melting at 171° C, the other at 240° C. The latter turned out to be dimorphous giving an α modification stable below 90—95° C, and a β modification stable at higher temperature. The β modification is isomorphous with the β modification of $1\varkappa$, $2\varkappa$, $4\varkappa$, $5\varkappa$-tetrachlorocyclohexane and with the corresponding tetrabromocyclohexane prepared by Haak. The α modification, stable at lower temperatures, on the other hand, is isomorphous with the α modification of the higher melting tetrachlorocyclohexane. This is obvious from X-ray diagrams and even from the macroscopical properties of the crystals. Crystals of the β modifications both of the tetrachlorocyclohexane and the dichloro-dibromocyclohexane may be kept at room temperature for a considerable length of time without showing any tendency to be transformed into the α modification.

Table 1. Crystallographic data and calculated densities of $1\varkappa$, $2\varkappa$, $4\varkappa$, $5\varkappa$-tetrahalogenocyclohexanes.

			a	b	c	β	Density
α	$C_6H_8Cl_4$	226° C	6.13	6.74	10.72	97.8°	1.68
α	$C_6H_8Cl_2Br_2$	240° C	6.29	6.93	10.81	98°	2.21
β	$C_6H_8Cl_4$	226° C	6.40	11.41	6.20	110°	1.73
β	$C_6H_8Cl_2Br_2$	240° C	6.79	11.75	6.20	110°	2.22
	$C_6H_8Br_4$	218° C [3]	7.23	12.32	6.36	110.5°	2.49

Table 1 contains the axial lengths and monoclinic angles of both forms of the higher melting tetrachloro- and dichloro-dibromocyclohexanes. The values reported by Haak have also been included for comparison. The densities calculated under the assumption that the unit cells all contain *two* molecules are in good agreement with those observed for other tetrahalogenocyclohexanes. The space group is $C_{2h}^5 - P2_1/c$ in all cases, from which follows that the molecules should have a point symmetry corresponding to a center of symmetry. This is of course in full agreement with the conclusion drawn above that the molecules have the $\varkappa,\varkappa,\varkappa,\varkappa$ configuration as long as the halogen atoms are all of the same kind. In the two modifications of 1,2-dichloro-4,5-dibromocyclohexane, however, it would imply that the structures are of a

disordered type. The molecules, although identical in shape, would have to be statistically orientated in space; positions occupied in some cases by chlorine atoms, would in other cases be occupied by bromine atoms. An analogous case was discussed in 1933 by Hendricks [4] who found *p*-chloro-bromobenzene to be isomorphous with *p*-dichloro- and *p*-dibromobenzene, all substances crystallizing in the space group $P2_1/c$ with *two* molecules in the unit cell. One of the present authors (E. W. L.) will publish a detailed report on the crystal structure work he has carried out on the two substances under discussion. So much may already be stated, however: Fourier projections of the α modification of the dichloro-dibromocyclohexane show halogen peaks which are all of the same height, and the distances from the center of the carbon ring to the halogen atoms are intermediate between the distances to be expected in the case of chlorine and bromine atoms.

The crystals of dichloro-dibromocyclohexane of m.p. 171° C were found to be isomorphous with the crystals of 1,2,4,5-tetrachlorocyclohexane and tetrabromocyclohexane of m.p. 176° and 187° C respectively, in which the halogen atoms are known to occupy the positions 1ε, 2ε, $4\varkappa$, $5\varkappa$. As a matter of fact our main purpose when starting the work described in the present communication was to prepare the substance in question and to decide which of the two possible configurations of the molecule is the energetically more stable: that in which the chlorine atoms occupy ε positions (Fig. 1a) or that in which they are in $\varkappa$ positions (Fig. 1b). This question has now been definitively settled on the basis of interferometric measurements using X-ray crystallographic methods [5] and also using the electron diffraction technique based on a rotating sector [6]. It was found that the chlorine atoms are in ε positions, the bromine atoms in $\varkappa$ positions (Fig. 1a) both in the crystalline state and in the vapour. The explanation of this result seems rather straightforward: As pointed out already in 1943 [7] the distance from a chlorine or bromine atom in ε position to the two nearest (ε bonded) hydrogen atoms in an undistorted structure is so small that a considerable repulsion must exist between them. This repulsion effect is likely to be greater in the case of a bromine atom than in the case of a chlorine atom. The repulsion between two $\varkappa$ halogen atoms attached to neighbouring carbon atoms will probably be of minor importance to the energy of the molecule than the halogen hydrogen interaction.

An observation which seems worth while mentioning regards the transformation of the higher melting isomer of 1,2-dichloro-4,5-dibromocyclohexane into the lower melting isomer. This transformation takes place to a considerable extent when the 240° isomer is heated for some time above its melting point. As hydrogen halides are evolved during the process it is possible that the transformation involves a S_{N_i} reaction. We have not, however, been able

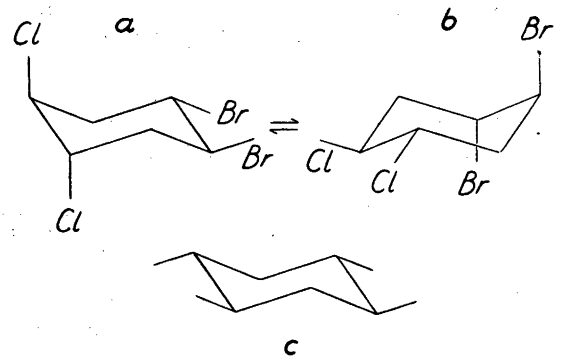


Fig. 1. a) and b). The two possible configurations of the 1,2-dichloro-4,5-dibromocyclohexane molecule (m.p. 171° C). c) Molecular configuration of 1κ, 2κ, 4κ, 5κ-tetrahalogenocyclohexane.

to perform a transformation at lower temperature by adding alkali halides to an alcoholic solution of the 240° substance.

The melting point of 218° C reported by Wibaut and Haak for the 1κ, 2κ, 4κ, 5κ-tetrabromocyclohexane appears to be rather low when compared with the melting point of the corresponding crystals containing chlorine. It may be due to a decomposition similar to that observed in the case of the higher melting dichloro-dibromocyclohexane.

Iodo-monochloride reacts both with 1,4-cyclohexadiene and with 1,2-dichlorocyclohexene(4). In the first case 1,4-dichloro-2,5-diiodocyclohexane (or 1,5-dichloro-2,4-diiodocyclohexane) of m.p. 121° C is formed, and in the second case 1,2,4-trichloro-5-iodo-cyclohexane having a melting point of 128° C. If a solution containing equimolecular amounts of chlorine and bromine, after being exposed to light, contains appreciable quantities of bromo-monochloride as has occasionally been claimed [8–10], one would expect it to react with the diene giving 1,4-dichloro-2,5-dibromocyclohexane (or 1,5-dichloro-2,4-dibromocyclohexane). However, we have not been able to obtain other products from the reaction than the dichloro-dibromo-cyclohexanes melting at 171° C and 240° C respectively. The reaction was carried out at low temperatures where the quantity of bromomonochloride present in the solution has been assumed to be considerable.

EXPERIMENTAL

1,2-Dichlorocyclohexene (4). Solutions in carbon tetrachloride containing 0.5 moles of 1,4-cyclohexadiene and 0.5 moles of chlorine respectively, were mixed at 0° C. After evaporation of the solvent *in vacuo* the product was distilled at a pressure of 0.5 mm.

Nearly all the substance distilled at 45° C. The crystal mass thus obtained was recrystallized from ethyl alcohol containing some water. M. p. 42° C:

($C_6H_8Cl_2$)	Calc.	C	47.71	H	5.34	Cl	46.95
	Found	»	47.58	»	5.50	»	46.65

1,2,4,5-Tetrachlorocyclohexanes. To a solution of 0.1 moles of 1,2-dichlorocyclohexene(4) (m.p. 42° C) in carbon tetrachloride a solution of 0.1 moles of chlorine in the same solvent was added at 0° C. Some crystals were deposited and isolated (m.p. 226° C). The solid substance remaining after evaporation of the carbon tetrachloride from the solution was separated into its two components by alternating crystallization from carbon tetrachloride and ethyl acetate. Besides further quantities of the substance melting at 226° C and crystallizing in fine needles appreciable quantities of 1,2,4,5-tetrachlorocyclohexane of m.p. 176° C were obtained. The higher melting compound is also a tetrachlorocyclohexane:

($C_6H_8Cl_4$)	Calc.	Cl	63.89
	Found	»	63.45

1,2-Dichloro-4,5-dibromocyclohexanes. When bromine (0.1 moles) was added to dichlorocyclohexene of m.p. 42° C (0.1 moles) under the conditions just described in the case of chlorine, crystals were also deposited. Further crystals were obtained from the remaining liquid on evaporation of the carbon tetrachloride. The same technique as described in the case of the tetrachloro-compounds was used in order to separate the two substances formed in the addition reaction. In this case also, the higher melting substance — m.p. 240°-formed thin needle-shaped crystals, whereas the crystals of the lower melting substance — m.p. 171° C — were rather compact and some times they were obtained in the form of twins forming regularly shaped crosses (Fig. 2). The melting point 240° C for the higher melting compound is only obtained by rapid heating, in other cases a lower value is found, probably as a result of beginning decomposition.

($C_6H_8Cl_2Br_2$ — 240° C)	Found	Cl	23.19	Br	50.75				
	Calc.	»	22.81	»	51.41				
($C_6H_8Cl_2Br_2$ — 171° C)	Found	Cl	21.85	Br	51.92	Mol. w.	Found		305
	Calc.	»	22.81	»	51.41	» »	Calc.		311

Dichloro-diiodocyclohexane. To 0.2 moles of 1,4-cyclohexadiene dissolved in 200 ml of ethyl ether was added a solution of 65 g of iodo-monochloride dissolved in ethyl ether, the temperature being kept below room temperature. After some hours considerable quantities of crystals had been deposited. The crystals were washed with pure solvent and recrystallized from chloroform. They were well-developed rhombic plates of m.p. 121° C:

($C_6H_8Cl_2J_2$)	Calc.	Cl	17.52	I	62.70
	Found	»	17.15	»	63.20

Trichloro-idodocyclohexane. To 0.01 moles of dichlorocyclohexene (m.p. 42° C) dissolved in ethyl ether an etheric solution of 0.01 moles of iodo-monochloride was added. After a few days the solvent was evaporated and the crystal mass thus obtained recrystallized from alcohol containing some water. Needle-formed crystals were obtained melting at 128° C:

($C_6H_8Cl_3J$)	Calc.	Cl	33.95	I	40.49
	Found	»	34.05	»	40.34

Transition phenomena. In order to decide which of the two modifications of the higher melting dichloro-dibromocyclohexane is stable at room temperature, a mixture containing both α and β crystals was kept in contact with a saturated solution in ethyl acetate

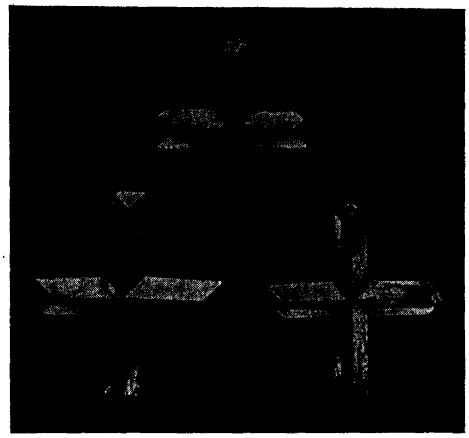


Fig. 2. Twin crystals of 1,2-dichloro-4,5-dibromocyclohexane, m. p. 171° C.

for some days. Growth of the α crystals at the expense of β crystals was then observed. At temperatures above 120° C the transition of α crystals into β crystals takes place at a considerable rate. A more exact determination of the transition point could be carried out by keeping crystals for a considerable time at temperatures varying between 90° and 110° C. It was found that the transition temperature lies between 90° and 95° C.

Using the same procedure the transition temperature for the higher melting tetrachloro-compound was found to lie between the limits 60° and 65° C, the α modification being the form stable at lower temperatures.

SUMMARY

The following substances have been prepared from 1,4-cyclohexadiene and chlorine, bromine or iodo-monochloride:

1,2-dichlorocyclohexene(4), m.p. 42° C
1,2,4,5-tetrachlorocyclohexane, m.p. 226° C
1,2,4,5-tetrachlorocyclohexane, m.p. 176° C
1,2-dichloro-4,5-dibromocyclohexane, m.p. 240° C
1,2-dichloro-4,5-dibromocyclohexane, m.p. 171° C
1,2,4-trichloro-5-iodocyclohexane, m.p. 128° C
dichloro-diiodocyclohexane, m.p. 121° C

In the 1,2-dichloro-4,5-dibromocyclohexane of m.p. 171° C the chlorine atoms occupy ε positions, the bromine atoms $\varkappa$ positions. Even in the gaseous state no indications of the inverted form with chlorine in $\varkappa$ positions, bromine in ε positions could be detected. An explanation of this observation is given. The $1\varkappa$, $2\varkappa$-dichloro-$4\varkappa$, $5\varkappa$-dibromocyclohexane (m.p. 240°) is dimorphous, the higher temperature form (β) is isomorphous with the tetrabromocyclo-

hexane prepared by Haak. Both forms, however, crystallize in the space group $P2_1/c$ with two molecules in the unit cell which would require the molecule to have a center of symmetry. It must be assumed, therefore, that the molecules are statistically orientated in the crystal. In the case of the low temperature form (α) the correctness of this conclusion has been substantiated by the working out of electron density maps of the structure.

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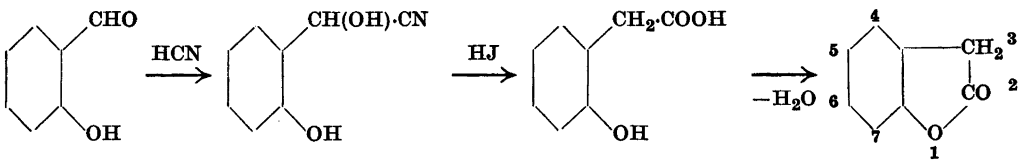
Some New Derivatives of Isocoumaranone

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It has been found previously in this institute [1,2] that certain derivatives of isocoumaranone will undergo some interesting reactions. In the first place they show a pronounced tendency to pass into mostly yellow-coloured enolic forms of varying stability. The enolization may take place either by the action of alkali, when the enol form will be present as the salt, or by the action of diazomethane, in which case the methyl ether of the enol form is formed. Secondly, the isocoumaranone derivatives are easily oxidized to intensely red-coloured bimolecular compounds with an indigoid structure. Up to the present relatively few simple derivatives of isocoumaranone are known, and we have therefore found it of interest to prepare some new compounds of this type. It ought to be remarked that isocoumaranone in the literature will be found under several other names, *e.g.* 2-benzofuranone and 2-keto-2.3-dihydrobenzofuran.

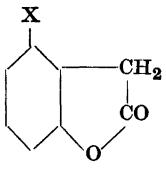
Isocoumaranone and its derivatives can be regarded as the lactones of *o*-hydroxyphenylacetic acid and its derivatives respectively. Isocoumaranone itself (III) was first prepared by Baeyer and Fritsch [3] by destillation of *o*-hydroxyphenylacetic acid (II). A convenient method for the preparation of the latter was introduced by Czaplicki, v. Kostanecki and Lampe [4] consisting in the addition of hydrogen cyanide to salicylic aldehyde and boiling the hydroxymandelonitrile (I) with hydriodic acid. This method has been used by us, but instead of salicylic aldehyde its homologues and carboxylic acids formed the starting materials. The latter substances were prepared from the



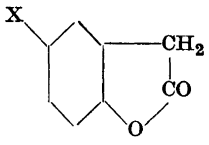
I II III

isomeric cresols and hydroxybenzoic acids respectively by introducing an aldehyde group according to the well-known method of Reimer-Tiemann. In some cases it was found advantageous to methylate the hydroxyaldehydes before the addition of hydrogen cyanide. The details are given in the experimental part.

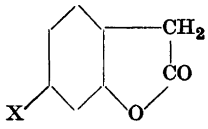
The following 8 new derivatives of isocoumaranone have been prepared, *viz.* the monomethyl-isocoumaranones with methyl in the positions 4,5,6, and 7 (IV—VII, X = CH_3) and the corresponding carboxylic acids (IV—VII, X = COOH)



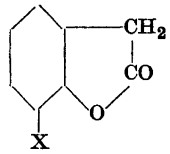
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V



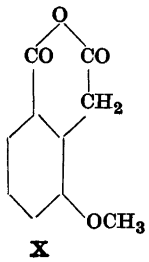
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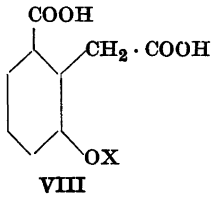
VII

The corresponding substituted hydroxyphenylacetic acids are also as far as we know described here for the first time. The transformation of these acids into the isocoumaranones was in most cases effected very easily by sublimation either at ordinary pressure or in a vacuum. In one case, however, there was a considerable resistance against the splitting off of water, *viz.* by the 2-hydroxy-3-carboxyphenylacetic acid leading to the isocoumaranone derivative VII (X = COOH). Obviously there is a steric hindrance due to the carboxylic group in ortho position to hydroxyl. In order to obtain this isocoumaranone it was therefore necessary to treat the hydroxy-acid with acetic anhydride.

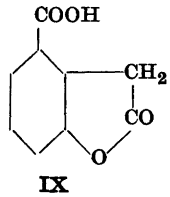
One of the isocoumaranone carboxylic acids, *viz.* that one with carboxyl in the 4-position was prepared in a different way than the others. The 2-methoxy-6-methylphenylacetic acid was oxidized to 2-methoxy-6-carboxyphenylacetic acid (VIII, X = CH_3) which was demethylated to the corresponding hydroxy-acid (VIII, X = H). This could theoretically split off water in two



X



VIII



IX

ways, giving either the lactone (isocoumaranone) or, being a derivative of homophtalic acid, an acid anhydride. It could, however, be shown that on sublimation in a vacuum the isocoumaranone IX only was formed as the product gave no colour with ferric chloride. On the other hand the methoxy-acid (VIII, X = CH_3) by sublimation gave the methoxyhomophtalic anhydride (X). As in the case of homophtalic anhydride itself X gave a yellow colour with alkali due to an enolization as first described by Dieckmann [5]. The yellow colour was in this case stronger and more persistent than that occurring when alkali was added to the isocoumaranone carboxylic acids described in this paper. Obviously the enolic form of the 5-ring is less stable than that of the 6-ring. It ought, however, to be remarked that one of us [1] previously has described very stable enolic forms of isocoumaranone derivatives. Thus in one case the free enol form could be isolated in the crystalline state, as could the methyl ether of the enol form. We are of the opinion that the stability of these enolic forms is dependent upon how many substituents the isocoumaranone carries in the benzene nucleus and of the character of the substituents. We shall return to this question on a later occasion.

EXPERIMENTAL

(Melting points not corrected)

The methylisocoumaranones

The four isomeric methylsalicylic aldehydes were prepared from the isomeric cresols according to Tiemann and Schotten [6]. They were separated from the *p*-hydroxyaldehydes by distillation with steam. The two isomers obtained from m-cresol had to be separated by the method given by Anselmino [7]. All four methylsalicylic aldehydes were methylated with methyl sulphate according to Simonsen [8].

Preparation of the nitriles. The sodium bisulphite compound of a methylsalicylic aldehyde was shaken with a concentrated solution of potassium cyanide, when the nitrile separated as an oil. An etheral solution of this was shaken with a solution of sodium bisulphite in order to remove excess of aldehyde. After drying the ethereal solution the ether was distilled off in a vacuum.

2-Methoxy-3-methylmandelonitrile could only be obtained as a viscous yellow oil and had to be used as such.

2-Methoxy-4-methylmandelonitrile crystallized but could not be purified sufficiently for analysis.

The two other nitriles were obtained pure by repeated crystallization from ether-ligroin.

2-Methoxy-5-methylmandelonitrile, m.p. 37.5°. Yield 80 % (from methylated aldehyde).

	Found	C 67.63, 67.72	H 6.26, 6.24	N 7.79, 7.75
$C_{10}H_{11}O_2N$	Calc.	» 67.78	» 6.26	» 7.91

2-Methoxy-6-methylmandelonitrile, m.p. 68°, yield 60 %.

	Found	C 67.87, 67.79	H 6.38, 6.37	N 7.80, 7.78

The *hydroxy-methylphenylacetic acids* were prepared by boiling the corresponding nitriles four hours with ten times their weight of hydriodic acid (d = 1.7) in the presence of a little red phosphorus. After diluting with water the acids were extracted with ether and free iodine removed by shaking with a solution of sodium thiosulphate. From the dried solutions the acids were obtained as crystalline masses. In one case (2-hydroxy-6-methylphenylacetic acid) the crude product was a mixture with the corresponding isocoumaranone. It was therefore dissolved in alkali and the free phenylacetic acid extracted with ether after adding hydrochloric acid. All four isomeric acids were easily soluble in ether, ethanol, acetone, and ethyl acetate, but only slightly soluble in ligroin. But for the 2-hydroxy-3-methyl isomer they were very little soluble in cold benzene. The 2-hydroxy-6-methyl isomer was considerably more soluble in water than the others.

2-Hydroxy-3-methylphenylacetic acid (A) was crystallized from ligroin. M.p. 88°. Yield 45 % (from the nitrile).

	Found	C 64.95, 65.01	H 6.20, 6.18	M 165.1, 165.0
$C_9H_{10}O_3$	Calc.	» 65.05	» 6.07	» 166.2

2-Hydroxy-4-methylphenylacetic acid (B) was purified by repeatedly dissolving in ether and precipitating by adding ligroin. M.p. 124°; yield 45 %.

Found C 65.15, 65.18 H 6.06, 6.13 M 165.4, 166.0

2-Hydroxy-5-methylphenylacetic acid (C). Recrystallized from benzene, m.p. 123.5°. Yield 70 %.

Found C 65.18, 65.03 H 6.15, 6.04 M 165.3, 165.7

2-Hydroxy-6-methylphenylacetic acid (D) was purified by crystallization from ether-ligroin. M.p. 110.5°; yield 65 %.

Found C 65.10, 65.04 H 6.22, 6.17 M 165.8, 165.4

The *methylisocoumaranones* were obtained directly from the described phenylacetic acids by splitting off water.

7-Methylisocoumaranone, by sublimation of A in a vacuum, m.p. 98°.

	Found	C 72.97, 72.73	H 5.56, 5.46
$C_9H_8O_2$	Calc.	» 72.96	» 5.44

6-Methylisocoumaranone, by sublimation of B at atmospheric pressure, had about the same solubility as B. M.p. 73°.

Found C 72.98, 72.85 H 5.40, 5.39

5-Methylisocoumaranone, by sublimation of C in a vacuum, was more soluble in ligroin than C. M.p. 74°.

Found C 72.81, 72.90 H 5.56, 5.45

4-Methylisocoumaranone, by sublimation of D at atmospheric pressure, was less soluble in water than D. Recrystallized from petroleum ether, m.p. 93.5°.

Found C 73.00, 72.86 H 5.36, 5.42

The methylisocoumaranones gave as expected no colour reaction with ferric chloride. On boiling with water a partial hydrolysis took place, and the solution gave a blue colour with ferric chloride. The hydrolysis became complete by dissolving in sodium hydroxide. From the acidified solutions the phenylacetic acids were extracted with ether. After recrystallization they had the same m.p. as the original acids.

The isocoumaranone carboxylic acids

The carboxy-hydroxybenzaldehydes were prepared from the three isomeric hydroxybenzoic acids by current methods [9] (in the parenthesis m.p. from literature).

3-Carboxy-2-hydroxybenzaldehyde				m.p.	179° (179°)
4-	»	2-	»	»	233—34° (234°)
5-	»	2-	»	»	244—45° (243—44°)
3-	»	4-	»	»	248° (248—49°)

Addition of hydrogen cyanide. The crystalline sodium bisulphite compound of one of the aldehydes was added to a saturated solution of potassium cyanide and the solution stirred until the reaction was complete. After acidifying with mineral acid the nitrile was extracted with ether, the ethereal solution dried with sodium sulphate, and most of the ether distilled off. The rest of the ether was removed in a vacuum desiccator when the nitrile was obtained in the crystalline state. Only one of the nitriles, *viz. 5-carboxy-2-hydroxymandelonitrile*, was purified sufficiently for analysis by crystallizing several times from ether-ligroin.

	Found	C 56.02, 55.98	H 3.65, 3.60	
$C_9H_7O_4N$	Calc.	» 55.96	» 3.65	

Preparation of the *carboxy-hydroxyphenylacetic acids.* The nitriles were boiled for five hours with 20 times their weight of hydriodic acid (1.7) and a little red phosphorus. In two cases the phenylacetic acid separated already on diluting with water. It was filtered off together with excess of red phosphorus, the mixture washed with a cold solution of potassium iodide in order to remove iodine, and then separated from the phosphorus by means of ethanol. In two other cases the phenylacetic acid had to be extracted with ether and the ethereal solution freed from iodine by shaking with sodium thiosulphate. The acids were soluble in ether, ethanol, and acetone, slightly soluble in cold water and very slightly soluble in benzene.

3-Carboxy-2-hydroxyphenylacetic acid. Crystallized from dilute ethanol, m.p. 251—52°. Violet colour with ferric chloride.

	Found	C 54.94, 55.08	H 4.31, 4.15	M 197.0, 197.2	
$C_9H_8O_5$	Calc.	» 55.11	» 4.11	» 196.15	

4-Carboxy-2-hydroxyphenylacetic acid was recrystallized from dilute hydrochloric acid and obtained as colourless needles with m.p. 215—16°. It gave red colour with ferric chloride.

Found	C 54.93, 55.01	H 4.15, 4.26	M 194.4, 193.2	

5-Carboxy-2-hydroxyphenylacetic acid was crystallized alternately from dilute hydrochloric acid and ether-ligroin. M.p. 192—93°. Violet colour with ferric chloride.

Found	C 54.84, 55.03	H 4.23, 4.02	M 194.2, 195.8

3-Carboxy-4-hydroxyphenylacetic acid was obtained as small colourless needles on crystallizing alternately from water and dilute ethanol. M.p. 204—205°. Violet colour with ferric chloride.

Found	C 55.18, 55.24	H 4.17, 4.15	M 198.8

The 6-carboxy-2-hydroxyphenylacetic acid was prepared in the following way. To 2-hydroxy-6-methylphenylacetic acid (3 g) was added 30 % sodium hydroxide (9 ml) and dimethylsulphate (6 ml) and the mixture shaken for some time at 50°. The methoxy-ester which separated as an oil was not isolated but saponified at once by adding sodium hydroxide (10 ml) and boiling till the oil had dissolved. By adding mineral acid the *2-methoxy-6-methylphenylacetic acid* separated and was recrystallized from dilute ethanol, at the same time decolourizing with active carbon. M.p. 150—152°. Yield 80 %.

	Found	C 66.61, 66.71	H 6.78, 6.76	CH_3O	17.30, 17.20	M	181.1, 180.7	
$C_{10}H_{12}O_3$	Calc.	» 66.65	» 6.71	»	17.22	»	180.2	

A solution of 2-methoxy-6-phenylacetic acid (2 g) in 2N sodium hydroxide (7 ml), diluted to 100 ml, was heated to 80° and 120 ml 5 % potassium permanganate added in small portions during two hours. After reducing excess of permanganate and filtering off the manganese dioxide, the solution was concentrated to a small volume and sufficient 5N sulphuric acid added. The precipitate consisted of a mixture of the starting material and *6-carboxy-2-methoxyphenylacetic acid*. The latter could be obtained in a pure state by fractional crystallization from acetone. M.p. 191°. It was also soluble in ether, ethano and glacial acetic acid, but very little soluble in benzene and ligroin.

	Found	C 57.15, 57.03	H 4.88, 4.86	CH_3O 14.75, 14.78	M 212.0, 211.0
$C_{10}H_{10}O_5$	Calc.	» 57.14	» 4.80	» 14.76	» 210.18

6-Carboxy-2-hydroxyphenylacetic acid was obtained from the preceding methoxy-acid by boiling with hydriodic acid as described above. It was crystallized from ether-ligroin. M.p. 158°.

	Found	C 54.98, 55.25	H 4.27, 4.23	M 195.2, 195.0
$C_9H_8O_5$	Calc.	» 55.11	» 4.11	» 196.15

The isocoumaranone carboxylic acids. Of the four isomeric carboxy-2-hydroxyphenylacetic acids the three very easily split off water on sublimation in a vacuum, giving the corresponding isocoumaranone carboxylic acids. When the fourth, 3-carboxy-2-hydroxyphenylacetic acid, was sublimated in a vacuum only a partial dehydration took place. Even after sublimating seven times the product still gave a violet colour with ferric chloride. The acid was therefore boiled for two hours with 15 times its weight of acetic anhydride. Having removed excess of acetic anhydride the residue was sublimated in a vacuum, giving the pure isocoumaranone-7-carboxylic acid, which gave no colour with ferric chloride. The isocoumaranone carboxylic acids were soluble in ether, ethanol, and acetone, but not in cold water. Analyses are given below. Their acidities were determined by dissolving in a sufficient amount of alkali for opening the lactone ring and titrating excess of alkali.

	M.p.	C	H	M
Isocoumaranone-7-carboxylic acid	266°	60.59, 60.56	3.42, 3.48	181.6,
» *6-* » »	210°	60.47, 60.40	3.32, 3.47	180.8, 180.0
» *5-* » »	232°	60.64, 60.66	3.48, 3.50	173.4, 177.0
» *4-* » »	191°	60.71, 60.49	3.56, 3.57	176.9, 179.7
» $C_9H_6O_4$ Calc.		60.68	3.40	178.14

o-Methoxyhomophtalic anhydride, by sublimation of 6-carboxy-2-methoxyphenylacetic acid in a vacuum, had m.p. 153°.

	Found	C 62.40, 62.30	H 4.35, 4.27
$C_{10}H_8O_4$	Calc.	» 62.50	» 4.19

SUMMARY

Four isomeric methylisocoumaranones and four isomeric isocoumaranone carboxylic acids, all with the substituents in the benzene nucleus, have been prepared from the corresponding substituted 2-hydroxyphenylacetic acids which are also described. In the case of the carboxy-2-hydroxyphenylacetic acid with the carboxyl group in ortho position to hydroxyl there is a marked steric hindrance against the formation of the corresponding isocoumaranone

derivative. The tendency of isocoumaranone derivatives to pass into enol forms by the action of alkali or of diazomethane in the presence of methanol as well as the stability of the enol forms seem to increase when more substituents are introduced in the benzene nucleus.

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Short Communications

The Solubilization of Hydrocarbons by Association Colloids Below and Above the Second Critical Concentration for Micelle Formation

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In general the opinion prevails that the curves representing the solubilities of hydrocarbons such as hexane, benzene etc. in solutions of association colloids rise with increasing colloid concentration and are convex toward the concentration axis (see, for example, the review entitled Solubilization by Klevens [1]). One of the present authors, however, proved in 1947 that in the case of several paraffin chain salts the solubility curves for hydrocarbons follow linear courses over a wide concentration range [2]. This means that after a limited transition range immediately above the critical concentration for micelle formation the saturation capacity of the micellar substance is practically constant over the whole of the so-called small micelle concentration range. In the paper mentioned [2] it was, however, indicated that the saturation capacity has a higher value above the so-called roentgengraphic concentration for micelle formation than below this concentration limit. These observations have now been confirmed.

In the experiments saturated solutions of *p*-xylene in aqueous solutions of sodium oleate and sodium myristyl sulphate were prepared at 40° C and the amounts of solubilized *p*-xylene were determined with a Zeiss-Löwe interferometer for liquids. Fig. 1 shows the forms of the solubility curves. In oleate solutions the solubility of xylene increases linearly up to an oleate concentration of 0.19 *M*. At this concentration there is a break point in the curve and above this point the solubility increases more rapidly than below. Below 0.19 *M* the saturation capacity of the micellar substance is 189 ml xylene per mol oleate and above this concentration it rapidly increases to 300–330 ml xylene per mol oleate. A similar behaviour is observed in the case of the sodium myristyl sulphate solutions. In the latter case the break point occurs at a concentration of 0.19–0.20 *M*. Below this concentration the saturation capacity for xylene is 127 ml per mol myristyl sulphate and above this concentration 184 ml xylene per mol myristyl sulphate.

These experimental results show that the power of the micellar substance to solubilize xylene differs on both sides of a definite concentration limit, *i.e.* the micelles have different properties in this respect on both sides of this limit.

A comparision of our results with those of earlier X-ray studies shows that it was in the same concentration region that the so-called roentgenographic concentration for micelle formation was believed to lie [3–6]. The existence of such a concentration limit is however queried by recent X-ray studies of American investigators [7,8]. Nevertheless, the so-called I-band which seems to

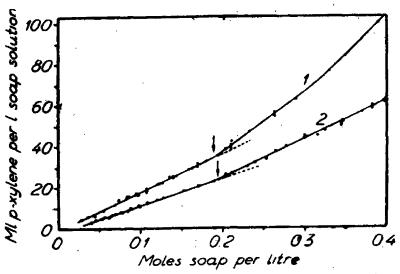


Fig. 1. The solubilization of p-xylene in sodium oleate and sodium myristyl sulphate solutions below and above the 2nd critical concentration.

1. The solubility of xylene in sodium oleate solutions.
2. The solubility of xylene in sodium myristyl sulphate solutions.

represent the distance between the centres of similar micelles or between similar layers within the micelles has never been observed below this concentration region.

It was in this same concentration range that an equivalent conductivity minimum was observed already long ago. The equivalent conductivity was observed to increase above a concentration of approximately 0.2 *M* in the case of sodium oleate (18° C)[9] and above a concentration of about 0.1 *M* in the case of sodium myristyl sulphate (60° C)[10]. One of the present authors has shown [2] that this increase in the equivalent conductivity can in the case of sodium oleate be explained by assuming that increasing amounts of water are immobilized between the hydrophilic interfaces within "large micelles" of some kind.

Many investigators are now of the opinion that large micelles do not exist. Among those who believe that there is such a concentration limit separating two different micellar regions this limit is known as the second critical micelle concentration (2*nd* C.M.C.). The present study provides proof that such a concentration limit actually exists.

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The Specific Volumes of Association Colloid Solutions Below and Above the Second Critical Concentration

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Previous studies have shown that the partial specific volumes of association colloids undergo marked changes in the region of the first critical concentration at which the molecules or ions associate to form micelles [1–3]. As far as we have been able to determine, no data are available in the literature concerning any changes undergone by the partial specific volume in the region of the second critical concentration. We have now studied the variation of the specific volume of sodium oleate and sodium myristyl sulphate solutions in this concentration range. As shown by curves 1 a and 2 a of Fig. 1, the specific volumes vary linearly with the colloid concentration over the whole of the concentration range studied. Thus no changes are observed in the partial specific volumes of these colloids when their 2nd critical concentrations at 0.19—0.20 *M* are exceeded.

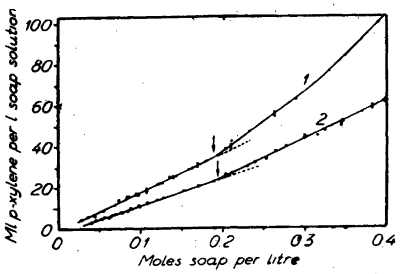


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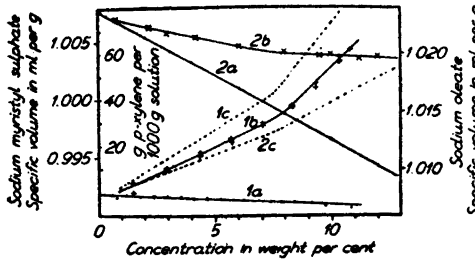


Fig. 1. The specific volumes of sodium oleate and myristyl sulphate solutions.
1 a. Pure sodium oleate solutions.
1 b. Sodium oleate solutions saturated with p-xylene.
1 c. The amounts of p-xylene solubilized by sodium oleate solutions.
2 a. Pure sodium myristyl sulphate solutions.
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We have also measured the specific volumes of aqueous solutions of these colloids in which *p*-xylene had been solubilized. The specific volumes of series of colloid solutions saturated with *p*-xylene are shown in Fig. 1, curves 1 b and 2 b. The curves are seen to be formed of two almost straight lines which meet at an angle. Calculations based on the measured values show that the break points are due to the fact that the micellar substance is able to solubilize more xylene per mol colloid above than below this concentrations (1 c and 2 c). The break point occurs in the oleate solutions at an oleate concentration of about 7 % (0.23 *M*) and in the case of sodium myristyl sulphate at about 7.2 % (0.22 *M*). These concentrations are approximately the same as those which according to earlier solubilization studies [4] represent the 2nd critical concentrations of these associationscolloids.

The data thus confirm that the micelles present below and above the 2nd critical concentration possess different properties as far as their ability to solubilize hydrocarbons is concerned. Since the partial specific volumes of the colloids in their pure aqueous solutions (without added xylene) are not found to undergo any marked changes in this region, it must be assumed that the packing within the micelles remains unaltered when the mentioned concentration limit is exceeded even though the structures and/or sizes of the micelles may undergo changes.

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Dipole Moment Measurements of α, β, γ, δ and ε Benzene Tetrachloride

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By the photochlorination of benzene in the presence of catalytic quantities of iodine, G. Galingaert, A. J. Kolka, H. D. Orloff *et al.* of the Ethyl Corporation, Detroit, have succeeded in isolating five compounds having the structure 3,4,5,6-tetrachlorocyclohexene-1 [1,2]. These five benzene tetrachlorides (BTC) were designated as α, β, γ, δ and ε, in the order of their characterization, and the difference between the various isomers was believed to be of the same kind as that known from the benzene hexachlorides (BHC) [3,4]. Adopting the ideas and designations used for cyclohexane derivatives [5] the possible configurations of the BTCs can be characterized by the scheme given in Table 1. The "conversion forms" containing more than two ε bonds (in parentheses in Table

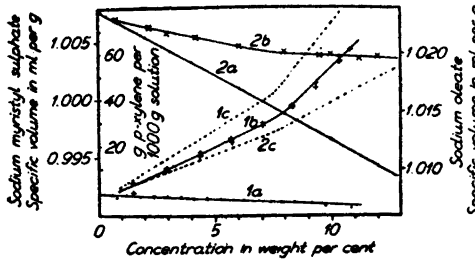


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Table 1. Possible configurations for the BTCs. Calculated dipole moments in Debye.

I	$\varkappa\varkappa\varkappa\varkappa \rightleftharpoons (\varepsilon\varepsilon\varepsilon\varepsilon)$	3.06
II	$\varepsilon\varkappa\varkappa\varkappa \rightleftharpoons (\varkappa\varepsilon\varepsilon\varepsilon)$	4.88
III	$\varkappa\varepsilon\varkappa\varkappa \rightleftharpoons (\varepsilon\varkappa\varepsilon\varepsilon)$	2.89
IV	$\varepsilon\varepsilon\varkappa\varkappa \rightleftharpoons \varkappa\varkappa\varepsilon\varepsilon$	3.10
V	$\varepsilon\varkappa\varkappa\varepsilon \rightleftharpoons \varkappa\varepsilon\varepsilon\varkappa$	4.03 $\rightleftharpoons$ 0.39
VI	$\varepsilon\varkappa\varepsilon\varkappa \rightleftharpoons \varkappa\varepsilon\varkappa\varepsilon$	5.74

1) are rather improbable according to experiences from cyclohexane chemistry [6]. Because of the $1\varepsilon-3\varepsilon$-repulsion also known from cyclohexane chemistry the isomer (VI) is less probable than the other five. We shall, therefore in the following exclude the forms inclosed within parentheses in Table 1 and suppose that the five known isomers are to be found among the first five possibilities listed in the table.

In the last column of Table 1 we have listed the dipole moments calculated for the free undistorted molecules. The dipole moments of the forms in parentheses are not given. In the undistorted molecules we have assumed that the $\rangle C-CHCl-CHCl-C\langle$ group of the molecules is arranged as a corresponding group in the BHCs and that the $\rangle -CCH = CH-C\langle$ group is planar. The partial moments of the C—Cl bonds are for the sake of simplicity assumed to be the same in all positions and forms. We have chosen for the partial moment the value 2.10 D as found for monochlorocyclohexane in benzene solution [7].

In Table 2 we have listed the dipole moments of the BTCs measured in benzene solution. In accordance with common experience we find that the calculated values on an average are greater than the measured. However, if we assume that the sequence of the measured and the calculated values is the same we can conclude that the known isomers have the following forms α $(\varepsilon\varepsilon\varkappa\varkappa)$, β $(\varepsilon\varkappa\varkappa\varepsilon)$, γ $(\varepsilon\varkappa\varkappa\varkappa)$, δ $(\varkappa\varkappa\varkappa\varkappa)$ and ε $\varkappa\varepsilon\varkappa\varkappa)$.

The dipole moment measurements indicate that the β isomer has the form V

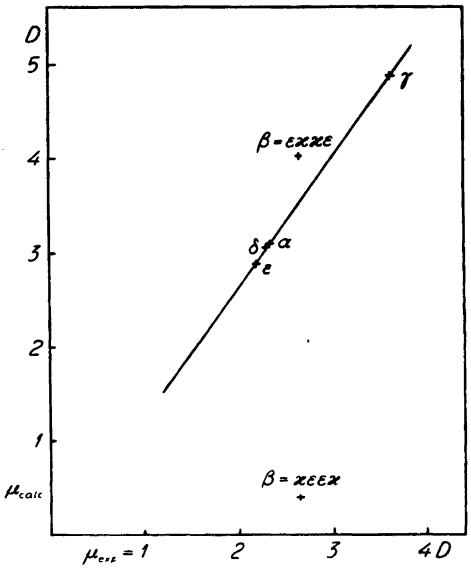


Fig. 1. Calculated dipole moments of α, β, γ, δ and ε BTC plotted against the experimental dipole moments.

Table 2. Measured dipole moments for α, β, γ, δ and ε BTC.

Isomers	Measured dipole moments in Debye
α	2.34
β	2.65
γ	3.62
δ	2.30
ε	2.19

(Table 1) and that the configuration $\varepsilon\varkappa\varkappa\varepsilon$ is the predominating one. We can not, however, exclude the possibility of some contribution from the $\varkappa\varepsilon\varepsilon\varkappa$ form. As a matter of fact the dipole moment measurements do indeed indicate the presence of an equilibrium with some contribution from the latter form. In Fig. 1 we have plotted the measured dipole moment as the abscissa and the corresponding calculated value as the ordinate. We see that all points except that of the β isomer lie practically on a straight line indicating

that the measured and calculated values are approximately proportional for the α, γ, δ and ε isomer. To bring the point of the β isomer onto the straight line we have to assume an equilibrium $\varepsilon\varkappa\varkappa\varepsilon \rightleftharpoons \varkappa\varepsilon\varepsilon\varkappa$ with approximately 87 % contribution from the former and about 13 % contribution from the latter form.

Because of the small differences between the dipole moments of the α, δ and ε isomers and because of the rather uncertain assumption of the undistorted molecules the dipole moments measurements must not be regarded as conclusive for the structure of these three isomers. The results are, however, in accordance both with the results of the Ethyl Corporation team [2] and with the electron diffraction work done at our institute. The electron diffraction work which is going to be published soon in this journal establishes the structure of the α, δ and ε isomers and excludes the presence of the $\varepsilon\varkappa\varepsilon\varkappa$ form and any appreciable contribution from the forms containing more than two ε bonds. The electron diffraction work thus demonstrates the validity of the assumptions on which the present discussion is based.

Acknowledgements. The authors wish to express their gratitude to the Ethyl Corporation, Detroit, for having supplied them with samples of the five isomers of BTC. One of the authors (J. M.) wishes to acknowledge Det Grevelige Hielmstierne Rosencronske legat for financial aid.

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On the Tantalum — Oxygen System

STIG LAGERGREN and ARNE MAGNÉLI

Institute of Chemistry, University of Uppsala, Uppsala, Sweden

Through the chemical and X-ray investigations on the niobium-oxygen system, carried out by Brauer [1] a decade ago, the occurrence of the phases NbO, NbO_2, and Nb_2O_5 (three modifications) was established while the oxides Nb_2O, Nb_2O_3, and Nb_3O_5 reported by previous authors were shown not to exist. There are also a number of tantalum oxides described in literature, *viz.* TaO, Ta_2O_3, TaO_2, and Ta_2O_5. Among these compounds, however, the pentoxide is the only one, whose existence has been firmly established. As a systematic investigation of the tantalum-oxygen system was lacking, we decided to study this matter.

The starting materials for the investigation were tantalum powder and tantalum pentoxide (both of Fansteel manufacture), weighed amounts of which were intimately mixed, pressed to pastilles and heated in vacuo. The products were investigated by taking X-ray powder photographs using a Guinier focusing camera with monochromatized Cu-$K\alpha$ radiation.

It has recently been found that niobium and niobium pentoxide readily react to form intermediate oxides when heated for one or two days at 1 000—1 100° C in sealed off silica tubes *. When mixtures of tantalum and tantalum pentoxide were correspondingly treated, no reaction could be observed even after rather intense heating (1 100° C for ten days or 1 250° C for two days).

In order to obtain higher temperatures a vacuum high frequency induction fur-

* Private communication from Dr. Beryl M. Oughton.

that the measured and calculated values are approximately proportional for the α, γ, δ and ε isomer. To bring the point of the β isomer onto the straight line we have to assume an equilibrium $\varepsilon\varkappa\varkappa\varepsilon \rightleftharpoons \varkappa\varepsilon\varepsilon\varkappa$ with approximately 87 % contribution from the former and about 13 % contribution from the latter form.

Because of the small differences between the dipole moments of the α, δ and ε isomers and because of the rather uncertain assumption of the undistorted molecules the dipole moments measurements must not be regarded as conclusive for the structure of these three isomers. The results are, however, in accordance both with the results of the Ethyl Corporation team [2] and with the electron diffraction work done at our institute. The electron diffraction work which is going to be published soon in this journal establishes the structure of the α, δ and ε isomers and excludes the presence of the $\varepsilon\varkappa\varepsilon\varkappa$ form and any appreciable contribution from the forms containing more than two ε bonds. The electron diffraction work thus demonstrates the validity of the assumptions on which the present discussion is based.

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In order to obtain higher temperatures a vacuum high frequency induction fur-

* Private communication from Dr. Beryl M. Oughton.

nace [2] was used. The samples were kept in covered zirconia crucibles, which were externally wound with spirals of tantalum wire in order to produce a favourable heating effect. In this way a maximum temperature within the crucibles of about 1 800° C could be obtained. The samples were allowed to cool down in the furnace immediately from the highest temperature applied. Due to the low heat capacity of the furnace this process was rather rapid. Powder photographs of samples heated in this way showed the lines of tantalum at unaltered positions as well as lines of another phase, which was not identical with the tantalum pentoxide used. (The diffraction pattern of the latter was in fair agreement with the drawing given by Brauer [1].) Tantalum metal evidently does not dissolve significant quantities of oxygen under these circumstances. For niobium Brauer states a solubility of less than 0.05 oxygen atoms/metal atom.

Attempts to prepare the new phase in a pure state by heating various mixtures of tantalum and tantalum pentoxide were fruitless. However, a pastille of pure tantalum pentoxide when heated for a short time in the vacuum furnace changed its colour from white to grey and powder photographs of the product showed only the reflections of the new phase and no trace of the tantalum lines. In order to establish the composition a weighed sample of the grey substance was heated in a stream of oxygen at 1 000° C for several hours. The colour turned white but the weight remained constant after the heating and powder photographs showed the ordinary pattern of tantalum pentoxide. It was thus obvious that the new phase represents a previously unknown high temperature modification of tantalum pentoxide. Accordingly it could also be prepared by heating a pastille of the original pentoxide specimen on a platinum disk in the gas-oxygen torch, however, the product obtained in this way was white. This is the true colour of the substance while the grey colour of the specimens prepared in vacuo is due to the presence of minute amounts of tantalum metal. Powder photographs of pentoxide samples that had been very intensely heated in the vacuum furnace showed namely faint tantalum lines in addition to those of the high temperature modification.

In order to find the transition temperature of the two modifications pastilles of tantalum pentoxide were heated in an uncovered tantalum crucible in the vacuum high frequency induction furnace. The temperature was read on an optical pyrometer. A sample heated at 1 310° C for one hour remained white and gave exclusively the powder lines of the low temperature modification while a preparation similarly treated at 1 330° C turned grey and was found to contain both forms of tantalum pentoxide. The transition point may thus be estimated to be 1 320° C with a probable error of ± 20°. The grey colour of the product indicates that the high temperature modification has a considerable oxygen pressure even at or slightly above the transition temperature. This is in agreement with the statement by von Bolton [3] that metallic tantalum may be prepared by pumping off the oxygen from tantalum oxide at a high temperature. On the other hand the pronounced stability of the low temperature modification was also strikingly demonstrated by the negative result when attempts were made to reduce this phase in a stream of hydrogen at about 1 250° C.

While the low temperature modification of tantalum pentoxide is isostructural with the corresponding form of niobium pentoxide [1,4], the powder photographs of the high temperature tantalum pentoxide indicate this structure to be quite different from those of the other two niobium pentoxide modifications. The investigation is continuing with the special purpose of finding the crystal structure of the high

temperature modification of tantalum pentoxide.

The authors wish to thank professor G. Hägg for his kind interest in the investigation and for the facilities put at our disposal. This work forms part of a research program on oxides and oxide systems financially supported by the Swedish Natural Science Research Council.

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On the Occurrence of Sedoheptulose in Certain Species and Genera of the Plant Family *Saxifragaceae*

ARNOLD NORDAL and DAGRUN ÖISETH

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The heptose sedoheptulose (D-altro-heptulose) had, until quite recently, been detected in only a few species and genera of the plant family *Crassulaceae*, and investigators were of the opinion that where this sugar occurred fermentable sugars were absent [1].

Nordal and Klevstrand [2, 3] recently demonstrated the presence of both sedoheptulose and fermentable sugars in representatives of all the five sub-families of the plant family *Crassulaceae*.

Benson, Bassham and Calvin [4] identified monophosphate esters of sedoheptulose in *Chlorella*, *Scenedesmus*, and *Rhodospirillum rubrum*, and in the leaves of barley seedlings, soybean, alfalfa, sugar beet, spinach and geranium.

The present authors succeeded in isolating sedoheptulose from the rhizome of *Primula elatior* (L.) Hill.[5] and more recent, unpublished results have shown that the rhizome of *Primula vulgaris* Huds. also contains sedoheptulose, as does *Primula veris* (L.) Huds. In the case of the latter plant, extracts from the flowers, leaves and rhizomes were examined separately, and it was found that the aerial portion of the plant, especially the flowers, contained considerable quantities of sedoheptulose, as well as sucrose and fructose. In the rhizome, on the other hand, the latter two sugars predominated and sedoheptulose could hardly be detected. The material was not tested for aldoses.

These results, together with others not yet published, obtained with extracts from representatives of certain other plant families lend credence to our assumption that sedoheptulose is widely distributed in the plant kingdom, and that this sugar plays a far more important part in the metabolism of plants than has been considered hitherto.

The plant family *Saxifragaceae* gave some of the most encouraging results in our screening investigations, and as this family is closely related to the *Crassulaceae*, we thought it would be worth while to examine some specimens of the family more closely.

Sedoheptulose was identified by means of paper chromatography in eight of ten species and in general as the chief component of the sugar mixture (*cf.* Table 1). No sedoheptulose could be detected in *Ribes alpinum* L. or *Saxifraga stellaris* L. by the method used.

Fresh plant material which was fixed with boiling alcohol was used in all the analysis, except in the case of *Parnassia palustris* L. and *Saxifraga stellaris* L., where the air dried material was extracted with water.

The aqueous extracts were purified by means of ion exchange resins. The alcohol extracts were evaporated and freed from chlorophyll after the addition of water, and then all the extracts were concentra-

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The aqueous extracts were purified by means of ion exchange resins. The alcohol extracts were evaporated and freed from chlorophyll after the addition of water, and then all the extracts were concentra-

Table 1. Sedoheptulose, sucrose, fructose and glucose in certain Saxifragaceaen plants. Where sedoheptulose was the predominating sugar on the chromatograms this is indicated by (++).

Species	Sedo-heptulose	Glucose	Fructose	Sucrose
Chrysosplenium alternifolium L.	++	–	(trace)	(trace)
Parnassia palustris L.	++	+	+	+
Ribes alpinum L.	–		+	+
Saxifraga decipiens Ehrh.	++	+	+	+
×*Saxifraga arendsii* Engl.	++	+	+	+
Saxifraga hypnoides L.	++	+	+	+
Saxifraga umbrosa L.	+	+	+	+
Saxifraga aizoon Jacq.	+	+	+	+
Saxifraga cotyledon L.	+	+	+	+
Saxifraga stellaris L.	–		+	+

ted at temperatures not exceeding 40°, and subjected to paper chromatographic examination.

Ethyl acetate – acetic acid – water [6] was used as solvent, and the chromatograms were run for about forty eight hours, concurrently with pure sedoheptulose, fructose, sucrose and glucose. For detection of the ketoses, orcinol-trichloroacetic acid reagent [7] was used. This reagent gives a brownish green colour with fructose and sucrose, and a bluish green colour with sedoheptulose. The latter colour is very stable and characteristic, and we are of opinion that, in conjunction with the position of the spot on the chromatogram, this gives sufficient certainty to the identification. For the identification of glucose we used *m*-phenylenediamine hydrochloride.

In order to isolate and characterize the sugar, a sirup was prepared from fixed *Chrysosplenium alternifolium* L., and purified by ion exchange. The dibenzylidene derivative of sedoheptulosan [8] was prepared from 1.5 g of the sirup: m.p. (micro) 244–245°.

Crystallographic investigation gave the following data [9]:

"Rods showing parallel extinction, some rectangular with indices 1.614 and 1.625, some (the majority) acicular and more birefringent with the higher index, approximately 1.640."

These values are in agreement with the constants which have previously been found for this derivative of sedoheptulosan.

Besides sedoheptulose, the sugars sucrose, fructose and glucose were detected in most of the plants examined, in addition to substances with very low R_f-values which were not identified. In some cases more or less distinct spots, the colour of which resembled that given by sedoheptulose, appeared on development with orcinol reagent. Some had smaller, others larger R_f-values than sedoheptulose. We are at present engaged in identifying these and similar compounds which have appeared in the course of our work on heptoses and heptose derivatives.

The authors are indebted to Amanuensis Dr. Alf Wickström, Department of Pharmaceutical Chemistry, Pharmaceutical Institute of the University of Oslo, for the crystallographic measurements.

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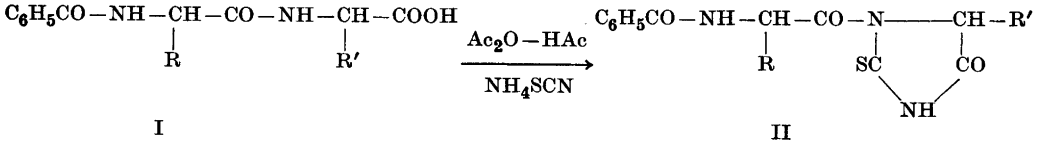
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On the Alkaline Deacylation of 1-Acyl-2-thiohydantoins

ANDERS KJÆR and POVL ERIKSEN

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The recent interest in the stepwise degradation of peptides [1–3] aroused our interest in the method of Schlack and Kumpf [4], involving the facile formation of 1-acylated-2-thiohydantoins (II) on treating suitable N-benzoylated peptides (I) with ammonium thiocyanate in mixtures of acetic anhydride and acetic acid. This reaction represents an extension of the Komatsu-Johnson-procedure [5, 6] for converting N-acylated α-amino acids into 2-thiohydantoins.

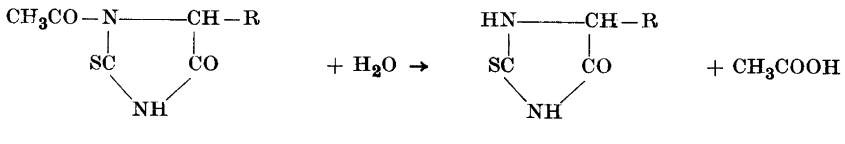


I II

While Johnson and coworkers consistently employed a fairly vigorous treatment with strong acid in order to detach the acyl-group located in 1-position of the thiohydantoin, Schlack and Kumpf (*l.c.*) demonstrated the hydrolytic fission to take place with 1 *N* sodium hydroxide at room temperature.

With a view to applying the method on higher peptides, still milder conditions were desired and found through spectrophotometric studies. The UV-spectra of 1-acylated- and unsubstituted 2-thiohydantoins, respectively, appear to be very different and quite characteristic, the former having two peaks located at *ca.* 235 mμ and 275–280 mμ with high extinctions (ε_{mol} : 15–20000), while the thiohydantoins show maximum absorption at *ca.* 225 mμ and 260 mμ with large differences in extinction (ε_{mol} : 10000 and 17000, respectively). These measurements, made in 0.001 *N* hydrochloric acid solution (Fig. 1), are in good agreement with the results obtained in alcoholic solution during the penicillin programme [7].

Samples of different 1-acetyl-2-thiohydantoins (III, R = *iso*-C_4H_9, $C_6H_5CH_2$ and H) were dissolved in 2 equivalents of 0.1 *N* sodium hydroxide at room temperature and immediately upon dissolution an aliquot was withdrawn, diluted and made 0.001 *N* in hydrochloric acid. Much to our surprise, the absorption data disclosed that the fission had proceeded practically to hundred per cent in less than five minutes. Parallel experiments in 0.01 *N* sodium hydroxide revealed that even this extraordinary mild treatment achieves the complete fission in about 30 minutes. Again, the benzoylglycyl-residue in 1-hippuryl-2-thiohydantoin (II, R = R′ = H) was detached completely in less than five minutes upon treatment with 0.1 *N* sodium hydroxide as seen from absorption measurements. These findings, adopted to a synthetical scale, resulted in a quantita-



III IV

R	M.p.	Nitrogen % Calc.	Nitrogen % Found	Sulphur % Calc.	Sulphur % Found
$(CH_3)_2CH$	114°	13.99	13.87	16.01	15.90
$CH_3(C_2H_5)CH$	153–158° *	13.08	13.25	14.96	14.90
$CH_3SCH_2CH_2$	105–106°	12.06	12.54	27.59	27.84

* The wide melting range indicates a mixture of stereoisomides.

tive yield of 2-thiohydantoin (IV, R = H) after dissolution of 1-acetyl-2-thiohydantoin, (III, R = H) in two equivalents of 0.1 *N* sodium hydroxyde, followed by immediate acidification and extraction with *n*-butanol.

An interesting observation was made when a freshly prepared solution of 1-benzoyl-2-thiohydantoin in water was followed spectrophotometrically. The starting curve (Fig. 2), which is reminiscent of the

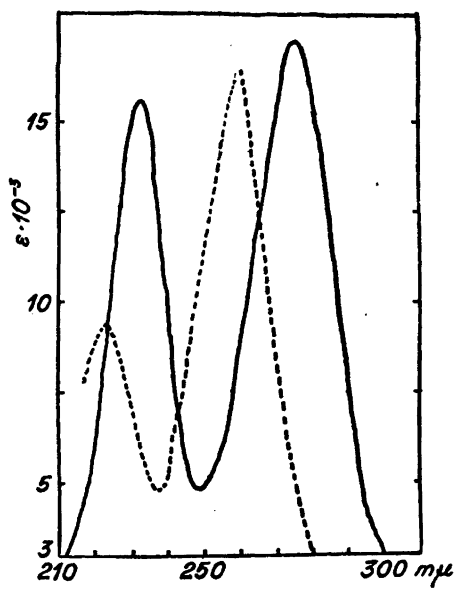


Fig. 1. Ultraviolet absorption spectra in 0.001 N HCl of ——— 1-acetyl-2-thiohydantoin and — — — — 2-thiohydantoin.

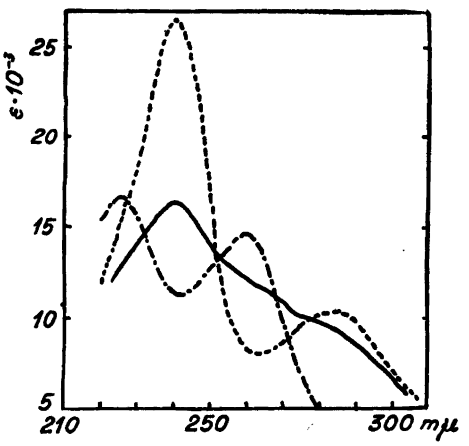
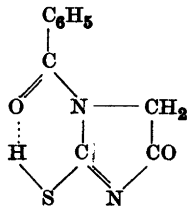


Fig. 2. Ultraviolet absorption spectra of 1-benzoyl-2-thiohydantoin in: — — — — 95 % EtOH; ——— freshly prepared aqueous solution; — · — · — · — water after standing for 18 hours.

absorption data in ethanol, changes quite rapidly into a final curve, practically identical with the absorption data obtained in dilute acid solution and not differing principally from the curve in dilute alkali. As an explanation for this behaviour we tentatively suggest the presence in the neutral alcoholic solution of hydrogen-bonded molecules such as (V), whereas acid or alkali may break up the labile bond under simultaneous displacement of the absorption peak by about 15 mμ towards shorter wave lengths.



V

During the present work the following new DL-1-acetyl-2-thiohydantoins (III) were prepared from the appropriate α-amino acids according to the usual procedure [6].

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Studies on Lignin. XII.* Alkaline Desulphonation of Radioactive Lignin Sulphonic Acid

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When lignin sulphonic acids are heated with aqueous alkali, they gradually undergo desulphonation [1,2], with the formation of sulphite. Under suitable conditions this process can be effected stepwise [3], and it was considered that a detailed study of the reaction might throw much light on the problem of the nature of the sulphonatable groups in lignin.

This preliminary communication describes some desulphonation experiments with a spruce "Kullgren acid" (S = 5.5 %, Ba/S = 0.50, S/OCH_3 = 0.49) which was further sulphonated with sulphite containing S^{35}, and then analyzed as follows: S = 6.8 %, Ba/S = 0.53, S/OCH_3 = 0.68. The data obtained during desulphonation are listed in Table 1.

Table 1. Alkaline desulphonation of lignin sulphonic acid.

Time of alkali treatment minutes	Sulphur removed as SO_2 (*c*), % of total	Specific S^{35} activity (relative values)
15	26.1	100 ± 0.5
30	30.2	97.7 ± 0.9
50	32.0	95.0 ± 0.7
110	36.7	91.3 ± 0.7
170	39.9	89.0 ± 0.4

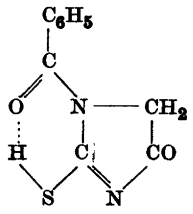
The figures of the last column are averages of 4 measurements.
The statistical error is 0.4—0.5 %.

Initially, sulphite is formed rapidly, but subsequently much more slowly as is evident from Fig. 1, in which ln (100-*c*) is plotted against time.

The graph shows clearly that the desulphonation involves two reactions, one relatively fast and one relatively slow. In the rapid reaction about 30 % of the total sulphur is liberated. (This figure, obtained by extrapolation, changes only little if it be assumed that only *e.g.* 75 % of the total sulphur may be liberated.)

The specific activity of the liberated SO_2 decreases regularly with time, showing that the sulphur introduced in the second phase of the sulphonation is most readily

* Part XI. *Svensk Kem. Tid.* **64** (1952) 18.



V

During the present work the following new DL-1-acetyl-2-thiohydantoins (III) were prepared from the appropriate α-amino acids according to the usual procedure [6].

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Studies on Lignin. XII.* Alkaline Desulphonation of Radioactive Lignin Sulphonic Acid

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When lignin sulphonic acids are heated with aqueous alkali, they gradually undergo desulphonation [1,2], with the formation of sulphite. Under suitable conditions this process can be effected stepwise [3], and it was considered that a detailed study of the reaction might throw much light on the problem of the nature of the sulphonatable groups in lignin.

This preliminary communication describes some desulphonation experiments with a spruce "Kullgren acid" (S = 5.5 %, Ba/S = 0.50, S/OCH_3 = 0.49) which was further sulphonated with sulphite containing S^{35}, and then analyzed as follows: S = 6.8 %, Ba/S = 0.53, S/OCH_3 = 0.68. The data obtained during desulphonation are listed in Table 1.

Table 1. Alkaline desulphonation of lignin sulphonic acid.

Time of alkali treatment minutes	Sulphur removed as SO_2 (*c*), % of total	Specific S^{35} activity (relative values)
15	26.1	100 ± 0.5
30	30.2	97.7 ± 0.9
50	32.0	95.0 ± 0.7
110	36.7	91.3 ± 0.7
170	39.9	89.0 ± 0.4

The figures of the last column are averages of 4 measurements.
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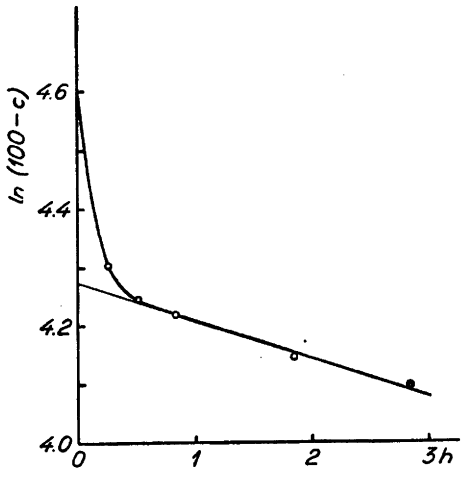


Fig. 1. ln (100−c) as a function of time.

removed. In theory it is possible to calculate the specific activities of the sulphur liberated in the two stages of the desulphonation, but to do so on the basis of the present incomplete data would be premature.

Experimental. Active BaS (from Oak Ridge) was diluted with inactive sulphide and the sulphide ions were precipitated as CdS. Hydrogen sulphide, liberated by treatment with conc. orthophosphoric acid, was combusted to sulphur dioxide which was absorbed in 2 *N* sodium hydroxide. The active sulphite solution was acidified to pH 1.5 with inactive sulphur dioxide and then used for the sulphonation of the Kullgren acid [4]. (Temperature 135°, time 6 hours.) The product was isolated in the usual manner [4]. Sulphur was determined by the method of Salvesen and Hogan [5].

For desulphonation, the active barium lignin sulphonate (0.8 g) was dissolved in water and the solution was heated to 100°. Sodium hydroxide solution (0.2 mol), preheated to 100°, was then added (final volume 80 ml, nitrogen atmosphere). The reaction was stopped by the addition of conc. hydrochloric acid. The sulphur dioxide was expelled by a current of nitrogen and absorbed in a Grote-Krekeler apparatus containing dilute hydrogen peroxide solution. The sulphuric acid formed was determined by precipitation with barium chloride, and the barium sulphate precipitate prepared for the measurement of the S^{35} activity by centrifugation in tubes with removable bottoms, using a refined procedure developed by G. Aniansson and A. Edhborg (*cf.* Larson *et al.*[6]).

1. Pearl, I. A., and Benson, H. K. *Paper Trade J* **111** (1940) no. 19, 29.
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Studies on the Chemistry of Oil Tanning. I. Formation of Peroxide Groups in the Oils

J. GLAVIND and J. WISMER PEDERSEN

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The very old method of tanning, oil tanning, is, in this country, carried out in the following way. After the skins, mostly from sheep or deer, have been split, the flesh layers are impregnated with oil which is squeezed into the skins with heavy beaters in a stamping mill. This milling is repeated about ten times. Between every treatment the skins are exposed to the sun for about one hour and then packed close together until the next milling, new oil being sometimes added. Between the last millings the skins are hung in heated rooms where the tanning process proceeds rapidly with the liberation of heat and the skins assuming a yellowish colour. When heat ceases to be evolved the tanning process has finished.

The oils used in the tanning are highly unsaturated, usually marine oils, such as

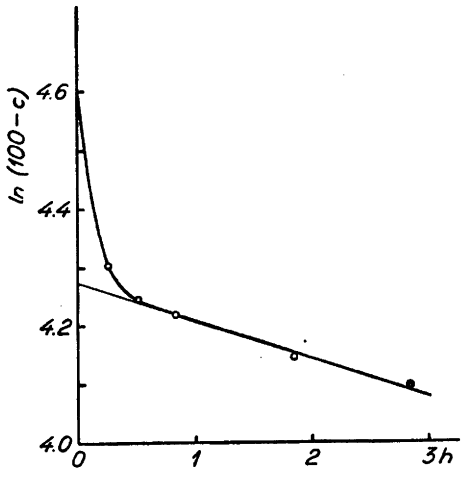


Fig. 1. ln (100−c) as a function of time.

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Experimental. Active BaS (from Oak Ridge) was diluted with inactive sulphide and the sulphide ions were precipitated as CdS. Hydrogen sulphide, liberated by treatment with conc. orthophosphoric acid, was combusted to sulphur dioxide which was absorbed in 2 *N* sodium hydroxide. The active sulphite solution was acidified to pH 1.5 with inactive sulphur dioxide and then used for the sulphonation of the Kullgren acid [4]. (Temperature 135°, time 6 hours.) The product was isolated in the usual manner [4]. Sulphur was determined by the method of Salvesen and Hogan [5].

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The oils used in the tanning are highly unsaturated, usually marine oils, such as

cod liver and seal oils, preferably having an iodine number about 120—160 and an acid number about 20—40. Fahrion [1] suggested that the reactive groups involved in the tanning process are peroxide groups formed by the action of atmospheric oxygen on the unsaturated constituents of the oils. This theory has been questioned by other authors (for a discussion, see Gnamm [2]), but seems to be generally accepted today. Probably because reliable methods for the determination of peroxides are of fairly recent origin, it seems that the oil tanning process has never been studied by following it by means of peroxide determinations. As a contribution to the chemistry of oil tanning, this paper reports the results of a series of such studies.

The material used was a batch of sheep skins tanned by "Dansk Chromlaederfabrik", Copenhagen, by the method outlined above, samples being taken out after each milling. The oil was extracted from each skin sample by shaking with xylene for an hour and then centrifuging for 5 minutes. The acid numbers, iodine numbers, and peroxide values of the resulting solutions were then determined. The values were calculated on the basis of the dry substance in the xylene solution. The acid number was determined by titration with 0.1 *N* KOH, the iodine number by the method of Rosenmund & Kuhnhenn [3], and the peroxide value by the method [4] described by the present authors. The results are summarized in Table 1.

Table 1.

Days after 1st milling	Number of millings	Acid number	Iodine number	Peroxide value
0	0 (fresh oil)	34.0	134.0	22
0	1	20.0	115.5	135
3	2	21.8	126.7	182
5	3	18.5	110.0	251
6	4	20.5	105.3	646
8	5	22.2	91.1	72
9	6	21.6	103.8	314
10	7	28.7	103.1	2 340
12	8	32.9	94.5	2 150
14	9	36.3	63.2	900
29	9	34.8	71.9	568

It will be seen that the values are somewhat erratic. This is due partly to the fact that the heterogenity of the material makes the sampling difficult, and especially to the addition of fresh oil carried out between the millings.

The results show, however, that the acid number decreases sharply initially, from 34 for the oil before the tanning to 20 after the first milling. Probably the oil is partly neutralized by residual alkali from the limeyard. After the 7th milling when the tanning process proceeds rapidly, the acid number increases.

It was to be expected that the iodine number of the oil would decrease as the processes related to peroxidation of unsaturated compounds normally lead to saturation of the compounds. In this study, the iodine number was found to decrease slowly at first, then more rapidly as the peroxide formation became more vigorous.

The peroxide values, finally, increase steadily during the course of the first few days, then quite violently after the 7th or 8th milling when the tanning process, as judged by the heat formation, is at its maximum, and then decrease slowly as the process ceases. These observations indicate very definitely that peroxide formation plays a decisive role in the chemical processes underlying oil tanning.

A preliminary study of this problem was carried out jointly by J. Glavind and a student of his, N. Klenow. This preliminary work was published by Klenow [5], on his own initiative, in connection with certain other investigations.

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5. Klenow, N. *J. Soc. Leather Trades' Chem.* **35** (1951) 67.

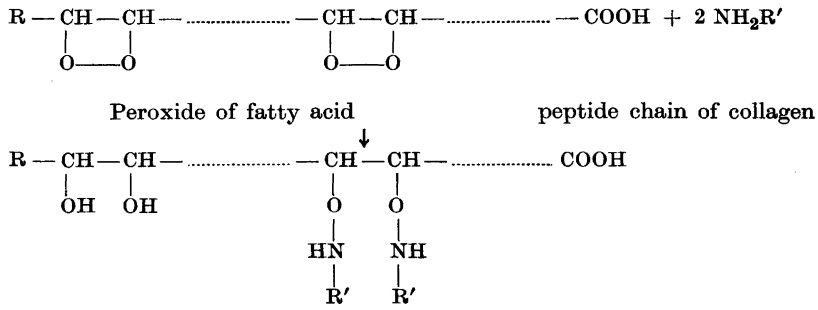
Received June 22, 1951.

Studies on the Chemistry of Oil Tanning. II. On the Chemical Nature of the Bonds between Peroxidized Fats and Collagen

J. WISMER PEDERSEN and J. GLAVIND

Department of Biochemistry and Nutrition, Polytechnic Institute, Copenhagen, Denmark

As mentioned in the preceeding paper, the most generally accepted theory of the chemistry of oil tanning was advanced by Fahrion [1], who suggested that peroxide groups in the fats are the reactive groups which combine with the collagen of the skin. In our experiments we were able to confirm Fahrion's views. As to the nature of the bonds between the peroxidized fats and collagen, Fahrion thinks that peroxides react with collagen ($R'NH_2$) in a manner which can be summarized as follows:



On this theory oil tanned leather should be a derivative of hydroxylamine. This seems *a priori* very improbable, and, as has been pointed out by Gnamm [2], has never been proved. Furthermore, it is a well-known fact that the tanning process takes place only at an acid pH. For instance, L. Klenow [3] followed the pH during oil tanning and found that it decreased from 12 to 3.5 and that the tanning did not start until the pH had decreased to 5.5. In the preceeding paper we reported on a similar observation: that it was not until the acid number began to increase rapidly, *i.e.*, the alkali in the skin had been neutralized, that vigorous peroxidation and the tanning process itself commenced. These facts make it appear highly improbable that the peroxide groups of the fats do react at all with the amino-groups of the collagen since, on the acid side of the isoelectric point of collagen (about pH 4.8) only a very small proportion of free amino-groups should be found.

Estimation of the amount of fixed fatty acids. Samples of skin and leather were extracted with petrol ether and ethyl ether to remove free fat. The ash contents of the dried samples were determined by incineration and the nitrogen contents by the Kjeldahl method. The skin contained 3.3 % ash and 17.27 % N, corresponding to 17.80 % N on the basis of the organic matter. The leather contained 3.3 % ash

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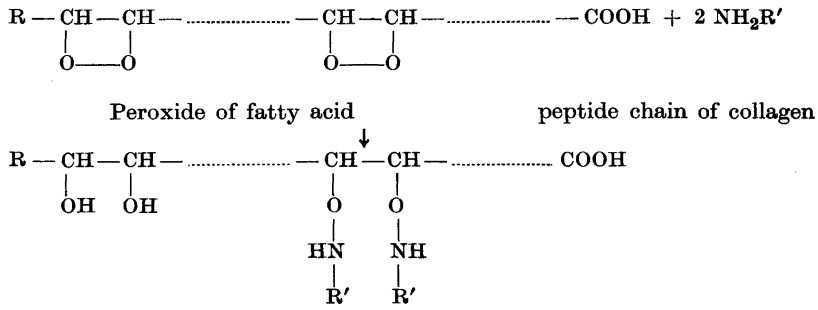
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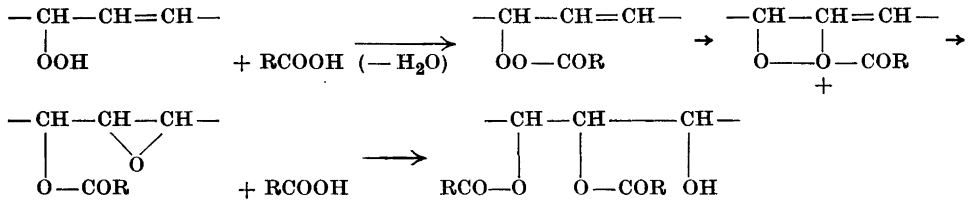
and 16.49 % N, or 17.05 % N in the organic matter. The lower nitrogen content of the leather must be accounted for by the fatty acids bound firmly to the skin, and from the equation $17.05 \times 100 = 17.80\ (100-x)$, the percentage of oil may be calculated to be 4.2. From the saponification value of the oil, 185, it can be calculated, further, that this corresponds to about 0.14 mmol of fatty acids per gram of leather.

Determination of amino-N in skins and leather. If the tanning process involved a reaction with free amino-groups the number of such groups should decrease during the process. We therefore made determinations of amino-N in samples of untanned skin and of leather, using the Van Slyke manometric method. The samples of the skin and leather were treated in exactly the same way. They were dried carefully, then mixed with dry ice and milled three times in a small coffee-mill whereby a fine powder was easily obtained. For the determinations, 0.5 g of powder was suspended in 10 ml of freshly boiled distilled water and introduced into the reaction vessel of the Van Slyke apparatus through the dropping funnel which was afterwards rinsed with 10 ml of water. The reaction vessel was shaken occasionally and the nitrogen evolved during two hours was measured.

In the samples of sheep skin 0.35, 0.40, and 0.39 (average 0.38) mmol of amino-N per g of skin was found. This value is of the same order as those found by earlier investigators [4].

In three corresponding samples of leather 0.35, 0.38, and 0.32 (average 0.35) mmol of amino-N per g of leather was found. The values vary appreciably probably due to the fact that the powders had a great tendency to adhere to the walls of the funnel and form lumps. On the other hand, it is clear that there has been no significant decrease in the number of amino-groups, or at least a much smaller one than the mole fraction of fatty acid fixed.

The considerations mentioned above make it probable that the hydroperoxide groups react with the carboxyl groups of collagen. For the reaction between fat peroxides and an organic acid, Farmer *et al.*[5] have proposed the following mechanism:



An analogous reaction might take place in oil tanning as a result of which a fatty acid molecule would become attached to one or to two peptide chains, in the latter case forming a bridge which would account for the stabilization of the collagen molecule.

Attempts to determine the fixed fatty acids. According to the above scheme, the linkages between the fatty acids and collagen are of an ester character and thus should be saponifiable. An attempt to titrate them was made in the following way. Samples of defatted leather weighing 1—2 g were treated in 100 ml Erlenmeyer flasks equipped with reflux condensers with 20 ml of 0.1 *N* NaOH for two hours at 50—52° C, a temperature well below the shrinkage temperature of collagen. After cooling, 20 ml of 0.1 *N* HCl were added, the flasks were vigorously

shaken for 15 minutes, and the contents were titrated with 0.1 *N* NaOH using phenolphthalein as indicator. An average of 4.49 ml of 0.1 *N* NaOH per g of leather was required, while, without heating, 1,41 ml was used to titrate the acid groups of 1 g leather. From the difference between these two figures it can be calculated that saponification liberates 0.31 mmol carboxyl per g of leather. This figure is of the same order as that expected if one mole of fatty acid combines with two carboxyl groups (2 × 0.14 = 0.28). It should be mentioned that during the alkali treatment the leather lost its characteristic yellow colour, and on drying got the same hard consistency as untanned collagen. Thus it appears that the leather has probably become detanned, in accord with the theory of formation of ester-like bonds during the tanning process.

Conclusion. Our observations point to the conclusion that the essential chemical process in oil tanning is the formation of peroxide groups which then combine with the carboxyl groups of the peptide chains of the collagen molecule. Newer concepts of tanning require the formation of bridges between the peptide chains for the stabilization of the collagen molecule. The following suggests a hypothesis which is in accordance with our observations:

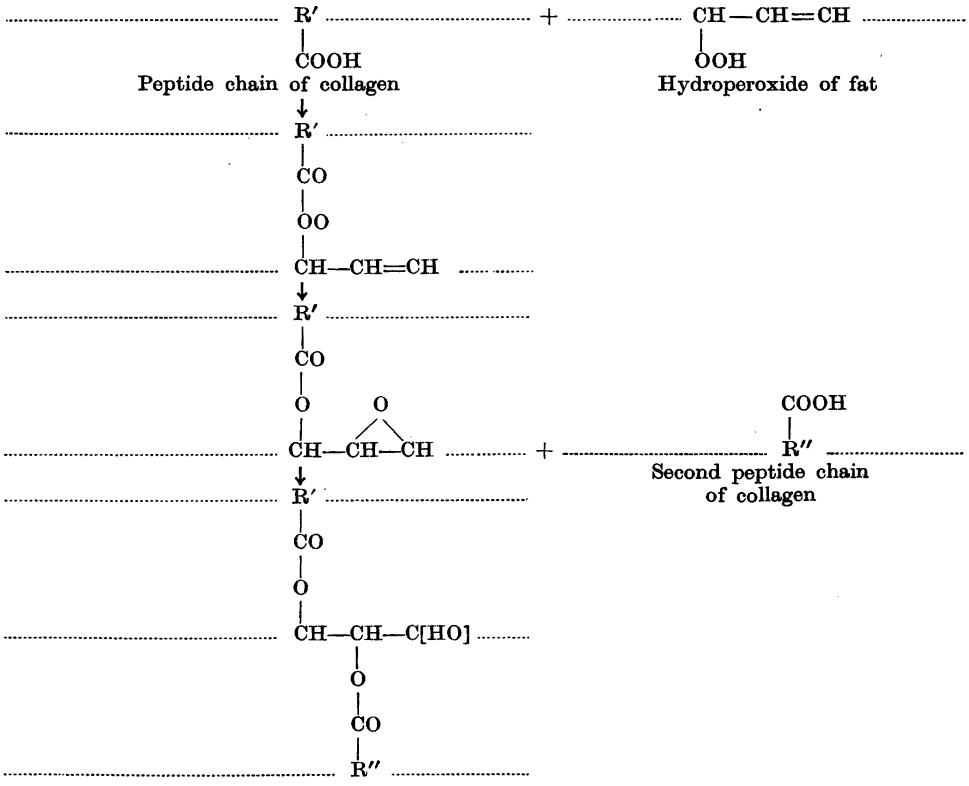


Fig. 1. Tentative structure of oil tanned skin.

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Die Diffusion von radioaktivem Zink in Zink-Eisen-Spinell und Zinkoxyd

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(Unter experimenteller Mitarbeit von D. Campbell und Å. Åkerström)

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Die Entstehung von $ZnO \cdot Fe_2O_3$ aus den Einzeloxyden im festen Zustand ist als Prototyp einer Spinellbildungsreaktion häufig untersucht worden[1–4]. Dennoch ist der Mechanismus dieser Reaktion nicht aufgeklärt, insbesondere ist die Frage nach der Art der diffundierenden Teilchen noch nicht beantwortet, und der theoretische Ansatz von Wagner [5,6] nicht experimentell auf seine Brauchbarkeit in diesem Falle geprüft worden.

Dazu ist offenbar nötig die Kenntnis der Beweglichkeit von Zink und Eisen in der Reaktionsschicht Zink-Eisen-Spinell. Hierzu wird in vorliegender Untersuchung mit der Messung der Selbstdiffusion von radioaktivem Zink in $ZnO \cdot Fe_2O_3$ ein Beitrag geliefert, (entsprechende Untersuchungen mit radioaktivem Eisen sind im Gange). Ausserdem wurde die Diffusion von Zink in Zinkoxyd untersucht, was von Bedeutung ist für die Frage der Oxydation von Zinkmetall, wenngleich die bei den hier vorliegenden hohen Temperaturen gewonnenen Werte nicht ohne weiteres auf die Verhältnisse bei den erheblich tieferen Temperaturen der Metalloxydation übertragen werden dürfen.

Schliesslich soll durch die Ermittlung der Temperaturabhängigkeit des Diffusionskoeffizienten zur Kenntnis der gitterenergetischen Verhältnisse und der Fehlordnung des betreffenden Stoffes beigetragen werden [7].

DIE METHODIK

Es wurden aus analytisch reinem ZnO und $ZnO \cdot Fe_2O_3$ Presstabletten (von 10 mm Durchmesser und 1–2 mm Dicke) hergestellt und zwecks Er-

reichen hoher Dichte getempert. Der radioaktive Indikator (Zn-65) wurde dem Diffusionsmedium zugefügt, und die Diffusionskoeffizienten bei verschiedenen Temperaturen bestimmt, wie im folgenden näher beschrieben wird.

DIE PRÄPARATE

Zinkeisenspinell $ZnO \cdot Fe_2O_3$ hat normale Spinellstruktur [8] und ist nicht ferromagnetisch. Die Dichte wird zu 5,35—5,40 [8], der Schmelzpunkt zu 1590 [9] bzw. 1720 °C [4] angenommen. Die elektrische Leitfähigkeit ist als elektronisch festgestellt und nimmt mit zunehmenden Sauerstoffdruck ab [10].

Unsere Präparate wurden durch gemeinsame Ausfällung äquimolarer Mengen Zink und Eisen mit Ammoniak und anschliessendes Erhitzen des sorgfältig (mit ammoniakhaltigem Wasser) gewaschenen Niederschlages erhalten. (Diese Herstellungsart erwies sich als zweckmässig, da ein aus den Oxyden durch Reaktion im festen Zustand bei 1200° hergestellter Spinell keine haltbaren Tabletten ergab.)

Die Fällung wurde im allgemeinen auf 800—1000° erhitzt, was sich als die günstigste Vorhandlungstemperatur mit Hinblick auf die Haltbarkeit der daraus herzustellenden Presstabletten erwies. Es gelangten verschiedene Präparate zur Anwendung.

Die Dichten der Pulver wurden pyknometrisch bestimmt (unter Zuhilfenahme der Vakuumdestillation von Benzol [8]) und ergaben sich zu 5,18—5,26. Analytisch wurde ein Eisenüberschuss festgestellt; der Gehalt an Fe_2O_3 betrug in einem Fall 52—53 Molprozent.

Aus den Pulvern wurden Tabletten mittels Handpresse gepresst und zur Sinterung erhitzt. Hierbei erwiesen sich Temperaturen von 1100° als optimal, höhere Temperaturen hatten Schwellung der Tabletten und Verringerung des Raumerfüllungsgrades zur Folge (wobei an Verdampfung von ZnO sowie an die Abgabe von O_2 und die Entstehung von Magnetit Fe_3O_4 gedacht werden muss [10] — die entsprechenden Tabletten waren ferromagnetisch). (Die Tablettendichten lagen zwischen 4,3 und 5,2.)

Zinkoxyd zeigt in der Literatur die folgenden Konstanten: Dichte 5,70 [11] Verdampfungswärme ca. 125 Cal [11]. Verdampfungsgeschwindigkeit (in g $cm^{-1}h^{-2}$) $6{,}3 \cdot 10^9$ exp $(-95000/RT)$ [21]. Zinkoxyd wird als Elektronenleiter gekennzeichnet, die Leitfähigkeit nimmt mit zunehmendem Sauerstoffdruck ab [10]. Die Frage der zum Elektronenüberschuss gehörenden Gitterbausteine, Zinkionen auf Zwischengitterplätzen oder Leerstellen im Sauerstoffteilgitter ist noch nicht geklärt [13].

Bei unseren Versuchen wurden drei verschiedene Präparate verwendet: das erste Präparat (A) wurde durch Erhitzen eines aus $ZnCl_2$- und Na_2CO_3-Lösungen erhaltenen und intensiv gewaschenen Niederschlages hergestellt [14]. Das zweite Präparat (B) ergab sich durch langsame Verbrennung von Zinkstaub p. a. an der Luft, und als drittes Präparat wurde Zinkoxyd p. a. der Powers-Weightman-Rosengarten Corporation (Philadelphia USA) verwendet. Alle Pulver wurden bei 900° vorerhitzt, und die mittels einer Handpresse erhaltenen Tabletten bei 1300° gesintert. Die Dichte der Pulver wurde pyknometrisch bestimmt zu 5,67; 5,60; 5,63. Die Dichte der gesinterten Tabletten lag im allgemeinen zwischen 4,6 und 5,5, was einem Raumerfüllungsgrad von 82 bis 97 % entspricht.

Als *radioaktiver Indikator* wurde Zn-65 verwendet, das 250 Tage Halbwertszeit hat *. Dieses Isotop wandelt sich hauptsächlich durch K-Einfang in den Folgekern Cu-65 um, nur 2,2 % der Zerfälle treten als Positronenstrahlung von 0,47 MeV Maximalenergie in Erscheinung. Der K-Einfang führt zu 45 % zu einer γ-Strahlung von 1,14 MeV [15].

Der Massenabsorptionskoeffizient der Positronenstrahlung wurde in unserer Messanordnung durch Auflegen von Aluminiumfolien zu 80,5 cm g^{-1} festgestellt (Halbwertsdicke in Al : $32 \cdot 10^{-4}$ cm). In Zählrohren mit Glimmerfenstern ergab sich das Verhältnis von β- zu γ-Strahlung zu 3 : 1. (Zählrohre vom Typ EHM 2 der General Electric Company, London.) Gemessen wurde mit einem GM-Zählverstärker mit bis zu 64-facher Untersetzung, stets unter Verwendung eines radioaktiven Zinkpräparates als »Standard«, um auf etwaige Schwankungen im Zählvermögen der Apparatur korrigieren zu können.

DIE METHODEN ZUR BESTIMMUNG DER DIFFUSION

Das vorliegende Problem war schon 1946 am hiesigen Institut durch Zimen aufgegriffen worden, wobei sich zeigte, dass die Methode nach dem Vorgang von Stefan-Kawalki [16] zu unempfindlich war. Daher wurde beim Beginn der vorliegenden Untersuchung zunächst auf die Methode der aktiven dünnen Schicht [16] zurückgegriffen, wozu allerdings wegen der verhältnismässig starken Absorption der weichen β-Strahlung dünnwandige Zählrohre nötig waren, die seinerzeit noch nicht zur Verfügung gestanden hatten.

Die Methode der radioaktiven dünnen Schicht bei Zinkverbindungen

Bei der Verwendung von Zn-65 sind wegen der komplexen Strahlung zweckmässigerweise stets zwei Aktivitätsmessungen durchzuführen, um die Menge ausgesendeter β-Strahlung zu erhalten. Einmal wird das Präparat mit aufgelegter Absorptionsfolie (etwa 0,5 mm Al) gemessen, was die Menge der γ-Strahlung ergibt. Das andere Mal wird das Präparat ohne Folie gemessen, wobei β- und γ-Strahlung zur Wirkung kommen. Die Differenz beider Messwerte ergibt die Menge der β-Strahlung, deren Schwächung durch zunehmende Absorption bei zunehmender Eindringtiefe der diffundierenden radioaktiven Atome zur Ermittlung der Diffusionskonstanten dient. Hierzu verwendet man zweckmässigerweise eine graphische Darstellung $A/A_0 = f(\mu\sqrt{Dt})$, die andererorts wiedergegeben ist [16]. Was die γ-Strahlung des Zn-65 angeht, so lässt sich hier aus der Not eine Tugend machen, insofern, als durch eine etwaige Verminderung der γ-Aktivität unerwünschte Verluste radioaktiver Substanz angezeigt werden.

* Für die ersten Versuche wurde ein Präparat aus Berkeley (USA) (Prof. Hamilton), für die Hauptversuche eine Lieferung des AERE. (Harwell/England) verwendet.

Solche Verluste treten besonders im Falle des Zinkoxydes bei hohen Temperaturen infolge Verdampfung ein, können allerdings experimentell weitgehend durch Aufeinanderlegen zweier Tabletten mit den (gleich stark) aktiven Schichten zurückgedrängt — oder notfalls durch entsprechende Korrekturen bei der Auswertung des Versuches berücksichtigt werden.

Die Aufbringung der dünnen radioaktiven Schicht von ZnO auf die gesinterten Presstabletten von Zinkeisenspinell und Zinkoxyd geschah auf folgende Art:

Radioaktives Zinkmetall, entweder auf Platin elektrolytisch niedergeschlagen oder in Form von Zinkfeilicht, wurde beim Vakuum einer Ölkapselpumpe in einer geeigneten Apparatur verdampft [17] und auf der Oberfläche der entsprechenden Tablette kondensiert. Es ergaben sich so dünne Schichten von Zinkmetall im Gewicht von ca. 1 mg. (Gelegentlich auftretende gröbere Körner liessen sich durch vorsichtiges Abpinseln entfernen.) Die Schichten sind durch kurzes Erhitzen (800°, 2—3 min.) leicht zu oxydieren.

Ausser durch Messung der im Laufe des Versuches durch Diffusion und Absorption eintretenden Aktivitätsminderung lässt sich die Diffusionskonstante nach durchgeführtem Versuch durch direkte Bestimmung des Konzentrationsgradienten für das aktive Material mittels Abschleifen dünner Schichten bestimmen. Hieraus lässt sich klarer als aus der zeitlichen Inkonstanz des Diffusionskoeffizienten bei der Absorptionsmethode die etwaige Überlagerung verschiedener Diffusionswege entnehmen [18].

Die obere Grenze der Anwendbarkeit der Methode der radioaktiven dünnen Schicht liegt hier bei etwa 10^{-8}, die untere Grenze bei etwa 10^{-12}cm^{2}sec^{-1}. Für noch geringere Diffusionskonstanten wurde die folgende Methode benutzt.

Die Kontaktmethode

Wie an anderer Stelle ausführlich behandelt [19], lässt die Bestimmung der Diffusionskonstante auf Grund des Überganges von radioaktivem Material von einer stark aktiven auf eine dagegen gepresste, ursprünglich inaktive Tablette die Ermittlung recht kleiner Diffusionswerte zu (bis hinab zu 10^{-16}cm^{2}sec^{-1}). Wir benutzten dazu einen Pastillhalter, der in Fig. 1 wiedergegeben ist.

Bei (1) befindet sich das Tablettpaar, durch einen Ausschnitt in den Alundumtiegel (2) eingeführt und zwischen zwei Platinplatten zusammengepresst. An der oberen Platinplatte sind Drähte aus Platin bzw. Platin-Rhodium angeschweisst, zwischen denen bei (3) und (4) die Thermospannung abgenommen werden kann. Der an der unteren Platinplatte befestigte Draht dient zur Messung des Tablettwiderstandes, der so zwischen (3) bzw. (4) und (5) ermittelt werden kann. Die Tabletten werden zusammengepresst durch

den Druck der Feder (6), der durch die Schraube (7) variiert werden kann und auf das Sillimanitrohr (8) wirkt. Der Zusammenhalt des Apparates wird bewirkt durch einen Ring aus Chromnickelstahl (9), der einerseits durch Stahlstäbe mit der Kopfplatte verschraubt ist, andererseits mit Stiften in Aussparungen des Alundumtiegels nach Art eines Bajonettverschlusses eingreift.

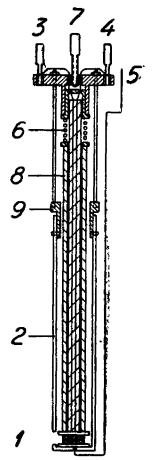


Fig. 1. Apparat zur Durchführung von Diffusionsversuchen nach der »Kontaktmethode» bei hohen Temperaturen. (Oberteil stark verkürzt wiedergegeben.)

Die Ermittlung der Diffusionskonstante geschieht auf Grund der Beziehung $A = qC_0\sqrt{Dt/\pi}$, wobei A die überdiffundierte Aktivität, q die Diffusionskonstante und t die Zeit bedeuten.

Die Gültigkeit dieser Beziehung wird zweckmässigerweise durch wiederholte Aktivitätsmessung nach verschiedenen Diffusionszeiten überprüft; die so erhaltenen Werte müssen, gegen die Wurzel aus der Diffusionszeit aufgetragen, auf einer Geraden liegen, was sich bei den vorliegenden Versuchen ausnahmslos bewahrheitete. Wichtig ist bei dieser Methode eine genaue Bestimmung der Aktivitätskonzentration, welche ja quadratisch in den Ausdruck für die Diffusionskonstante eingeht. Es wurden daher zahlreiche Bestimmungen vorgenommen, teils durch Messung bekannter Lösungsmengen bekannten Gehaltes, auf entsprechender Unterlage, z. B. Spinelltabletten, eingedunstet, teils (und besser) durch Wägung und radioaktive Messung einigermassen kräftiger Schichten (2—3 mg) radioaktiven Zinkmetalls und Zinkoxyds, durch Aufdampfen auf entsprechende Tabletten hergestellt.

DIE VERSUCHE UND IHRE ERGEBNISSE

Die vorliegenden Untersuchungen wurden schon Anfang 1948 begonnen in Form orientierender Versuche mit der Methode der β-Absorption (dünne Schicht) [20], konnten aber infolge äusserer Umstände (Mangel an radioaktiver Substanz, die gerade bei den Kontaktversuchen in reichlicher Menge benötigt wird) erst 1950 wieder aufgenommen werden.

Die Erhitzung der Präparate beim Diffusionsversuch geschah in Platinschiffen bzw. geeigneten Pastillhaltern in Platin-Rhodium bewickelten Alundum-Rohröfen. Die Gasatmosphäre war im allgemeinen Luft. Die Ofentemperatur wurde mittels Eisen-Wasserstoffwiderständen im Heizkreis oder mit einem Cambridge Regulator (Typ D, Zweipunktsregulator und -Schreiber) stabilisiert und registriert.

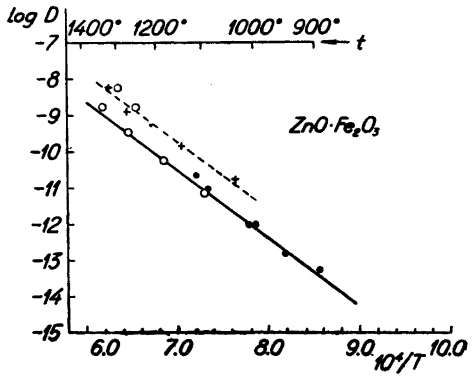


Fig. 2. Die Diffusionskonstante von Zink in Zink-Eisen-Spinell, logarithmisch gegen 1/T aufgetragen. Die obere Linie bezieht sich auf Presskörper geringerer Dichte.

Die Temperaturmessung wurde mit kalibrierten Platin/Platin-Rhodium-Thermoelementen durchgeführt.

ZINKEISENSPINELL

Zunächst seien die Messungen am Spinell behandelt, die ein einigermassen klares Bild geben und zusammenfassend in Fig. 2 wiedergegeben sind.

Dabei sind zur Konstruktion der Kurve: $\log D = f(1/T)$ insgesamt 10 Messwerte herangezogen worden, von denen 4 mittels der Methode der β-Absorption (dünne Schicht) (offene Kreise) und 6 mittels der Kontaktmethode (volle Kreise) erhalten worden.

Die Lage der Punkte lässt sich durch eine Gerade wiedergeben, die der Beziehung äquivalent ist: $D = 8{,}8 \cdot 10^2 \exp(-86000/RT)$ cm^2sec^{-1}.

Dies ist also der Ausdruck für die Temperaturabhängigkeit der Diffusion von Zink in Zinkeisenspinell-Presstabletten von 80—90 % Raumerfüllungsgrad.

Dabei ist aber folgendes zu beachten:

Bei hohen Temperaturen (> 1 200°) werden die Werte schlecht reproduzierbar und mitunter zu gross. Wir möchten dies auf die eingangs erwähnte Instabilität des Spinells [10] (Entweichen von ZnO, Zerfall von Fe_2O_3 zu Fe_3O_4 und O_2) zurückführen.

Weiter ist bei derartigen Presstabletten mit einer »Verteilung« der Diffusion auf verschiedene Wege zu rechnen, die als Gitterdiffusion und Diffusion auf inneren Grenzflächen zu bezeichnen sind. Dies kann durch Analyse der durch Abschleifen erhaltenen Konzentrationsverteilungskurven qualitativ und quantitativ nachgewiesen werden.[18] Bei den spröden und zerbrechlichen Zinkspinelltabletten waren solche Versuche mit Schwierigkeiten verbunden, liessen aber immerhin Konstanten für die Gitterdiffusion erkennen, die im

allgemeinen etwas unterhalb der Werte der Kurve in Fig. 2 lagen. Ausserdem ergaben sich für die Diffusion auf inneren Grenzflächen Werte in der Nähe der in Fig. 2 wiedergegebenen gestrichelten Linie. Jene Linie verbindet ausserdem vier durch Kreuze wiedergegebene Messwerte, die in Vorversuchen an verhältnismässig porösem Material mittels der β-Absorptionsmethode gewonnen wurden [20]. (Etwa 75 % Raumerfüllungsgrad statt der sonst vorliegenden 80—90 %).

All dies zeigt, dass der genannte Ausdruck für die Temperaturabhängigkeit nur mit gewissen Einschränkungen als für die Konstante der Gitterdiffusion kennzeichnend anzusehen ist. In der Gleichung $D = A \exp(-E/RT)$ ist offenbar der präexponentielle Faktor A (hier $8{,}8 \cdot 10^2$) für die Gitterdiffusion niedriger anzusetzen (etwa 1/2-1 Zehnerpotenz), während die Temperaturinkremente E sich für Gitter- und Grenzflächendiffusion offenbar nicht nennenswert voneinander unterscheiden.

Natürlich wäre prinzipiell eine Untersuchung an Einkristallen wünschenswert, doch erschien die Untersuchung von polykristallinen gesinterten Schichten ausreichend für die Frage des Mechanismus von Pulverreaktionen, bei denen ähnliche Verhältnisse vorliegen dürfen.

ZINKOXYD

Erheblich undurchsichtiger als beim Spinell waren die Ergebnisse der Versuche am Zinkoxyd.

Die Methode der aktiven dünnen Schicht liess zunächst wieder den Einfluss der Dichte der Presstabletten auf die resultierende Diffusionskonstante erkennen. In Fig. 3 gibt die obere — gestrichelte — Linie die Temperaturfunktion von Messwerten (Kreuze) wieder, die an Material geringerer Dichte gewonnen wurden. Presstabletten aus dem gleichen Material (Präparat A), zu höherer Dichte gesintert, ergeben Werte der gleichen Temperaturfunktion, die aber beinahe eine Zehnerpotenz tiefer liegen.

Die Gleichung lautet hier: $D = 1{,}3 \cdot \exp(-73700/RT)$ $\mathrm{cm^2 sec^{-1}}$.

Ausserdem finden sich in der Figur noch einige tief liegende Messwerte (gestrichelte Kreise), die zu Presstabletten extrem hoher Dichte (5,3—5,4), aus Präparat C gewonnen, gehören. Durch Abschleifen und Feststellen der Konzentrationsverteilung in den entsprechenden Tabletten ergibt sich — wie erwartet — dass der Anteil der Diffusion an inneren Grenzflächen im weniger dichtem Material höher ist.

Darüber hinaus aber sind die so erhaltenen Diffusionskonstanten auch einzeln höher als in dem dichteren Material. Es liegt nahe, dies auf spurenweise Verunreinigung des Materiales A zurückzuführen. (Eine Prüfung in der

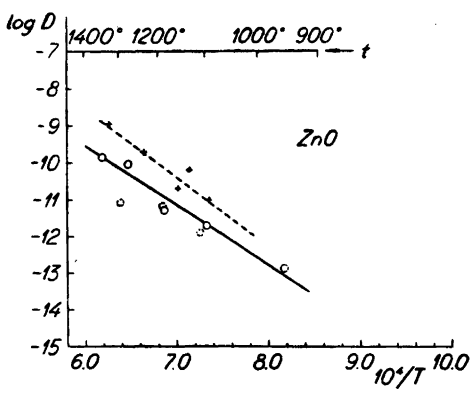


Fig. 3. Die Diffusionskonstante von Zink in Zinkoxyd nach der Methode der dünnen radioaktiven Schicht, logarithmisch gegen 1/T aufgetragen.

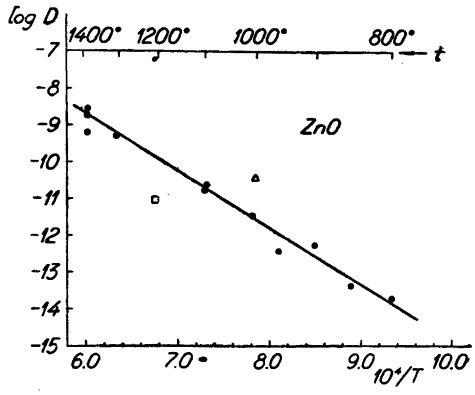


Fig. 4. Die Diffusionskonstante von Zink in Zinkoxyd nach der »Kontaktmethode», logarithmisch gegen 1/T aufgetragen.

Bunsenflamme nach der Diffusion ergab hier eine deutliche Na-Reaktion im Gegensatz zu Material C.) Hiernach wäre aber für reines Zinkoxyd der präexponentielle Faktor in obiger Gleichung noch tiefer anzunehmen.

Die Kontaktmethode

Hier ergaben sich recht gut reproduzierbare Werte, die zwischen 1 370° und 655° der Beziehung folgen: $D = 3{,}5 \exp(-70400/RT)$ cm^2sec^{-1}. Fig. 4.

Dies bedeutet aber, dass bis hinauf zu 1 300° die Werte etwa um eine Zehnerpotenz höher liegen als die nach der β-Absorptionsmethode erhaltenen. Zur Besprechung dieser Diskrepanz eine kurze Betrachtung der hauptsächlichen Faktoren, die auf die Methoden im entsprechenden Sinne einwirken könnten:

1. Verringerung der nach der β-Absorptionsmethode erhaltenen Werte kann eintreten durch: *Kontaktwiderstände* zwischen der aktiven Schicht und dem Presskörper.

Dies erscheint unwahrscheinlich im vorliegenden Fall, da die Schichten sehr gut hafteten und keine zeitliche Erhöhung der Diffusionskonstanten (infolge allmählicher Verbesserung des Kontaktes) festzustellen war.

Die *endliche Dicke der* als unendlich dünn behandelten *Schicht* kann, wie eine Überschlagsrechnung zeigt, nicht für so grosse Effekte verantwortlich sein. Eine weitere Ausbreitung der Schicht als Folge des Verdampfungs- und Kondensationsprozesses sowie Oxydationsprozesses konnte nicht in nennenswertem Masse nachgewiesen werden. (Ausserdem hätten ähnliche Erscheinungen sich ja auch im Falle des Spinells in vergleichbarem Ausmasse bemerkbar machen müssen. Dies war aber nicht der Fall, und es sind keine ausreichenden experimentellen Gründe dafür vorhanden, dass ein solcher

Effekt etwa beim Spinell durch die lokale Abweichung vom stöchiometrischen Verhältnis (Überschuss von Zinkoxyd durch die aufgedampfte Schicht) kompensiert würde.)

2. Es sind also offenbar die Faktoren zu betrachten, die zu zu hohen Werten mittels der Kontaktmethode führen könnten. Hier ergibt sich an physikalisch-chemischen Faktoren eigentlich nur ein einziger, der Beachtung verdient (Anhaften aktiven Materials kann nach Augenschein ausgeschlossen werden) und das ist die Einwirkung der radioaktiven Strahlung auf den Diffusionsprozess.

Man darf nämlich nicht vergessen, dass die aktive Tablette im Verhältnis zur dünnen Schicht eine ungleich kräftigere Strahlungsquelle darstellt. (Gewichtsverhältnis des aktiven Materials etwa 400 : 1.) Es wäre in erster Linie an die Wirkung der γ-Strahlung bzw. der von ihr verursachten Sekundärelektronen zu denken, da — nach erfolgtem Diffusionsversuch — Abschleifungen von Kontakttabletten keinen nennenswerten Unterschied im spiegelbildlichen Konzentrationsverlauf für die aktive und die ehemals inaktive Tablette zeigten.

Es bieten sich offenbar zunächst zwei Wege der experimentellen Untersuchung dar: Zunächst kann die Aktivitätskonzentration des aktiven Materials bei Kontaktversuchen verringert werden. Eine Verringerung auf 20 % ergab allerdings keine markierte Senkung der Diffusionswerte, wohin gegen eine Verringerung auf etwa 8 % einen erheblich tieferen Wert ergab, in Fig. 4 als Quadrat wiedergegeben. (Versuch bei 1200°.) (Das Dreieck in der gleichen Figur gibt den einzigen Wert wieder, den wir der Literatur entnehmen konnten [21].)

Dann könnte man versuchen, durch Einwirkung einer äusseren γ-Strahlungsquelle auf dünne aktivierte Tabletten während der Diffusion diese zu beeinflussen. Zu diesem Zwecke wurden bei einem Diffusionsversuch nach gewisser Zeit die Pastillen der Einwirkung von 15 Millicurie Co-60 ausgesetzt. Von diesem Zeitpunkt ab schien ausserhalb der Streuung der Masspunkte eine beschleunigte Abnahme der an der Tablettenoberfläche gemessenen β-Aktivität vorzuliegen.

Zusammenfassend können wir sagen, dass zumindest qualitativ eine diffusionsbefördernde Wirkung von γ-Strahlung bei Zinkoxyd wahrscheinlich erscheint. Dies hat für unsere Probleme (Mechanismus von Festkörperreaktionen) zunächst nur die Folge, dass im Falle des Zinkoxydes die mit der β-Absorptionsmethode gewonnenen tieferen Werte als die anwendbaren erscheinen. Der Erscheinung im »strahlungschemischen» Sinne weiter nachzugeben ist hier nicht die Aufgabe, wenn auch die Einwirkung radioaktiver Strahlung auf die Fehlordnung fester Stoffe im Zeitalter der Kernenergiegewinnung von erheblicher Bedeutung ist, daher aber auch sicher an den betreffenden Stellen nicht nur theoretisch [22], sondern auch in vielen, wenn auch unzugänglichen, Experimenten untersucht werden dürfte.

DIE FRAGE DER OXYDATION VON METALLISCHEM ZINK

Es scheint die Annahme berechtigt, die Diffusion von Zink durch Zinkoxyd als den zeitbestimmenden Teilprozess der Oxydation von Zinkmetall anzusehen. Doch hat der Oxydationsprocess eine erheblich verschiedene

Temperaturabhängigkeit (45 Cal [23] statt 74, wie hier für die Diffusion gefunden). Darüber hinaus liegt der von Wagner und Grünewald [24] gefundene Wert für die Diffusionskonstante (aus der Anlaufkonstante berechnet) viele Zehnerpotenzen höher als der aus unseren Werten zu extrapolierende. Dafür kann offenbar nicht eine Umwandlung von ZnO verantwortlich gemacht werden, jedenfalls nicht oberhalb 655°, der tiefsten Temperatur, bei der uns ein Diffusionsversuch möglich war, der auch den erwarteten Wert zeigte (allerdings bei unverhältnismässig geringer elektrischer Leitfähigkeit, was auf unzureichenden Kontakt deutet).

Die Untersuchung der Diffusion *während* der Oxydation des Metalles (von Moore [23] am Kupfer durchgeführt) stösst hier auf Schwierigkeiten wegen des niedrigen Schmelzpunktes von Zinkmetall.

ZUSAMMENFASSUNG

Es wurde nach verschiedenen Methoden die Diffusion von radioaktivem Zink in Presskörpern aus Zink-Eisen-Spinell (zwischen 900° und 1350°) und Zinkoxyd (zwischen 800° und 1370°) untersucht.

Die Ergebnisse der Messungen lassen sich durch folgende Ausdrücke wiedergeben:

$ZnO \cdot Fe_2O_3$:	$D = 8{,}8 \cdot 10^2 \exp$	$(-86000/RT)$ cm^2sec^{-1}
ZnO:	$D = 1{,}3 \exp$	$(-73700/RT)$ cm^2sec^{-1}

(Diese Ausdrücke sind aber nicht ohne weiteres als für die Gitterdiffusion massgeblich anzusehen, da in den untersuchten Probekörpern eine Überlagerung der Diffusionen im Gitter und auf Grenzflächen vorliegt. Doch ergab sich kein Anhalt dafür, dass die experimentellen Aktivierungsenergien für die beiden Fällen sich nennenswert voneinander unterscheiden. — Die Spinellpräparate wiesen einen geringen Überschuss an Eisenoxyd auf, während eins der Zinkoxydpräparate Spuren von Natrium enthielt.)

Es ergaben sich Anhaltspunkte für die diffusionsbefördernde Wirkung radioaktiver Strahlung im Falle des Zinkoxyds.

Die experimentelle Aktivierungsenergie für die Diffusion von Zink in Zinkoxyd im Temperaturbereich 800—1300° unterscheidet sich erheblich von derjenigen des zeitbestimmenden Teilprozesses bei der Oxydation von Zinkmetall (unterhalb 419°).

Herrn Prof. *J. A. Hedvall* danke ich für sein Interesse an der Untersuchung, *Statens Tekniska Forskningsråd* sowie der Fa *Werner und Carlström, Göteborg*, für die Bereitstellung von Mitteln.

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Die Kinetik im Kalziumoxyd

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Im Zusammenhang mit Untersuchungen des Mechanismus von Festkörperreaktionen ergab sich u.a. die Aufgabe, Beweglichkeit und elektrischen Charakter der im Kalziumoxyd diffundierenden Gitterbausteine zu bestimmen. (Solche Beobachtungen an Erdkalioxyden scheinen darüber hinaus auch von Interesse zu sein im Zusammenhang mit der Frage der »Aktivierung» von Oxydkathoden [1].)

In vorliegender Arbeit wird berichtet über Messungen der Selbstdiffusion und der Überführungszahl von radioaktivem Kalzium in Kalziumoxyd.

DIE VERSUCHSMETHODIK

Kalziumoxyd (CaO) zeigt in der Literatur [1] folgende Konstanten: Dichte: 3,4; Schmelzpunkt: 2 572° C; Verdampfungswärme: 128 kcal/mol.

Wir gingen aus von Kalziumkarbonat p. A. Schering-Kahlbaum, das bei 900° vorerhitzt, dann zu Tabletten von 1 cm Durchmesser und 1–2 mm Höhe gepresst wurde, die bei ca 1 450° C etwa 10 Stunden lang erhitzt wurden, wonach sie Dichten von durchschnittlich 3,1 (maximal 3,25), also 91 % Raumerfüllungsgrad aufwiesen.

Als radioaktiver Indikator wurde Ca-45 verwendet (geliefert von der Isotope Division, Atomic Energy Research Establishment, Harwell, England), für das eine Halbwertszeit von 152 Tagen und eine maximale β-Energie von 0,254 MeV angegeben wird.

Der Absorptionskoeffizient der β-Strahlung wurde von uns bestimmt in Kalziumglas (der Dichte 2,405) sowie in Aluminium. Für die Diffusionsversuche nach der Methode der β-Absorption (mit einer dünnen radioaktiven Schicht als Ausgangsbedingung) und die Tablettendichte 3,1 wurde mit einem Wert des Absorptionskoeffizienten von 430 cm^{-1} gerechnet.

BESTIMMUNG DER DIFFUSION

(Eine ausführliche Besprechung der Methoden findet sich in den zitierten Arbeiten, daher hier nur eine summarische Besprechung.)

A. *Die Methode der aktiven Teiltablette* [2,3] (anwendbar für Diffusionskonstanten bis minimal 10^{-10}cm^{2}sec^{-1}). Hierbei wird eine Presstablette verwendet, die aus einer radioaktiven und einer unaktiven Schicht besteht; und die im Laufe der Diffusion (durch Absorption im untersuchten Material) eintretende Aktivitätsabnahme auf der »aktiven» sowie die Aktivitätszunahme auf der vordem »inaktiven» Seite beobachtet. Aus dem zeitlichen Aktivitätsverlauf lässt sich mit Hilfe einer graphischen Darstellung der für diesen Fall ausgewerteten Diffusionsgleichung die Diffusionskonstante entnehmen.

B. *Die Methode der radioaktiven dünnen Schicht* [2,3]. (Anwendungsbereich etwa: D-Werte von 10^{-4} bis 10^{-12}cm^{2}sec^{-1}). Es wird auf geeignete Art (z.B. durch Kondensation im Vakuum) eine sehr dünne Schicht des entsprechenden radioaktiven Materials auf die Stirnseite des Probekörpers aufgebracht. Nach erfolgter Diffusion kann der Diffusionskoeffizient aus der durch Absorption verminderten, auf der Stirnseite der Tablette gemessenen Radioaktivität entnommen oder aber durch direkte Ermittlung des Konzentrationsgradienten mittels Abschmirgeln dünner Schichten bestimmt werden.

C. *Die Kontaktmethode* [3,4]. (Anwendungsbereich etwa: D-Werte von 10^{-12} bis 10^{-16}cm^{2}sec^{-1}). Eine homogen stark radioaktiv indizierte Tablette wird in idealem Kontakt gegen eine inaktive Tablette gepresst. Nach dem Diffusionsversuch wird die »übergegangene» Radioaktivität gemessen, woraus die Diffusionskonstante bestimmt werden kann.

DIE MESSUNG VON LEITFÄHIGKEIT UND ÜBERFÜHRUNGSZAHL

Der bei der Kontaktmethode verwendete Pastillhalter (andererorts eingehend beschrieben [5]) wurde auch für die Messung von Leitfähigkeit und Überführungszahl benutzt. Alle Messungen wurden in Luft durchgeführt (entsprechend den üblichen Bedingungen bei Pulverreaktionen). Die Bestimmung der Leitfähigkeitswerte geschah mit einer Messbrücke »Philoskop». Der Kontakt zwischen Platinelektroden und Tabletten wurde durch 0,01 mm dicke Platinfolien ausreichend hergestellt.

Die Bestimmung der Überführungszahl wurde in enger Anlehnung an die eben erwähnte »Kontaktmethode» durchgeführt. Auch hier wurde eine (bzw. zwei — auf jeder Seite eine —) inaktive gegen eine aktive Tablette gepresst, an die Elektroden jedoch eine Spannung von grössenordnungsmässig 1000V gelegt, was bei Kalziumoxyd im untersuchten Temperaturbereich zu Strömen von der Grössenordnung 1 mA führte, die mit einem Angus-Esterline Milli-Ampèremeter registriert wurden.

Es zeigte sich eine erhebliche Erhöhung der übergehenden Aktivität durch die Einwirkung des elektrischen Feldes; und durch Analyse der Aktivität-Zeitkurve (weiter unter an einem Beispiel beschrieben) lassen sich auch recht kleine Überführungszahlen ermitteln, die der klassischen *Tubandt*schen Wägemethode nicht mehr recht zugänglich sind.

DIE VERSUCHE UND IHRE ERGEBNISSE

Die getemperten und falls nötig plangeschliffenen Tabletten wurden in geeigneten Öfen erhitzt. (Wärmekörper: Platin-Rhodiumdraht auf Alundumrohren bzw. „Kanthal"-Band auf Pythagorasrohren.) Die Ofentemperatur wurde mit in den Heizkreis geschalteten Eisen-Wasserstoff-widerständen oder — in der Mehrzahl der Versuche — mit einem schreibenden »Cambridge»-Regulator konstant gehalten. Die Thermomessung geschah mit kalibrierten Pt/Pt-Rh-Thermoelementen.

Bei den radioaktiven Messungen wurden Zählrohre des Typ EHM2 der General Electric Co. Ltd. sowie ein Zählrohrverstärker mit bis zu 64-facher Untersetzung verwendet. Die Konstanz der Zählapparatur wurde in üblicher Weise durch Messung eines »Standard-Präparates» in Form einer mit radioaktivem Kalzium aktivierten Tablette kontrolliert.

1. *Die Selbstdiffusionskonstante.* Die Methode der *aktiven Teiltablette* erwies sich in dem uns zur Verfügung stehenden Temperaturbereich als zu unempfindlich. Vorversuche hatten zwar ansehnliche Aktivitätsänderungen ergeben, doch waren diese Werte schlecht reproduzierbar und offenbar durch unzureichende Sorgfalt bei der Herstellung der aktiven Teiltabletten bedingt.

Es genügt nämlich in vorliegendem Fall nicht, den einen Teil der Tablette als Pulver auf den andern Teil aufzupressen und dann den Diffusionsversuch zu beginnen, da in diesem Fall zunächst eine Grenzflächendiffusion in dem verhältnismässig porösen Material vorzuherrschen scheint, die jedoch mit der Dichtezunahme der Tablette während des Diffusionsversuches geringer wird, so dass die gemessenen Aktivitätswerte einem konstanten Grenzwert zustreben. Es ist also nötig, die aktive und die inaktive Teiltablette bis zum Erreichen einer möglichst hohen Dichte zu sintern, ehe sie zusammengefügt werden. Zwei derartige Versuche bei 1640° C (Molybdänofen) und 20 Stunden Diffusionszeit zeigten nur sehr geringe Aktivitätsänderungen von etwa 1 %, woraus sich die ungefähre Diffusionskonstante von $3 \cdot 10^{-10} \mathrm{cm^2 sec^{-1}}$ abschätzen lässt (in der Figur 1 als Quadrat wiedergegeben). — Die Methode der *aktiven dünnen Schicht* stiess in diesem Fall auf die Schwierigkeit, jene Schicht auf die Tablette aufzubringen, da eine Verdampfung von CaO mit den uns zur Verfügung stehenden Mitteln nicht möglich war.

Es wurde zunächst der folgende Versuch gemacht, die dünne Schicht durch Ausfällen auf der Tablette herzustellen: Als die geeigneten Reagenzien * erwiesen sich eine Lösung von Ammoniumformiat in Alkohol, mit der die Tablettenoberfläche »imprägniert» wurde, sowie eine Lösung radioaktiven Kalziumchlorides in Alkohol, von der ein

* Für die Angabe der Reagenzien sind wir Civ. Ing. N. Hartler zu Dank verpflichtet.

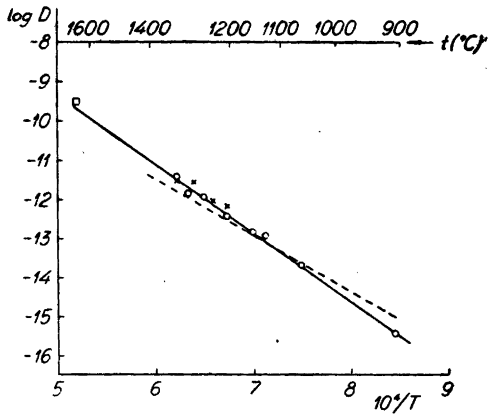


Fig. 1. Die Konstante D der Diffusion von radioaktivem Kalzium in Kalziumoxyd, logarithmisch aufgetragen als Funktion der reziproken absoluten Temperatur.

Tropfen auf die »imprägnierte» Oberfläche gebracht wurde, worauf Ausfällung einer Schicht radioaktiven Kalziumformiates eintrat, das durch Erhitzen in Kalziumoxyd übergeführt wurde. Wie jedoch die Betrachtung im Mikroskop zeigt, kommen in der so erhaltenen Schicht Körner bis zu 0,05 mm Grösse vor, deren Kontakt mit der Tablette unzulänglich sein dürfte. Es ist daher nicht verwunderlich, dass auf diese Art keine reproduzierbaren Diffusionswerte erhalten wurden, und die Diffusionskonstante so nur der ungefähren Grössenordnung nach festgestellt werden konnte.

Aussichtsreicher schien es, die dünne radioaktive Schicht durch Überführung im elektrischen Felde herzustellen wie in vorliegender Mitteilung beschrieben. (Dies scheint eine allgemein brauchbare Methode zu sein, falls die Überführungszahl nicht allzu klein ist ($>10^{-4}$), da sonst Störungen durch die Joule'sche Wärme des Elektronenstroms bei den zur Überführung ausreichender Mengen radioaktiver Substanz nötigen Feldstärken auftreten. Trotz der beim Überführungsversuch eintretenden »Verwischung» der aktiven Schicht durch gleichzeitige Diffusion dürfte die Schicht ausreichend die Anfangsbedingungen für einen Diffusionsversuch bei höheren Temperaturen erfüllen.)

So konnten also die durch Überführungsversuch aktivierten Tabletten später zu Diffusionsversuchen nach der Methode der dünnen aktiven Schicht verwendet werden. Die so erhaltenen Werte sind als Kreuze in Figur 1 eingezeichnet.

Die meisten befriedigenden Ergebnisse lieferte jedoch die *Kontaktmethode*, die hier im Temperaturbereich 850 bis nahezu 1 400° C angewendet werden konnte und gut reproduzierbare Werte ergab (in der Fig. 1 als Kreise eingezeichnet).

Die Summe der Messwerte lässt sich durch folgenden Ausdruck wiedergeben: $D = 0{,}4\ exp\ (-81\,000\,/\,RT)$ cm^{-2} sec^{-1}. In diesem Ausdruck dürfte hauptsächlich die Gitterdiffusion zum Ausdruck kommen; (wie durch direkte

Ermittlung des Konzentrationsgradienten, durch fraktioniertes Abschleifen von Tabletten [6] nach der Diffusion kontrolliert wurde).

2. *Die Überführungszahl.* Wie erwähnt, wurden geringe Mengen von im elektrischen Feld überführter Substanz durch Radioaktivitätsmessung statt durch Wägung bestimmt. Dabei ist zu beachten, dass gleichzeitig ein Aktivitätsübergang stattfindet, der auf Austausch an den Phasengrenzen und Diffusion zurückzuführen (Kontaktmethode, s. oben) und mit keiner Gewichtsänderung verbunden ist.

Dies wird am einfachsten auf folgende Art berücksichtigt:

Die auf die entsprechende (im Falle der positiven Ca-Ionen an der Kathode befindliche) Tablette übergegangene Radioaktivität wird graphisch als Funktion der Versuchszeit t dargestellt. Die so resultierende Kurve zeigt zunächst parabolischen Verlauf (Diffusion proportional $\sqrt{t}$) um später in eine praktisch gerade Linie umzubiegen (elektrische Überführung proportional t bei konstanter Stromstärke). Aus der Neigung dieser Geraden lässt sich die pro Strommenge transportierte Aktivität und bei Kenntnis der Aktivität pro Äquivalent die Überführungszahl entnehmen.

(Als eine Art Kontrolle und zur qualitativen Veranschaulichung der Überführung kann in gleicher Weise die Aktivität aufgetragen werden, welche auf die an der anderen Elektrode gelegenen Tablette übergegangen ist. Die Differenzwerte der beiden Kurven können jedoch offenbar nicht zur Berechnung der Überführungszahl verwendet werden, da ja bei der zuletzt genannten Tablette eine Aktivitätsverringerung durch elektrische Überführung stattfindet und darüber hinaus die Ausgangsbedingungen für die Diffusion geändert werden durch Belegung der entsprechenden Seite der aktiven Tablette mit einer inaktiven Schicht.)

Das ganze sei veranschaulicht an dem Beispiel eines Überführungsversuches an CaO bei 990° C, wiedergegeben in Fig. 2.

Die obere Kurve verbindet die Messwerte für die an der Kathode gelegene Tablette und zeigt nach anfänglich parabolischem Verlauf den für die Überführung der Ca^{++}-Jonen charakteristischen linearen Anstieg. Dieser entspricht der Überführung einer Aktivität von 48 imp/min pro mAh. Da die spezifische Aktivität des Präparates $3{,}4 \cdot 10^4$ imp/min pro mg Ca war, entspräche einer Überführungszahl 1 eine Aktivität von $3{,}4 \cdot 10^4 \cdot 20{,}04 \cdot 3{,}73 \cdot 10^{-2} = 2{,}56 \cdot 10^4$ imp / min pro mAh.

(20,04 = Ca-Äquivalentgewicht; $3{,}73 \cdot 10^{-2}$ = mF (Millifaraday) / mAh.

Die vorliegende Überführungszahl ist also: $48/25\,600 = 1{,}9 \cdot 10^{-3}$.

Die Ergebnisse der Überführungsmessungen sind in Fig. 3 zusammengefasst und zeigen annähernd exponentielle Temperaturabhängigkeit.

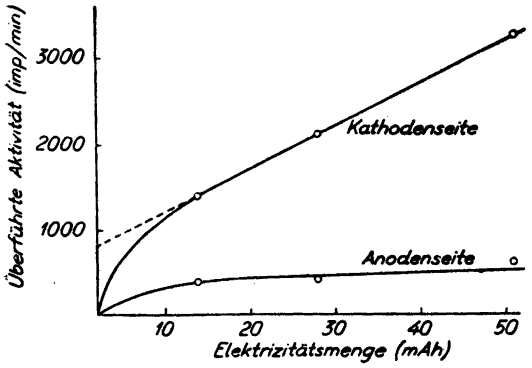


Fig. 2. Überführung von Kalzium in Kalziumoxyd bei 990° C.

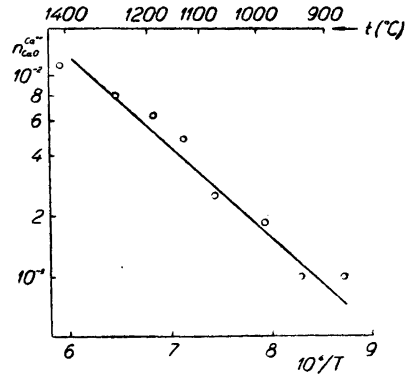


Fig. 3. Die Überführungszahl n für Kalzium in Kalziumoxyd, logarithmisch aufgetragen als Funktion der reziproken absoluten Temperatur.

3. *Die Ionenpartialleitfähigkeit und die indirekte Ermittlung der Selbstdiffusionskonstante.* Bekanntlich lässt sich die Selbstdiffusionskonstante nach folgendem Ausdruck berechnen:

$$D = \frac{n \cdot \varkappa}{Fzce} kT,$$

(n = Überführungszahl, $\varkappa$ = spezifische Leitfähigkeit, F = Faradaykonstante, z = Wertigkeit, c = Konzentration in Äquivalent/cm³, e = Elementarladung, k = Boltzmannkonstante, T = absolute Temperatur).

Die Durchführung der Rechnung unter Verwendung der in Fig. 3 wiedergegebenen Werte für die Überführungszahl sowie der bei den gleichen Versuchen gewonnenen Werte für die spezifische Leitfähigkeit führt zu Werten für die Diffusionskonstante, deren Temperaturabhängigkeit durch die gestrichelte Linie in Fig. 1 wiedergegeben ist. Aus der Verschiedenheit in der Neigung der beiden Geraden dürfen angesichts der Fehlergrenzen unser bisherigen Leitfähigkeitsmessungen keine übereilten Schlüsse gezogen werden. Die Übereinstimmung erscheint uns ausreichend, um praktisch ausschliesslich Kalziumionen-Diffusion im Kalziumoxyd anzunehmen.

ZUSAMMENFASSUNG

Die Diffusion von radioaktiven Kalzium in Kalziumoxyd wurde gemessen im Temperaturbereich von etwa 850 bis etwa 1 600° C und gehorcht dem Ausdruck: $D = 0{,}4 \exp(-81\,000/RT)$ cm²sec⁻¹.

Die Überführungszahl für Kalzium in Kalziumoxyd, nach einer neuem radioaktiven Methode gemessen, liegt im Temperaturbereich 900—1 400 °C in der Grössenordnung 10^{-3} bis 10^{-2}.

Die aus der Ionenpartialleitfähigkeit berechnete Selbstdiffusionskonstante stimmt mit der radioaktiv gemessenen so gut überein, dass auf ausschliessliche Ionendiffusion geschlossen werden kann.

Herrn Prof. J. A. Hedvall danke ich für sein Interesse an der Untersuchung, *Statens Tekniska Forskningsråd* sowie *Nobelstiftelsen* für die Bereitstellung von Mitteln.

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Die Dissoziationskonstanten von Hämoglobin und Hämatin und die Pufferkapazität des Blutes

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Die Säure-Basen-Titrationskurve von Hämoglobin hat zwei deutliche Inflexionspunkte mit Pufferkapazitätsminima [1], nämlich bei p(aH) = 5,8 und bei p(aH) = 8,7. Die zwischen diesen Inflexionspunkten wirkenden Dissoziationskonstanten sind leichter bestimmbar als die übrigen, weil die betreffenden Äquivalentpunkte durch die Inflexionspunkte gegeben sind. Diese Konstanten sind auch die in physiologischer Hinsicht wichtigsten, weil beim p(aH) des Blutes (7,3) die Pufferwirkung hauptsächlich von ihnen abhängt. Die Bestimmung der anderen Dissoziationskonstanten ist unsicherer, da nicht nur die betreffenden Inflexionspunkte fehlen, sondern auch in sauren bzw. alkalischen Lösungen Hydrolyse stattfindet, und weil sich in den ersteren bisweilen die Messungen erschwerende Fällungen bilden. Dies war der Fall insbesondere beim Untersuchen von gefälltem, fabriksmässig hergestelltem Hämoglobin [2]. Die nachstehend mitgeteilten Messungsergebnisse sind mit nativem Hämoglobin erhalten worden. Ausserdem wurden die Säure-Basen-Dissoziationskonstanten von Hämin gemessen, weil es von Interesse war, diese Konstanten mit denen des Hämoglobins zu vergleichen.

DIE BEREITUNG DER HÄMOGLOBINLÖSUNG

Die Hämoglobinlösungen wurden aus Menschen-, Pferde- und Rinderblut bereitet. Das frische Blut wurde unmittelbar durch kräftiges Schütteln defibriniert. Darauf wurde das Hämoglobin durch Einleiten von CO in das eisgekühlte Blut in CO-Hämoglobin übergeführt, die Serumlösung nach Zentrifugieren von den roten Blutkörperchen abgesaugt und das Sediment mit 0,1 N Natriumchloridlösung dreimal durch Zentrifugieren gewaschen. Das Sediment wurde dann in destilliertes Wasser gebracht, um die Zellwände

zu zerstören. Nach Zentrifugieren erhielt man so eine weinrote, klare Hämoglobinlösung. Die in bezug auf NaCl etwa 0,1 normal gemachten Hämoglobinlösungen wurden bei 0—5° C aufbewahrt.

Zur Bestimmung der NaCl-Konzentration wurde aus 50 ml Lösung das Hämoglobin durch Koagulieren ausgefällt. Nach Verdünnen des Filtrats auf 200 cm^3 wurde in 50 ml das Chlor gravimetrisch bestimmt.

Zur Bestimmung der Konzentration des Hämoglobins in den in obiger Weise bereiteten Hämoglobinlösungen wurden 50 ml der Lösung auf dem Wasserbad eingedampft, bis das Hämoglobin vollständig koaguliert war. Die Lösung wurde von dem ausgefällten Hämoglobin durch einen Filtertiegel abfiltiert und die Fällung mit warmem Wasser gewaschen. Sie wurde bei 100—102° C bzw. im Vakuumexsikkator getrocknet und gewogen. Vergleichsweise wurden 50 ml der Hämoglobinlösung auf dem Wasserbad bis zur Trockne eingedampft und bei 100—102° C getrocknet. Von der gewogenen Menge wurde dabei die bekannte NaCl-Menge abgezogen. Die verschiedenen Bestimmungen gaben miteinander übereinstimmende Werte für den Hämoglobingehalt.

Der Stickstoffgehalt des getrockneten Hämoglobins wurde durch Verbrennungsanalyse ermittelt.

Die Analysenresultate sind in Tabelle 1 wiedergegeben.

Tabelle 1.

Ursprung des Hämoglobins	Hämogl.-% der Lösung	% N im Hämoglobin	NaCl-Konz. Mol/l.
Menschenblut	1,176	16,20	0,1016
Pferdeblut	0,828	16,28	0,1019
Rinderblut	0,520	16,16	0,1006
»	0,861	15,70	0,1011

DIE BERECHNUNG DER DISSOZIATIONSKONSTANTEN

Die Dissoziationskonstanten wurden aus den potentiometrisch gemessenen p(aH)-Werten der Säure-Basen-Titration berechnet. In den folgenden Gleichungen, die für Konzentrationswerte abgeleitet worden sind, wird statt [aH^+] die H^+-Ionenkonzentration verwendet werden. Der zu deren Berechnung erforderliche Aktivitätskoeffizient wurde nach der Debye-Hückelschen Theorie berechnet (S. 381).

Der Übersichtlichkeit halber wurde bei den Berechnungen als Anfangspunkt der Titration der kleinste p(aH)-Wert der Titrationskurve verwendet, der zugleich der Endpunkt beim Titrieren von Hämoglobin mit einer starken Säure ist. [B] bezeichnet im folgenden die Konzentration der zuzufügenden Menge einer starken Base, berechnet von diesem Punkt aus. Dieser Anfangspunkt wurde so gewählt, dass der gemessene p(aH)-Wert an demselben mit dem mit Hilfe der erhaltenen Konstanten für diesen Punkt berechneten p(aH)-Wert übereinstimmt (S. 380).

Die Säure-Basen-Dissoziationskonstanten bzw. die Hydrolysekonstanten der Basen-Kationen [3] sowie deren Konzentrationen seien bezeichnet:

$$\begin{aligned} &K_1 > K_2 > K_3 \ldots\ldots\ldots \\ &C_1 = [HA_1] + [A_1^-] \\ &C_2 = [HA_2] + [A_2^-] \\ &C_3 = [HA_3] + [A_3^-] \\ &- - - - - - - - \\ &- - - - - - - - \end{aligned}$$

Aus den Gleichgewichtsgleichungen:

$$\begin{aligned} &\frac{[H^+][A_1^-]}{[HA_1]} = K_1 \\ &\frac{[H^+][A_2^-]}{[HA_2]} = K_2 \\ &- - - - - - - - \\ &- - - - - - - - \end{aligned}$$

und aus der nach dem Neutralisationsprinzip sich ergebenden Gleichung:

$$[B] + [H^+] = [A_1^-] + [A_2^-] + \ldots\ldots + [OH^-]$$

erhält man für die Berechnung von K die Gleichungen:

$$\left.\begin{aligned} K_\nu &= [H^+]\,\frac{[B] - \sum_{i=1}^{\nu-1}[A_i^-] - \sum_{i=\nu+1}^{n}[A_i^-] + [H^+] - [OH^-]}{C - \left\{[B] - \sum_{i=1}^{\nu-1}[A_1^-] - \sum_{i=\nu+1}^{n}[A_i^-] + [H^+] - [OH^-]\right\}} \\ [A_i^-] &= \frac{K_i\ C_i}{K_i + [H^+]} \end{aligned}\right\} \quad (1)$$

die sich zur Berechnung der Dissoziationskonstanten durch Annäherungsrechnung eignen. Bei der Berechnung von K_ν mit Hilfe der p(aH)-Werte zwischen den Äquivalentpunkten $\nu-1$ und ν ist:

$$\begin{aligned}[H^+] &\ll K_{\nu-1} < K_{\nu-2} \ldots\ldots \\ [H^+] &\gg K_{\nu+1} > K_{\nu+2} \ldots\ldots\end{aligned} \tag{2}$$

weshalb, wenn $K_1 \ldots K_{\nu-1}$, $K_{\nu+1}$, $K_{\nu+2} \ldots\ldots$ unbekannt sind, zur Berechnung des ersten Näherungswertes von K_ν gesetzt werden darf:

$$\begin{aligned}[A_{\nu-1}^-] &\sim C_{\nu-1} \\ [A_{\nu-2}^-] &\sim C_{\nu-2} \\ &- - - - - - \\ [A_{\nu+1}^-] &\sim 0 \\ [A_{\nu+2}^-] &\sim 0 \\ &- - - - - - \\ &- - - - - -\end{aligned}$$

Die obigen Gleichungen sind für ein Säure- bzw. Basen-Gemisch abgeleitet worden. Sie gelten deshalb nicht exakt für mehrbasische Säuren bzw. mehrsäurige Basen, die in einem Molekül mehrere Säure- bzw. Basenfunktionen haben, wie dies betreffs des unhydrolysierten Hämoglobins der Fall ist. Die Gleichungen (1) liefern indessen praktisch genaue Werte in dem Fall, dass $K_\nu/K_{\nu+1} \geqq 10^2$. Beim Hämoglobin liegen die Konstanten näher einander. Aber die Messungsfehler sind in diesem Fall so gross, dass die Gleichungen (1) trotzdem verwendbar sind. Um auf jedem Fall die Grösse des möglichen Fehlers zu prüfen, wurden K_1, K_2, K_3' und K_3'' des menschlichen Hämoglobins ausser mit Hilfe der Gleichungen (1) auch mit Hilfe der für mehrbasische Säuren geltenden weniger übersichtlichen Gleichungen:

$$\left.\begin{aligned}
K_\nu &= [H^+]\,\frac{([B]+[H^+]-[OH^-])\ M - N}{O-([B]+[H^+]-[OH^-])\ R} \\
M &= 1+\frac{[H^+]}{K_{\nu-1}}+\frac{[H^+]^2}{K_{\nu-1}\cdot K_{\nu-2}}+\ldots\ldots+\frac{[H^+]^{\nu-1}}{K_{\nu-1}\cdot K_{\nu-2}\ldots K_1} \\
N &= \sum_{i=1}^{\nu-1} C_i+\frac{[H^+]}{K_{\nu-1}}\sum_{i=1}^{\nu-2} C_i+\frac{[H^+]^2}{K_{\nu-1}\cdot K_{\nu-2}}\sum_{i=1}^{\nu-3} C_i+\ldots+\frac{[H^+]^{\nu-2}}{K_{\nu-1}\ldots K_2}\,C_1 \\
O &= \sum_{i=1}^{\nu} C_i+\frac{K_{\nu+1}}{[H^+]}\sum_{i=1}^{\nu+1} C_i+\frac{K_{\nu+1}\cdot K_{\nu+2}}{[H^+]^2}\sum_{i=1}^{\nu+2} C_i+\ldots+\frac{K_{\nu+1}\cdot K_{\nu+2}\ldots K_n}{[H^+]^{n-\nu}}\sum_{i=1}^{n} C_i \\
R &= 1+\frac{K_{\nu+1}}{[H^+]}+\frac{K_{\nu+1}\cdot K_{\nu+2}}{[H^+]^2}+\ldots+\frac{K_{\nu+1}\cdot K_{\nu+2}\ldots K_n}{[H^+]^{n-\nu}}
\end{aligned}\right\} \tag{3}$$

berechnet (vgl. Tabelle 6a). Die Übereinstimmung ist für den vorliegenden Zweck genügend, denn der Unterschied steigt nicht über die Versuchsfehler und hat keinen prinzipiellen Einfluss auf die Resultate. Der verhältnismässig grössere Unterschied bei K_4, K_5 und K_6 wird verursacht durch die Vergrösserung der Faktoren $\sum_{i=1}^{\nu-1} C_i$ und $\sum_{i=1}^{\nu+1} C_i$; denn die nach (3) zu berechnenden Werte von K werden dadurch stärker von den möglichen Fehlern in C_i und von den Fehlern in den naheliegenden Werten von K beeinflusst.

Die Gleichungen (3) wurden durch Verallgemeinerung der für eine vierbasische Säure geltenden Gleichung abgeleitet. Es sei hier die Ableitung der betreffenden Gleichungen für eine zweibasische Säure dargestellt *), weil dieselben zur Berechnung der Dissoziationskonstanten von Hämin verwendet wurden.

Aus den Gleichungen:

$$\frac{[H^+][HA^-]}{[H_2A]} = K_1; \quad \frac{[H^+][A^=]}{[HA^-]} = K_2$$

$$[H_2A] + [HA^-] + [A^=] = C; \quad [B] + [H^+] = [HA^-] + 2[A^=] + [OH^-]$$

ergeben sich für eine zweibasische Säure die Gleichungen:

$$\left.\begin{aligned} K_1 &= [H^+]\,\frac{([B] + [H^+] - [OH^-])}{C\,(1 + \frac{2K_2}{[H^+]}) - ([B] + [H^+] - [OH^-])\,(1 + \frac{K_2}{[H^+]})} \\ K_2 &= [H^+]\,\frac{([B] + [H^+] - [OH^-])\,(1 + \frac{[H^+]}{K_1}) - C}{2C - ([B] + [H^+] - [OH^-])} \end{aligned}\right\} \quad (4)$$

die sich zur Berechnug von K_1 und K_2 durch Annäherungsrechnung eignen. Im vorliegenden Fall sind $[H^+]$ und $[OH^-]$ im Vergleich zu [B] so klein, dass sie neben [B] unberücksichtigt bleiben können. Sie sind jedoch der Vollständigkeit halber in die Gleichungen mitgenommen worden, weil sie bei sehr starken bzw. sehr schwachen Säuren zu berücksichtigen sind. Bei der Berechnung des ersten Näherungswertes von K_1 darf $2K_2/[H^+]$ neben 1 vernachlässigt werden und ebenso $[H^+]/K_1$ neben 1 bei der Berechnung des ersten Näherungswertes von K_2.

* Die früher in anderem Zusammenhang für zweibasische Säuren mitgeteilte Gleichung (Kilpi, S., und Harjanne, P. *Suomen Kemistilehti* **B, 21**, 1948, 14–18) ist fehlerhaft; doch wirkt der betreffende Fehler auf das Berechnungsresultat nur ein, wenn K_1 und K_2 nahe einander liegen. In dem zitierten Fall ist der Einfluss des fehlerhaften Ausdrucks auf das Berechnungsresultat von untergeordneter Bedeutung.

DIE DISSOZIATIONSKONSTANTEN VON HÄMOGLOBIN

Von den Dissoziationskonstanten des Hämoglobins wurden die zwischen den Inflexionspunkten wirkenden zuerst berechnet, weil deren Berechnung verhältnismässig wenig von den übrigen Dissoziationskonstanten abhängig ist. Beim Untersuchen von fabriksmässig hergestelltem Hämoglobin wurde für dieses Intervall nur eine einzige Dissoziationskonstante ermittelt, deren Grösse etwa 10^{-7} betrug [2]. Von derselben Grössenordnung ist der sich nach den nachstehend dargestellten Messungen ergebende Mittelwert der im Zwischengebiet der Inflexionspunkte gefundenen Werte der Dissoziationskonstante beim nativen Hämoglobin, wenn vorausgesetzt wird, dass in diesem Bereich nur eine Dissoziationskonstante vorkommt. Die ermittelten Werte der Dissoziationskonstante zeigten jedoch die Neigung, bei zunehmender Wasserstoffionenkonzentration zu wachsen. Beim fabriksmässig hergestellten Hämoglobin konnte dies nicht beobachtet werden wegen der Koagulation des Hämoglobins bei $p(\alpha H) = 7{,}2$, wobei die Fällung beim Wachsen der Wasserstoffionenkonzentration fortwährend zunahm. Beim nativen Hämoglobin ergab sich die beste Konstanz der Dissoziationskonstante bei Annahme von zwei Dissoziationskonstanten zwischen den Inflexionspunkten und mit der Annahme, dass der Äquivalentpunkt beim »Neutralpunkt» ($p\alpha H = 7{,}5$) liegt, d.h. in der ursprünglichen Lösung, die weder mit Säure noch mit Base versetzt worden war.

Die Werte aller übrigen Dissoziationskonstanten wurden mit Hilfe der Gleichungen (1) durch Annäherungsrechnung ermittelt. Der Endpunkt der Titration auf der sauren Seite, der als »Anfangspunkt» in den Berechnungen verwendet wurde, wurde ermittelt durch Ausprobieren mit Hilfe der Gleichung [3]:

$$[H^+] = \sqrt{\sum_{i=1}^{n} K_i C_i / (1 + \frac{K_i}{[H^+]})} \qquad (5)$$

wobei die gemessene H^+-Konzentration mit der nach dieser Gleichung berechneten übereinstimmen soll. Die Bestimmung des Endpunktes der Titration auf der alkalischen Seite beim Titrieren mit einer Base geschah in entsprechender Weise mit Hilfe der Gleichung [3]:

$$[OH^-] = \sqrt{\sum_{i=n}^{1} \frac{K_W}{K_i} C_i / (1 + \frac{[H^+]}{K_i})} \qquad (6)$$

Die Äquivalentpunkte wurden durch Ausprobieren so gewählt, dass der Gang der Dissoziationskonstanten zwischen ihnen, welcher bei unrichtig gewählten Äquivalentpunkten vorkommt, möglichst ausblieb (vgl. Tab. 6).

In den Berechnungen wurden folgende für 0,1 *N* NaCl-Lösungen früher ermittelte Beziehungen verwendet:

$$p(aH) = p(cH) + 0{,}085\ ^{4}$$
$$[H^+][OH^-] = 1{,}65 \cdot 10^{-14}\ ^{5} \qquad (7)$$

Die für Menschen-, Pferde- und Rinderhämoglobin bei 25° C in 0,1 *N* NaCl-Lösung erhaltenen Werte der Dissoziationskonstanten, die Konzentrationen *C* der verschiedenen Säure- bzw. Basenfunktionen und deren Äquivalentgewichte $\ddot{A}\left(=\frac{\text{g-Hämogl./l}}{C}\right)$ sowie die Zahl *n* der verschiedenen Säure- bzw. Basenfunktionen im Hämoglobinmolekül $\left(n=\frac{67000}{\ddot{A}}\right)$ befinden sich in den Tabellen 2—4. In diesen sind weiter die Konzentration des Hämoglobins in der Lösung und die p(*a*H)-Werte der obengenannten Inflexionspunkte angegeben. Die Werte der Inflexionspunkte wurden in dieser Arbeit mit Hilfe der von Meretoja hergeleiteten Gleichung [13] berechnet.

Tabelle 2. Menschliches Hämoglobin, 5,88 g/l. Die Inflexionspunkte bei p(aH) = 5,76 und 8,70.

	HA_1	HA_2	HA_3'	HA_3''	HA_4	HA_5	HA_6
$C \cdot 10^3$	2.95	3,81	1,676	0,884	1,1	2,743	4,86
p*K* (Gl. 1)	3,15	4,35	6,69	7,63	9,82	10,73	11,37
pK (Gl. 3)	3,09	4,32	6,65	7,61	9,63	10,56	11,45
Ä	2000	1550	3500	6650	5350	2150	1210
n	33	43	19	10	13	31	55

Tabelle 3. Pferdehämoglobin, 8,28 g/l. Die Inflexionspunkte bei p(aH) = 5,77 und 8,79.

	HA_1	HA_2	HA_3'	HA_3''	HA_4	HA_5	HA_6
$C \cdot 10^3$	4,23	3,5	2,1	1,4	1,5	3,75	4
p*K*	3,21	4,28	6,70	7,66	9,87	10,73	11,38
Ä	1960	2370	3940	5,900	5500	2210	2070
n	34	28	17	11	12	30	32

Tabelle 4. Rinderhämoglobin, 8,61 g/l. Die Inflexionspunkte bei p(aH) = 5,77 und 8,72.

	HA_1	HA_2	HA_3'	HA_3''	HA_4	HA_5	HA_6
$C \cdot 10^3$	7,95	3,715	2,0	1,0	1,6	4,15	4,25
p*K*	3,46	4,23	6,71	7,71	10,04	10,66	11,45
Ä	1080	23,20	4280	8450	5380	2075	2025
n	62	29	16	8	12,5	32	33

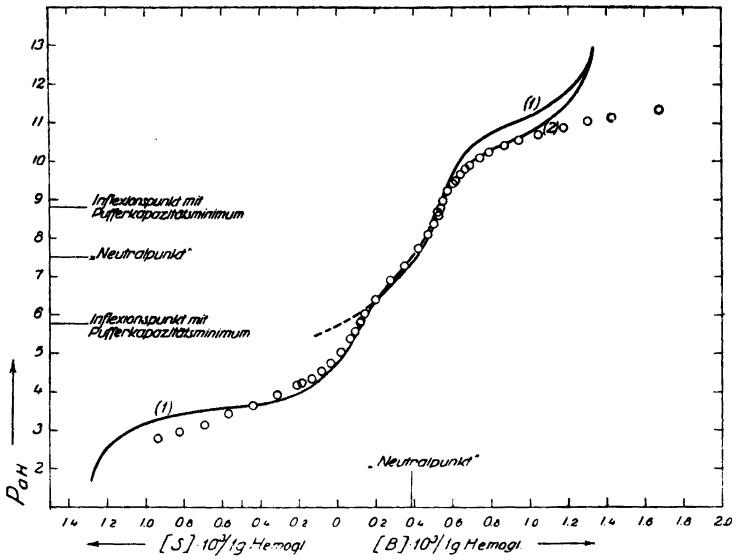


Abbildung 1. Titrierkurven von Pferdehämoglobin.

Kurve (1) nach Cohn und Mitarb. ohne NaCl-Zusatz.
» (2) » » » » in $^1/_1$ N NaCl.
○ Titrierungswerte von Kilpi in 0,1 N NaCl; bei diesen ist $[OH^-]$ bzw. $[H^+]$ nicht von dem bezüglichen Basen- bzw. Säureverbrauch abgezogen.

Den Tabellen gemäss sind die für Menschen-, Pferde- und Rinderhämoglobin erhaltenen, einander entsprechenden Dissoziationskonstanten von der gleichen Grössenordnug. Zwischen den Inflexionspunkten ist nach den Tabellen die Zahl der Säure-Basen-Funktionen in einem Hämoglobinmolekül am kleinsten beim Rinderhämoglobin. Damit hängt zusammen der aus Abb. 2 ersichtliche, im Vergleich zu den anderen Titrationskurven grössere Anstieg der Titrationskurve des Rinderhämoglobins zwischen den Inflexionspunkten, wonach die Pufferkapazität des Rinderhämoglobins zwischen den Inflexionspunkten etwas kleiner ist als die Pufferkapazität des Menschen- und Pferdehämoglobins.

Abb. 1 zeigt, wie sich die gemessenen p(aH)-Werte beim Titrieren von Pferdehämoglobin der von Cohn, Green und Blanchard gegebenen Titrationskurve von Pferdehämoglobin (CO-Hämoglobin) [6] anpassen. Als 0-Punkt der Titration ist in der Abbildung der von Cohn und Mitarb. verwendete angenommen. Obgleich die Hämoglobinlösungen in ganz verschiedener Weise bereitet worden sind, ist die Übereinstimmung innerhalb des p(aH)-Bereichs

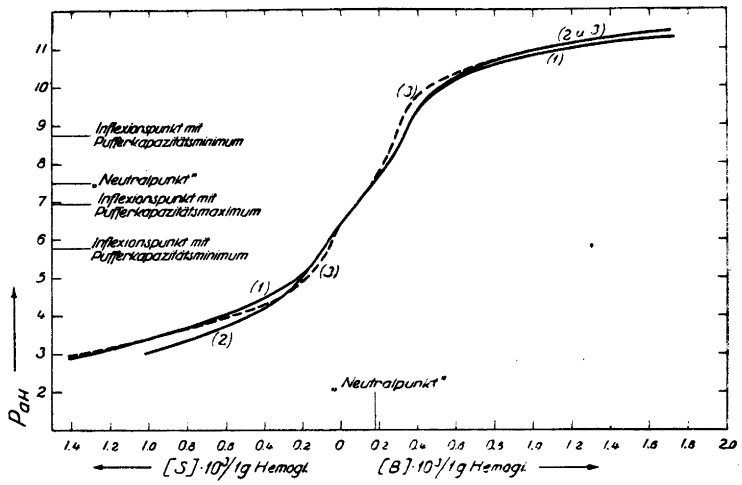


Abbildung 2. Titrierkurven von Menschen (1)-, Pferde (2)- und Rinderhämoglobin (3) in 0,1 N NaCl.

4—9,5 gut, am besten in dem physiologisch wichtigsten Bereich zwischen den Inflexionspunkten. Die in den stärker sauren bzw. stärker basischen Lösungen eintretende Abweichung unserer Werte von der Cohn'schen Titrationskurve dürfte weningstens zum Teil durch die ungleiche Hydrolyse des Hämoglobins verursacht sein.

Während unsere Titrationskurve in ihrem wichtigsten Teil mit der von Cohn und Mitarb. übereinstimmt, weichen die erhaltenen Konstanten mehr voneinander ab. Cohn und Mitarb. teilen folgende Werte für die Säure-Dissoziationskonstanten des Hämoglobins mit:

pK_1	pK_2	pK_3	pK_4	pK_5	pK_6	pK_7
3,7	4,0	4,8	5,7	7,5	10,8	11,6

Es möge bemerkt werden, dass beim Berechnen von p(aH) mit Hilfe der Gleichungen (1) und (7) sich Werte ergeben, die sich mit den Titrationskurven in Abb. 2 decken, sofern man sich bei den Berechnungen der in den Tabellen 2—4 angegebenen Dissoziationskonstanten und Konzentrationen bedient.

DIE PUFFERWIRKUNG DES BLUTES

Sind die Konzentrationen und die Dissoziationskonstanten der in der Lösung befindlichen Säuren bzw. Basen bekannt, kann die Pufferkapazität

bei verschiedenen H^+-Konzentrationen mit Hilfe der van Slyke'schen [7] Gleichung:

$$\frac{d\,[B]}{d\,pH} = P = 2{,}303 \left\{ \sum_i \frac{K_i C_i\,[H^+]}{(K_i + [H^+])^2} + [H^+] + [OH^-] \right\}$$

berechnet werden, wo K_i die Konzentrationsdissoziationskonstanten der Säuren bzw. die Hydrolysekonstanten [3] der Basen-Kationen sind.

Mit Hilfe dieser Gleichung wurde die Pufferkapazität des Blutes bei p(aH) = 7,3 und 6,5 berechnet, unter der Annahme, dass die Pufferkapazität vom Hämoglobin sowie von Kohlensäure und Phosphorsäure des Blutes herrührt. Die Pufferwirkung des Hämoglobins bei p(aH) = 7,3 and 6,5 ist hauptsächlich von den zwischen den Inflexionspunkten wirkenden Dissoziationskonstanten K_3' und K_3'' abhängig, während die Pufferwirkung der Kohlensäure bei diesen p(aH)-Werten hauptsächlich von der ersten Dissoziationskonstante und die der Phosphorsäure hauptsächlich von der zweiten abhängig ist. Betreffs der Kohlensäure wurde die zweite Dissoziation gleichfalls berücksichtigt, obgleich deren Wirkung von untergeordneter Bedeutung ist, weshalb zur Berechnung der betreffenden Pufferwirkung ein angenäherter Wert dieser Dissoziationskonstante genügt.

Die betreffenden Konzentrationsdissoziationskonstanten wurden berechnet für die Ionenstärke $I = 0{,}1$ mit NaCl als hauptsächlicher Elektrolyt, weil die Ionenstärke des Blutes annähernd von dieser Grösse ist. Die nach der Debye-Hückelschen Theorie berechneten Konzentrationsdissoziationskonstanten der Kohlensäure [8] und der Phosphorsäure [9] und die stöchiometrischen molaren Konzentrationen C derselben im Blut sind:

	K_1	K_2	C
Kohlensäure	$7{,}76 \cdot 10^{-7}$	10^{-10}	0,0245
Phosphorsäure		$1{,}8 \cdot 10^{-7}$	0,0013

Bei der Berechnung der Pufferkapazität des Hämoglobins wurde das Blut als 16 %ig in bezug auf das Hämoglobin angenommen. Man erhält dann die Konzentrationen der verschiedenen Säurefunktionen des Hämoglobins im Blut durch Multiplizieren der in den Tabellen 2—4 angegebenen Konzentrationen C beim menschlichen Hämoglobin mit 16/0,584, beim Pferdehämoglobin mit 16/0,828 und beim Rinderhämoglobin mit 16/0,861. Die Rechnung ergab für die Pufferkapazität bei p(aH) = 7,3 und 6,5 die Werte der Tabelle 5.

Tabelle 5. Pufferkapazität (P).

	In Menschenblut		In Pferdeblut		In Rinderblut	
	$p_{aH} = 7{,}3$	$p_{aH} = 6{,}5$	$p_{aH} = 7{,}3$	$p_{aH} = 6{,}5$	$p_{aH} = 7{,}3$	$p_{aH} = 6{,}5$
Hämoglobin	$3{,}04 \cdot 10^{-2}$	$2{,}85 \cdot 10^{-2}$	$2{,}90 \cdot 10^{-2}$	$2{,}60 \cdot 10^{-2}$	$2{,}37 \cdot 10^{-2}$	$2{,}22 \cdot 10^{-2}$
Kohlensäure	$0{,}48 \cdot 10^{-2}$	$1{,}27 \cdot 10^{-2}$	$0{,}48 \cdot 10^{-2}$	$1{,}27 \cdot 10^{-2}$	$0{,}48 \cdot 10^{-2}$	$1{,}27 \cdot 10^{-2}$
Phosphorsäure	$0{,}06 \cdot 10^{-2}$	$0{,}06 \cdot 10^{-2}$	$0{,}06 \cdot 10^{-2}$	$0{,}06 \cdot 10^{-2}$	$0{,}06 \cdot 10^{-2}$	$0{,}06 \cdot 10^{-2}$
P des Blutes:						
berechnet	$3{,}58 \cdot 10^{-2}$	$4{,}18 \cdot 10^{-2}$	$3{,}44 \cdot 10^{-2}$	$3{,}93 \cdot 10^{-2}$	$2{,}91 \cdot 10^{-2}$	$3{,}55 \cdot 10^{-2}$
gemessen	$3{,}61 \cdot 10^{-2}$	$4{,}25 \cdot 10^{-2}$	$3{,}26 \cdot 10^{-2}$	$4{,}00 \cdot 10^{-2}$	$2{,}97 \cdot 10^{-2}$	$3{,}59 \cdot 10^{-2}$

Gemäss der Tabelle 5 wird die Pufferkapazität des Blutes hauptsächlich durch das Hämoglobin verursacht. Der Anteil der Kohlensäure an der Pufferwirkung variiert jedoch stark mit der Veränderung der H^+-Konzentration der Lösung.

Zum Vergleich mit den mit Hilfe der erhaltenen Dissoziationskonstanten berechneten Werten wurde die Pufferkapazität des Blutes direkt durch Titration gemessen. Dazu wurden im Verhältnis von 1/10 verdünnte und in bezug auf NaCl 0,1-normale Blutlösungen verwendet, nachdem durch Titration gezeigt worden war, dass sich die Pufferkapazität des Blutes innerhalb dieses Intervalls linear mit der Konzentration verändert.

Das $p(aH)$ des »Neutralpunktes» des Blutes, zu dem keine Säure bzw. Base zugesetzt worden war, war $7{,}5 \pm 0{,}1$, wie beim »Neutralpunkt» des Hämoglobins (vgl. Abb. 1 und 2). Auch hat die Titrationskurve des Blutes (vgl. Abb. 3 und die Tabellen 8—10) der Titrationskurve des Hämoglobins entsprechend zwei Inflexionspunkte mit Pufferkapazitätsminima, deren $p(aH)$-Werte annähernd gleich den $p(aH)$-Werten der Inflexionspunkte des Hämoglobins sind.

In der Tabelle 5 sind die berechneten Pufferkapazitätswerte bei $p(aH) = 7{,}3$ und bei $p(aH) = 6{,}5$ den sich nach den Titrationskurven ergebenden Werten nebengestellt. Die berechneten und gemessenen Werte der Pufferkapazität zeigen eine gute Übereinstimmung. Die Titrationskurven des Menschen- und Rinderblutes waren gut reproduzierbar. Beim Pferdeblut war die Reproduzierbarkeit schlechter, wahrscheinlich wegen der Neigung zur Sedimentation. In bezug auf das Rinderblut wurde erwiesen, dass der Gang der Titrationskurve von dem Alter des Tieres unabhängig ist. Die aus der Abb. 3 und nach der Tabelle 5 ersichtliche geringe gegenseitige Abweichung der Pufferkapazitätswerte des Menschen-, Pferde- und Rinderblutes voneinander (Menschenblut > Pferdeblut > Rinderblut) dürfte deshalb durch die Titrationen mit ziemlich

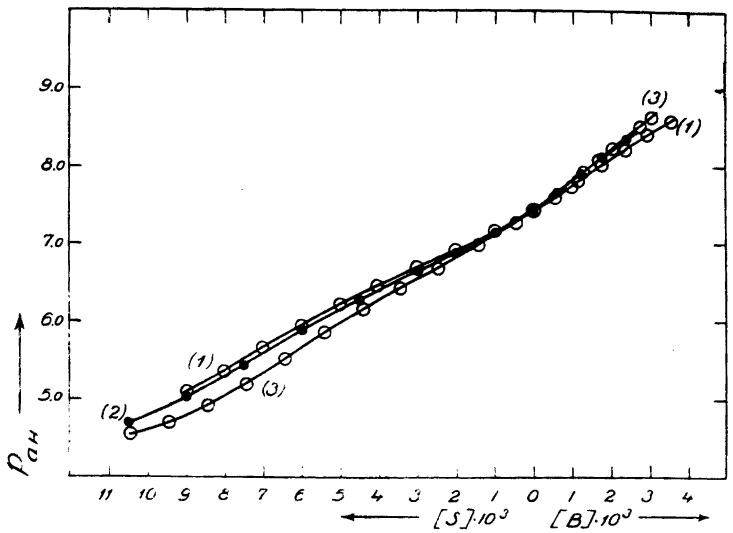


Abbildung 3. Titrierkurven von in Verhältnis von $^1/_{10}$ verdünnten Menschen (1)-, Pferde (2)- und Rinderblutlösungen (3) in 0,1 N NaCl.

grosser Sicherheit erwiesen sein. Diese Abweichung würde durch den verschiedenen Gehalt des Hämoglobins an Säure-Basenfunktionen mit den Dissoziationskonstanten K_3' und K_3'' verursacht. Die Grössen dieser Konstanten waren nämlich gemäss den Messungen gleich, unabhängig von der Herkunft des Hämoglobins.

DIE DISSOZIATIONSKONSTANTEN VON HÄMATIN IM VERGLEICH MIT DENEN DES HÄMOGLOBINS

Weil der Hämatingehalt des Hämoglobins ziemlich gross ist (vier Hämatingruppen pro Hämoglobinmolekül [10]), war es von Interesse, die Säure-Basen-Dissoziationskonstanten des Hämatins mit denen des Hämoglobins zu vergleichen.

Bei der Bestimmung der Dissoziationskonstanten von Hämatin wurde zuerst ein Hämatin-Präparat von Fraenkel & Landau verwendet [2]. Dieses löste sich doch nur teilweise in Natriumhydroxyd. Die folgenden Messungsresultate sind mit in Natriumhydroxyd gelöstem Hämin (krist. reinst, Fraenkel & Landau) erhalten, wobei die Titrationskurve wie bei Anwendug von Hämatin zwei Inflexionspunkte hatte.

Für die Dissoziationskonstanten konnten nur angenäherte Werte erhalten werden. Die Unsicherheit der Messungen beruht hauptsächlich darauf, dass wegen der Schwerlöslichkeit des Hämins nur äusserst verdünnte Lösungen (0,68 g/l) verwendet werden konnten.

Den Hämoglobinlösungen entsprechend hatte die Titrierungskurve des Hämins zwei deutliche Inflexionspunkte, nämlich bei p(aH) = 5,51 und 9,13 bei obiger Konzentration der Lösung. Beim sauren Inflexionspunkt fiel das Hämin aus. Die stöchiometrische Konzentration der Basenmenge, die bei der Titrierung von dem ersten Inflexionspunkt zum anderen zuzusetzen war, betrug $2 \cdot 10^{-3}$. Daraus berechnet sich für das Äquivalentgewicht der Hämins $0{,}68/2 \cdot 10^{-3} = 340$, welcher Wert innerhalb der Titrationsfehler mit dem halben Molekülgewicht des Hämins 325,8 übereinstimmt. Dieses ist damit in Übereinstimmung, dass das Hämin zwei Caboxylgruppen enthält. Für die pK_A-Werte der Dissoziationskonstanten wurde 6,63 bzw. 7,76 erhalten, welche Werte von derselben Grössenordnung sind wie die erhaltenen pK_3' und pK_3'' des Hämoglobins.

Unter der Annahme, dass die Konstanten des Hämins gleich denen des Hämatins sind, ist deshalb die Pufferwirkung des Hämoglobins zwischen den Inflexionspunkten von dem Hämatingehalt des Blutes abhängig, wie auch auf Grund der Titration von Hämatin zu schliessen war [2]. Weil ein Hämoglobinmolekül vier Hämatingruppen enthält [10], ist nach unseren Titrationsresultaten (vgl. Tabellen 2—4) zwischen den Inflexionspunkten der Anteil des Hämatins ca. 4/10 der gesamten Pufferwirkung des Hämoglobins. Die Pufferwirkung zwischen den Inflexionspunkten dürfte daneben von Histidylhistidin verursacht sein. Denn das Histidylhistidin hat nach Greenstein [11] vier Dissoziationskonstanten, von denen zwei mit den pK_A-Werten 6,80 bzw. 7,80 von ähnlicher Grössenordnung sind.

DIE MESSUNGEN

50 ml der betreffenden Hämoglobin-, bzw. Häminlösung wurden mit 0,1 N NaOH- bzw. 0,1 N HCl-Lösung titriert.

Die p(aH)-Werte der Hämoglobin- und Häminlösungen wurden bestimmt durch Messen des Potentials der Kette:

(8) $\mathrm{Pt/H_2}\,\left|\begin{matrix}0.09\ N\ \mathrm{KCl}\\ 0.01\ N\ \mathrm{HCl}\end{matrix}\right|\begin{matrix}\text{Gesätt.}\\ \text{KCl-Lös.}\end{matrix}\Big|0.1\ N\ \mathrm{KCl}\Big|\mathrm{Hg_2Cl_2Hg}\Big|0.1\ N\ \mathrm{KCl}\Big|\begin{matrix}\text{Gesätt.}\\ \text{KCl-Lös.}\end{matrix}\Big|\begin{matrix}\text{Titr.-}\\ \text{flüss.}\end{matrix}\Big|\mathrm{Pt/H_2}$

und unter Anwendung von p(aH) = 2,075 für die Veibelsche Lösung (0,09 N KCl + 0,01 N HCl).

Beim Titrieren von Blut wurden die p(aH)-Werte mit einer Glaselektrode gemessen. Die Kettenanordnung war dieselbe wie in (8), jedoch wurde statt

der Veibelschen Lösung ein Acetat-Puffer mit p(aH) = 4,65 als Vergleichslösung [12] verwendet.

(8′) | Glas-elektr. | Acetat-Puffer | Gesätt. KCl-Lös. | 0.1 N KCl | Hg_2Cl_2, Hg | 0.1 N KCl | Gesätt. KCl-Lös. | Titr.-flüss. | Glas-elektr.

Beim Pferdehämoglobin wurde die Ausführung der Messungen in sauren Lösungen durch die Bildung einer Fällung bei p(aH) = 4,2 erschwert. Die

Tabelle 6a. Menschen-Hämoglobin, 5,88 g/l.

(Die Titrationen sind von A. A. O. Kuosmanen ausgeführt.)

E mV	p(aH)	$[H^+]$	$[OH^-]$	$[B] \cdot 10^3$	V ml	pK Gl. (1)	pK Gl. (3)
		(ber.) $1{,}15 \cdot 10^{-3}$					
57,0	3,040	$1{,}12 \cdot 10^{-3}$		0	55,40		
58,9	3,172	$8{,}23 \cdot 10^{-4}$		0,440	54,40	3,30	3,24
68,4	3,232	$7{,}16 \cdot 10^{-4}$		0,835	55,80	3,20	3,15
71,0	3,276	$6{,}47 \cdot 10^{-4}$		1,277	54,80	3,05	3,00
81,0	3,446	$4{,}38 \cdot 10^{-4}$		1,673	56,15	3,17	3,12
84,6	3,507	$3{,}80 \cdot 10^{-4}$		2,110	55,15	3,04	2,98
94,6	3,676	$2{,}58 \cdot 10^{-4}$		2,507	56,52	3,14	3,06
Äquiv.							
99,1	3,752	$2{,}16 \cdot 10^{-4}$		2,949	55,50	*3,15*	*3,09* im Mittel
112,6	3,980	$1{,}28 \cdot 10^{-4}$		3,343	56,90	4,35	4,34
115,2	4,024	$8{,}65 \cdot 10^{-5}$		3,783	55,92	4,38	4,38
131,5	4,300	$6{,}12 \cdot 10^{-5}$		4,180	57,32	4,39	4,39
136,9	4,391	$4{,}97 \cdot 10^{-5}$		4,629	56,45	4,30	4,31
151,5	4,640	$2{,}80 \cdot 10^{-5}$		5,020	57,78	4,41	4,43
152,7	4,659	$2{,}68 \cdot 10^{-5}$		5,466	56,85	4,23	4,26
188,2	5,259	$6{,}73 \cdot 10^{-6}$		6,310	57,30	4,39	4,37
197,2	5,412	$4{,}73 \cdot 10^{-6}$		6,520	57,40	4,38	4,28
207,5	5,586	$3{,}17 \cdot 10^{-6}$		6,720	57,45	4,34	4,10
214,6	5,706	$2{,}33 \cdot 10^{-6}$		6,730	54,88	*4,35*	*4,32* im Mittel
Äquiv.	5,762			6,755	54,80		
231,8	5,997	$1{,}19 \cdot 10^{-6}$		6,932	54,72	6,69	6,66
247,3	6,259	$6{,}53 \cdot 10^{-7}$		7,166	54,68	6,67	6,65
260,8	6,488	$3{,}86 \cdot 10^{-7}$		7,378	54,60	6,69	6,66
286,3	6,922	$1{,}42 \cdot 10^{-7}$		7,790	54,32	6,77	6,78
312,0	7,354	$5{,}25 \cdot 10^{-8}$		8,420	54,00	6,61	6,69
Äquiv.						*6,69*	*6,65* im Mittel
358,5	8,141	$8{,}57 \cdot 10^{-9}$	$2 \cdot 10^{-6}$	9,050	53,72	7,63	7,67
382,5	8,547	$3{,}39 \cdot 10^{-9}$	$4 \cdot 10^{-6}$	9,272	53,70	7,62	7,55
Äquiv.	8,698			9,320		*7,63*	*7,61* im Mittel

Tabelle 6b. Menschen-Hämoglobin 5,88 g/l.

E mV	p(aH)	[H^+]	[OH^-]	[B] · 10^3	V ml	pK (Gl. 1)
420,9	9,333	$5{,}68 \cdot 10^{-10}$	$2{,}9 \cdot 10^{-5}$	9,656	50,50	9,88
439,3	9,508	$3{,}80 \cdot 10^{-10}$	$4{,}3 \cdot 10^{-5}$	9,878	50,60	9,76
449,6	9,682	$2{,}54 \cdot 10^{-10}$	$6{,}5 \cdot 10^{-5}$	10,070	50,65	9,79
459,4	9,848	$1{,}73 \cdot 10^{-10}$	$9{,}5 \cdot 10^{-5}$	10,205	50,80	9,83
Äquiv.						*9,82* im Mittel
466,0	9,960	$1{,}34 \cdot 10^{-10}$	$1{,}23 \cdot 10^{-4}$	10,504	50,90	10,75
477,2	10,149	$8{,}67 \cdot 10^{-11}$	$1{,}90 \cdot 10^{-4}$	10,911	51,10	10,75
486,6	10,309	$6{,}00 \cdot 10^{-11}$	$2{,}75 \cdot 10^{-4}$	11,346	51,26	10,75
493,5	10,425	$4{,}59 \cdot 10^{-11}$	$3{,}59 \cdot 10^{-4}$	11,767	51,48	10,71
499,6	10,529	$3{,}61 \cdot 10^{-11}$	$4{,}57 \cdot 10^{-4}$	12,188	51,70	10,69
508,3	10,676	$2{,}58 \cdot 10^{-11}$	$6{,}41 \cdot 10^{-4}$	12,784	51,95	10,72
Äquiv.						*10,73* im Mittel
521,2	10,894	$1{,}56 \cdot 10^{-11}$	$1{,}058 \cdot 10^{-3}$	13,841	52,48	11,42
529,3	11,031	$1{,}14 \cdot 10^{-11}$	$1{,}450 \cdot 10^{-3}$	14,867	52,92	11,37
536,1	11,146	$8{,}73 \cdot 10^{-12}$	$1{,}890 \cdot 10^{-3}$	15,957	53,55	11,32
547,3	11,336	$5{,}64 \cdot 10^{-12}$	$2{,}94 \cdot 10^{-3}$ $3{,}04 \cdot 10^{-3}$ (ber.)	18,023	54,50	11,37 im Mittel

Fällung bildete sich an dieser Stelle sowohl beim Titrieren mit Chlorwasserstoffsäure als mit Natriumhydroxyd. Im letzteren Falle war zu der Lösung zuerst Chlorwasserstoffsäure in einem solchen Überschuss zugefügt worden, dass die Fällung ausblieb. Beim Menschen- und Rinderblut verblieb die Lösung während der ganzen Titration klar sowohl in sauren als in alkalischen Lösungen.

In den folgenden Tabellen bezeichnet E das Potential der Kette (8) bzw. (8′) und V das Volumen der Lösung in ml. Die in den Tabellen 2—4 angegebenen Konzentrationen C_1, C_2, C_3', C_3'', C_4, C_5, und C_6 sowie die stöchiometrische Konzentration [B] der vom Beginn der Titration an zugesetzten Basenmenge sind auf das ursprüngliche Volumen der Lösung, nämlich 50 ml, bezogen. In den Berechnungen ist die tatsächliche Konzentration während der Titration, $C\,\frac{50}{V}$, verwendet worden. [S] der Tabellen bezeichnet die Konzentration der zugesetzten Säure, weshalb [B] = — [S].

Von den ausgeführten Titrationen des Menschen-, Pferde- und Rinderhämoglobins ist hier als Beispiel nur die des menschlichen Hämoglobins vorgelegt worden. Ausserdem sind im folgenden die Titrationen von Hämin sowie die Titrationen des Menschen-, Pferde- und Rinderblutes wiedergegeben.

Alle Titrationen sind bei 25,0° C ausgeführt worden. Die Lösungen waren in bezug auf NaCl 0,1-normal.

Tabelle 7. Hämin, 0,680 g/l in 0,1 N NaCl-Lösung.

(Die Titrationen sind von Elsa Keränen ausgeführt.)

E mV	p(aH)	[H^+]	[OH^-]	[B] 10^3	V ml	pK Gl. (1)	pK Gl. (4)
165,6	4,875	$1{,}62 \cdot 10^{-5}$			52,80		
Äquiv.	5,51			0	52,66		
252,5	6,347	$5{,}50 \cdot 10^{-7}$		0,340	52,50	6,61	6,58
303,5	7,207	$7{,}59 \cdot 10^{-8}$		0,934	52,20	6,64	6,61
Äquiv.				1,000		*6,63*	*6,60* im Mittel
328,0	7,625	$2{,}88 \cdot 10^{-8}$		1,260	52,22	7,78	7,80
360,9	8,180	$8{,}04 \cdot 10^{-9}$	$2 \cdot 10^{-6}$	1,661	52,02	7,74	7,77
Äquiv.	9,13			2,000	51,85	*7,76*	*7,79* im Mittel
426,5	9,285	$6{,}31 \cdot 10^{-10}$	$2{,}7 \cdot 10^{-5}$		51,82		

Tabelle 8. Menschenblut.

Die Titrationen von Blut wurden mit Anwendung der Kette (8') ausgeführt. Die Blutlösungen waren im Verhältnis von 1/10 verdünnt und in bezug auf NaCl 0,1-normal.
(Die Titrationen sind von P. J. Väänänen ausgeführt.)

E	p(aH)	[S] $\cdot 10^3$	V (ml)
234,75	8,62	— 3,55	206
223,90	8,44	— 2,96	205
212,50	8,24	— 2,37	204
199,65	8,03	— 1,78	203
187,85	7,83	— 1,18	202
176,35	7,63	— 0,59	201
166,90	7,47	0,00	200
150,10	7,19	1,00	202
135,35	6,94	2,01	204
121,85	6,71	3,01	206
108,50	6,48	4,01	208
94,05	6,24	5,01	210
77,30	5,96	6,01	212
60,45	5,67	7,01	214
43,10	5,38	8,01	216
26,15	5,09	9,01	218

Die Inflexionspunkte bei p(aH) = 5,51 und 8,11.

Tabelle 9. Pferdeblut.

E	p(aH)	$[S] \cdot 10^3$	V (ml)
219,10	8,35	— 2,37	204
206,10	8,13	— 1,78	203
192,10	7,90	— 1,18	202
180,00	7,69	— 0,59	201
167,80	7,49	0,00	200
148,45	7,16	1,00	202
132,05	6,88	2,00	204
120,10	6,68	3,01	206
98,35	6,31	4,52	209
75,25	5,92	6,02	212
48,15	5,46	7,52	215
22,70	5,03	9,02	218
3,30	4,71	10,52	221

Die Inflexionspunkte bei p(aH) = 5,78 und 8,06.

Tabelle 10. Rinderblut.

E	p(aH)	$[S] \cdot 10^3$	V (ml)
238,10	8,68	— 3,07	204,2
230,90	8,55	— 2,72	203,6
222,30	8,41	— 2,36	203,0
214,70	8,28	— 2,01	202,4
204,55	8,11	— 1,65	201,8
194,55	7,94	— 1,30	201,2
184,75	7,77	— 0,94	200,6
177,55	7,65	— 0,59	200
156,10	7,29	+ 0,41	202
140,35	7,02	+ 1,42	204
120,05	6,68	2,42	206
106,55	6,45	3,42	208
90,80	6,19	4,42	210
72,65	5,88	5,42	212
53,40	5,55	6,43	214
32,45	5,20	7,43	216
16,75	4,93	8,43	218
3,95	4,72	9,43	220
— 6,10	4,55	10,43	222

Die Inflexionspunkte bei p(aH) = 5,48 und 8,11.

ZUSAMMENFASSUNG

Es wurden die Säure-Basen-Dissoziationskonstanten des Hämoglobins von Menschen-, Pferde- und Rinderblut durch potentiometrische Titration bestimmt. Die erhaltenen, einander entsprechenden Dissoziationskonstanten waren von dem Ursprung des Hämoglobins unabhängig und von der gleicher Grössenordnung.

Die Titrationskurven haben zwei Inflexionspunkte mit Pufferkapazitätsminima, nämlich bei p(aH) = 5,8 bzw. 8,7. Die Pufferkapazität des Rinderhämoglobins ist in dem physiologisch wichtigsten Bereich, nämlich zwischen den Inflexionspunkten, kleiner als die des menschlichen und des Pferdehämoglobins. Dieses beruht darauf, dass der Gehalt des Hämoglobins an Säure-Basenfunktionen mit den Dissoziationskonstanten K'_3 und K''_3, von denen die Pufferkapazität zwischen den Inflexionspunkten hauptsächlich abhängig ist, beim Rinderhämoglobin kleiner ist als beim Menschen- und Pferdehämoglobin.

Die Titrationskurven des Menschen-, Pferde- und Rinderblutes haben zwei den Inflexionspunkten der Titrationskurve des Hämoglobins entsprechende Inflexionspunkte. Die Pufferkapazität zwischen den Inflexionspunkten vermindert sich in der Reihenfolge: Menschen- > Pferde- > Rinderblut.

Mit Anwendung der erhaltenen Dissoziationskonstanten des Hämoglobins berechnen sich Pufferkapazitätswerte, welche mit Berücksichtigung der Pufferkapazität von Kohlensäure und Phosphorsäure des Blutes mit den experimentell nach der Titrationskurve des Blutes gemessenen Werten übereinstimmen. Die Pufferkapazität des Blutes zwischen den genannten Inflexionspunkten lässt sich hauptsächlich auf das Hämoglobin zurückführen.

Betreffend des Hämoglobins scheint die Pufferwirkung zwischen den Inflexionspunkten hauptsächlich von Hämin und Histidylhistidin des Hämoglobins verursacht zu sein.

Für ein Staatsstipendium zur Förderung der höchsten Geistesbildung sowie für ein Stipendium des Staatlichen Naturwissenschaftlichen Kommission, die mir zur Ausführung der vorliegenden Arbeit bewilligt wurden, bringe ich hier meinen Dank zum Ausdruck.

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Properties of Monolayers of Rosin Acids *

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The properties of monolayers of rosin acids spread on water are relatively little known. A study of *d*-pimaric acid and its hydrogenated derivative tetrahydro-*d*-pimaric acid has been published by Harkins, Ries and Carman [1]. Recently Ekwall and Bruun [2] studied the properties of tall oil rosin acid, a mixture of different rosin acids, in monolayers on substrates of varying pH and of different aluminium ion contents.

From the surface-chemical viewpoint the rosin acids are interesting substances for investigation. The carbon skeleton and the site of the carboxyl group are the same in most of the rosin acids. They differ, however, from each other in the number of double bonds and the location of the latter in the molecule. It is of interest to determine whether the differences in the structures of the rosin acids become evident in the properties of their monolayers. It is only now that this aspect can be closer studied since the methods of purification of the rosin acids have been greatly improved and it is possible to prepare several of the rosin acids in high purity.

The author has studied the properties of the monolayers of abietic acid, dehydro-, dihydro-, and tetrahydroabietic acids and isodextropimaric acid.

EXPERIMENTAL

The monolayers were investigated with a recording surface balance of the Wilhelmy-Dervichian type as improved by Andersson-Groth, Ställberg-Stenhagen and Stenhagen [3]. The monolayer was compressed at a rate of 5 cm per minute and the compression was commenced two minutes after the spreading of the substance under study. The rosin acids were added to the surface of the substrate in a benzene solution (concentration $2 \cdot 10^{18}$ molecules per millilitre) with an "Agla" micrometer syringe.

* Preliminary communication read at the VII Nordiska Kemistmötet in Helsingfors 1950. Abstract in the Proceedings of the Meeting, Helsingfors 1951, p. 164 and in *Teknisk Tidskrift* **80** (1950) 790.

The twice distilled water used in the experiments had a specific conductance of approximately $0.6 \cdot 10^{-6}$ ohm$^{-1} \cdot$ cm^{-1}. The required pH was obtained by adding hydrochloric acid. When studying the influence of the pH of the substrate on the monolayers, the substrate was first brought to pH 3.8, and then the pH was increased by successive additions of sodium hydroxide. The pH measurements were carried out on aliquots of the aqueous solution with a electronic potentiometer (Radiometer Type PHM 3), fitted with a glass electrode and a saturated calomel reference electrode. In order to avoid oxidation in the case of abietic acid, the surface balance measurements with this acid were conducted in a nitrogen atmosphere. The substrate was saturated with nitrogen and contained 10—15 mg hydroquinone (Merck, pro analysi) per litre.

The rosin acids studied were obtained from the Research Laboratory of the Hercules Powder Company, Wilmington, Delaware. According to information received from Dr. G. C. Harris of that laboratory the abietic acid and dehydroabietic acid were pure and free from isomeric acids. The dihydro- and tetrahydroabietic acids were prepared by partial and complete hydrogenation of abietic acid. The resulting products were mixtures of isomeric acids, but were otherwise pure. Isodextropimaric acid was prepared according to the method described by Harris and Sandermann [4].

Owing to its tendency to oxidize easily, the abietic acid was stored in a closed evacuated glass tube. The preparation was recrystallized immediately before use from acetone to which 1 g hydroquinone per litre had been added. Another sample of pure abietic acid in the form of its butylamine salt was obtained from the Svenska Träforskningsinstitutet, Stockholm. The abietic acid was liberated from its salt with boric acid, shaken with ether and recrystallized as described immediately before use.

The corrected melting points of the rosin acids and their specific rotations in absolute alcohol were:

Abietic acid (both samples)	m. p. 170.2—172.7° C,	$[\alpha]_D^{20} = -103°$ to $-104°$;
dehydroabietic acid	m. p. 173.3—174.8° C,	$[\alpha]_D^{20} = +65°$ to $+67.5°$;
dihydroabietic acid	m. p. 133.8—136.8° C,	$[\alpha]_D^{20} = +17°$ to $+21°$;
tetrahydroabietic acid	m. p. 149.4—152.4° C,	$[\alpha]_D^{20} = +9°$ to $+13°$;
isodextropimaric acid	m. p. 162.9—164.9° C,	$[\alpha]_D^{20} = 0°$.

RESULTS

The pressure-area curves of the pure rosin acid monolayers

a. *Experiments at pH 3.0.* Fig. 1 shows the pressure-area curves for tetrahydroabietic acid (TAbH), dihydroabietic acid (DiAbH), abietic acid (AbH), isodextropimaric acid (IdPH) and dehydroabietic acid (DeAbH) monolayers on water with a pH of 3.0 at 20° C ($\pm$ 0.3°). Certain characteristic data obtained from these pressure-area curves are collected in Table 1. The values given are average values from six curves recorded in succession for each acid. The different values for A_K and A_{15} deviated less than $\pm$ 0.2 sq.

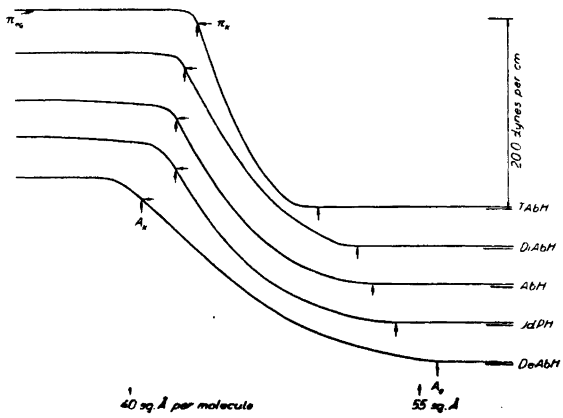


Fig. 1. Pressure-area curves for monolayers of tetrahydroabietic (TAbH), dihydroabietic (DiAbH), abietic (AbH), isodextropimaric (IdPH) and dehydroabietic (DeAbH) acids. Aqueous substrate containing hydrochloric acid (pH = 3.0).

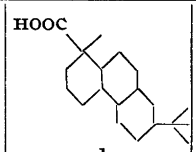
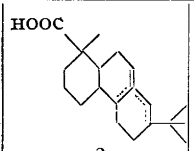
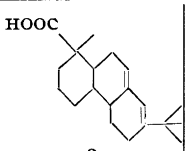
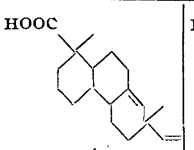
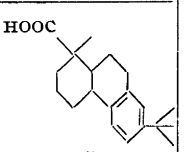
Å from the average. In the table the acids are listed in the order of their degree of unsaturation as indicated by the schematic structural formulae. The skeleton of isodextropimaric acid differs somewhat from those of the abietic acids, but has been included for comparison.

From Fig. 1 it is seen that the pressure-area curves for the different rosin acids are very similar. Both the areas per molecule and the collapse pressures of the monolayers of these acids are approximately the same for the acids investigated. Compression of the monolayers occurs between the molecular areas 56 and 40 sq.Å, while the surface pressures at which the films collapse (π_K) are within the range 16 to 20 dynes per centimetre. The acids form liquid layers on compression. The properties of the rosin acids studied resemble greatly those of *d*-pimaric acid and tetrahydro-d-pimaric acid described by Harkins *et al.*[1], when the differences in method of study and temperature are taken into account.

A closer inspection of the values of Table 1, however, reveals certain gradual differences between the rosin acids investigated. Both A_K and A_{15} decrease in value in the order TAbH, DiAbH, AbH, IdPH, DeAbH. The difference in the values of A_K for TAbH and DeAbH is 3.0 sq.Å. The corresponding difference for A_{15} is 2.3 sq.Å and for π_K about 2.2 dynes per cm (excluding IDP). A_0, however, undergoes an increase of 6—7 sq.Å in the order of acids given.

The gradual changes noted in the case of A and π appear to be connected with the structures of the rosin acid molecules, particularly with the number of double bonds. It is clearly evident that the TAbH molecules which do not

Table 1. Data relating to the pressure-area curves for rosin acid monolayers shown in Fig. 1.

Rosin acid		1 Tetra-hydro-abietic acid TAbH	2 Dihydro-abietic acid DiAbH	3 Abietic acid AbH	4 Iso-dextro-pimaric acid IdPH	5 Dehydro-abietic acid DeAbH
Area at the collapse point of the monolayer (sq.Å per molecule)	A_K	43.4	42.9	42.0	42.6	40.4
Area when the surface pressure is 15 dynes per cm	A_{15}	44.2	43.9	42.8	42.8	41.9
Area when the first increase in pressure is observed	A_0	49.6	51.9	52.2	54.1	55.9
Surface pressure at the collapse point of the monolayer (dynes per cm)	π_K	19.5	19.2	17.9	15.8	17.3
Surface pressure when equilibrium pressure is attained after the collapse of the monolayer	π_{Keq}	20.8	20.3	19.5	19.7	19.6
Compressibility of the monolayer just before collapse $K = -\frac{\delta A}{\delta \pi} \cdot \frac{1}{A}$	K	0.0041	0.0058	0.0066	0.0077	0.013
Schematic structural formulae		 1	 2	 3	 4	 5

contain double bonds in their ring system take up the largest area in the compressed monolayer. With increase in the number of double bonds A_K decreases in the series DiAbH, AbH, IdPH, DeAbH, the latter occupying the smallest area. It is also seen that the double bonds increase the affinity of the molecules for water. The larger the number of double bonds, the earlier

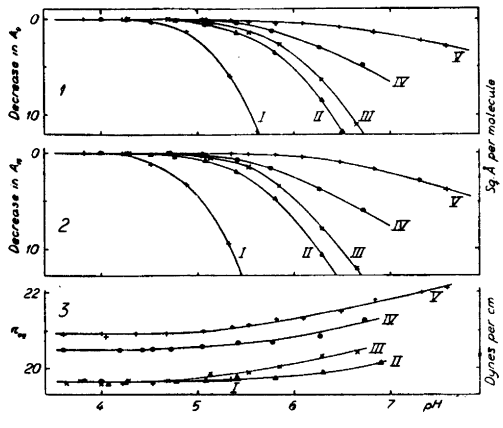


Fig. 2. The values of π_{Keq} (diagram 3) and the decrease in the values of A_0 (d. 1) and A_{15} (d. 2) observed in the pressure-area curves for various rosin acids at increasing pH values of the substrate at 20° C. I DeAbH, II AbH, III IdPH, IV DiAbH, V TAbH.

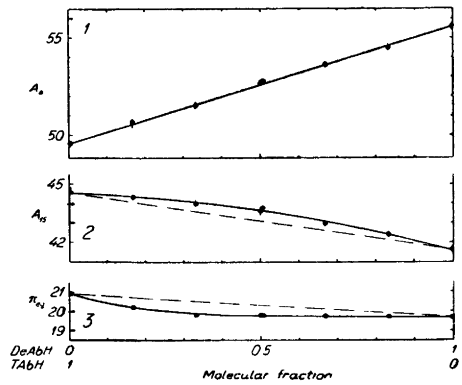


Fig. 3. The values of A_0 (1), A_{15} (2), and π_{Keq} (3) from the pressure-area curves of monolayers containing TAbH and DeAbH. pH = 3.0 20° C.

the surface pressure begins to increase on compression and the higher the value of A_0. This value is largest in the case of DeAbH and smallest for TAbH.

The values of most of the properties of the monolayer of IdPH with two double bonds, one of these in the side chain, lie between those of DeAbH and AbH. The monolayer of IdPH shows, however, the lowest π_K value of all these acids.

b. *Experiments at varying pH.* Pressure-area curves were also recorded for the monolayers of the rosin acids mentioned above on substrates of different pH values (2 to 8) to determine to what extent the dependence of the properties of the monolayers on the pH value of the underlying water is determined by the molecular structure.

Fig 2 illustrates the relation between the pH of the substrate and the quantities A_0, A_{15} and π_{Keq} (Table 1). These quantities remain unaltered up to pH 4.2; above this value the areas taken up by the rosin acids begin to decrease, first that of DeAbH, followed by AbH, IdPH, DiAbH and THAB. π_{Keq} undergoes an increase with increasing pH. This increase is smallest for DeAbH.

The decrease in area with increasing pH of the substrate coincides with the beginning of ionization in the rosin acid monolayer and with the accompanying increase in the solubility of the monolayer substance. This solubility is highest in the case of DeAbH and AbH and is weakest with TAbH.

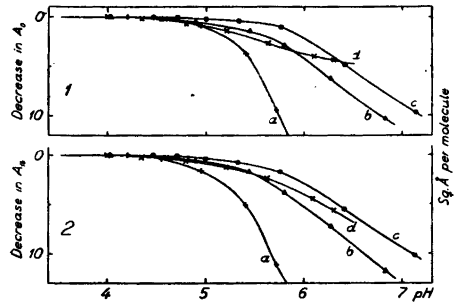


Fig. 4. The decrease in the values of A_0 (1) and A_{15} (2) in the pressure-area curves of binary rosin acid mixtures with increasing pH of the substrate. Molar ratio of components 1 : 1.20° C. a) TAbH + DeAbH; b) TAbH + AbH; c) TAbH + IdPH; d) DiAbH + IdPH.

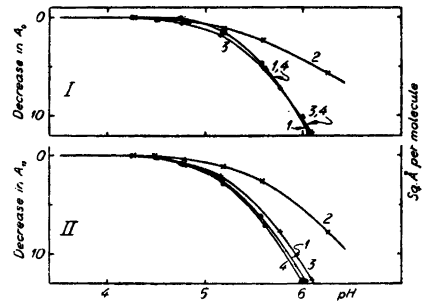


Fig. 5. The decrease in the values of A_0 (I) and A_{15} (II) in the pressure-area curves of monolayers of a purified Finnish tall oil rosin and of certain ternary rosin acid mixtures with increasing pH. 20° C. 1 (●) tall oil rosin, 2 (x) AbH 50 %, IdPH 25 %, DiAbH 25 %, 3 (+) AbH 50 %, IdPH 25 %, DeAbH 25 %, 4 (Δ) AbH 33 1/3 %, IdPH 33 1/3 %, DeAbH 33 1/3 %.

The differences in the structures of rosin acids are clearly evident also in the properties of the monolayers at different acidities of the substrate.

Monolayers of binary rosin acid mixtures

A number of binary mixtures of the rosin acids were studied to determine whether the components affect each other's properties in the mixed monolayer or whether the individual properties of the component acids become evident in the mixtures.

a. *Experiments at pH 3.0.* Fig. 3 shows the variation of A_0, A_{15} and π_{Keq} for monolayers containing TAbH and DeAbH at pH 3.8. On the left side are given the values for the pure TAbH layer and on the right those for the pure DeAbH layer. Each point in the curves is an average value from 4 to 5 successive measurements which fall within the range indicated. A_0 is seen to vary linearly with the ratio of the components in the layer. Also A_{15} varies in a similar manner but the values of A_{15} are higher than would have been expected. The molecules thus appear to take up larger areas in the mixed monolayers than in the simple monolayers. This is accompanied by a decreased strength of the monolayer as shown by the values for the surface pressures π_{Keq} given by the lowest curve in Fig. 3.

b. *Substrates of different pH.* Four binary rosin acid mixtures were studied with varying pH values of the substrate, *viz.*, TAbH + DeAbH (Fig. 4, curves a), TAbH + AbH (curves b), TAbH + IdPH (curves c) and DiAbH + IdPH (curves d). In all cases the mole ratio of the components was unity. Marked differences are noted between the properties of the mixed monolayers. A comparison of curves for the mixtures and the single acids (Fig. 2) shows that the components behave to a large degree independently of each other; the curves of Fig. 4 correspond to curves calculated for the mixtures from the curves of the pure components given in Fig. 2.

Tall oil rosin acid

According to Sandermann [5] purified tall oil rosin acid contains approximately 50 per cent abietic acid, 25 per cent dextropimaric acid and 25 per cent dehydro-, dihydro-, and tetrahydroabietic acids. According to Harva [6] purified Finnish tall oil rosin contains 30 to 40 per cent abietic acid. The compositions of tall oil rosin acids vary, however, considerably owing to various factors. For special uses, *e.g.*, in the paper industry, a method for defining tall oil rosin acid is of importance. An attempt has been made to determine whether the surface balance can be employed to indicate variations in the composition of the tall oil rosin.

The properties of the monolayer of a purified Finnish tall oil rosin ($[\alpha]_D^{20}$ = −44 to −45° in absolute alcohol) are shown in Fig. 5, curves 1.[7] Curves 2 apply to a rosin acid mixture containing 50 % AbH, 25 % IdPH, 25 % DiAbH, curves 3 to a mixture containing 50 % AbH, 25 % IdPH, 25 % DeAbH, and curves 4 to a mixture containing 33 1/3 % AbH, 33 1/3 % IdPH, and 33 1/3 % DeAbH. The latter curves (4) correspond fairly well with those obtained for the purified tall oil rosin (1). Curves 3 relating to a mixture containing more AbH and less DeAbH are seen to differ clearly from the former curves (4). Substitution of DeAbH for DiAbH in the mixtures represented by curves 3 leads to different values of A_0 and A_{15} as shown by curves 2. It is hardly possible to prepare a mixture containing known rosin acids that would exactly correspond to tall oil rosin in its monolayer properties. It seems, however, that with the surface balance it is possible to differentiate between various tall oil rosins and other rosin acid mixtures.

SUMMARY

The monolayers of abietic, dehydro-, dihydro- and tetrahydroabietic acids have been studied with a surface balance. The pressure-area curves of the monolayers are of the same form. The areas per molecule and the collapse

pressures of the monolayers show differences. These differences appear to be connected with the structure of the rosin acid molecules, particularly with the number of double bonds in the molecules.

The properties of the rosin acid monolayers on substrates of different acidities have also been studied. The beginning of the ionization of the rosin acids with accompanying increase in the solubility of the monolayer substance is seen from the increase in the collapse pressure and from the decrease in the area per molecule. Differences are noted also in this respect between the various rosin acids.

The properties of isodextropimaric acid resemble most closely those of abietic acid. The pressure-area curve of the monolayer shows a lower collapse pressure than the curves for the acids of the abietic series.

A study of binary mixtures of rosin acids indicated that in most cases the individual monolayer properties of the components become evident in the properties of the mixed monolayers.

An investigation of ternary rosin acid mixtures and of a Finnish tall oil rosin showed that the surface balance can be employed to indicate differences in composition of rosin acid mixtures.

This work is connected with studies of the theory of paper sizing with rosin which are being conducted at the Institute of Physical Chemistry of Åbo Akademi in collaboration with *Ab Centrallaboratorium* of Helsingfors.

I am indebted to Professor Per Ekwall, the Head of the Institute, for helpful discussions and for the opportunity of using the facilities of the Institute.

I wish to thank Dr. C. G. Harris of the Hercules Powder Company for the pure rosin acids which he has put at the disposal of the Institute and to Mr. E. Back, Civ. E., of the Svenska Träforskningsinstitutet for the sample of butylamine abietate.

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The Reaction between Diphenylchloroacetyl Chloride and Methylurea

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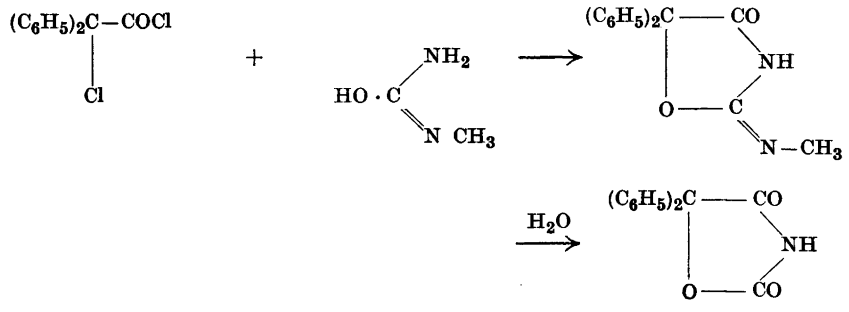
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Some years ago Aspelund and Holmberg [1] investigated the reactions between diphenylchloroacetyl chloride and substituted ureas. Only one aliphatic urea, acetylmethylurea, was studied at that time and it was established that following reaction took place.

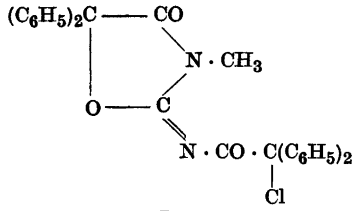
$$(C_6H_5)_2C(Cl)-COCl + CH_3CO-NH-CO-NH-CH_3 \longrightarrow$$

$$CH_3COCl + (C_6H_5)_2C(Cl)-CO-NH-CO-NH-CH_3$$

As it was of some interest to extend the investigations to methylurea, experiments were performed with this substance and diphenylchloroacetyl chloride both in the absence of and in the presence of pyridine. In the first case 5,5-diphenyloxazolidione was formed, apparently by the following mechanism.



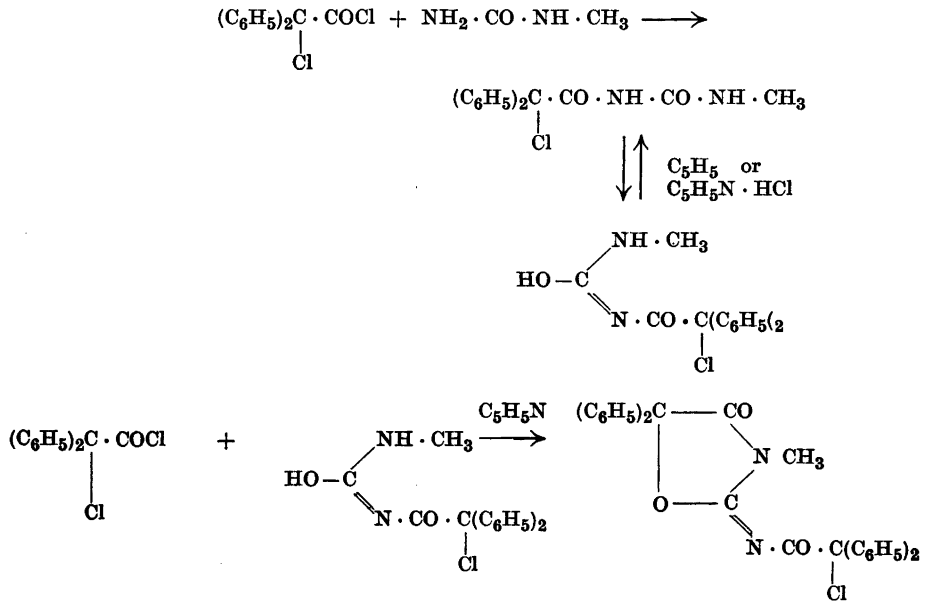
When the reaction was carried out in the presence of pyridine, a crystalline substance, $C_{30}H_{23}O_3N_2Cl$, was obtained, which was hydrolyzed to 3-methyl-5,5-diphenyloxazolidione by treatment with alcoholic hydrochloric acid. The initial product is therefore 2-(diphenylchloroacetylimino)-3-methyl-5,5-diphenyloxazolidone, I.



I

In addition to these substances, considerable quantities of resinous oils were obtained.

N-methyl-*N'*-phenylchloroacetylurea and *N*-methyl-*N'*-chloroacetylurea respectively are formed when phenylchloroacetyl chloride and chloroacetyl chloride are allowed to react with methylurea or acetylmethylurea [2], *i.e.* the reaction products are the same whether methylurea or its acetyl derivative is used. If diphenylchloroacetyl chloride reacts in the same manner the formation of 2-(diphenylchloroacetylimino)-3-methyl-5,5-diphenyloxazolidone could be explained as follows:



This, however, proved to be incorrect, because 5,5-diphenyloxazolidione and benzilic acid and not the acylated iminoöxazolidone were isolated when diphenylchloroacetyl chloride and *N*-methyl-*N'*-diphenylchloroacetylurea were heated together in the presence of pyridine.

Aspelund [2] showed that *N*-phenyl-*N'*-phenylchloroacetylurea is formed when phenylchloroacetyl chloride reacts with phenylurea, *i.e.* a derivative of type *A* is formed, but when Aspelund and Holmberg [1] treated diphenylchloroacetyl chloride with phenylurea they isolated 3,5,5-triphenylhydantoin and 3,5,5-triphenyloxazolidione, *i.e.* compounds of type B. A similar change in the point of attack of the acyl chlorine atom is observed in the reactions between

$-CO-NH-$	$-CO-\overset{\vert}{N}-R$	$-CO-\overset{\vert}{N}-CH_3$	$-CO-\overset{\vert}{N}-C_6H_5$
A	*B*	*C*	*D*

methylphenylurea on one hand and phenylchloroacetyl chloride, chloroacetyl chloride, and diphenylchloroacetyl chloride on the other. Chloroacetyl chloride and methylphenylurea give *N*-phenyl-*N'*-methyl-*N'*-chloroacetyl urea [2] while phenylchloroacetyl chloride and methylphenylurea give 2-phenylimino-3-methyl-5-phenyloxazolidone and 1,5-diphenyl-3-methylhydantoin [2]. All these reaction products contain the same group, *viz. C*. However, diphenylchloroacetyl chloride and methylphenylurea give 3,5,5-triphenyloxazolidione [1], containing the group *D*.

As, in the absence of pyridine, diphenylchloroacetyl chloride and methylurea give only small amounts of 5,5-diphenyloxazolidione (type *A*) it could be possible that methylurea represents a borderline case, and that the main product would be 2-imino-3-methyl-5,5-diphenyloxazolidone (type *B*). But if this is the case, 2-methylimino-5,5-diphenyloxazolidone must have polymerized or decomposed in the presence of pyridine. However, when *N*-methyl-*N'*-diphenyl-chloroacetylurea and a small quantity of pyridine are heated, a very good yield of 5,5-diphenyloxazolidione is obtained. This shows that the 2-methylimino derivative does not undergo any change under the conditions of the experiment.

In order to prove that the 2-methylimino and 2-imino derivatives are not formed simultaneously, diphenylchloroacetyl chloride and methylurea were allowed to react in the absence of pyridine. Unreacted methylurea was then removed and the heating was continued after the addition of diphenylchloroacetyl chloride and pyridine. If the 2-imino derivative had formed in the absence of pyridine it should have been possible to isolate the acylated imino compound from the reaction mixture, but only a very small amount of 5,5-diphenyloxazolidione was, however, obtained.

These two experiments show that pyridine or its hydrochloride has a directing influence on the reaction between diphenylchloroacetyl chloride and methylurea. It is difficult to decide its role in the reaction mechanism, but it seems possible that it forms a complex with either diphenylchloroacetyl chloride or methylurea, the complex then reacting with the other component. It is, however, evident that diphenylchloroacetyl chloride reacts with methylurea in the absence of pyridine in the same way as phenylchloroacetyl chloride and chloroacetylchloride, *i.e.* the acyl group becomes attached to the primary amino group of the urea.

EXPERIMENTAL

The reaction in the absence of pyridine

A mixture of methylurea (0.74 g) and diphenylchloroacetyl chloride (3.18 g, 1.20 equiv.) in dry benzene (25 ml) was heated for 8 hours on the boiling water bath. The following day the solvent was evaporated, and the residue was treated with a mixture of ether and water. The ether solution was separated and shaken with aqueous sodium bicarbonate for 45 minutes and then with 1 *N* sodium hydroxide solution (12 ml + 3 × 4 ml). After this treatment the ether solution yielded a tarry resinous oil (1.58 g) that did not crystallize.

The sodium bicarbonate solution was acidified, whereupon benzilic acid (0.16 g, m.p. 150°) separated.

The first sodium hydroxide extract was slowly acidified with 1 *N* hydrochloric acid and the material which separated when the solution was still slightly alkaline to litmus was extracted with ether and the material recovered from the ether solution was treated with benzene, yielding impure 5,5-diphenyloxazolidione (0.06 g, m.p. 129—130°). On further addition of hydrochloric acid to the aqueous solution an oil separated and this after working up as before gave more 5,5-diphenyloxazolidione (0.06 g, m.p. 129—131°).

The second alkaline extract gave 0.20 g of the same substance (m.p. 130—132°) after the solution had been made slightly acid to litmus. When the acidification was continued until the solution was acid to congo red, only 0.16 g of a resinous substance was isolated.

The two last extracts gave only small amounts of resinous substances after acidification.

The reaction in the presence of pyridine

A mixture of methylurea (0.74 g), diphenylchloroacetyl chloride (3.18 g, 1.20 equiv.), and dry pyridine (1.90 g) in dry benzene (20 ml) was heated on the boiling water bath for 8 hours. The benzene was distilled off and the residue was treated with water and ether. After some time crystals began to separate from the ether solution and at the interface. After three hours the crystals (1.01 g) were collected and purified by solution in benzene and precipitation with ethyl alcohol (m.p. 172—173°).

$C_{30}H_{23}O_3N_2Cl$	C	72.79	H	4.68	N	5.66	Cl 7.16
	»	72.65	»	4.67	»	5.74	» 7.29

This substance, 2-(diphenylchloroacetylimino-3-methyl-5,5-diphenyloxazolidone, is readly soluble in benzene but sparingly soluble in alcohol and ether.

The separated ether layer was shaken for two hours with 1 *N* sodium hydroxide (60 ml). The alkaline aqueous solution was acidified with hydrochloric acid and the oil that separated was dissolved in ether. The substance dissolved in this ether solution was completely extracted with solutions of sodium bicarbonate and sodium carbonate. When these solutions were acidified 0.18 g of acids with a melting range between 80 and 140° were isolated.

Evaporation of the ether solution that had been separated from the 60 ml quantity of 1 *N* sodium hydroxide yielded a tarry resinous oil (0.97 g). In order to hydrolyze any imino or acylated compounds the oil was boiled with a mixture of alcohol (50 ml) and conc. hydrochloric acid (20 ml) for one hour. The alcohol was distilled off and the remaining acid solution was extracted with ether, and the ether solution was shaken with dilute sodium hydroxide. Evaporation of the ether yielded a tarry resinous oil (0.90 g, probably unchanged material). When the alkaline aqueous solution was acidified, only a very small amount of a resinous oil separated.

Hydrolysis of the acylated iminoöxazolidone

2-(Diphenylchloroacetylimino)-3-methyl-5,5-diphenyloxazolidone (0.5 g) was boiled with a mixture of ethyl alcohol (50 ml) and conc. hydrochloric acid (20 ml) for 1.5 hours, *i.e.* half an hour after the substance had dissolved. The alcohol was evaporated *in vacuo* and the oil remaining was treated with ether and water. The ether phase was shaken with 1 *N* sodium hydroxide (10 ml) and evaporated giving an oil (0.48 g) from which 3-methyl-5,5-diphenyloxazolidione (0.20 g, m.p. 97—98°) was isolated by treatment with ethyl alcohol.

The acid aqueous solution was neutralized and the alkaline solution was acidified, but no material separated from either solution.

Attempts to prepare the acylated iminoöxazolidone in other ways

Experiment 1. A mixture of *N*-methyl-*N'*-diphenylchloroacetylurea (1.25 g), diphenylchloroacetyl chloride (1.10 g), and pyridine (0.9 g) dissolved in dry benzene (20 ml) was heated for three hours on the boiling water bath. The benzene was evaporated *in vacuo* and the residue was dissolved in ether and water to which a small quantity of conc. hydrochloric acid had been added. The mixture was set aside over night, but no crystals were deposited. The ether solution was shaken with dilute sodium hydroxide, and dilute hydrochloric acid was added to the latter until a small amount of an oil had separated. The oil (0.22 g) recovered by ether extraction did not crystallize. The addition of hydrochloric acid to the aqueous solution was continued until the latter was neutral to congo red. The oil which separated (1.40 g) was recovered with ether and then treated with benzene. The solid so obtained (0.96 g, m.p. 109—130°) was dissolved in dilute sodium hydroxide, and dilute hydrochloric acid was added to the mixture. When it was slightly alkaline to litmus 5,5-diphenyloxazolidione (0.04 g, m.p. 129—130°) separated. After further addition of hydrochloric acid another sample of the same substance (0.35 g, m.p. 133—133.5°) was obtained when the solution was acid to litmus but not to congo red. A mixture of substances (0.41 g, melting range 109—134°) separated finally when more hydrochloric acid was added. This last mixture was boiled with water and the clear hot

solution was decanted. From this solution slightly impure benzilic acid (0.29 g, m.p. 146—148°) crystallized.

Experiment 2. A mixture of methylurea (0.74 g) and diphenylchloroacetyl chloride (3.18 g) in dry benzene (20 ml) was heated on the boiling water bath for 8 hours. The next morning the clear solution was decanted from unreacted methylurea which is almost insoluble in benzene. Diphenylchloroacetyl chloride (2.00 g) and dry pyridine (4 ml) were added to the solution, and the mixture was heated for 8 hours on the boiling water bath. The solvent was evaporated, and the residue was treated with ether and water to which a small quantity of hydrochloric acid had been added. The solutions remained clear and the ether layer was extracted with dilute sodium hydroxide, then evaporated, yielding a tarry resinous oil (4.27 g) which did not crystallize. The aqueous solution was acidified and the oil that separated (0.72 g) was collected by ether extraction and dissolved in dilute sodium hydroxide. Dilute hydrochloric acid was gradually added to the solution. When an oil began to separate the solution was extracted with ether and in this way 0.05 g of resinous substance was removed. When the solution was neutral to litmus an oil (0.14 g) was obtained by ether extraction. This sample, however, crystallized after it had been treated with benzene, yielding 5,5-diphenyloxazolidione (m.p. 128—130°). The addition of hydrochloric acid was continued and when the solution was acid to litmus but not to congo red another sample of the same substance (0.20 g, m.p. 130—131°) was obtained by ether extraction and treatment with benzene. On further addition of hydrochloric acid only oil precipitated.

Ring closure of *N*-methyl-*N'*-diphenylchloroacetylurea

N-Methyl-*N'*-diphenylchloroacetyl urea (1.00 g) was dissolved in dry benzene (20 ml) and pyridine (0.9 g) was added. The mixture was heated on the boiling water bath for three hours. The benzene was evaporated, and the residue was dissolved in ether and water to which a small quantity of hydrochloric acid had been added. After some time the clear solutions were separated and the organic phase was shaken with dilute sodium hydroxide. After acidifying the alkaline solution, 5,5-diphenyloxazolidione (0.76 g, 91 per cent of the theoretical amount, m.p. 128—130°) separated.

Evaporation of the ether yielded oily material (0.03 g).

SUMMARY

The reaction between diphenylchloroacetyl chloride and methylurea has been studied both in the presence of and in the absence of pyridine. The reaction mechanisms have been discussed.

The author wishes to express his gratitude to Professor Helge Aspelund for kind advice and criticism in connection with this work.

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Aromatic Sulphinates of Divalent Selenium

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This article describes the syntheses and some properties of the selenium benzene- and *p*-toluenesulphonyl derivatives:

$$Se(SO_2R)_2; \qquad Se_2(SO_2R)_2; \qquad Se_3(SO_2R)_2$$

These represent new types of selenium compounds *. The sulphur analogues, *viz.*, the so-called bisphenyl- and bis-*p*-tolylsulphonyl sulphide, disulphide and trisulphide, are known [3,4] since 1870–91.

In reactions with nucleophilic reagents, the new compounds eliminate the sulphonyl groups as sulphinate anions. The di- and triselenium derivatives in some cases do so without break-down of the selenium groupings. The compounds thus behave as selenium, diselenium and triselenium disulphinates.

The crystals of the *p*-toluene compounds and selenium di(benzenesulphinate) are, according to X-ray data, isomorphous with those of the corresponding sulphur compounds.

The 'sulphide' nomenclature for the sulphur analogues is misleading, inasmuch as the 'sulphide' terminology usually refers to sulphur bearing an excess of electronegative charge, whereas the divalent sulphur of the sulphonyl compounds is electropositively polarized. The 'trisulphides', in reactions with nucleophilic reagents, behave [5,6] as thiosulphonates of divalent sulphur, S^{++}, and may conveniently be named as such, in analogy with the divalent selenium and tellurium thiosulphonates [6,7]. The 'disulphides' possess, as a prominent characteristic, relatively strong oxidizing properties, thus giving measurable equilibria with iodide-iodine [5,8,9]. These 'thiosulphonatogens' [8,9] are in this article termed disulphur disulphinates, and the 'sulphides' correspondingly, sulphur disulphinates. The last species behave as derivatives of divalent electropositive sulphur in reactions with sodium 2-naphtoxide [10].

* For preliminary accounts, see Ref. 1 and 2.

SELENIUM DISULPHINATES

The compounds arise when sodium benzene- or *p*-toluenesulphinate, suspended in an inert solvent, reacts with selenium oxychloride or selenium tetrachloride:

$$SeOCl_2 + 3\ RSO_2Na = Se(SO_2R)_2 + RSO_3Na + 2\ NaCl \quad (1)$$
$$SeCl_4 + 3\ RSO_2Na = Se(SO_2R)_2 + RSO_2Cl + 3\ NaCl \quad (2)$$

The crystals of the selenium disulphinates have a very pale greenish colour. When pure, the crystals are stable for months; in sunlight, or in an acid atmosphere, they liberate selenium fairly rapidly. The crystals decompose on heating; in preheated bath the benzenesulphinate melts at 132—133° C and the *p*-toluenesulphinate at 121—122° C to green liquids which ultimately become red. The compounds are insoluble in water, slightly soluble in warm ligroin, moderately soluble in warm ether, ethanol and acetic acid, and readily soluble in chloroform and in warm benzene and carbon tetrachloride.

The following reactions serve to characterize the disulphinates as derivatives of divalent electropositive selenium, Se^{++}. The nucleophilic reagents, sodium dimethyldithiocarbamate and sodium thiosulphate, act rapidly and completely to give selenium *bis*(dimethyldithiocarbamate) and selenopentathionate, respectively:

$$Se(SO_2R)_2 + 2\ Me_2NCS_2^- = Se(S_2CNMe_2)_2 + 2\ RSO_2^- \quad (3)$$
$$Se(SO_2R)_2 + 2\ S_2O_3^{--} = Se(S_2O_3)_2^{--} + 2\ RSO_2^- \quad (4)$$

Potassium *p*-toluenethiosulphonate reacts similarly, to displace the sulphinate groups:

$$Se(SO_2R)_2 + 2\ R'SO_2S^- \rightleftharpoons Se(S_2O_2R')_2 + 2\ RSO_2^- \quad (5)$$

The last change is comparable with the equilibrium of the inorganic analogue, *viz.*, selenotrithionate, with sodium thiosulphate [11]:

$$Se(SO_3)_2^{--} + 2\ S_2^*O_3^{--} \rightleftharpoons Se(S_2^*O_3)_2^{--} + 2\ SO_3^{--} \quad (6)$$

Eq. (5) may, likewise, represent an equilibrium, which however, proceeds to the right because of the low solubility of the product, selenium di(*p*-toluenethiosulphonate).

EXPERIMENTAL

Sodium benzene- and *p*-toluenesulphinate dihydrates, crystallized from water, were powdered, dried for two hours at 110° C, and finely powdered once more.

Ether and benzene, dried over sodium, were used as solvents for the preparations. The ligroin employed boiled at 60—90° C.

Selenium determinations were made gravimetrically by digesting with sulphuric acid--nitric acid, and precipitation of the selenium by means of hydrazine sulphate [12]. Removal

of the excess of nitric acid by means of urea and titration of the selenious acid according to Norris and Fay [13] does sometimes give unreliable results.

These details also apply to the experiments on di- and triselenium disulphinates described later in this article.

Selenium oxychloride. To 9 g (about 20 % excess) of sodium benzene- or *p*-toluenesulphinate, suspended in 50 ml of ether and cooled in ice, was added 1 ml of selenium oxychloride. The beaker was removed from the ice, and the contents were stirred with a glass rod for a few minutes. Then 50 ml of ether were added, and the mixture was heated, and filtered while warm. The pale greenish filtrate was evaporated to 10—20 ml on the water bath, and subsequently cooled in ice-sodium chloride. Yields of greenish crystals, about 3 g. Further, more impure crops were obtained by treatment of the sodium salt residue with hot chloroform, combining the filtrate with the ether mother liquor, and evaporation to dryness under reduced pressure.

For recrystallizations *, 1 g of $SeBs_2$ was dissolved in 15 ml of hot glacial acetic acid, and 1 g of $SeTs_2$ in 25 ml of hot 80 % acetic acid, and the solutions were allowed to cool slowly. Yields, about 70 % based on the crude products.

0.5178 g substance: 0.1136 g Se.

$Se(SO_2C_6H_5)_2$ (361.3) Calc. Se 21.9 Found Se 21.9.

0.6841 g substance: 0.1379 g Se.

$Se(SO_2C_7H_7)_2$ (389.3) Calc. Se 20.3. Found Se 20.2.

From the sodium salts residue of a selenium di(*p*-toluenesulphinate) preparation was isolated ** 1.9 g of sodium *p*-toluenesulphonate; theoretically, according to Eq. (1), 2.8 g.

Selenium tetrachloride. Equivalent amounts (Eq. 2) of sodium sulphinates and selenium tetrachloride were employed, since an excess of the salts may react [14] with the sulphonyl chloride formed, to give disulphones.

4.4 g of selenium tetrachloride and 9.8 g of sodium benzenesulphinate (or 10.7 g of sodium *p*-toluenesulphinate) in 40 ml of benzene were triturated for a few minutes at room temperature. The mixture was then heated rapidly, and filtered while hot, giving a greenish filtrate. The solid residue was treated with 20 ml of hot benzene, and filtered once more. The filtrates were combined and heated, and 60 ml of ligroin were added. On cooling, crystals of selenium disulphinates separated out; yields, 4—6 g. The crude products were recrystallized as described above.

From the mother liquors, on treatment with aqueous ammonia, were isolated benzene- and *p*-toluenesulphonamide, respectively, formed from the sulphonyl chlorides.

Sodium dimethyldithiocarbamate. 0.3625 g of $SeBs_2$ were dissolved in 15 ml of ethylacetate, and the solution was cooled in ice. 0.75 g (about 100 % excess) of sodium dimethyldithiocarbamate, dissolved in 15 ml of methanol, were added while stirring. A greenish solid immediately separated out. The product was filtered off, washed with

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* The symbols Bs and Ts are sometimes used to designate the benzenesulphonyl and *p*-toluenesulphonyl group, respectively.

** Excess of sulphinate was precipitated by means of ferric chloride, and the excess of ferric iron, after filtering, was removed as hydroxide by means of ammonia. To the filtrate was added sodium hydroxide, the ammonia was expelled by boiling, and the solution was subsequently neutralized by means of hydrochloric acid and evaporated to dryness. The residue was extracted with boiling abs. ethanol, and the solvent evaporated off. The product was dried for one hour at 110° C, and identified as sulphonyl chloride and amide.

water and methanol, and dried *in vacuo* over sulphuric acid. Yield, 0.3145 g (98 %) of selenium *bis*(dimethyldithiocarbamate), identified by melting point and mixed melting point with an authentic sample [15].

An analogous experiment with $SeTs_2$ gave a yield of 96 % of the theoretical amount of the same product.

Sodium thiosulphate. 0.1590 g of $SeTs_2$ were dissolved in 10 ml of chloroform in a 100 ml volumetric flask, and 10 ml of 0.1037 *N* sodium thiosulphate were added. On swirling, the pale greenish colour of the chloroform layer passed rapidly into the aqueous layer. After shaking for one minute, the volume was made up to 100 ml with water, and 25 ml of the aqueous layer (total volume, 90 ml) were pipetted out. 1 g of potassium iodide and 5 ml of 10 % acetic acid were added to the sample, and the excess of thiosulphate was titrated with 0.01130 *N* iodine: 7.3 ml, corresponding to a comsumption of 0.740 millimoles of sodium thiosulphate; theoretically, 0.817 millimoles. However, in thiosulphate titrations sulphinate ions are partly oxidized by iodine, even in presence of potassium iodide [5]. The chloroform layer was devoid of selenium compounds, which indicates a quantitative change. The aqueous layer gave, with sodium dimethyldithiocarbamate and with alkalies, qualitative reactions on selenopentathionate.

Potassium p-*toluenethiosulphonate.* About 1 millimole of selenium disulphinate and 4 millimoles (about 100 % excess) of potassium *p*-toluenethiosulphonate hydrate were dissolved in 5 ml of chloroform and 5 ml of water, respectively. To a two-layer mixture of the two solutions, cooled in ice, were added 20 ml of methanol while stirring. A homogeneous solution resulted, from which crystals of selenium *p*-toluenethiosulphonate, $Se(STs)_2$, rapidly began to separate out. After standing for 5 minutes in ice, the product was filtered off, washed with water, and dried *in vacuo* over sulphuric acid. The crystals sometimes had a faint reddish colour due to a slight catalytic decomposition of the selenium compounds.

0.3760 g of $SeBs_2$ gave 0.3770 g of $Se(STs)_2$ (80 %) and 0.4034 g of $SeTs_2$ gave 0.4028 g of $Se(STs)_2$ (86 %). The crude products consisted of tiny tetragonal bipyramides and decomposed at about 180° C. Recrystallization from chloroform as described in a following article [7] gave in both cases about 75 % yields of pale greenish bipyramides, decomposing at about 200° C and containing 17.4 % and 17.2 % of Se, respectively; calc. for $Se(STs)_2$, 17.4 %.

DISELENIUM AND TRISELENIUM DISULPHINATES

The compounds were prepared from diselenium dichloride, by reactions with an excess of sodium benzene- or *p*-toluenesulphinate suspended in benzene or ether. The course of the process is dependent upon the nature of the solvent. In benzene, diselenium disulphinates are the major products:

$$Se_2Cl_2 + 2\ RSO_2Na = Se_2(SO_2R)_2 + 2\ NaCl \qquad (7)$$

whereas in ether, selenium and triselenium disulphinates result almost exclusively:

$$2\ Se_2Cl_2 + 4\ RSO_2Na = Se(SO_2R)_2 + Se_3(SO_2R)_2 + 4\ NaCl \qquad (8)$$

Ethanol-free chloroform, according to one single experiment with sodium *p*-toluenesulphinate, gave equal amounts of di- and triselenium disulphinates,

whereas carbon tetrachloride was like benzene, causing only a slight degree of rearrangement.

The disulphinates arise from diselenium dichloride through nucleophilic displacements of the chloride by sulphinate groups. No attempts have been made to establish the mechanism by which selenium and triselenium disulphinates result instead of diselenium disulphinates. It appears, however, that the different course is not due to a rearrangement of primarily formed diselenium disulphinate, since diselenium di(*p*-toluenesulphinate) is relatively stable when heated in ether or carbon tetrachloride. Catalysts may, though, play a role. Nor is it likely that diselenium dichloride, in ether and partly in chloroform, rearranges into selenium and triselenium dichloride, judging from the fact that diselenium dichloride [16] and dibromide [17] with organo-magnesium reagents in ether give rise to organic diselenides.

The analogous reactions of disulphur dichloride with sodium benzene- and *p*-toluenesulphinate in carbon tetrachloride lead to disulphur disulphinates beside small amounts of rearrangement products [18]. In presence of water, sodium benzenesulphinate gave the tri- and pentathionic compounds and no disulphur disulphinate. Rearrangements of this type take place in various reactions of disulphur dichloride. Thus, silver cyanide reacts [19] to give a mixture of sulphur dicyanide and sulphur di(thiocyanate). Likewise [20], diselenium dibromide, with silver cyanide in ether, gives selenium dicyanide and selenium di(selenocyanate). Possible mechanisms have been discussed by Lecher and Goebel [21] and Kaufmann and Kögler [20].

The di- and triselenium disulphinates form pale greenish crystals having only a slight yellow tinge. The green colour deepens slightly with increasing number of selenium atoms, from selenium to triselenium disulphinates. When pure, and kept in a dry, neutral atmosphere, shielded from light, the crystals remain unchanged for months. The compounds liberate selenium when heated, and so the melting points depend upon the rate of heating. In preheated bath, diselenium di(benzenesulphinate) and di(*p*-toluenesulphinate) melt at 79—80° C and 104—106° C, respectively, to green liquids which become red after about five minutes and half a minute. Likewise, triselenium di(benzenesulphinate) melts and decomposes rapidly at 101—102° C, and triselenium di(*p*-toluenesulphinate) decomposes at 136—140° C. The last compound is the least soluble (1 g dissolves in about 30 ml of chloroform at boiling temperature). The diselenium disulphinates and triselenium di(benzenesulphinate) are readily soluble in chloroform and relatively so in boiling benzene. They are appreciably soluble in ethanol and methanol, and insoluble in ligroin and water.

When heated in solutions, the diselenium disulphinates rearrange slowly into selenium and triselenium disulphinates, in analogy with the behaviour of the sulphur analogoues:

$$2\ Se_2(SO_2R)_2 = Se(SO_2R)_2 + Se_3(SO_2R)_2 \qquad (9)$$

The compounds are, though, relatively stable. Thus, 2 g of diselenium di(*p*-toluenesulphinate), dissolved in 50 ml of carbon tetrachloride and kept at boiling temperature of the solvent for one hour, rearranged to an extent of about 10 %. Disulphur di(*p*-toluenesulphinate), at the same molar concentration in carbon tetrachloride and treated in the same way, was recovered unchanged, and so was the diselenium compound from a saturated solution in warm ether (1.5 g per 100 ml) after 15 minutes' heating under reflux. In boiling acetic acid, according to Otto and Troeger [4], disulphur di(*p*-toluenesulphinate) undergoes rearrangement, though less readily than does the benzene compound.

Similar rearrangements are those of thiocyanogen and selenocyanogen [20], and of the tetrathionate ion to give trithionate and pentathionate.

Solutions of the di- and triselenium disulphinates in chloroform, on treatment with aqueous potassium ethylxanthate, acquire a deeper green colour, and two moles of ethylxanthate are consumed per mole of disulphinate. No selenium is set free during the process. The chloroform solutions of the new compounds are not stable, and so no attempts have been made to isolate the products. The reactions can hardly be anything else than nucleophilic displacements, as follows:

$$Se_2(SO_2R)_2 + 2\ C_2H_5OCS_2^- = Se_2(S_2COC_2H_5)_2 + 2\ RSO_2^- \quad (10)$$

$$Se_3(SO_2R)_2 + 2\ C_2H_5OCS_2^- = Se_3(S_2COC_2H_5)_2 + 2\ RSO_2^- \quad (11)$$

The sulphonyl groups are eliminated as sulphinate ions, the di- and triselenium groupings thereby remaining intact.

More strikingly, triselenium di(benzenesulphinate) reacts with sodium *p*-toluenesulphinate to give triselenium di(*p*-toluenesulphinate). The product is less soluble and separates as crystals, thus displacing a possible equilibrium completely to the right:

$$Se_3(SO_2R)_2 + 2\ R'SO_2^- = Se_3(SO_2R')_2 + 2\ RSO_2^- \quad (12)$$

Corresponding experiments with the diselenium disulphinates were not made, since there is no appreciable solubility difference between the benzene and *p*-toluene compounds.

With aqueous sodium hydroxide, the di- and triselenium disulphinates in chloroform-ethanol solutions are rapidly and completely hydrolyzed. The net reactions are:

$$2\ Se_2(SO_2R)_2 + 6\ OH^- = 3\ Se + SeO_3^{--} + 4\ RSO_2^- + 3\ H_2O \quad (13)$$

$$2\ Se_3(SO_2R)_2 + 6\ OH^- = 5\ Se + SeO_3^{--} + 4\ RSO_2^- + 3\ H_2O \quad (14)$$

In view of the sodium *p*-toluenesulphinate and potassium ethylxanthate reactions (Eqs. 10—12) it appears reasonable to assume that the first steps are displacements of sulphinate by hydroxide, to give unstable di- and triselenium dihydroxides as intermediates:

$$Se_2(SO_2R)_2 + 2\ OH^- = Se_2(OH)_2 + 2\ RSO_2^- \quad (15)$$
$$Se_3(SO_2R)_2 + 2\ OH^- = Se_3(OH)_2 + 2\ RSO_2^- \quad (16)$$

The subsequent rearrangements would be:

$$2\ Se_2(OH)_2 + 2\ OH^- = 3\ Se + SeO_3^{--} + 3\ H_2O \quad (17)$$
$$2\ Se_3(OH)_2 + 2\ OH^- = 5\ Se + SeO_3^{--} + 3\ H_2O \quad (18)$$

The diselenium dihalides hydrolyze in an analogous way, whereas hydrolysis reactions of derivatives of Se_3^{++} have not been studied before, the triselenium disulphinates being the first representatives of this type.

The fact that the latter compounds behave as triselenium disulphinates, $Se_3(SO_2R)_2$, and not as selenium selenosulphonates, $Se(SeSO_2R)_2$, whereas the corresponding sulphur compounds react as sulphur thiosulphonates, $S(S_2O_2R)_2$, is in accordance with the existence of thiosulphonate and non-existence of selenosulphonate ions. Selenocyanate ions are relatively stable, and so the cyanide analogue, $Se(SeCN)_2$, behaves as a selenium di(selenocyanate)[22].

EXPERIMENTAL

Diselenium disulphinates. The following proportions of reagents and solvent were employed: 10 g of sodium benzenesulphinate in 20 ml of benzene, or 12 g of sodium *p*-toluenesulphinate in 40 ml of benzene, 2 ml of diselenium dichloride. The suspensions were cooled in ice, and the dichloride was added from a pipette while stirring. The beaker was subsequently removed from the ice, and the contents were triturated for a few minutes, until the diselenium dichloride colour disappeared. The mixture was then heated rapidly, and the sodium chloride and the excess of sodium sulphinate were filtered off, giving a greenish yellow filtrate. The sodium salt residue was treated with 10 ml of hot benzene, filtered once more, and the filtrates were combined *. 40—50 ml of ligroin were added to the mixture, and the beaker was placed in ice. The resulting, pale greenish crystals of diselenium disulphinate were filtered off, washed with ligroin and small amounts of ether, and dried *in vacuo* over sulphuric acid. Yields, 5—7 g.

The recrystallizations were made as follows.

* The *p*-toluenesulphinate solutions, on standing, deposited 0.5—1.0 g of the less soluble triselenium disulphinate. These crystals may be filtered off at this stage, or later at the recrystallization.

2 g of Se_2Bs_2 were dissolved in 3 ml of chloroform at room temperature. 10 ml of benzene and 10 ml of ligroin were added, and the mixture was cooled, ultimately in ice-sodium chloride. Yield, 1.3—1.5 g of pale green prisms.

2 g of Se_2Ts_2 were dissolved in 5 ml of chloroform at room temperature, and 5 ml of ether were added. The triselenium disulphinate, if present, is slightly soluble in this mixture, and may be filtered off, whereas the diselenium disulphinate is soluble. 10 ml of ether were added to the filtrate, and the beaker was ultimately placed in ice-sodium chloride; yield, as above.

0.3413 g substance: 0.1229 g Se.

$Se_2(SO_2C_6H_5)_2$ (440.3) Calc. Se 35.9. Found Se 36.0.

0.3206 g substance: 0.1086 g Se.

$Se_2(SO_2C_7H_7)_2$ (468.3) Calc. Se 33.7. Found Se 33.9.

Triselenium disulphinates. To 10 g of sodium benzenesulphinate (or 12 g of sodium *p*-toluenesulphinate) suspended in 50 ml of ether and cooled in ice, were added 2 ml of diselenium dichloride while stirring. The beaker was removed from the ice, and the solid particles were treated with a glass rod for a few minutes, until the diselenium dichloride colour vanished from the liquid phase. The insoluble substance, which had a faint reddish colour, was filtered off, treated with 20 ml of ether, drained well, and dried *in vacuo* over sulphuric acid. The solid was subsequently treated repeatedly with water to remove the sodium salts, drained, and dried as above.

(1) Benzenesulphinate. The yield was about 3 g. A further crop of about 3 g was obtained from the ether filtrate on evaporation on the water bath to 30—40 ml, and cooling in ice-sodium chloride. Uusually, a reddish oil first separated out, which on stirring changed to greenish yellow crystals.

The crude product was recrystallized by dissolving 3 g in 15—20 ml of hot benzene, filtering, if necessary, and addition of 10—15 ml of ligroin to the filtrate while still hot. On cooling, triselenium di(benzenesulphinate) separated as yellowish green plates. Yield, 2.0—2.3 g. Better developed crystals were obtained by slow crystallization from chloroform-ether.

(2) *p*-Toluenesulphinate. The residue from the water treatment was extracted with two times 10 ml of chloroform, drained, and dried once more. Yield, about 8 g. The substance was dissolved in boiling chloroform (30 ml per g) and the solution filtered. About two thirds of the chloroform were subsequently boiled off, and the solution was then allowed to cool to room temperature. Yield, about 4 g of triselenium di(*p*-toluenesulphinate).

0.2867 g substance: 0.1303 g Se.

$Se_3(SO_2C_6H_5)_2$ (519.2) Calc. Se 45.6. Found Se 45.4.

0.2806 g substance: 0.1215 g Se.

$Se_3(SO_2C_7H_7)_2$ (547.3) Calc. Se 43.3. Found Se 43.3.

The ether filtrate from a triselenium di(*p*-toluenesulphinate) preparation, on partial evaporation and addition of ligroin, gave 1.5 g of almost colourless crystals which contained 24.2 % of Se. The substance was treated with boiling ligroin, and the extract, on cooling, gave long, thin needles of selenium disulphinate, $SeTs_2$, identified by analysis (found, 20.5 % of Se) and by an X-ray oscillation photograph around the needle axis.

Potassium ethylxanthate. About 0.8 millimoles of di- or triselenium disulphinate were dissolved in 20 ml of chloroform, and the solution was cooled in ice. 50 ml of 0.04 *M* potassium ethylxanthate (5 ml of which consumed 19.64 ml of 0.01130 *N* iodine) were

added, and the two-layer mixture was stirred for two minutes under continued cooling, the chloroform layer thereby acquiring a deeper green colour. 5 ml of the aqueous layer were subsequently pipetted out, and the excess of potassium ethylxanthate was titrated with 0.01130 *N* iodine after addition of 1 g of potassium iodide and 50 ml of water.

0.3884 g (0.8294 millimoles) of Se_2Ts_2: 5.27 ml of 0.01130 *N* iodine. Amount of potassium ethylxanthate thus consumed by the disulphinate: 1.624 millimoles, *i. e.*, 97.9 % of theory (Eq. 10).

0.4410 g (0.8492 millimoles) of Se_3Bs_2: 4.76 ml of 0.01130 *N* iodine. Amount of potassium ethylxanthate thus consumed by the disulphinate: 1.682 millimoles, *i. e.*, 99.0 % of theory (Eq. 11).

After 15 minutes, the chloroform layer from the Se_2Ts_2 experiment had become faintly yellow, while that from the Se_3Bs_2 experiment was reddish and had deposited small amounts (8 mg) of selenium. These chloroform solutions of, supposedly, di- and triselenium di(ethylxanthate) gave, with sodium dimethyldithiocarbamate in methanol, precipitates of selenium and selenium *bis*(dimethyldithiocarbamate). The aqueous layers, after destruction of the excess of potassium ethylxanthate by means of hydrochloric acid, gave a red colour with ferric chloride and thus contained sulphinate ions.

Sodium p-*toluenesulphinate.* A two-layer mixture of 0.5583 g of Se_3Bs_2 in 5 ml of chloroform, and 0.85 g (about 100 % excess) of sodium *p*-toluenesulphinate dihydrate in 5 ml of water, was cooled in ice, and 20 ml of methanol were added while stirring. A homogeneous solution resulted, from which pale greenish crystals rapidly began to separate out; however, a reddish colour soon appeared, indicating a slight degree of decomposition. After standing for five minutes in the ice the product was filtered off, washed with water and dried *in vacuo* over sulphuric acid. Yield, 0.5400 g, *i. e.*, 91.9 % of theory (Eq. 12). The substance consisted, when viewed in a microscope, of tetragonal bipyramides, having a greenish colour with a faint reddish tinge. The crude product was dissolved in 15 ml of boiling chloroform, and the solution was filtered, leaving 6 mg of selenium on the filter. The greenish filtrate, on evaporation and cooling, gave 0.40 g of Se_3Ts_2 crystals, decomposing at 135—140° C and containing, by analysis, 43.2 % of Se.

Hydrolysis reactions. About 1 millimole of di- or triselenium disulphinate was dissolved in 5 ml of chloroform and 25 ml of ethanol, and 5 ml of 1 *M* sodium hydroxide were added. Selenium immediately separated out, and was after five minutes filtered off, washed with water, methanol and ether, dried *in vacuo* over sulphuric acid, and weighed. The filtrate was evaporated to dryness on the water bath. The residue was, in order to destroy the sodium sulphinate, treated with nitric acid, and the selenium was finally precipitated by means of hydrazine sulphate, dried and weighed.

0.4430 g (1.006 millimole) of Se_2Bs_2 gave 0.1185 g of Se and, from the filtrate, 0.0388 g of Se, corresponding to 1.49 and 0.49 g atoms of Se, respectively, per mole of diselenium disulphinate.

0.5536 g (1.066 millimole) of Se_3Bs_2 gave 0.2126 g of Se and, from the filtrate, 0.0399 g of Se, corresponding to 2.53 and 0.47 g atoms of Se, respectively, per mole of triselenium disulphinate.

Non-existence of selenosulphonate ions. 10 g of sodium *p*-toluenesulphinate dihydrate were dissolved in 10 ml of water. 4 g of freshly precipitated, red selenium, moistened with ethanol, were added, and the mixture was heated under reflux for one hour. 10 ml of water were subsequently added, and the mixture was filtered. 1 ml of the colourless filtrate, diluted to 10 ml with water and 1 ml of concentrated hydrochloric acid added, remained colourless and slowly deposited crystals of *p*-toluenesulphinic acid.

Disulphur di(p-toluenesulphinate), S_2Ts_2, has previously been prepared from aqueous potassium *p*-toluenethiosulphonate and iodine [4], and from sodium *p*-toluenesulphinate, suspended in carbon tetrachloride, and disulphur dichloride [18]. For the rearrangement experiment and X-ray measurements of the present work, the compound was obtained from potassium *p*-toluenethiosulphonate and bromine, as follows.

A suspension of 10 g (about 20 % excess) of dry, powdered potassium *p*-toluenethiosulphonate in 25 ml of carbon tetrachloride was cooled in ice, and 1 ml of bromine was added drop-wise with stirring. The bromine colour disappeared rapidly, and the mixture was heated and filtered. The residue was treated with 25 ml of boiling chloroform, filtered once more, and the filtrates were combined. On cooling in ice, 0.8 g of sulphur di(*p*-toluenethiosulphonate), $S(STs)_2$, separated out and was filtered off. The filtrate was evaporated under reduced pressure to a volume of about 20 ml, then 40 ml of ligroin were added, and the mixture was cooled in ice. Yield, 5.0 g. 4 g of the crude product were dissolved in 6 ml of chloroform, small amounts of $S(STs)_2$ were filtered off, and 15 ml of ligroin were added to the filtrate. Yield, 2.5 g of S_2Ts_2. The substance melted at 114° C as reported by Christiansen [23].

THE STRUCTURE OF THE SELENIUM AND DISELENIUM DISULPHINATES

A search was made for a possible isomorphism relationship between the crystals of the selenium, diselenium and triselenium disulphinates and the parent sulphur compounds. These are, as mentioned before, the sulphur and disulphur disulphinates, $S(SO_2R)_2$ and $S_2(SO_2R)_2$, and the sulphur thiosulphonates, $S(S_2O_2R)_2$. For reasons of analogy with the corresponding thiosulphonates of divalent selenium and tellurium, $Se(S_2O_2R)_2$ and $Te(S_2O_2R)_2$, crystallographic data on the triselenium disulphinates will be reported in a subsequent article [7], the present discussion being concerned with the selenium and diselenium compounds only.

X-ray measurements (oscillation and Weissenberg photographs) were carried out using FeK_α radiation, $\lambda = 1.934$ Å, and in some cases CuK_α radiation, $\lambda = 1.54$ Å. Density determinations were made by flotation in suitable solvents.

The unit cells and space group of the sulphur disulphinates, SBs_2 and STs_2, and a detailed structure analysis of the former substance, have been reported recently by Robertson and co-workers [24,25]. The crystals are monoclinic prismatic, with the unit cell dimensions: SBs_2, $a = 15.88$ Å, $b = 5.52$ Å, $c = 15.88$ Å, $\beta = 112.9°$. STs_2, $a = 16.50$ Å, $b = 5.85$ Å, $c = 18.88$ Å, $\beta = 119.9°$. There are four molecules per unit cell. Absent reflections, (hkl) when $k + l$ is odd, $(h0l)$ when h is odd or when l is odd. These absences are characteristic for the space groups C_s^4-Aa and C_{2h}^6-A2/a, of which the latter, from studies of the intensities, was found to be the correct one. The molecules possess a twofold axis of symmetry parallel to the b crystal axis.

The crystals of the selenium disulphinates, $SeBs_2$ and $SeTs_2$, appear as flat prisms or long needles, elongated along the b axis. They are isomorphous with those of the sulphur disulphinates.

Table 1. Unit cells of selenium di(benzenesulphinate) and di(p-toluenesulphinate).

	$a \pm 0.06$ Å	$b \pm 0.02$ Å	$c \pm 0.06$ Å	β	Density calc.	Density found
$SeBs_2$	16.14	5.60	15.84	112°	1.81	1.79
$SeTs_2$	16.81	5.76	19.07	119°	1.61	1.60

There are four molecules per unit cell. The absent reflections, for both compounds, are the same as for the sulphur disulphinates, referred to above. Analogy with the latter compounds indicates the space group C_{2h}^6-A2/a, and this has been made probable by a comparison of the intensities of the $(h0l)$ zones of sulphur and selenium di(benzenesulphinate).

The crystals of disulphur and diselenium di(benzenesulphinate) are not mutually isomorphous, nor is any of the crystals isomorphous with those of disulphur and diselenium di(p-toluenesulphinate). The latter compounds, however, are mutually isomorphous, which shows that they have analogous structures. Since there are no chemical indications of a structural difference between the benzene and p-toluene compounds, it may be inferred that disulphur and diselenium di(benzenesulphinate) possess the same structure.

The isomorphous p-toluene compounds, S_2Ts_2 and Se_2Ts_2, crystallize as flat prisms, elongated along the c axis and flattened along the b axis.

Table 2. Unit cells of disulphur and diselenium di(p-toluenesulphinate).

	$a \pm 0.04$ Å	$b \pm 0.03$ Å	$c \pm 0.03$ Å	β	Density calc.	Density found
S_2Ts_2	9.96	15.07	11.24	95°	1.48	1.46
Se_2Ts_2	10.15	15.48	11.26	94°	1.76	1.76

There are four molecules per unit cell. Absent reflections, $(h0l)$ when l is odd, $(0k0)$ when k is odd. Space group, for both compounds, $C_{2h}^5-P2_1/c$.

No structure analysis, by X-ray or other physical methods, is available for the aromatic disulphur disulphinates. However, the methane analogue [9], $S_2(SO_2CH_3)_2$, possesses an unbranched sulphur chain structure [26]. The chemical evidence indicates structural analogy between the aliphatic and the aromatic

disulphur disulphinates. Thus, the compounds arise from the sodium or potassium thiosulphonates by reactions with bromine:

$$2\ CH_3SO_2SNa + Br_2 = S_2(SO_2CH_3)_2 + 2\ NaCl \quad (19)$$
$$2\ ArSO_2SK + Br_2 = S_2(SO_2Ar)_2 + 2\ KCl \quad (20)$$

and give reversible equilibria with iodide-iodine [5,8,9]:

$$S_2(SO_2CH_3)_2 + 3\ J^- \rightleftharpoons 2\ CH_3SO_2S^- + J_3^- \quad (21)$$
$$S_2(SO_2Ar)_2 + 3\ J^- \rightleftharpoons 2\ ArSO_2S^- + J_3^- \quad (22)$$

The evidence, therefore, points towards the presence of unbranched S—Se—Se—S chains in the diselenium disulphinates.

Isomorphism of disulphur and diselenium compounds has been reported in earlier cases, *e. g.*, for *bis*(diacetylmethyl) disulphide and diselenide [27], and diphenyl and dibenzyl disulphide and diselenide [28].

SUMMARY

Selenium, diselenium and triselenium di(benzenesulphinate) and di(*p*-toluenesulphinate) have been prepared for the first time. Reactions are demonstrated, in which the compounds behave as derivatives of Se^{++}, Se_2^{++} and Se_3^{++}, respectively.

The structure of the selenium and diselenium disulphinates have been discussed on the basis of isomorphism of the crystals with those of the corresponding sulphur compounds.

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Isomorphous Series of Pentathionic Compounds

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In a previous article [1], a new type of pentathionic compounds was described, *viz.*, triselenium disulphinates. The representatives investigated were those derived from benzene and *p*-toluene. The triselenium disulphinates arise from the corresponding thiosulphonates of divalent sulphur [2–4] by substitution of three selenium atoms for sulphur atoms. One purpose of the present article is to describe benzene- and *p*-toluenethiosulphonates of divalent selenium and tellurium, so as to complete a four-membered series of aromatic pentathionic compounds.

Within the series, the crystals of the *p*-toluene compounds are mutually isomorphous, and the compounds thus possess analogous structures.

The thiosulphates of divalent sulphur, selenium and tellurium, *viz.*, the pentathionate, selenopentathionate and telluropentathionate anions, give isomorphous salts [5], and the methanethiosulphonates of the same divalent elements are, likewise, mutually isomorphous [6]. We thus have the following three isomorphous series of pentathionic compounds.

Alkali salts with the anions:	*p*-Toluenethiosulphonates, and triselenium di(*p*-toluenesulphinate):	Methane-thiosulphonates:
$S(S_2O_3)_2^{--}$	$S(S_2O_2R)_2$	$S(S_2O_2CH_3)_2$
$Se(S_2O_3)_2^{--}$	$Se(S_2O_2R)_2$	$Se(S_2O_2CH_3)_2$
$Te(S_2O_3)_2^{--}$	$Te(S_2O_2R)_2$	$Te(S_2O_2CH_3)_2$
	$Se_3(SO_2R)_2$	

The chemical properties of members of the three series are closely analogous, and furthermore, the series are interconvertible in the order of increasing nucleophilic reactivities of the electronegative components. Thus, the methanethiosulphonates react with *p*-toluenethiosulphonate and thiosulphate anions to

give the corresponding members of the *p*-toluenethiosulphonate and thiosulphate series. The chemical evidence therefore strongly indicates that the three series possess the same type of structure.

BENZENE- AND *p*-TOLUENETHIOSULPHONATES OF DIVALENT SELENIUM AND TELLURIUM *

The selenium compounds result when diselenium dichloride reacts with an excess of the potassium thiosulphonates suspended in ether. Half of the selenium is set free:

$$2\ Se_2Cl_2 + 2\ RSO_2SK = Se(S_2O_2R)_2 + Se + 2\ KCl \qquad (1)$$

The same method was recently [6] employed for the preparation of selenium di(methanethiosulphonate).

The tellurium compounds were obtained from the potassium thiosulphonates, suspended in ethanol-free chloroform, by reactions with tellurium tetrachloride:

$$TeCl_4 + 4\ RSO_2SK = Te(S_2O_2R)_2 + S_2(SO_2R)_2 + 4\ KCl \qquad (2)$$

Here, the corresponding disulphur disulphinate is formed in an equimolar amount. The procedure used for the methane analogue [6], *viz.*, tellurium dioxide dissolved in hydrochloric acid and aqueous sodium or potassium thiosulphonate, gives the same products. However, this method is less suitable for the aromatic thiosulphonates, because the disulphur disulphinates under aqueous conditions undergo rearrangement, and a mixture of sulphur and tellurium thiosulphonates results. This mixture is, especially in the case of the *p*-toluene compounds, difficult to separate into the components. By the tellurium tetrachloride-chloroform method, on filtering, the disulphur disulphinate occurs in the filtrate and most of the tellurium thiosulphonate in the residue.

The crystals of the selenium benzene- and *p*-toluenethiosulphonates are pale greenish. Those of the tellurium compounds are yellow, and so are dilute solutions, whereas more concentrated solutions have an orange tinge. The crystals are relatively stable, like those of the methanethiosulphonates [6].

The compounds ** liberate selenium or tellurium on heating, $Se(SBs)_2$ being more stable in this respect. In preheated bath, $Se(SBs)_2$ melts at 154 —155° C to a greenish liquid which becomes red after a few minutes, while $Se(STs)_2$ melts and decomposes at about 200° C. The tellurium compounds,

* For a preliminary account, see Ref. 7.

** The symbols Bs and Ts are sometimes used to designate the benzenesulphonyl and *p*-toluenesulphonyl group, respectively.

$Te(SBs)_2$ and $Te(STs)_2$, melt at about 170° C and about 215° C, respectively, to red liquids which liberate tellurium after a few seconds.

The benzene compounds are more soluble than the *p*-toluene compounds, $Se(SBs)_2$ being fairly readily and $Te(SBs)_2$ moderately soluble in warm ethylacetate, benzene and chloroform. The solubilities of the four isomorphous *p*-toluene derivatives, Se_3Ts_2, $S(STs)_2$, $Se(STs)_2$ and $Te(STs)_2$, decrease in the order of mentioning. 1 g of $Se(STs)_2$ and $Te(STs)_2$ dissolve in about 120 ml and about 160 ml, respectively, of boiling chloroform, the hot solutions being quite stable. The compounds are insoluble in water.

EXPERIMENTAL

Potassium benzene- and *p*-toluenethiosulphonate were crystallized from 96 % ethanol and from water, respectively. The salts were dried for two hours at 110° C, and subsequently powdered finely.

Selenium compounds. To 14 g of potassium benzenethiosulphonate, or 15 g of potassium *p*-toluenethiosulphonate, suspended in 50 ml of dry ether and cooled in ice, were added 2 ml of diselenium dichloride. On stirring, the dichloride colour vanished rapidly from the liquid phase, and selenium was liberated on the solid substance. The mixture was filtered, and the residue on the filter was dried *in vacuo* over sulphuric acid, while the filtrate was discarded. The residue was subsequently treated several times with water, drained, and dried once more.

The benzenethiosulphonate residue (about 10 g) was heated with 100 ml of ethylacetate, elemental selenium was filtered off, and the pale green filtrate was cooled in ice. Yield, 5—6 g of selenium di(benzenethiosulphonate).

The *p*-toluenethiosulphonate residue (about 12 g) was treated with boiling chloroform (100—120 ml per g) and the mixture was filtered. About three fourths of the chloroform were subsequently boiled off, until crystals began to separate out, and the solution was then allowed to cool slowly. Yield of selenium di(*p*-toluenethiosulphonate), about 50 % based on the crude product. Larger crystals were obtained by addition of an equal volume of carbon tetrachloride to the concentrated chloroform solution before cooling.

0.5392 g substance: 0.1008 g Se.

$Se(S_2O_2C_6H_5)_2$ (425.4) Calc. Se 18.6. Found Se 18.7.

0.6522 g substance: 0.1143 g Se.

$Se(S_2O_2C_7H_7)_2$ (453.5) Calc. Se 17.4. Found Se 17.5.

Tellurium compounds. The chloroform employed for the tellurium tetrachloride reactions was freed from ethanol by treatment, successively, with water, concentrated sulphuric acid, water, and anhydrous sodium sulphate, and was dried by distillation over phosphorus pentoxide and stabilized by addition of two parts per thousand of ligroin [8]. Ordinary, ethanol-stabilized chloroform was used for recrystallizations.

5.4 g of tellurium tetrachloride were suspended in 30 ml of chloroform and cooled in ice. To this mixture were added, in portions while stirring, 20 g of potassium benzenethiosulphonate, and the stirring and cooling were continued for 10 minutes. The yellow suspension was then filtered while cold, drained well, and the filtrate was discarded. The residue on the filter was treated with ice-cold chloroform (2 times 10 ml) and with 10 ml of ether, and was dried *in vacuo* over sulphuric acid. The substance was subsequently

treated with water (3 times 30 ml), methanol (2 times 5 ml) and ether (2 times 5 ml), and was dried once more. Yield of yellow substance, about 8 g, containing, in various preparations, 22.5—24.5 % of Te. The crude product was treated with 80 ml of boiling chloroform, and the mixture was filtered. Half of the chloroform was subsequently boiled off, and the solution was cooled in ice and stirred. Yield, about 5 g of practically pure tellurium di(benzenethiosulphonate). Further recrystallizations were made by dissolving 2 g in 25 ml of boiling chloroform and slow cooling.

The *p*-toluene compound was prepared in the same way, from 5.4 g of tellurium tetrachloride, 50 ml of chloroform, and 22 g of potassium *p*-toluenethiosulphonate. Tellurium di(*p*-toluenethiosulphonate) is less soluble than the benzene compound, and so the residue may be treated with larger amounts of chloroform for removal of the disulphur disulphinate. Yield of water-treated, dry product, about 12 g, containing about 20 % of Te. The yellow substance was treated with boiling chloroform (100—120 ml per g) as described above for selenium di(*p*-toluenethiosulphonate). Yield, 40—50 %. A further recrystallization was necessary to remove small amounts of the isomorphous sulphur analogue (1 g dissolved in 160 ml of boiling chloroform, about three fourths subsequently boiled off). The compound is more soluble in dibromoethane and tetrachloroethane at boiling temperature, but the hot solutions are less stable.

0.4572 g substance: 0.1222 g Te.

$Te(S_2O_2C_6H_5)_2$ (474.1) Calc. Te 26.9. Found Te 26.7.

0.5193 g substance: 0.1313 g Te.

$Te(S_2O_2C_7H_7)_2$ (502.1) Calc. Te 25.4. Found Te 25.3.

PRELIMINARY X-RAY DATA ON AROMATIC SELENO- AND TELLUROPENTATHIONIC COMPOUNDS

The crystals of the three *p*-toluene compounds, $Se(STs)_2$, $Te(STs)_2$ and Se_3Ts_2, are tetragonal trapezohedral, and are isomorphous with those of the sulphur analogue, $S(STs)_2$, the unit cell and space group of which has been reported by Dawson, Mathieson and Robertson [4]. The crystals appear as beautifully developed bipyramides {101}.

The dimensions of the unit cells were obtained from oscillation and Weissenberg photographs, using FeK_α radiation, $\lambda = 1.934$ Å, and in some cases CuK_α radiation, $\lambda = 1.54$ Å. Density determinations were made by flotation in suitable solvents.

In the table below, data for the sulphur analogue, from the paper by Robertson and co-workers [4], have been included for comparison.

Table 1. Unit cells of isomorphous p-*toluenesulphonyl compounds.*

	$a \pm 0.02$ Å	$c \pm 0.06$ Å	Density calc.	Density found
$S(STs)_2$	7.70	29.50	1.53	1.55
$Se(STs)_2$	7.70	29.60	1.72	1.72
$Te(STs)_2$	7.73	29.88	1.87	1.85
Se_3Ts_2	7.79	29.75	2.01	2.00

There are four molecules per unit cell. Absent reflections, ($h00$) when h is odd, ($0k0$) when k is odd, ($00l$) except when $l = 4n$. Space group, D_4^4—$P4_12_1$ (or the enantiomorph, D_4^8—$P4_32_1$). Since the general position of the space group is eightfold, each molecule must possess twofold symmetry; this can only be the twofold axis which lies in the only fourfold positions:

$$x, x, 0; \quad \bar{x}, \bar{x}, \tfrac{1}{2}; \quad \tfrac{1}{2}-x, \tfrac{1}{2}+x, \tfrac{1}{4}; \quad \tfrac{1}{2}+x, \tfrac{1}{2}-x, \tfrac{3}{4}.$$

The middle atoms of the molecules, *e.g.*, the tellurium atoms of the $Te(STs)_2$ molecules, thus lie in these positions.

The crystals of selenium di(benzenethiosulphonate), $Se(SBs)_2$, are, likewise, tetragonal trapezohedral, and possess, from absent reflections, the same space group as the *p*-toluene compounds of Table 1. They show the form $\{101\}$ and frequently also $\{001\}$; in the latter case they are sometimes elongated along the a axis. The axial lengths are, $a = 7.80 \pm 0.02$ Å, $c = 26.54 \pm 0.06$ Å. Density, calculated on the basis of four molecules per unit cell, 1.75, found 1.77. According to Dawson, Mathieson and Robertson [4], the sulphur analogue, $S(SBs)_2$, has a four-molecule unit cell with the dimensions, $a = 7.80$ Å, $c = 26.29$ Å, and the same tetragonal trapezohedral space group as the compounds above.

The two remaining benzene compounds, $Te(SBs)_2$ and Se_3Bs_2, do not belong to the isomorphous series.

The crystals of tellurium di(benzenethiosulphonate), $Te(SBs)_2$, are orthorhombic bipyramidal, and show the forms $\{111\}$ and $\{100\}$. The latter is frequently the more predominant, thus giving the crystals the appearance of plates. The axial lengths are, $a = 14.46 \pm 0.04$ Å, $b = 11.18 \pm 0.02$ Å, $c = 10.48 \pm 0.02$ Å. There are four molecules per unit cell; density, calc. 1.86, found 1.88. Absent reflections, ($hk0$) when $h + k$ is odd, ($h0l$) when l is odd, ($0kl$) when k is odd. The space group is therefore D_{2h}^{14}—$Pbcn$. The general position of this space group is eightfold, and there are three sets of fourfold positions, two of which have a centre and one has a twofold axis of symmetry. Since the molecule cannot reasonably have a centre of symmetry, it must possess a twofold axis, which thus passes through the tellurium atoms in the positions:

$$0, y, \tfrac{1}{4}; \quad 0, \bar{y}, \tfrac{3}{4}; \quad \tfrac{1}{2}, \tfrac{1}{2}+y, \tfrac{1}{4}; \quad \tfrac{1}{2}, \tfrac{1}{2}-y, \tfrac{3}{4}.$$

The crystals of triselenium di(benzenesulphinate), Se_3Bs_2, are triclinic. They appear as plates or prisms with (001) as the most predominant face. The unit cell dimensions are, $a = 8.24 \pm 0.03$ Å, $b = 8.54 \pm 0.03$ Å, $c = 12.80 \pm 0.06$ Å, $\alpha = 105°$, $\beta = 95°$, $\gamma = 101°$. No absent spectra.

Space group, C_1^1-P1 or $C_i^1-P\bar{1}$. Two molecules per unit cell; density, calc. 2.04, found 2.01. No molecular symmetry is required by any of the two possible space groups.

Thus, among the eight compounds discussed above, seven compounds possess, by space group requirements, a twofold axis of symmetry. As pointed out by Dawson, Mathieson and Robertson [4] in their study of the sulphur thiosulphonates, this property limits the number of possible molecular configurations, and is consistent with the presence of unbranched chains.

ANALOGOUS REACTIONS AND INTERCONVERTIBILITY OF THE ISOMORPHOUS SERIES

In the following, attempts are made to demonstrate, on chemical basis, that the three isomorphous series of pentathionic compounds possess analogous structures. Part of the reactions have been reported previously; the remaining ones are described in this article.

The sulphur thiosulphate [9], methanethiosulphonate [6] and aromatic thiosulphonates [3] arise from sulphur dichloride by analogous reactions:

$$SCl_2 + 2\ S_2O_3^{--} = S(S_2O_3)_2^{--} + 2\ Cl^- \quad (3)$$
$$SCl_2 + 2\ CH_3SO_2S^- = S(S_2O_2CH_3)_2 + 2\ Cl^- \quad (4)$$
$$SCl_2 + 2\ ArSO_2S^- = S(S_2O_2Ar)_2 + 2\ Cl^- \quad (5)$$

The products react with piperidine to give sulphur dipiperidide [6,9]:

$$S(S_2O_3)_2^{--} + 4\ C_5H_{10}NH = S(NC_5H_{10})_2 + 2\ C_5H_{10}NH_2^+ + 2\ S_2O_3^{--} \quad (6)$$
$$S(S_2O_2CH_3)_2 + 4\ C_5H_{10}NH = S(NC_5H_{10})_2 + 2\ C_5H_{10}NH_2^+ + 2\ CH_3SO_2S^- \quad (7)$$
$$S(S_2O_2Ar)_2 + 4\ C_5H_{10}NH = S(NC_5H_{10})_2 + 2\ C_5H_{10}NH_2^+ + 2\ ArSO_2S^- \quad (8)$$

The selenium and tellurium thiosulphates [10], methanethiosulphonates [6] and aromatic thiosulphonates react with sodium dimethyldithiocarbamate to give selenium and tellurium *bis*(dimethyldithiocarbamate):

$$Se(S_2O_3)_2^{--} + 2\ Me_2NCS_2^- = Se(S_2CNMe_2)_2 + 2\ S_2O_3^{--} \quad (9)$$
$$Se(S_2O_2CH_3)_2 + 2\ Me_2NCS_2^- = Se(S_2CNMe_2)_2 + 2\ CH_3SO_2S^- \quad (10)$$
$$Se(S_2O_2Ar)_2 + 2\ Me_2NCS_2^- = Se(S_2CNMe_2)_2 + 2\ ArSO_2S^- \quad (11)$$

with analogous equations for the tellurium compounds.

The sulphur, selenium and tellurium thiosulphates [10] and thiosulphonates, under the catalytic influence of thiosulphate and thiosulphonate ions, respectively, decompose to give the free elements and the tetrathionic compounds. These phenomena appear so characteristic for thio derivatives of these divalent elements as, alone, to constitute evidence for analogous structures.

The nucleophilic reactivities of the electronegative components, in displacements on divalent sulphur, selenium and tellurium, increase in the order methanethiosulphonate, aromatic thiosulphonate, and thiosulphate anions. Thus the following interconversions take place.

The sulphur, selenium and tellurium methane- and benzenethiosulphonates react with potassium *p*-toluenethiosulphonate to give the sulphur, selenium and tellurium *p*-toluenethiosulphonates:

$$S(S_2O_2CH_3)_2 + 2\ ArSO_2S^- = S(S_2O_2Ar)_2 + 2\ CH_3SO_2S^- \quad (12)$$
$$Se(S_2O_2CH_3)_2 + 2\ ArSO_2S^- = Se(S_2O_2Ar)_2 + 2\ CH_3SO_2S^- \quad (13)$$
$$Te(S_2O_2CH_3)_2 + 2\ ArSO_2S^- = Te(S_2O_2Ar)_2 + 2\ CH_3SO_2S^- \quad (14)$$

with analogous equations for the benzenethiosulphonates. The same compounds react with sodium thiosulphate to give the sulphur, selenium and tellurium thiosulphates, as do also the *p*-toluenethiosulphonates:

$$S(S_2O_2CH_3)_2 + 2\ S_2O_3^{--} = S(S_2O_3)_2^{--} + 2\ CH_3SO_2S^- \quad (15)$$
$$Se(S_2O_2CH_3)_2 + 2\ S_3O_3^{--} = Se(S_2O_3)_2^{--} + 2\ CH_3SO_2S^- \quad (16)$$
$$Te(S_2O_2CH_3)_2 + 2\ S_2O_3^{--} = Te(S_2O_3)_2^{--} + 2\ CH_3SO_2S^- \quad (17)$$

with analogous formulations for the benzene and *p*-toluene compounds.

It appears that members of the three isomorphous series possess analogous chemical properties, and that members of one series, through nucleophilic displacement reactions, may be converted into the corresponding member of another series. On this basis, it is highly unlikely that the three series should have different structures.

The structural problem of particular interest is that of branched or unbranched chains [11]. The methanethiosulphonate series having been found by X-ray methods to possess an unbranched structure [12], we conclude that the sulphur, selenium and tellurium thiosulphates and aromatic thiosulphonates, and the triselenium disulphinates, are also built up of unbranched chains.

EXPERIMENTAL

Reactions with sodium dimethyldithiocarbamate and sodium thiosulphate. About 1 millimole of selenium or tellurium di(benzenethiosulphonate) was dissolved in 5—10 ml of chloroform and 15 ml of ethylacetate, and the solution was cooled in ice. 0.75 g (about 100 % excess) of sodium dimethyldithiocarbamate, dissolved in 15 ml of methanol, were added while stirring. A solid immediately separated out, which was filtered off, washed with methanol and water, and dried *in vacuo* over sulphuric acid.

(1) 0.4528 g of $Se(SBs)_2$ gave 0.3372 g of a greenish yellow substance; theoretically, 0.3400 g of selenium *bis*(dimethyldithiocarbamate). The product was identified by melting point (180—181° C) and mixed melting point with an authentic sample [10].

(2) 0.5006 g of $Te(SBs)_2$ gave 0.3899 g of a pale red substance; theoretically, 0.3886 g of tellurium *bis*(dimethyldithiocarbamate). M. p., above 250° C [10].

Selenium and tellurium di(*p*-toluenethiosulphonate) react analogously with sodium dimethyldithiocarbamate. The corresponding reactions of the methanethiosulphonates have been described previously [6], and so have the reactions of sulphur, selenium and tellurium di(methanethiosulphonate) with sodium thiosulphate [6]. The aromatic thiosulphonates react similarly with the last reagent. Experiments with sodium thiosulphate and sulphur and selenium di(benzenethiosulphonate) were performed exactly as described for the methane compounds [6]:

(3) 0.1578 g of $S(SBs)_2$ [3] in 5 ml of ethylacetate and 20 ml of ethanol, 10 ml of 0.1033 *N* sodium thiosulphate. Volume made up to 100 ml with water, and 25 ml pipetted out and titrated with 0.01130 *N* iodine, after addition of 1 g of potassium iodide and 5 ml of 10 % acetic acid: 5.00 ml; theoretically, 4.41 ml.

(4) 0.1771 g of $Se(SBs)_2$ in 10 ml of chloroform, 10 ml of 0.1037 *N* sodium thiosulphate. On shaking, the greenish colour of the chloroform layer passed into the aqueous layer. Volume made up to 100 ml with water, and 25 ml of the aqueous layer (total volume, 90 ml) titrated as above: 5.63 ml of 0.01130 *N* iodine; theoretically, 5.02 ml. The aqueous layer contained selenopentathionate, as shown by reactions with sodium dimethyldithiocarbamate and sodium hydroxide, and the chloroform layer was devoid of selenium compounds. The too high iodine values found above are, partly at least, due to a slight oxidation of thiosulphonate ions by iodine [9].

In the case of the tellurium compounds the excess of sodium thiosulphate cannot be back-titrated with iodine, since the product, telluropentathionate, is not indifferent to iodine [13]. Instead, the amount of telluropentathionate formed was estimated by means of sodium dimethyldithiocarbamate:

(5) 0.4806 g of $Te(SBs)_2$ were dissolved in 20 ml of chloroform in a 100 ml volumetric flask, and 25 ml of 0.1 *N* sodium thiosulphate were added. On shaking, the yellow, faintly orange colour of the chloroform layer rapidly vanished, reappearing in the aqueous layer. The volume was made up to 100 ml with water, and 50 ml of the aqueous layer (total volume, 90 ml) were pipetted out. An excess of aqueous sodium dimethyldithiocarbamate was added, and the resulting reddish solid was filtered off, washed with water and methanol, and dried *in vacuo* over sulphuric acid. Yield, 0.2346 g; theoretically, 0.2331 g of tellurium *bis*(dimethyldithiocarbamate). M. p., above 250° C [10].

Catalytic decompositions. The decompositions of sulphur, selenium and tellurium di(thiosulphate) ions, into tetrathionate and the free elements, are strongly catalyzed by thiosulphate ions [10]. The corresponding decompositions of the thiosulphonates of these divalent elements are, analogously, susceptible to catalysis by thiosulphonate ions. As with the thiosulphates, the thiosulphonates of divalent tellurium are more stable in this respect than are the sulphur and selenium derivatives. Some experiments, at room temperature, with selenium thiosulphonates are described below.

0.01 *M* solutions of (a) selenium di(methanethiosulphonate) and (b) selenium di(benzenethiosulphonate) in chloroform are stable for several days. To 5 ml of these pale greenish solutions were added various amounts of 0.01 *M* solutions, in methanol, of (c) sodium methanethiosulphonate and (d) potassium benzenethiosulphonate. Time from mixing till appearance of red selenium colour:

(a); 5 ml of (c): 10 seconds. 2 ml of (c): 20 seconds.

(b); 5 ml of (d): 30 seconds. 2 ml of (d): 2½ minute.

The benzenethiosulphonate thus appears to be more stable than the methanethiosulphonate.

Sulphur, selenium and tellurium di(p-*toluenethiosulphonate*) *from the methane and benzene analogues.* The experiments were made with the following solutions: (1) 0.3917 g of $S(SBs)_2$, (2) 0.2592 g of $S(S_2O_2CH_3)_2$, (3) 0.4265 g of $Se(SBs)_2$, (4) 0.3159 g of $Se(S_2O_2CH_3)_2$, (5) 0.4721 g of $Te(SBs)_2$, in 5 ml, 5 ml, 10 ml, 15 ml and 20 ml of chloroform, respectively, and (6) 0.3592 g of $Te(S_2O_2CH_3)_2$ in 15 ml of bromoform. The solutions were cooled in ice, and 1 g (about 100 % excess) of potassium *p*-toluenethiosulphonate hydrate, dissolved in 5 ml of water, were added. On stirring, crystals rapidly began to separate out, except in the case of (1) where crystallization was effected by addition of 20 ml of methanol to produce a homogeneous mixture. After five minutes' stirring and cooling the products were filtered off, washed with water and dried *in vacuo* over sulphuric acid. The yields were as follows. Of $S(STs)_2$: (1) 0.3937 g or 94 %, (2) 0.3631 g or 89 %. Of $Se(STs)_2$: (3) 0.3981 g or 88 %, (4) 0.3913 g or 82 %. Of Te(STs): (5) 0.4840 g or 97 %, (6) 0.4702 g or 91 %. No selenium or tellurium and apparently no sulphur were set free in the experiments.

The crude products, when viewed in a microscope, consisted of tiny tetragonal bipyramides as characteristic for the sulphur, selenium and tellurium *p*-toluenethiosulphonates. The products were recrystallized from chloroform, and identified by crystal habit and by melting or decomposition points, and by selenium or tellurium determinations in the case of the $Se(STs)_2$ and $Te(STs)_2$ samples.

The percentage yields stated above refer to complete conversions of the methane- and benzenethiosulphonates to *p*-toluenethiosulphonates. The use of heterogeneous reaction mixtures (chloroform and water phases) serves to depress catalytic decompositions of the sulphur, selenium and tellurium thiosulphonates.

SUMMARY

Selenium and tellurium di(benzenethiosulphonate) and di(*p*-toluenethiosulphonate) have been described for the first time. Unit cells and space groups are reported for the crystals of these compounds and of the corresponding triselenium disulphinates. The *p*-toluene compounds, together with the previously known sulphur *p*-toluenethiosulphonate, constitute a four-membered isomorphous series of pentathionic compounds.

Evidence, based on interconvertibility and similarity of chemical behaviour, indicates that members of this series, and of the pentathionate, selenopentathionate and telluropentathionate series, possess the same type of structure as the sulphur, selenium and tellurium di(methanethiosulphonate) series.

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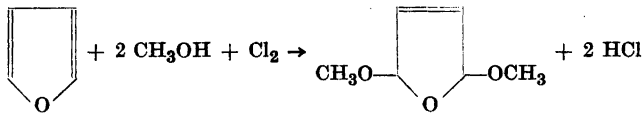
ACTA CHEMICA SCANDINAVICA 6 (1952) 531–534

Electrolytic Methoxylation of Furan

NIELS CLAUSON-KAAS, FRANZ LIMBORG and KIRSTEN GLENS

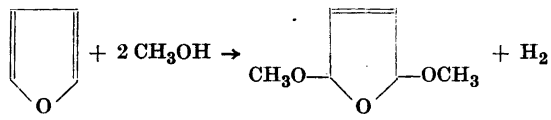
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When furan is treated with a methanolic solution of bromine or chlorine it adds two methoxy groups whereby 2,5-dimethoxy-2,5-dihydrofuran and two moles of hydrogen halide are formed. Since dimethoxydihydrofuran is sensitive to acids, the reaction is carried out in the presence of sodium



carbonate[1] or potassium acetate[2,3]. We have found that dimethoxydihydrofuran prepared by this method is often contaminated with a small amount of some halogen-containing impurity, which may cause the formation of hydrogen halide and therefore greatly influence the stability of the acid sensitive dimethoxydihydrofuran.

We have now developed a methoxylation method, which is simpler and cheaper than the one above, and which gives a halogen-free reaction product [4]. Furan is mixed with a methanolic solution of ammonium bromide and the mixture electrolyzed. At the cathode, hydrogen and ammonia, at the anode bromine are formed. The bromine reacts immediately with furan and methanol to give dimethoxydihydrofuran and hydrogen bromide. The hydrogen bromide and the ammonia from the cathode then regenerate the ammonium bromide. The net equation for the process is:



The yield of dimethoxydihydrofuran is 73 per cent and the current efficiency 86 per cent. As the boiling point and the refractive index of the product rise

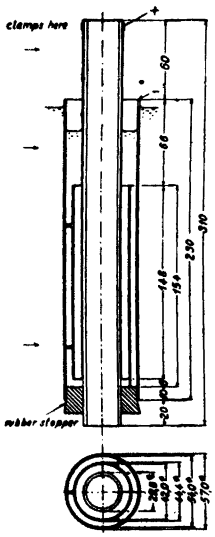
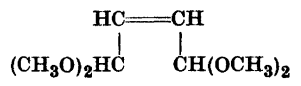


Fig. 1. Cell for electrolytic alkoxylations.

slightly during distillation, we believe that the dimethoxydihydrofuran is a mixture of the *cis* and the *trans* isomer.

A small amount of a higher boiling liquid is also formed by the electrolysis. This has been found to consist mainly of malealdehyde tetramethyl acetal (I).



I

EXPERIMENTAL

Apparatus. Fig. 1 gives a detailed design of the cell used for the electrolysis. The cathode consists of two brass cylinders electroplated with nickel, while the anode is a porcelain tube covered with sheet platinum (note 1). During electrolysis the electrolyte circulates continuously owing to the evolution of hydrogen at the inner surface of the inner cathode.

The cell is fed from a storage battery or from a rectifier and connected with an ammeter and a coulometer of the ordinary domestic type.

Procedure. 5.00 g of ammonium bromide (0.051 mole) is dissolved in a mixture of 230 ml of methanol (5.7 moles) and 68.0 g of freshly distilled furan (1.00 mole) (note 2) and the solution (total volume about 310 ml) poured into the cell. The cell is placed in a cooling-bath kept at − 22° and the voltage applied after some minutes. The data of the electrolysis are given below.

Hours	Current (ampere)	Potential across the cell during electrolysis (volt)	Temperature in the cell	Ampere hours (per cent of theoretical amount)
0.3	3.0	4.6	− 14°	1.0 (2 %)
5.8	3.0	4.8	− 14°	10.9 (20 %)
7.9	3.0	4.8	− 14°	23.6 (44 %)
15.5	2.4	5.2	− 14°	44.7 (83 %)
15.9	2.3	5.3	− 14°	45.6 (85 %)

After 45.6 ampere hours have passed through the cell (85 % of the theoretical amount), the electrolysis is stopped. The volume of the liquid in the cell has diminished 35 ml mainly due to contraction (note 3). The liquid, which is colourless in the upper part of the cell, but slightly yellow from unreacted bromine near the bottom, is poured into a solution of sodium methoxide (1.20 g of sodium (0.052 mole) in 20 ml of methanol) and the mixture distilled through a *Vigreux* column from a water-bath under atmospheric pressure. About 160 ml of distillate, containing ammonia and the exess of furan, is collected. The residue consists of a clear yellowish-brown liquid and a precipitate of sodium bromide, which is filtered off and washed with a little ether. The filtrate is then distilled through a 5 cm column packed with glass helices.

Fraction (g)	B. p.$_{760}$	n_D^{25}	OCH_3 Calc. 47.7 %
1 (2)	80—160°		
2 (26.4)	160°	1.4321	47.8
3 (25.4)	160—161°	1.4323	47.1
4 (24.3)	161°	1.4322	47.5
5 (19.3)	161—162°	1.4326	46.9
residue (5)			

All fractions show a negative Beilstein test for halogens. Yield (fractions 2—5) 95.5 g of dimethoxydihydrofuran = 73 %; current efficiency 86 %.

Dimethoxydihydrofuran has previously been prepared by methoxylation of furan by Jones [1] (yield 48 %, $n_D^{20} = 1.4353$) and by Clauson-Kaas, Limborg and Fakstorp [3] (yield 65 %, $n_D^{20} = 1.4328$).

Identification of malealdehyde tetramethyl acetal (I). By fractional distillation of the residue from an electrolytic preparation of dimethoxydihydrofuran about 2 g of a higher boiling liquid may be isolated. One sample boiling at 200—201° (760 mm), $n_D^{25} = 1.4247$, was analyzed. The microhydrogenation was performed in 2 ml of ethanol using 32 mg of

$C_4H_4(OCH_3)_4$ (176.2)	Calc.	C 54.6	H 9.2	OCH_3 70.4	/=1.00
	Found	» 54.7	» 9.4	» 67.9	» 0.92

substance and 30 mg of Adams platinum oxide catalyst. The hydrogen uptake was complete after about 2 hours.

65 mg of the same sample was left standing for 1 hour at room temperature with 0.25 ml of phenylhydrazine in 12 ml of 32 % aqueous acetic acid. The precipitate was then filtered off, washed with dilute acetic acid and water and dried. The crude product was crystallized from acetone-ligroin. Yield 65 mg of malealdehyde bis-phenylhydrazone = 63 %; m.p. 171° (Kofler stage, corr.); previously found [5] 171°.

$C_{16}H_{16}N_4$ (264.3) Calc. N 21.2
Found » 21.2

The above evidence shows, that the higher boiling product is rather pure malealdehyde tetramethyl acetal. This compound has previously been prepared by Wohl and Bernreuther [6] (b.p.$_{760}$ = 198.0—198.5°, n_D^{25} = 1.4282).

Note 1. Graphite may also be used as an anode.

Note 2. The ammonium bromide and the furan were technical products. The methanol was an analytical grade in this particular experiment, but it was found in other experiments that a good technical grade may equally well be used. It is not essential, that the methanol is perfectly dry.

Note 3. The contraction of the mixture due to the reaction is about 33 ml, while about 1 ml of methanol and 1 ml of furan are lost by evaporation owing to the liberation of hydrogen.

SUMMARY

Directions are given for the preparation of 2,5-dimethoxy-2,5-dihydrofuran by electrolysis. Malealdehyde tetramethyl acetal was isolated as a by-product of the reaction.

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The Acyloxylation of Furan with Lead Tetraacyloxylates; *Cis-Trans* Isomerism of 2,5-Diacyloxy-2,5-dihydrofurans and 2,5-Diacyloxytetrahydrofurans

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The formation of 2,5-diacetoxy-2,5-dihydrofuran from furan and lead tetraacetate has been previously reported (yield 45 per cent) [1]. We have now worked out new directions for this synthesis, in order that larger amounts of diacetoxydihydrofuran can be more easily prepared (yield 70 per cent).*

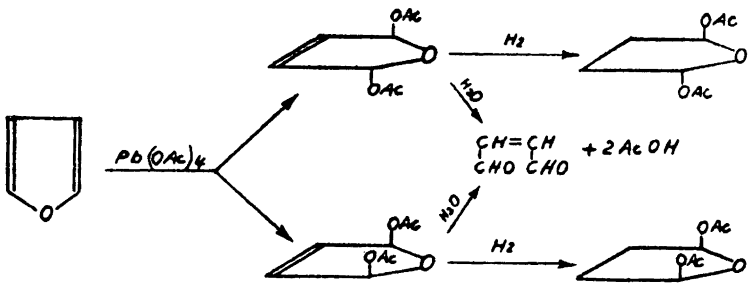
Diacetoxydihydrofuran, prepared by the lead salt method, is usually obtained as a liquid, but we have found that it consists 1/3 of a crystalline isomer and 2/3 of another isomer, which it was not possible to bring to crystallization. The two isomers can be easily separated by crystallization from methanol. It was shown by hydrolysis and identification of malealdehyde, that both compounds actually are 2,5-diacetoxy-2,5-dihydrofurans. They must therefore be *cis-trans* isomers. We do not yet know, which of the compounds is the *cis*, and which the *trans* isomer.

Diacetoxydihydrofuran, prepared by the bromine method [2], also crystallizes readily from methanol, and was found to consist about half of the crystalline isomer. No analytically pure liquid isomer could be isolated. It is quite reasonable that the two *cis-trans* isomers are formed in different proportions by the two different methods of preparation.

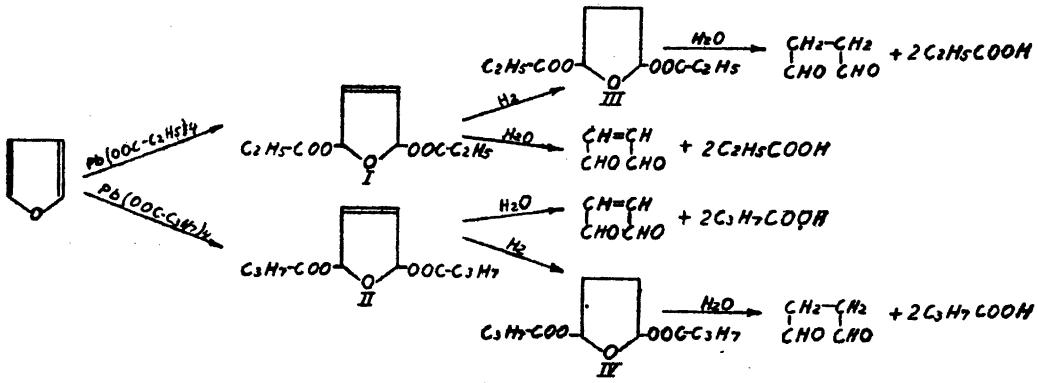
The diacetoxydihydrofurans were hydrogenated catalytically to the corresponding 2,5-diacetoxytetrahydrofurans (*cf.* [2]). Crystalline diacetoxydihydrofuran gave a crystalline tetrahydrofuran, and liquid diacetoxydihydrofuran gave a liquid tetrahydrofuran. Naturally the two diacetoxytetrahydrofurans must also be *cis-trans* isomers,

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* Patent pending.



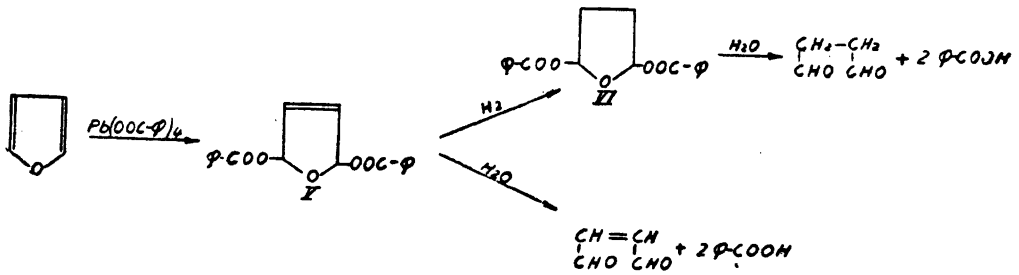
We have also synthesized the homologous 2,5-dipropionoxy- and 2,5-dibutyroxy-2,5-dihydrofurans (I and II) from furan and the corresponding lead tetraacyloxylates. These are all new compounds. Their structure was proved by hydrolysis and identification of malealdehyde and propionic, respectively butyric acid. Catalytic hydrogenation of I and II gave 2,5-dipropionoxy- and 2,5-dibutyroxytetrahydrofuran (III and IV), which are also new compounds. Hydrolysis of III and IV gave succinaldehyde.



Dipropionoxydihydrofuran was, like diacetoxydihydrofuran, found to consist of a liquid and a crystalline isomer (in proportion ca. 1 : 3), which were shown to be *cis-trans* isomers by hydrolysis to malealdehyde. Hydrogenation of the two isomers gave the two corresponding *cis-trans* isomeric dipropionoxytetrahydrofurans. The dipropionoxytetrahydrofuran obtained from crystalline dipropionoxydihydrofuran was also crystalline.

Dibutyroxydihydrofuran was obtained as a liquid. It crystallized after standing with a little ether at $-20°$ for several months (melting point -3 to $1°$) and appears to consist almost entirely of either the *cis* or the *trans* isomer.

Finally 2,5-dibenzoxy-2,5-dihydrofuran (V) was prepared from furan and lead tetrabenzoate. Hydrolysis gave benzoic acid, but no malealdehyde could be isolated, probably because the sensitive aldehyde was destroyed during the prolonged boiling, which was necessary to hydrolyze this dihydrofuran. Hydrogenation gave 2,5-dibenzoxytetrahydrofuran VI, which was hydrolyzed to succinaldehyde.



V and VI are both new compounds. Their structure is proved by the above reactions.

2,5-Dibenzoxy-2,5-dihydrofuran was obtained as a crystalline solid, which melted at 179—182° after one crystallization from dioxane. After two more crystallizations the melting point of the product remained constant (185—190°). The crude, as well as the pure product and the intermediate fractions were, however, analytically pure. Therefore dibenzoxydihydrofuran may also be a mixture of the *cis* and the *trans* isomer.

EXPERIMENTAL

Microanalyses by Franz Limborg

2,5-Diacetoxy-2,5-dihydrofuran

Preparation. 580 ml of glacial acetic acid and 230 ml of acetic anhydride are placed in a 1-liter 3-necked flask provided with a stirrer, a thermometer and a reflux condenser with a calcium chloride tube. 300 g of red lead oxide (0.44 mole) is added to the stirred solution in portions of 15—20 g[3]. The temperature is kept at 50° by external heating. The addition takes about 3 ½ hours and part of the lead tetraacetate formed, precipitates as white crystals. 29.8 g of furan (0.44 mole) (note 1) is added, the temperature raised to 60° and heating discontinued. In the course of the following 10 minutes the temperature rises to 65°. The reaction mixture is then kept at 60—65° for 75 minutes, first by occasional cooling, later by external heating. The mixture is now a clear, pale-yellow solution (note 2). The major part of the solvent is distilled under 10 mm from a water-bath of 60—65° and 400 ml of anhydrous ether is added to the pasty residue. The mixture is shaken for some minutes and then stirred vigorously whereby the precipitate becomes

readily filterable. The lead acetate is filtered off on a sintered glass-disk and washed with 100 ml of anhydrous ether. Yield 415 g of lead acetate = 97 %.

The yellowish-brown filtrate is distilled from a water-bath of 70°, finally in vacuum, and the residue distilled further in vacuum. After 15—20 g of a pale-yellow fore-run (b.p.$_{10}$ < 127°), the main fraction is collected. Yield 56.0 g of diacetoxydihydrofuran = 69 %; pale-yellow liquid (note 3), b.p.$_{0.5}$ = 89—93°; n_D^{25} = 1.4536 (all refractive indices reported in this communication are only accurate within ± 0.0008).

Separation into two isomers. Diacetoxydihydrofuran may be crystallized from various solvents. Crystals are formed rapidly from solutions in methanol, ethanol, ether and petroleum ether, and slowly from ethyl acetate and carbon tetrachloride. Methanol was found to be the best solvent for the separation of the liquid and the crystalline diacetoxydihydrofuran.

(1). 20.0 g of the above sample of diacetoxydihydrofuran was crystallized from 20 ml of anhydrous methanol at — 20° under anhydrous conditions; yield 7.0 g of crystals. Recrystallization from 7 ml of methanol gave 6.3 g of crystalline diacetoxydihydrofuran; m.p. 51—52° (Hershberg apparatus, corr.); n_D^{25} = 1.4541 (after melting).

$C_8H_{10}O_5$ (186.2)	Calc.	C	51.6	H	5.4
	Found	»	51.4	»	5.5

A sample of crystalline diacetoxydihydrofuran was stored in a sealed glass tube at room temperature. The crystals became slightly sticky after 3—4 months.

The methanol was distilled from the mother liquor from the first crystallization and the residue distilled in vacuum. Yield 12.0 g; b.p.$_{0.2}$ = 84—87°; n_D^{25} = 1.4542. The product was dissolved in 12 ml of methanol and kept for 3 hours at — 20°. No crystals were formed. Cooling for 2 hours at — 80° gave 0.4 g of crystals, which were filtered off. The methanol was distilled from the mother liquor and the residue distilled in vacuum. Yield 11.0 g of liquid diacetoxydihydrofuran; b.p.$_{0.2}$ = 84—87°; n_D^{25} = 1.4545.

Found C 51.7 H 5.4

A sample of liquid diacetoxydihydrofuran was kept for 4 months with occasional scratching. No crystals were formed.

(2). 20.0 g of diacetoxydihydrofuran, prepared by the bromine method [2] (positive Beilstein test for halogen) was crystallized from 20 ml of methanol as above. Yield after 3 hours 6.5 g of crystals; m.p. 49—51°. After 24 hours another 7 g of a partially crystalline product had separated; m.p. < 20°. Both fractions as well as their mother liquors gave a positive Beilstein test. The first crop of crystals (6.5 g) was recrystallized from 7 ml of methanol. Yield 5.9 g of crystalline diacetoxydihydrofuran, m.p. 50.5—52.0°. The product gave a negative Beilstein test. Repeated crystallization did not give a higher melting product.

Found C 51.7 H 5.4

Crystalline isomer; hydrolysis and identification of malealdehyde. 46.2 mg of crystalline diacetoxydihydrofuran was shaken for 30 minutes with 1 ml of *N*/10 sulfuric acid. A solution of 0.20 ml of phenylhydrazine and 0.50 ml of acetic acid in 10 ml of water was added and the mixture shaken vigorously. After 15 minutes the precipitate was filtered off, washed thoroughly with water and dried. The product was crystallized from acetone-benzene and dried (sulfuric acid, 0.05 mm, 20°, 0.5 hours). Yield 38.7 mg of malealdehyde bis-phenylhydrazone = 59 %; m.p. 168—170° (Kofler stage, corr.); previously found [4] 171°.

$C_{16}H_{16}N_4$ (264.3)	Calc.	N	21.2
	Found	»	20.9

Crystalline isomer; hydrogenation. 1.60 g of crystalline diacetoxydihydrofuran and 8 ml of anhydrous ethanol were shaken with 0.2 g of Raney nickel under 100 atmospheres of hydrogen for 2 hours at room temperature. After filtration the ethanol was distilled in vacuum from a water-bath and the residue distilled further in vacuum. After 0.29 g of fore-run (b.p.$_{0.3}$ = 84°) the main fraction is collected. Yield 1.14 g of diacetoxytetrahydrofuran = 71 %; b.p.$_{0.3}$ = 84— 86°; m.p. 18—21°; n_D^{25} = 1.4371.

$C_8H_{12}O_5$ (188.2)	Calc.	C	51.1	H	6.4
	Found	»	51.3	»	6.6

Liquid isomer; hydrolysis and identification of malealdehyde. 61.8 mg of liquid diacetoxydihydrofuran was hydrolyzed as described above. Yield 53.5 mg of malealdehyde bisphenylhydrazone = 61 %; m.p. 171—172°.

$C_{16}H_{16}N_4$ (264.3)	Calc.	N	21.2		
	Found	»	21.2		

Liquid isomer; hydrogenation. 2.00 g of liquid diacetoxydihydrofuran was hydrogenated as above. Fore-run 0.17 g; b.p.$_{0.3}$ = 84°. Yield 1.54 g of diacetoxytetrahydrofuran = 76 %; b.p.$_{0.3}$ = 84—87°; n_D^{25} = 1.4386. The product did not crystallize on standing at — 20° for several weeks.

$C_8H_{12}O_5$ (188.2)	Calc.	C	51.1	H	6.4
	Found	»	51.2	»	6.5

2,5-Dipropionoxy-2,5-dihydrofuran

Preparation; method 1. 58 g of lead tetrapropionate (0.115 mole) (note 4), 150 ml of propionic acid and 7.9 g of furan (0.115 mole) (note 5) are placed in a 3-necked round-bottomed flask provided with a stirrer, a thermometer and a reflux condenser with a calcium chloride tube. The temperature is raised to 55° and heating is discontinued. In the course of the following 10 minutes the temperature remains at 55°, and the reaction mixture is then kept at 55—60° for 35 minutes and at 60—65° for 45 minutes by external heating. The mixture is now a clear pale-yellow solution. The major part of the solvent is distilled under 10 mm from a water-bath of 60—70° and 250 ml of anhydrous ether is added to the residue. The mixture is shaken for some minutes and then stirred vigorously whereby the precipitate becomes readily filterable. The lead propionate is filtered off on a sintered glass-disk and washed with 100 ml of anhydrous ether.

The yellow filtrate is distilled in vacuum from a water-bath of 70° and the residue distilled further in vacuum. After 17.5 g of a pale-yellow fore-run (b.p.$_{0.4}$ < 97°), the main fraction is collected. Yield 19.0 g of dipropionoxydihydrofuran = 76 %; pale-yellow liquid, b.p.$_{0.4}$ = 97—103°; n_D^{25} = 1.4510.

18.5 g of the product was redistilled. After 1.0 g of fore-run (b.p.$_{0.3}$ = 94°) the main fraction was collected. Yield 17.0 g; pale-yellow liquid, b.p.$_{0.3}$ = 94—98°; n_D^{25} = 1.4517. The product crystallized almost completely after inoculation.

$C_{10}H_{14}O_5$ (214.2)	Calc.	C	56.1	H	6.6
	Found	»	56.3	»	6.8

Preparation; method 2. 150 ml of propionic acid and 54 ml of propionic anhydride (technical product) are placed in a 3-necked flask provided with a stirrer, a thermometer and a reflux-condenser with a calcium chloride tube. 68.6 g of red lead oxide (0.100 mole) is added in the following manner. 10 g is added at 50° and the mixture is stirred for 4 hours at 50°. Then another 10 g is added at room temperature, and stirring is continued for 4 hours at room temperature. Finally the rest of the red lead oxide is added and

stirring is continued overnight. The next day a white precipitate of lead tetrapropionate has formed. The supernatant liquid has a faintly pink colour. The temperature is raised to 55° and 6.8 g of furan (0.100 mole) is added. Heating is discontinued. In the course of the following 10 minutes the temperature remains at 55°. The reaction mixture is then kept at 55—60° for 35 minutes and at 60—65° for 45 minutes by external heating. The major part of the solvent is distilled under 10 mm from a water-bath of 60—70° and 300 ml of anhydrous ether is added to the residue. The mixture is shaken for some minutes and then stirred vigorously whereby the precipitate becomes readily filterable. The lead propionate is filtered off on a sintered glass-disk and washed with anhydrous ether. Yield 104 g of lead propionate = 98 %.

The yellow filtrate is distilled from a water-bath, finally in vacuum, and the residue distilled further in vacuum. After 27 g of fore-run ($b.p._{0.1} < 88°$), the main fraction is collected. Yield 11.5 g of dipropionoxydihydrofuran = 54 %; pale-yellow liquid, $b.p._{0.1} = 88—92°$; $n_D^{25} = 1.4520$. The product crystallizes almost completely after inoculation.

$C_{10}H_{14}O_5$ (214.2)	Calc.	C	56.1	H	6.6
	Found	»	55.9	»	6.4

Hydrolysis; identification of propionic acid. 214 mg of dipropionoxydihydrofuran was boiled for two minutes with 2 ml of *N*/10 sulfuric acid. After cooling the solution was neutralized (methyl red) with *N* sodium hydroxide and a solution of 420 mg of S-benzylthiuronium chloride in 2 ml of water was added. A white precipitate was formed at once. The mixture was left for 10 minutes in the ice-box, the precipitate filtered off, washed with 2 ml of ethanol and dried (calcium chloride, 10 mm, 20°, 4 hours). Yield 290 mg of S-benzylthiuronium propionate = 61 %. The product was crystallized from ethanol and dried (sulfuric acid, 0.05 mm, 60°, 4 hours). Yield 150 mg = 31 %; m.p. 146—148°; mixed melting point with an authentic specimen of S-benzylthiuronium propionate 146—147°; Donleavy [5] found 148°.

$C_{11}H_{16}O_2N_2S$ (240.3)	Calc.	N	11.7
	Found	»	11.5

Hydrogenation; identification of succinaldehyde. 3.00 g of dipropionoxydihydrofuran and 15 ml of anhydrous ethanol were shaken with 0.5 g of Raney nickel under 100 atmospheres for 4 hours at room temperature. The reaction product was isolated in the usual way by distillation. Fore-run 0.19 g ($b.p._{0.1} = 86—88°$); main fraction 2.48 g of dipropionoxytetrahydrofuran = 82 %; $b.p._{0.1} = 88—92°$; $n_D^{25} = 1.4378$. Redistillation gave 2.21 g = 73 %; colourless liquid, $b.p._{0.1} = 88—92°$; $n_D^{25} = 1.4375$.

$C_{10}H_{16}O_5$ (216.2)	Calc.	C	55.5	H	7.5
	Found	»	55.6	»	7.6

It was found that hydrogenation for 8 hours did not change the yield or the quality of the reaction product.

216 mg of dipropionoxytetrahydrofuran was refluxed with 3 ml of *N*/10 sulfuric acid for 15 minutes. After cooling 0.25 ml of phenylhydrazine in 5 ml of *N* acetic acid and 2 ml of ethanol was added. After 1 hour the precipitate of succinaldehyde bis-phenylhydrazone was filtered off, washed thoroughly with water and dried (calcium chloride, 10 mm, 20°, 16 hours); yield 226 mg = 85 %. Yield after crystallization from ethanol and drying (sulfuric acid, 0.1 mm, 50°, 18 hours) 120 mg = 45 %; m.p. 120—122°; Ciamician and Zanetti [6] found 124—125°.

$C_{16}H_{18}N_4$ (266.3)	Calc.	N	21.0
	Found	»	20.9

Separation into two isomers. 10.0 g of dipropionoxydihydrofuran, prepared by method 1, was crystallized from 10 ml of anhydrous methanol. The mixture was left at − 20° for 1 hour and then at − 80° for 30 minutes. The crystals were filtered off (7.6 g, m.p. 38−47°) and the mother liquor distilled in vacuum. Yield 1.8 g of liquid dipropionoxydihydrofuran; b.p.$_{0.1}$ = 88−91°; n_D^{25} = 1.4522.

$C_{10}H_{14}O_5$ (214.2)	Calc.	C	56.1	H	6.6
	Found	»	56.1	»	6.7

The crystalline product (7.6 g) was recrystallized four times from methanol, the melting points being 46−51°, 55.0−56.5°, 56.0−57.5° and 56.0−57.5°. Yield of final product 3.7 g.

	Found	C	56.3	H	6.6

It was attempted to measure the refractive index of the molten crystals at 25°, but the liquid always crystallized before a determination could be made.

Crystalline isomer; hydrolysis and identification of malealdehyde. 210 mg of crystalline dipropionoxydihydrofuran was shaken with 1 ml of ethanol and 3 ml of *N*/10 sulfuric acid. The solution became homogenous after 5 hours. After 6 hours 0.25 ml of phenylhydrazine in 10 ml of *N* acetic acid was added and the mixture shaken vigorously. After 1 hour the malealdehyde bis-phenylhydrazone was isolated as above. Yield 92 mg = 35 %; m.p. 171−173°.

$C_{16}H_{16}N_4$ (264.3)	Calc.	N	21.2
	Found	»	21.0

Crystalline isomer; hydrogenation. 1.00 g of crystalline dipropionoxydihydrofuran and 5 ml of anhydrous ethanol were shaken with 0.2 g of Raney nickel under 100 atmospheres for 4 hours and the reaction product isolated as above. Yield 0.78 g of crystalline dipropionoxytetrahydrofuran = 77 %; b.p.$_{0.1}$ = 88−91°; n_D^{25} = 1.4372. The product crystallized on cooling to − 20°; m.p. 11−14°.

$C_{10}H_{16}O_5$ (216.2)	Calc.	C	55.5	H	7.5
	Found	»	55.8	»	7.5

Liquid isomer; hydrolysis and identification of malealdehyde. 212 mg of liquid dipropionoxydihydrofuran was shaken with 1 ml of ethanol and 3 ml of *N*/10 sulfuric acid for 5 hours. The mixture became homogenous after 2 hours. The malealdehyde bis-phenylhydrazone was isolated as above. Yield 106 mg = 41 %; m.p. 171−173°.

$C_{16}H_{16}N_4$ (264.3)	Calc.	N	21.2
	Found	»	21.3

Liquid isomer; hydrogenation. 1.00 g of liquid dipropionoxydihydrofuran was hydrogenated as above. Yield 0.66 g of liquid dipropionoxytetrahydrofuran = 65 %; colourless liquid, b.p.$_{0.1}$ = 88−90°; n_D^{25} = 1.4388. The product did not crystallize on standing at − 20° for several weeks.

$C_{10}H_{16}O_5$ (216.2)	Calc.	C	55.5	H	7.5
	Found	»	55.6	»	7.4

2,5-Dibutyroxy-2,5-dihydrofuran

Preparation. 4.5 g of furan (0.066 mole) is added to a solution of 36.5 g of lead tetrabutyrate (0.066 mole) in 135 ml of butyric acid (note 6). The temperature is kept at 55−60° for 30 minutes, then at 65° for another 30 minutes and finally at 75−80° for 45 minutes. The mixture is now a clear pale-yellow solution. The major part of the solvent is distilled under 0.5 mm from a water-bath of 65−70° and 250 ml of anhydrous ether is

added to the residue. The mixture is shaken for some minutes and then stirred vigorously whereby the precipitate becomes readily filterable. The lead butyrate is filtered off on a sintered glass disk and washed with anhydrous ether.

The filtrate is evaporated in vacuum from a water-bath of 70° and the residue distilled further in vacuum. After 11 g of a pale-yellow fore-run ($b.p._{0.2} < 105°$) the main fraction is collected. Yield 12.0 g of dibutyroxydihydrofuran = 75 %; $b.p._{0.2} = 104-108°$; $n_D^{25} = 1.4492$.

11.5 g of the product was redistilled. After 0.5 g of fore-run ($b.p._{0.2} < 104°$) the main fraction is collected. Yield 10.5 g of a colourless liquid, $b.p._{0.2} = 104-107°$; $n_D^{25} = 1.4497$.

$C_{12}H_{18}O_5$ (242.3)	Calc.	C	59.5	H	7.5
	Found	»	59.2	»	7.5

A sample of dibutyroxydihydrofuran, which was left standing with a little ether at − 20°, crystallized after some months. The main fraction from the above preparation crystallized almost completely at − 20° after inoculation; m.p. − 3 to 1°.

Hydrolysis; identification of butyric acid. 484 mg of dibutyroxydihydrofuran was boiled with 4 ml of *N*/10 sulfuric acid until the mixture was homogeneous (10−15 minutes). The S-benzylthiuronium salt was then prepared in the usual way. Yield of crude product 790 mg = 78 %. Yield after crystallization from ethanol 350 mg = 35 %; m.p. 146.0−146.5°; mixed m.p. with an authentic specimen of S-benzylthiuronium butyrate 146−147°; Donleavy [5] found 146°.

$C_{12}H_{18}O_2N_2S$ (254.3)	Calc.	N	11.0
	Found	»	11.1

Hydrolysis; identification of malealdehyde. 205 mg of dibutyroxydihydrofuran was shaken for 22 hours with 1 ml of ethanol and 3 ml of *N*/10 sulfuric acid. 0.25 ml of phenylhydrazine in 10 ml of 3 *N* acetic acid was added and the mixture shaken vigorously. After standing for 1 hour the malealdehyde bis-phenylhydrazone was isolated as above. Yield 62 mg = 28 %; m.p. 171−173°.

$C_{16}H_{16}N_4$ (264.3)	Calc.	N	21.2
	Found	»	21.3

Hydrogenation; identification of succinaldehyde. 3.00 g of dibutyroxydihydrofuran and 15 ml of anhydrous ethanol were shaken with 0.2 g of Raney nickel under 100 atmospheres for 4 hours and the reaction product isolated in the usual way. Yield 2.26 g of dibutyroxytetrahydrofuran = 75 %; $b.p._{0.1} = 99-104°$; $n_D^{25} = 1.4396$. Redistillation gave 1.86 g = 62 %; colourless liquid, $b.p._{0.1} = 100-104°$; $n_D^{25} = 1.4392$. The product did not crystallize on standing at − 20° for several weeks..

$C_{12}H_{20}O_5$ (244.3)	Calc.	C	59.0	H	8.3
	Found	»	59.3	»	8.1

It was found that hydrogenation for 8 hours did not change the yield or the quality of the reaction product.

272 mg of dibutyroxytetrahydrofuran was refluxed with 3 ml of *N*/10 sulfuric acid for 30 minutes. After cooling 0.30 ml of phenylhydrazine in 5 ml of *N* acetic acid and 1 ml of ethanol was added and the succinaldehyde bis-phenylhydrazone isolated as above. Yield 240 mg = 80 %. Yield after two crystallizations from ethanol 105 mg = 35 %; m.p. 124−125°.

$C_{16}H_{18}N_4$ (266.3)	Calc.	N	21.0
	Found	»	21.3

2,5-Dibenzoxy-2,5-dihydrofuran

Preparation. Lead tetrabenzoate is prepared from 135 g of lead tetraacetate (0.30 mole) after Hurd and Austin [7] (*cf.* also Criegee [8]). This preparation is carried out in a 3-necked round-bottomed flask provided with a stirrer, a thermometer and a reflux condenser with a calcium chloride tube. The flask is placed in a water-bath and the yellow lead tetrabenzoate dissolved in 500 ml of anhydrous benzene. 20.5 g of furan (0.30 mole) is added. The temperature is kept at 50° for 1 hour and then at 75° for 3 hours. At this time the lead tetrabenzoate has disappeared. After cooling the lead benzoate is filtered off on a sintered glass disk and washed with 50 ml of benzene. The solvent is distilled in vacuum from a water-bath of 50°. 51.0 g of crystalline dibenzoxydihydrofuran = 54 % remains.

Dibenzoxydihydrofuran is slightly soluble in ether, methanol and ethanol and somewhat more soluble in acetone and dioxane. When boiled with an aqueous solution of dinitrophenylhydrazine in 2 *N* hydrochloric acid it forms a red precipitate (malealdehyde bis-dinitrophenylhydrazone).

A sample of the above crude product was crystallized from anhydrous methanol and the crystals dried (sulfuric acid, 0.1 mm, 60°, 5 hours); m.p. 165—167° after sintering at 160°.

$C_{18}H_{14}O_5$ (310.3) Calc. C 69.7 H 4.6
Found » 69.6 » 4.9

Another sample of the crude product was crystallized from dioxane and dried (sulfuric acid, 0.1 mm, 80°, 18 hours); m.p. 179—182°.

Found C 69.6 H 4.8

5.84 g of crude product was crystallized 4 times from dioxane, the melting points being 179—182°, 180—182°, 185—189° and 185—189°. All products sintered and turned faintly brown at about 170°. Yield of final product 1.41 g.

Found C 69.6 H 4.7

Hydrolysis; identification of benzoic acid. 310 mg of dibenzoxydihydrofuran (m.p. 185—189°) was refluxed with 3 ml of *N*/10 sulfuric acid and 2 ml of ethanol for 1 1/2 hours. The S-benzylthiuronium benzoate was then prepared in the usual way; yield 470 mg = 81 %. Yield after crystallization from ethanol and drying (sulfuric acid, 0.1 mm, 55°, 16 hours) 240 mg = 42 %; m.p. 163—165°; mixed melting point with an authentic specimen of S-benzylthiuronium benzoate 163—165°; Donleavy [5] found 166°.

$C_{15}H_{16}O_2N_2S$ (288.4) Calc. N 9.7
Found » 9.4

Hydrogenation; identification of succinaldehyde. 2.00 g of dibenzoxydihydrofuran (m.p. 185—189°) in 20 ml of dioxane was shaken with 0.4 g of Raney nickel at 105—115° under 120 atmospheres for 3 hours. After filtration the dioxane was distilled in vacuum. Residue 2.0 g of white crystals. Crystallization from methanol gave 1.17 g of dibenzoxytetrahydrofuran = 58 %; the product melted partly at 80—84° and completely at 125°. Another crystallization from methanol or acetone did not change the melting point. Yield after recrystallization from methanol 0.95 g = 81 %.

$C_{18}H_{16}O_5$ (312.3) Calc. C 69.2 H 5.2
Found » 69.2 » 5.2

350 mg of dibenzoxytetrahydrofuran was refluxed with 2 ml of *N*/10 sulfuric acid and 1 ml of ethanol for 30 minutes. After cooling 0.25 ml of phenylhydrazine in 5 ml of *N* acetic acid was added and the succinaldehyde bis-phenylhydrazone isolated as above;

yield 190 mg = 63 %. Yield after crystallization from ethanol and drying (sulfuric acid, 0.1 mm, 50°, 18 hours) 110 mg = 37 %; m.p. 121–123°.

$C_{16}H_{18}N_4$ (266.3) Calc. N 21.0
Found N 21.1

Note 1. All materials were technical products.

Note 2. At higher temperatures dark-coloured products are formed.

Note 3. The colour does not disappear on redistillation.

Note 4. Prepared after Criegee [8]. The crude product was used directly without crystallization. It contained propionic acid and the content of lead tetrapropionate was determined iodometrically.

Note 5. The propionic acid and the furan were technical products.

Note 6. The solution of lead tetrabutyrate in anhydrous butyric acid was prepared after Criegee [8], except that the chlorine treatment was omitted. The content of lead tetrabutyrate was determined iodometrically.

SUMMARY

Improved directions are given for the preparation of 2,5-diacetoxy-2,5-dihydrofuran from furan and lead tetraacetate. 2,5-Dipropionoxy-, 2,5-dibutyroxy- and 2,5-dibenzoxy-2,5-dihydrofuran were prepared by a similar reaction. These 2,5-dihydrofurans are new compounds. They were hydrogenated catalytically to the corresponding 2,5-diacyloxytetrahydrofurans, which also are new.

2,5-Diacetoxy-2,5-dihydrofuran and 2,5-dipropionoxy-2,5-dihydrofuran were separated into a liquid and a crystalline isomer, which were shown to be *cis-trans* isomers. Catalytic hydrogenation of the pure isomers gave the corresponding pairs of *cis-trans* isomeric 2,5-diacyloxytetrahydrofurans.

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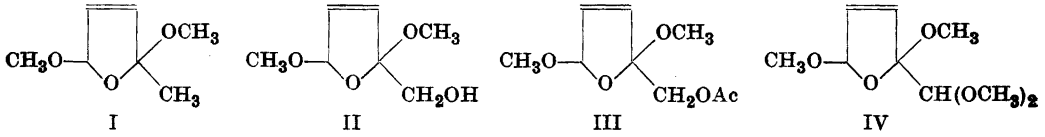
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Electrolytic Methoxylation of Some α-Substituted Furans

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An electrolytic method of methoxylating furan has recently been described[1]. Furan was mixed with a methanolic solution of ammonium bromide and the mixture electrolyzed. We have now methoxylated silvan, furfuryl alcohol, furfuryl acetate and furfural dimethyl acetal to the corresponding 2,5-dimethoxy-2,5-dihydrofurans (I—IV) in the same way. These compounds have all



I II III IV

been prepared previously, but the electrolytic method was in each case found more convenient than the old method of methoxylation.

EXPERIMENTAL

Microanalyses by Kirsten Glens

2,5-Dimethoxy-2,5-dihydrosilvan (I). 5.00 g of ammonium bromide (0.051 mole) is dissolved in 265 ml of methanol and 41.0 g of freshly distilled silvan (0.50 mole) (note 1) and the mixture (total volume about 310 ml) electrolyzed with the set-up used previously for the electrolytic methoxylation of furan [1].

Hours	Current (ampere)	Potential across the cell during electrolysis (volt)	Temperature in the cell	Ampere hours (per cent of theoretical amount)
0.2	3.3	4.4	− 16°	0.6 (2 %)
3.5	3.0	4.4	− 15°	11.3 (42 %)
6.1	2.8	4.5	− 15°	19.3 (72 %)
7.3	2.8	4.6	− 15°	22.8 (85 %)

After electrolysis the colourless liquid in the cell is poured into a solution of sodium methoxide (1.20 g of sodium (0.052 mole) in 20 ml of methanol) and the methanol containing the ammonia and the excess of silvan is distilled through a Vigreux column from a water-bath under atmospheric pressure. The residue consists of a clear yellowish-brown liquid and a precipitate of sodium bromide, which is filtered off and washed with a little ether. The filtrate is distilled through a 5 cm column packed with glass helices.

Fraction (g)	B. p. $_{760}$	n_D^{25}	OCH_3 Calc. 43.1
1 (0.2)	76–158°		
2 (10.7)	158°	1.4259	44.4
3 (23.2)	158–159°	1.4265	42.8
4 (13.7)	159°	1.4265	43.1
5 (1.9)	159°	1.4261	42.8
residue (3)			

All fractions show a negative Beilstein test for halogens. Yield (fractions 2–5) 49.5 g of dimethoxydihydrosilvan = 69 %; current efficiency 81 %.

Dimethoxydihydrosilvan has previously been prepared by methoxylation of silvan by Jones [2] (no experimental data) and by Clauson-Kaas and Limborg [3] (yield 65 %, $b.p._8$ = 46–56°).

2,5-Dimethoxy-2,5-dihydrofurfuryl alcohol (II). 5.00 g of ammonium bromide is dissolved in 265 ml of methanol and 39.2 g of freshly distilled furfuryl alcohol (0.40 mole) (note 2) and the mixture electrolyzed and poured (colourless liquid) into sodium methoxide as above.

Hours	Current (ampere)	Potential across the cell during electrolysis (volt)	Temperature in the cell	Ampere hours (per cent of theoretical amount)
0.3	3.3	4.7	– 14°	1.1 (5 %)
3.3	2.8	4.6	– 15°	10.0 (47 %)
5.9	2.6	4.8	– 15°	17.2 (80 %)
6.3	2.5	4.8	– 15°	18.3 (85 %)

The methanol and the ammonia are evaporated in vacuum from a water-bath and the clear yellowish-brown residue precipitated with 100 ml of ether. The precipitate of sodium bromide is removed by centrifugation and washed with a little ether. The supernatant liquid is then distilled in vacuum through a 5 cm column packed with glass helices.

Fraction (g)	B.p.$_{10-12}$	n_D^{25}	OCH_3 Calc. 38.8 %
1 (7.3)	76—106°	1.4712	
2 (4.9)	106—109°	1.4567	36.7
3 (33.2)	109—110°	1.4568	38.4
4 (3.9)	109°	1.4568	38.3
residue (6)			

All fractions show a negative Beilstein test for halogens. Yield (fractions 2—4) 42.0 g of dimethoxydihydrofurfuryl alcohol = 66 %; current efficiency 77 %.

Dimethoxydihydrofurfuryl alcohol has previously been prepared by methoxylation of furfuryl alcohol by Meinel [4] (yield 40 %; b.p.$_{0.4}$ = 86—87°; n_D^{25} = 1.4600). Meinel believed his product to be the 4,5-addition product, but it is almost certain that the methoxy groups add to the α-positions of furfuryl alcohol [3].

2,5-Dimethoxy-2,5-dihydrofurfuryl acetate (III). 5.00 g of ammonium bromide is dissolved in 260 ml of methanol and 42.0 g of furfuryl acetate (0.30 mole) (note 3) and the mixture electrolyzed and worked up as described for the preparation of dimethoxydihydrofurfuryl alcohol.

Hours	Current (ampere)	Potential across the cell during electrolysis (volt)	Temperature in the cell	Ampere hours (per cent of theoretical amount)
0.5	3.4	4.3	— 12°	1.8 (11 %)
1.5	3.2	4.3	— 13°	5.3 (33 %)
4.1	2.9	4.6	— 15°	13.4 (83 %)
5.2	2.3	5.0	— 15°	16.3 (101 %)

The final product is distilled without a column. Yield 45 g of a colourless liquid; b.p.$_{12}$ about 100—120° (note 4). 50 ml of acetic anhydride and 50 ml of pyridine are added and the mixture, which gets hot, left standing at room temperature for 20 hours. The low-boiling products are distilled in vacuum from a water-bath and the residue distilled further through a 5 cm column packed with glass helices.

Fraction (g)	B.p.$_{12}$	n_D^{25}	OCH_3 Calc. 30.7 %	$OCCH_3$ Calc. 21.3 %
1 (8.7)	117°			
2 (8.7)	117—119°	1.4431	30.2	21.2
3 (39.2)	119°	1.4433	30.5	20.9
4 (4.7)	119°	1.4433	30.2	20.8
residue (< 1)				

All fractions show a negative Beilstein test for halogens. Yield (fractions 2—4) 52.6 g of dimethoxydihydrofurfuryl acetate = 87 %; current efficiency = 86 %.

Dimethoxydihydrofurfuryl acetate has previously been prepared by methoxylation of furfuryl acetate by Clauson-Kaas and Limborg [3] (yield 66 %, b.p.$_{20}$ = 131°).

2,5-Dimethoxy-2,5-dihydrofurfural dimethyl acetal (IV). 5.00 g of ammonium bromide is dissolved in 270 ml of methanol and 35.5 g of furfural dimethyl acetal (0.25 mole) (note 5) and the mixture electrolyzed and worked up as described for the preparation of dimethoxydihydrofurfuryl alcohol.

Hours	Current (ampere)	Potential across the cell during electrolysis (volt)	Temperature in the cell	Ampere hours (per cent of theoretical amount)
0.5	3.1	4.7	— 13°	1.5 (11 %)
2.1	2.9	4.7	— 14°	6.6 (49 %)
4.8	2.2	5.2	— 16°	14.0 (104 %)

Fraction (g)	B.p.$_{13}$	n_D^{25}	OCH_3 Calc. 60.8 %
1 (1.8)	60—107°	1.4467	
2 (0.8)	107°	1.4429	59.0
3 (30.8)	107—110°	1.4428	60.0
4 (10.2)	110°	1.4431	59.5
residue (1)			

All fractions show a negative Beilstein test for halogens. Yield (fractions 2—4) 41.8 g of dimethoxydihydrofurfural dimethyl acetal = 82 %; current efficiency 79 %.

Dimethoxydihydrofurfural dimethyl acetal has previously been prepared by Meinel [4] by methoxylation of furfural (yield 50 %; b.p.$_{0.3}$ = 80—81°, n_D^{20} = 1.4451).

Note 1. All materials were technical products.

Note 2. All materials were technical products.

Note 3. The ammonium bromide and the methanol were technical products. The furfuryl acetate was prepared after directions given by Blatt, A. H. *Organic Synthesis, Collective Volume* **1** (1941) 285. New York: John Wiley & Sons Inc.

Note 4. This product contains 30—50 % of dimethoxydihydrofurfuryl alcohol, apparently formed by methanolysis during electrolysis. It is therefore acetylated in order to get pure dimethoxydihydrofurfuryl acetate.

Note 5. The ammonium bromide and the methanol were technical products. The methanol was dried with magnesium. Furfural dimethyl acetal was prepared from furfural and methyl orthoformate after Claisen. Yield 90 %; b.p.$_{14}$ = 57—58°; n_D^{25} = 1.4486. Previously found [5] b.p.$_{760}$ = 178°.

$C_5H_4O(OCH_3)_2$ (142.2)	Calc.	C	59.1	H	7.1	OCH_3	43.7
	Found	»	59.4	»	7.6	»	42.9

SUMMARY

Directions are given for the preparation of 2,5-dimethoxy-2,5-dihydrosilvan, 2,5-dimethoxy-2,5-dihydrofurfuryl alcohol, 2,5-dimethoxy-2,5-dihydrofurfuryl acetate and 2,5-dimethoxy-2,5-dihydrofurfural dimethyl acetal by electrolytic methoxylation of the corresponding furans.

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ACTA CHEMICA SCANDINAVICA 6 (1952) 551–555

The Alkoxylation of Furans With a Negative α-Substituent; Preparation of 2,5-Dimethoxy-2,5-dihydro-2-furoic Acid Methyl Ester and 2,5-Dimethoxytetrahydro-2-furoic Acid Methyl Ester

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The oxidation of furans to stable 2,5-disubstituted 2,5-dihydrofurans is a useful reaction since the dihydrofurans may serve as intermediates in the preparation of various types of compounds, which are otherwise difficult of accession. Several methods for the oxidation of furans to 2,5-dihydrofurans are known, Meinels method of methoxylation with halogens in methanol being by far the most convenient [1–3]. This method is, however, not generally applicable as furans with a negative α-substituent can usually not be methoxylated in this way. Of 2-furoic acid, ethyl 2-furoate, ethyl 2-furylacrylate, furfural and 2-acetylfuran only furfural is methoxylated by bromine in methanol under the usual reaction conditions [1,3]. Besides furfural, the only other furan with a negative substituent, which has been methoxylated, is methyl 2-furoate (Jones [2]). Chlorine in boiling methanol was used for the methoxylation. But the yield of dimethoxydihydro-2-furoic acid methyl ester was only 12 per cent and the product, of which no analyses were reported, can not have been very pure since it boiled from 120° to 140° at 20 mm.

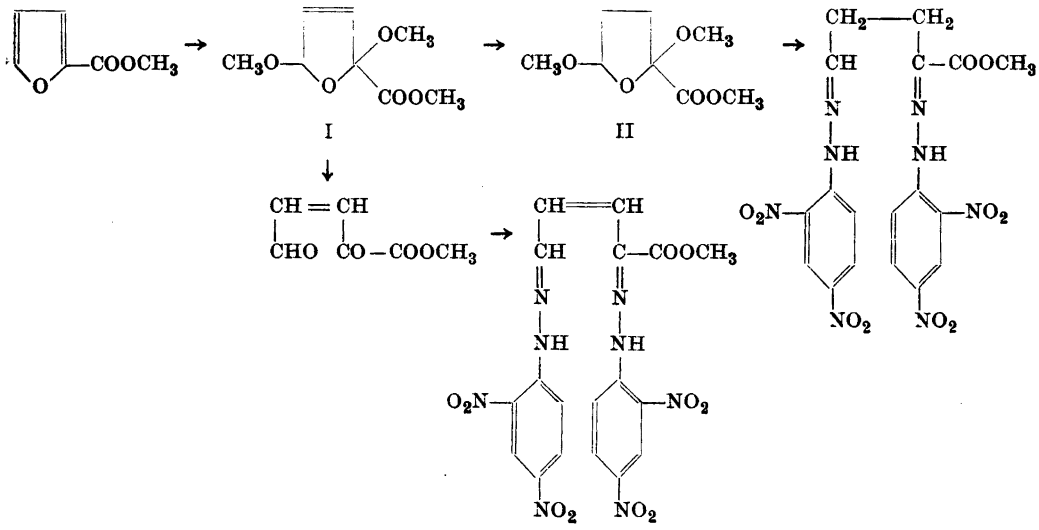
We have now found that a 68 per cent yield of analytically pure dimethoxydihydro-2-furoic acid methyl ester is obtained by electrolyzing a mixture of methyl furoate, methanol and sulfuric acid in the cell described previously for the methoxylation of furan [4]. It is possible that this new form of the alkoxylation method may also be used for the alkoxylation of other furans with a negative substituent *.

* Patent pending.

Dimethoxydihydrofuroic acid methyl ester was hydrogenated catalytically to dimethoxytetrahydrofuroic acid methyl ester, which is a new compound. Dimethoxytetrahydrofuroic acid methyl ester formed, with dinitrophenylhydrazine in methanol, a bis-dinitrophenylhydrazone with the formula $C_{17}H_{13}O_9N_8(OCH_3)$. This proves, that the methoxy groups in both dimethoxy compounds are in the 2,5-positions (formulas I and II).

The product obtained by treatment of methyl 2-furoate with chlorine in methanol gave, as reported by Jones [2], after hydrolysis and reaction with dinitrophenylhydrazine, a bis-dinitrophenylhydrazone with the formula $C_{18}H_{14}O_{10}N_8$. Jones' product must therefore also have the methoxy groups in the 2,5-positions.

The reactions starting from methyl 2-furoate so far carried out are shown below.



The structure of dialkoxydihydrofurans has, as a rule, been proved by identification of the compounds formed by hydrolysis [Cf.5]. Hydrogenation of the dialkoxydihydrofurans to dialkoxytetrahydrofurans and subsequent hydrolysis or alcoholysis, as described above, can likewise be used as a proof of structure, and it may now and then be easier to obtain, from the dialkoxytetrahydrofuran, a high yield of a derivative, which crystallizes well and melts sharply.

The individual dialkoxydihydrofurans and the dialkoxytetrahydrofurans are usually spoken of, as if they were pure compounds, but it should be remembered, that they may be mixtures of the *cis* and the *trans* isomers, as is probably

the case with electrolytically prepared 2,5-dimethoxy-2,5-dihydrofuran [4]. They may furthermore, as mentioned by Clauson-Kaas and Limborg [6], contain small amounts of structural isomers, *e. g.* 2,3-dialkoxy-2,3-dihydrofurans, which have escaped notice during the structure determination.

EXPERIMENTAL

Microanalyses in part by Kirsten Glens

2,5-Dimethoxy-2,5-dihydro-2-furoic acid methyl ester (I). A mixture of 63.0 g of methyl 2-furoate (0.50 mole), 230 ml of methanol (5.7 moles) and 1.00 ml of concentrated sulfuric acid (0.019 mole) (note 1) is electrolyzed with the set-up used previously for the methoxylation of furan[4].

Hours	Current (ampere)	Potential across the cell during electrolysis (volt)	Temperature in the cell	Ampere hours (per cent of theoretical amount)
0.5	2.3	6.4	− 13°	1.1 (4 %)
3.3	2.0	6.6	− 13°	7.2 (27 %)
9.1	1.6	7.0	− 15°	17.9 (67 %)
20.1	1.1	7.1	− 17°	32.0 (119 %)
	1.1	7.1	− 17°	33.5 (125 %)

After electrolysis the light-brown liquid in the cell is poured into a solution of sodium methoxide (0.95 g of sodium (0.041 mole) in 15 ml of methanol) and the methanol evaporated in vacuum from a water-bath. The oily dark-brown residue is precipitated with 200 ml of ether and the precipitate removed by centrifugation and washed with ether. The ether is then distilled from a water-bath and the residue distilled further in vacuum through a 5 cm column packed with glass helices.

Fraction (g)	B.p.$_{13-14}$	n_D^{25}	OCH_3 Calc. 49.4
1 (2.8)	72−119°	1.4549	
2 (20.7)	119−121°	1.4476	48.0
3 (43.0)	121	1.4476	49.2
residue (14)			

Yield (fractions 2—3) 63.7 g of dimethoxydihydrofuroic acid methyl ester = 68 %; current efficiency 54 %. Fraction 2 was also analyzed for carbon and hydrogen.

$C_5H_3O_2(OCH_3)_3$ (188.2) Calc. C 51.1 H 6.4
Found » 51.0 » 6.4

2,5-Dimethoxytetrahydro-2-furoic acid methyl ester (II). 63.1 g of dimethoxydihydrofuroic acid methyl ester ($b.p._{18}$ = 127—131°, n_D^{25} = 1.4474) and 100 ml of anhydrous methanol are shaken with 2.0 g of Raney nickel under 100 atmospheres of hydrogen for 5 hours. After filtration the methanol is evaporated in vacuum from a water-bath and the residue distilled further in vacuum.

Fraction (g)	$B.p._{13}$	n_D^{25}	OCH_3 Calc. 48.9 %
1 (5.2)	100—102°	1.4348	47.8
2 (20.2)	102—105°	1.4347	47.8
3 (27.5)	105—108°	1.4348	48.6
4 (5.1)	108—113°	1.4353	49.3
5 (3.7)	113—136°	1.4361	49.8
residue (1)			

Yield (fractions 1—4) 58.0 g of dimethoxytetrahydrofuroic acid methyl ester = 91 %. Fraction 3 was also analyzed for carbon and hydrogen.

$C_5H_5O_2(OCH_3)_3$ (190.2) Calc. C 50.5 H 7.4
Found » 50.7 » 7.3

Bis-dinitrophenylhydrazone of methyl α,δ-dioxo-n-valerate. 188 mg of dimethoxytetrahydrofuroic acid methyl ester in 4 ml of methanol was added to a solution of 500 mg of dinitrophenylhydrazine in 7 ml of methanol and 1 ml of concentrated sulfuric acid. The yellow hydrazone, which crystallized almost immediately, was filtered off after one hour and washed thoroughly with methanol; yield 364 mg of bis-dinitrophenylhydrazone = 73 %; m.p. (Hershberg apparatus, corr.) 246—248° (destruction). 50 mg was crystallized from nitromethane; yield 38 mg; m.p. 256—258°. Recrystallization gave 31 mg; m.p. 253—255°. This product was dried (sulfuric acid, 0.01 mm, 80°, 16 hours) and analyzed.

$C_{17}H_{13}O_9N_8(OCH_3)$ (504.4) Calc. C 42.9 H 3.2 N 22.2 OCH_3 6.2
Found » 42.8 » 3.3 » 21.7 » 6.2

Note 1. All materials were technical products.

SUMMARY

2,5-Dimethoxy-2,5-dihydro-2-furoic acid methyl ester has been prepared by electrolytic methoxylation of methyl 2-furoate. Catalytic hydrogenation gave 2,5-dimethoxytetrahydro-2-furoic acid methyl ester, which is a new compound.

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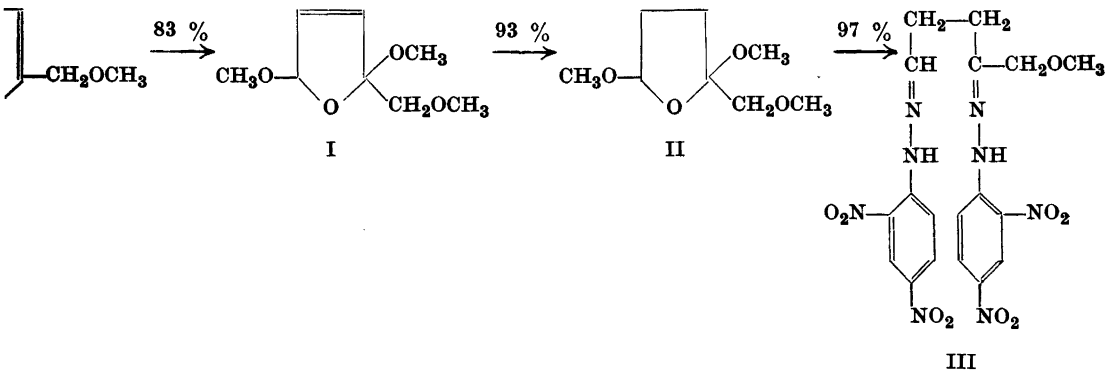
ACTA CHEMICA SCANDINAVICA 6 (1952) 556–559

Preparation of 2,5-Dimethoxy-2,5-dihydrofurfuryl Methyl Ether and 2,5-Dimethoxytetrahydrofurfuryl Methyl Ether

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A new electrolytic method for the methoxylation of furans has recently been described [1–3]. This method has now been used to methoxylate furfuryl methyl ether to dimethoxydihydrofurfuryl methyl ether, which is a new compound. Catalytic hydrogenation of dimethoxydihydrofurfuryl methyl ether gave the expected dimethoxytetrahydrofurfuryl methyl ether, which also is new. Dimethoxytetrahydrofurfuryl methyl ether gave, with dinitrophenylhydrazine in methanol, a bis-dinitrophenylhydrazone with the formula $C_{17}H_{15}O_8N_8(OCH_3)$. This proves that the methoxy groups in both dimethoxy compounds are in the 2,5-positions (formulas I and II) *Cf.* [3].



The bis-dinitrophenylhydrazone (structure III) is a derivative of δ-methoxylevulinaldehyde. Pummerer *et al.*[4,5] report having prepared the dimethyl acetal of δ-methoxylevulinaldehyde by acid methanolysis of furfuryl alcohol or furfuryl methyl ether. They obtained from their product a bis-dinitro-

phenylhydrazone with the formula $C_{18}H_{18}O_9N_8$. The bis-dinitrophenylhydrazone of Pummerer *et al.* is reported to melt at 192° and to be easily soluble in benzene, while the bis-dinitrophenylhydrazone from dimethoxytetrahydrofurfuryl methyl ether melts at 248—249° and is almost insoluble in benzene. The two hydrazones are thus apparently not identical. Since the hydrazone from dimethoxytetrahydrofurfuryl methyl ether is prepared in high yield by a series of well-known reactions it is almost certainly a bis-dinitrophenylhydrazone of δ-methoxylevulinaldehyde. The structure proposed by Pummerer *et al.* for their hydrazone should therefore not be accepted without further experimental evidence.

EXPERIMENTAL

Microanalyses by Franz Limborg and Kirsten Glens

2,5-Dimethoxy-2,5-dihydrofurfuryl methyl ether (I). 5.00 g of ammonium bromide (0.051 mole) is dissolved in 270 ml of methanol and 39.2 g of furfuryl methyl ether (0.35 mole) (note 1) and the mixture electrolyzed with the set-up used previously for the electrolytic methoxylation of furan [1].

Hours	Current (ampere)	Potential across the cell during electrolysis (volt)	Temperature in the cell	Ampere hours (per cent of theoretical amount)
0.5	2.9	4.5	— 14°	1.6 (8 %)
2.2	2.8	4.5	— 15°	6.5 (35 %)
6.3	2.3	4.8	— 15°	17.4 (93 %)
7.0	1.9	4.9	— 15°	18.8 (100 %)

After electrolysis the liquid in the cell is colourless in the upper part of the cell and slightly yellow from unreacted bromine near the bottom. The liquid is poured into a solution of sodium methoxide (1.20 g of sodium (0.052 mole) in 15 ml of methanol) and the methanol and the ammonia evaporated in vacuum from a water-bath. The residue is filtered and the precipitate of sodium bromide washed with a little ether. The filtrate is distilled through a 5 cm column packed with glass helices (see p. 558).

All fractions show a negative Beilstein test for halogens. Yield (fractions 2—4) 50.8 g of dimethoxydihydrofurfuryl methyl ether = 83 %; current efficiency 83 %.

Fraction 3 was further analyzed for carbon and hydrogen.

$C_5H_5O(OCH_3)_3$ (174.2) Calc. C 55.2 H 8.1
Found » 55.2 » 8.2

Fraction (g)	B. p.$_{10}$	n_D^{25}	OCH_3 Calc. 53.5 %
1 (0.4)	67–84°		
2 (37.8)	84–85°	1.4384	53.3
3 (8.0)	85°	1.4383	53.6
4 (5.0)	85°	1.4384	54.1
residue (2)			

2,5-Dimethoxytetrahydrofurfuryl methyl ether (II). 30.0 g of dimethoxydihydrofurfuryl methyl ether and 50 ml of anhydrous methanol are shaken with 4.0 g of Raney nickel under 100 atmospheres of hydrogen for 4 hours. After filtration the methanol is evaporated in vacuum and the residue distilled further in vacuum.

Fraction (g)	B. p.$_{12}$	n_D^{25}	OCH_3 Calc. 52.8 %
1 (3)	< 42°		
2 (7.8)	80–82°	1.4285	53.2
3 (7.2)	81–82°	1.4283	52.3
4 (9.0)	81–82°	1.4289	52.3
5 (4.2)	82–83°	1.4285	52.7
no residue			

Yield (fractions 2–5) 28.2 g of dimethoxytetrahydrofurfuryl methyl ether = 93 %. Fraction 4 was further analyzed for carbon and hydrogen.

$C_5H_7O(OCH_3)_3$ (176.2) Calc. C 54.5 H 9.2
Found » 54.5 » 9.2

Bis-dinitrophenylhydrazone of δ-methoxylevulinaldehyde (III). 176 mg of dimethoxytetrahydrofurfuryl methyl ether in 5 ml of anhydrous methanol was added to a solution of 495 mg of dinitrophenylhydrazine in 10 ml of methanol and 1 ml of concentrated sulfuric acid. The yellow hydrazone, which precipitated immediately, was filtered off after 1 hour, washed thoroughly with methanol and dried (sulfuric acid, 0.1 mm, 80°,

1 hour). Yield 477 mg of bis-dinitrophenylhydrazone = 97 %; m. p. (Hershberg apparatus, corr.) 227—229° (destruction).

$C_{17}H_{15}O_8N_8(OCH_3)$ (490.4)	Calc.	C	44.1	H	3.7	N	22.9	OCH_3 6.3
	Found	»	44.3	»	3.9	»	22.5	» 6.4

The hydrazone is almost insoluble in benzene and very slightly soluble in acetone. It crystallizes well from nitromethane. 50 mg gave 40 mg of recrystallized product; m. p. 249—250°. Recrystallization gave 30 mg; m. p. 248—249°. This product was dried (sulfuric acid, 0.01 mm, 80°, 16 hours) and analyzed.

Found	C	44.3	H	3.8	N	22.2	OCH_3 6.3

Note 1. Analytical grades of ammonium bromide and of methanol were used. The furfuryl methyl ether was prepared after Pummerer *et al.*[4] (b. $p._{760}$ = 136°, n_D^{25} = 1.4511).

SUMMARY

2,5-Dimethoxy-2,5-dihydrofurfuryl methyl ether has been prepared by electrolytic methoxylation of furfuryl methyl ether. Catalytic hydrogenation gave 2,5-dimethoxytetrahydrofurfuryl methyl ether. Both compounds are new.

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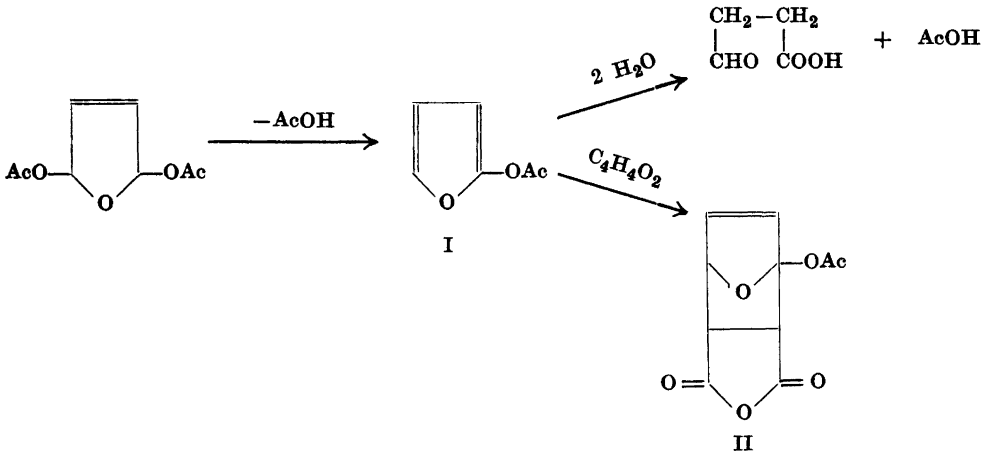
ACTA CHEMICA SCANDINAVICA 6 (1952) 560–564

2-Acetoxyfuran

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We have found that by pyrolysis of 2,5-diacetoxy-2,5-dihydrofuran a colourless liquid compound with the formula $C_4H_3O_2(COCH_3)$ is formed [1]. This compound reacts (1) with dinitrophenylhydrazine in 2 *N* aqueous hydrochloric acid to give the dinitrophenylhydrazone of β-formylpropionic acid; (2) with phenylhydrazine to give the hydrazone-hydrazide of β-formylpropionic acid and (3) with maleic anhydride to form an addition product. The compound must therefore be the hitherto unknown 2-acetoxyfuran I while the addition product probably has the structure II.

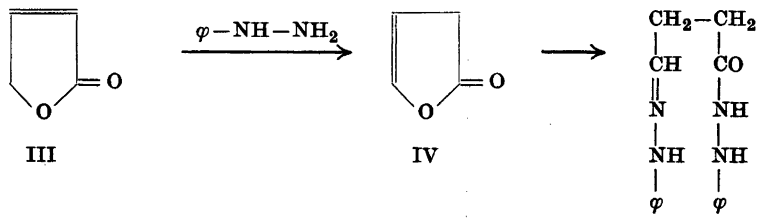


The course of the pyrolysis of diacetoxydihydrofuran depends upon its quality. Diacetoxydihydrofuran, prepared from furan and lead tetraacetate [2], gives a 35 per cent yield of 2-acetoxyfuran. If the diacetoxydihydrofuran is first roughly separated into the crystalline and the liquid isomer [2], and these pyrolyzed separately, the crystalline isomer gives 24 per cent of 2-acetoxyfuran,

while the liquid isomer gives 40 per cent. These results are consistent with our earlier findings [2] in that diacetoxydihydrofuran, prepared by the lead salt method, consists of about 1/3 of the crystalline and 2/3 of the liquid isomer.

Pyrolysis of diacetoxydihydrofuran, prepared by the bromine method [3], can also give about 35 per cent of 2-acetoxyfuran. But the yield is often considerably lower and at the same time varying amounts of γ-hydroxycrotonolactone (III) are formed. One experiment gave for instance 7 per cent of 2-acetoxyfuran and 19 per cent of γ-hydroxycrotonolactone. We believe that the variable behaviour of diacetoxydihydrofuran prepared by the bromine method, in the pyrolytic reaction, is due to the formation of small amounts of hydrogen bromide during the reaction *Cf.* [3]. Probably γ-hydroxycrotonolactone is formed from 2-acetoxyfuran and not directly from diacetoxydihydrofuran.

γ-Hydroxycrotonolactone gives no precipitate with dinitrophenylhydrazine in hydrochloric acid but, like 2-acetoxyfuran, gives the phenylhydrazone-hydrazide of β-formylpropionic acid by reaction with phenylhydrazine. This has previously been found by Glattfeld *et al.*[4], who, however, were unable to identify their product. Evidently γ-hydroxycrotonolactone is first transformed by phenylhydrazine into 2-oxo-2,3-dihydrofuran IV or its equivalent, which then reacts further to give the hydrazone-hydrazide. This is in agreement with what is known in general about the behaviour of α, β-unsaturated lactones (*cf.* Paist, Blout, Uhle and Elderfield [5]).



III IV

EXPERIMENTAL

Microanalyses by K. Glens and E. Boss

Pyrolysis of 2,5-diacetoxy-2,5-dihydrofuran. (1). 50.0 g of diacetoxydihydrofuran, prepared from furan and lead tetraacetate [1], was pyrolyzed under 10 mm in an apparatus of the Bouveault type [6] (Fig. 1).

The temperature of the oven was kept at 480—500°. The diacetoxydihydrofuran was kept boiling at such a rate that the temperature at the top of the condenser remained at 65—70°. The colour of the content of the distilling flask changed from pale-yellow to dark-brown during the reaction. After about 2 hours the reaction was stopped. 8 g of a black pitchy mass remained in the distilling flask. The cooling-trap contained 38 g of

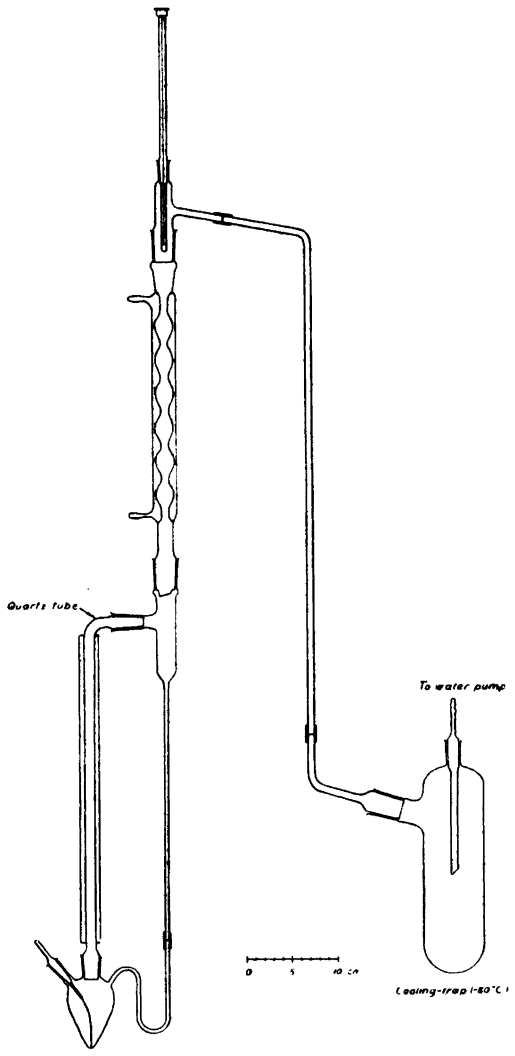


Fig. 1.

a partially crystalline product, which melted at room temperature to a pale-yellow liquid. Distillation under 15 mm through a short column gave 29 g of distillate, b. $p._{15} < 65°$. Further distillation of the residue without the column gave 5.10 g of regenerated diacetoxydihydrofuran = 10 %: b. $p._9 = 125-130°$; $n_D^{25} = 1.4544$. The product crystallized partly at room temperature after inoculation *Cf.* [2].

The distillate (29 g) was dissolved in 50 ml of ether and washed with 75 ml of water and then 4 times with 25 ml of 2 *M* potassium hydrogen carbonate. Drying and distillation of the etheral solution gave 12.0 g of 2-acetoxyfuran = 35 %; colourless liquid, b. $p._9 =$

49—50°; n_D^{25} = 1.4480; the UV-spectrum in 96 % ethanol showed a flat maximum at 212 mμ; log ε_{max} = 3.72.

$C_4H_3O_2(COCH_3)$ (126.1)	Calc.	C 57.1	H 4.8	$COCH_3$ 34.1
	Found	» 57.2	» 4.8	» 33.6

2-Acetoxyfuran, like furan itself, turns yellow on standing, probably due to autoxidation. By addition of ether to an old sample of 2-acetoxyfuran, a white amorphous substance separates.

(2). Diacetoxydihydrofuran, prepared from furan and lead tetraacetate, was separated roughly by decantation into the crystalline and the liquid isomer. Pyrolysis of the crystalline isomer as above gave 24 %, while pyrolysis of the liquid isomer gave 40 % of 2-acetoxyfuran.

(3). 50.0 g of diacetoxydihydrofuran, prepared by the bromine method [3], was pyrolyzed as above (reaction time 1.5 hours). Yield 12.5 g of 2-acetoxyfuran = 37 %; b. $p._{14}$ = 54—55°; n_D^{25} = 1.4480.

(4). Three 50 g-portions of another sample of diacetoxydihydrofuran, also prepared by the bromine method, gave by pyrolysis 114 g of liquid in the cooling-trap. Distillation through a short column gave first 84 g with b. $p._{15} < 65°$, from which 7 g of 2-acetoxyfuran = 7 % was isolated as above; b. $p._{13}$ = 53—54°; n_D^{25} = 1.4479. Further distillation gave 13 g of a pale-yellow liquid; b. $p._{18}$ = 95—98°; n_D^{25} = 1.4665. Redistillation after 2 months through a short column gave 10 g of a colourless liquid; b. $p._{18}$ = 100—102°; n_D^{25} = 1.4660. 5.0 g of this product was crystallized rapidly from 5 ml of ether and distilled. Yield 2.1 g; b. $p._{12}$ = 88—89°; m. p. 4—5°; n_D^{25} = 1.4662; previously found for γ-hydroxycrotonolactone b. $p._{12}$ = 92—93° [7], m. p. 5.0—5.3° [8].

$C_4H_4O_2$ (84.1)	Calc.	C 57.1	H 4.8	$COCH_3$ 0
	Found	» 57.1	» 4.8	» 0

The yield of γ-hydroxycrotonolactone (13 g) corresponds to 19 % of the theoretical amount. The crystallized sample gave no precipitate with dinitrophenylhydrazine in 2 *N* hydrochloric acid.

Reaction of 2-acetoxyfuran and γ-hydroxycrotonolactone with phenylhydrazine. 126 mg of 2-acetoxyfuran and 0.30 ml of phenylhydrazine were mixed and heated to 100° for 3 hours. After cooling the reaction mixture was washed 4 times with ether and the remaining crystals crystallized from ethanol. Yield 120 mg of β-formylpropionic acid phenylhydrazone-hydrazide = 43 %; m. p. 181—182° (Hershberg apparatus, corr.); previously found [9] 182°.

$C_{16}H_{18}ON_4$ (282.3)	Calc.	C 68.1	H 6.4	N 19.9
	Found	» 68.1	» 6.6	» 19.9

β-Formylpropionic acid phenylhydrazone-hydrazide was prepared from 84 mg of γ-hydroxycrotonolactone and 0.30 ml of phenylhydrazine as above. Yield 100 mg = 36 %; m. p. 182—183°; mixed melting point with a sample prepared from 2-acetoxyfuran 180—182°.

Found	C 68.1	H 6.5	N 20.0

Reaction of 2-acetoxyfuran with dinitrophenylhydrazine in 2 N hydrochloric acid. A few drops of 2-acetoxyfuran were added to an 0.2 % solution of dinitrophenylhydrazine in 2 *N* hydrochloric acid and the resulting dinitrophenylhydrazone of β-formylpropionic acid isolated in the usual way and crystallized from dioxane-petroleum ether; m. p. 197—203°; previously found [10] 198—200°.

$C_{10}H_{10}O_6N_4$ (282.2)	Calc.	C 42.6	H 3.6	N 19.9
	Found	» 42.3	» 4.0	» 19.7

Reaction of 2-acetoxyfuran with maleic anhydride. 0.63 g of 2-acetoxyfuran (0.005 mole) and 0.50 g of maleic anhydride (0.005 mole) were dissolved in 5 ml of benzene and the solution evaporated to 1—2 ml. On cooling the reaction mixture solidified to a mass of white crystals, which were washed 4 times with benzene. Yield 0.75 g of addition product = 67 %; m. p. 132—133°. Crystallization from benzene did not change the melting point.

$C_8H_5O_5(COCH_3)$ (224.2)	Calc.	C	53.6	H	3.6	$COCH_3$	19.2
	Found	»	54.0	»	3.9	»	18.9

SUMMARY

2-Acetoxyfuran, which is a new compound, has been prepared by pyrolysis of 2,5-diacetoxy-2,5-dihydrofuran. Pyrolysis of certain impure samples of diacetoxydihydrofuran gave varying amounts of 2-acetoxyfuran together with γ-hydroxycrotonolactone.

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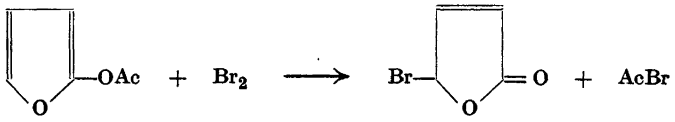
Preparation of Some 5-Substituted 2-Oxo-2,5-dihydrofurans from 2-Acetoxyfuran

NIELS ELMING and NIELS CLAUSON-KAAS

Centrallaboratoriet, Sadolin & Holmblad A/S, Copenhagen, Denmark

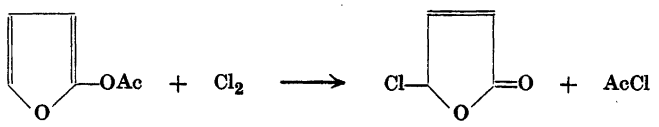
2-Acetoxyfuran has recently been prepared by pyrolysis of 2,5-diacetoxy-2,5-dihydrofuran [1]. We have now investigated the action of certain oxidizing agents on 2-acetoxyfuran.

2-Acetoxyfuran reacts with bromine to form 2-oxo-5-bromo-2,5-dihydrofuran I and acetyl bromide. The reaction takes place rapidly at — 5 to — 10°.



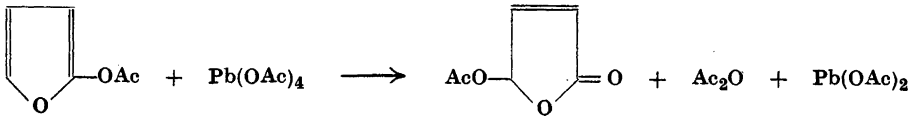
I

In the same way 2-acetoxyfuran and chlorine gives 2-oxo-5-chloro-2,5-dihydrofuran II and acetyl chloride.



II

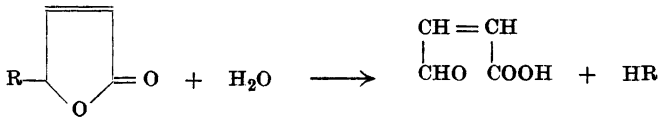
Finally 2-acetoxyfuran and lead tetraacetate in acetic acid at 20—45° gives 2-oxo-5-acetoxy-2,5-dihydrofuran III.



III

The similarity of the above reactions suggests that they are of the same type and either all three are electron-sharing reactions or all three are electron-pairing reactions.

The 5-substituted 2-oxo-2,5-dihydrofurans are new compounds. Their structure was proved by hydrolysis to *cis*-β-formylacrylic acid, which was identified as phenylhydrazone.



I (R = Br) II (R = Cl) III (R = CH_3COO)

EXPERIMENTAL

Microanalyses by K. Glens and E. Boss

Reaction of 2-acetoxyfuran with bromine. 5.00 ml of bromine (0.098 mole) in 20 ml of dry carbon tetrachloride was added during 8 minutes to a stirred solution of 12.5 g of 2-acetoxyfuran (0.099 mole) in 20 ml of carbon tetrachloride. The temperature was kept at − 5 to − 10°. The low boiling products were distilled in vacuum from a water-bath of 30−40° and collected in a receiver cooled to − 80°. The residue was distilled further in vacuum. Yield 14.7 g of a colourless liquid, which darkens rapidly; b. $p._{10}$ = 101−102°; n_D^{25} = 1.5343. Two redistillations, first under 10 mm and then under 0.1 mm, gave a product, which remained colourless. Yield 12.9 g of 2-oxo-5-bromo-2,5-dihydrofuran = 81 %; b. $p._{0.1}$ = 69−70°, n_D^{25} = 1.5348.

$C_4H_3O_2Br$ (163.0)	Calc.	C	29.5	H	1.9	Br	49.0
	Found	»	29.6	»	2.1	»	48.5

The low boiling products from the above synthesis consist of acetyl bromide and carbon tetrachloride. They were washed into a 100 ml volumetric flask with carbon tetrachloride. 2.00 ml was pipetted out and added to 0.50 ml of aniline in 10 ml of anhydrous ether. A white precipitate, which was formed immediately, was filtered off and washed with ether; yield 300 mg of aniline hydrobromide = 88 %. Dissolution in ethanol and precipitation with ether gave 260 mg = 76 %.

C_6H_8NBr (174.1)	Calc.	N	8.0	Br	45.9
	Found	»	7.8	»	46.4

The filtrate from the aniline hydrobromide was evaporated in vacuum and the residue crystallized from 10 ml of water and then from 5 ml of ethanol. Yield 90 mg of acetanilide = 34 %; m. p. 113−114° (Hershberg apparatus, corr.).

C_8H_9ON (135.2)	Calc.	N	10.4
	Found	»	10.5

Hydrolysis of 2-oxo-5-bromo-2,5-dihydrofuran. 190 mg of 2-oxo-5-bromo-2,5-dihydrofuran was boiled with 2 ml of water for a few seconds. 0.6 ml of phenylhydrazine in 2 ml of glacial acetic acid and 5 ml of water was added. The yellow precipitate was filtered off after 10 minutes, washed with water and dried. Yield 180 mg of *cis*-β-formylacrylic acid

phenylhydrazone = 81 %. Crystallization from acetone-benzene gave 105 mg = 47 %; m. p. 147—148° (compare Schenck and Appel [2] and Hellström [3]).

$C_{10}H_{10}N_2O_2$ (190.2)	Calc.	C	63.2	H	5.3	N 14.7
	Found	»	63.5	»	5.2	» 14.5

Reaction of 2-acetoxyfuran with chlorine. 2.35 ml of chlorine (measured at — 80°) (0.055 mole) was evaporated into 6.7 g of 2-acetoxyfuran (0.053 mole) in 10 ml of carbon tetrachloride at — 40 to — 50° during 10 minutes. Towards the end of the chlorination, the reaction mixture turned yellow. The mixture was heated to room temperature and left standing for 10 minutes. The reaction products were isolated as above.

Yield 4.81 g of 2-oxo-5-chloro-2,5-dihydrofuran = 77 %; colourless liquid; b. $p._{13}$ = 91—95°; n_D^{25} = 1.4884. Two redistillations gave 3.33 g = 53 %; b. $p._{14}$ = 91—93°; n_D^{25} = 1.4884.

$C_4H_3O_2Cl$ (118.5)	Calc.	C	40.5	H	2.6	Cl 29.9
	Found	»	40.1	»	2.8	» 31.0

It is apparent from the analytical evidence, that the product contains a certain amount of a chloro-compound with more chlorine and less carbon than 2-oxo-5-chloro-2,5-dihydrofuran.

Yield of reprecipitated aniline hydrochloride 91 mg = 66 %.

C_6H_8NCl (129.6)	Calc.	N	11.0	Cl	27.4
	Found	»	10.8	»	27.9

Yield of crystallized acetanilide 21 mg = 15 %; m. p. 112—114°.

Found N 10.4

Hydrolysis of 2-oxo-5-chloro-2,5-dihydrofuran. Cis-β-formylacrylic acid phenylhydrazone was prepared from 140 mg of 2-oxo-5-chloro-2,5-dihydrofuran as above. Yield of crude product 150 mg = 67 %. Yield after crystallization 80 mg = 36 %; m. p. 148—150°.

Found C 63.6 H 5.4 N 14.3

Reaction of 2-acetoxyfuran with lead tetraacetate. A suspension of lead tetraacetate was prepared in the usual way [4] from 55.0 g of read lead oxide (0.080 mole), 105 ml of glacial acetic acid and 41 ml of acetic anhydride. 10.1 g of 2-acetoxyfuran (0.080 mole) was added at once with stirring and the temperature kept at 20—25° for 30 minutes by external cooling and then raised to 40° and kept at 40—45° for 30 minutes. The reaction product was isolated as described previously for the preparation of 2,5-diacetoxy-2,5-dihydrofuran [5]. Yield 10.0 g of 2-oxo-5-acetoxy-2,5-dihydrofuran = 88 %; b. $p._{0.2}$ = 85—88°; n_D^{25} = 1.4593. The product was an almost colourless liquid. Redistillation gave 8.9 g; b. $p._{0.05}$ = 72—73°; n_D^{25} = 1.4594.

$C_4H_3O_3(CH_3CO)$ (142.1)	Calc.	C	50.7	H	4.3	CH_3CO 30.3
	Found	»	50.9	»	4.1	» 30.2

Hydrolysis of 2-oxo-5-acetoxy-2,5-dihydrofuran. Cis-β-formylacrylic acid phenylhydrazone was prepared from 340 mg of 2-oxo-5-acetoxy-2,5-dihydrofuran as above, except that the preliminary hydrolysis was carried out by boiling with 5 ml of *N*/10 sulfuric acid for 4 minutes. Yield of crude product 295 mg = 65 %. Yield after two crystallizations 130 mg = 29 %; m. p. 147—148°.

Found C 63.2 H 5.5 N 14.6

SUMMARY

2-Acetoxyfuran has been found to react (1) with bromine to give 2-oxo-5-bromo-2,5-dihydrofuran and acetyl bromide, (2) with chlorine to give 2-oxo-5-chloro-2,5-dihydrofuran and acetyl chloride, and (3) with lead tetraacetate to give 2-oxo-5-acetoxy-2,5-dihydrofuran. The dihydrofurans are all new compounds. They could be hydrolyzed to *cis*-β-formylacrylic acid.

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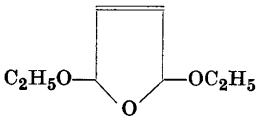
Electrolytic Ethoxylation of Furan

NIELS CLAUSON-KAAS

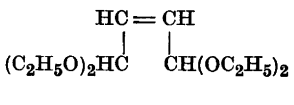
Centrallaboratoriet, Sadolin & Holmblad A/S, Copenhagen, Denmark

A new electrolytic method for the alkoxylation of furans with methanol, ethanol and the propanols has been described in a patent [1] and detailed directions were then worked out for the *methoxylation*, on a laboratory scale, of a number of the simple furans [2–5]. It has now been found that electrolytic *ethoxylation* of furans may be carried out with the same set-up as was used for the methoxylations and in this communication the preparation of 2,5-diethoxy-2,5-dihydrofuran I by electrolysis is given as an example. Diethoxydihydrofuran has been prepared previously, but the electrolytic method is more convenient than the known methods of alkoxylation.

In preparing diethoxydihydrofuran a small amount of a higher boiling liquid was formed. This has been found to consist mainly of malealdehyde tetraethyl acetal II *Cf.* [2].



I



II

EXPERIMENTAL

Microanalyses by G. Cornali and K. Glens

2,5-Diethoxy-2,5-dihydrofuran (I). 4.00 g of ammonium bromide (0.041 mole) is dissolved in 255 ml of absolute ethanol and 47.6 g of furan (0.70 mole) (all materials were technical products) and the mixture electrolyzed with the set-up used for the electrolytic methoxylation of furan [2].

After electrolysis the light-brown liquid in the cell is poured into a solution of sodium methoxide (0.96 g of sodium (0.042 mole) in 15 ml of methanol) and the diethoxydihydrofuran isolated as described for the preparation of dimethoxydihydrosilvan [2].

Hours	Current (ampere)	Potential across the cell during electrolysis (volt)	Temperature in the cell	Ampere hours (per cent of theoretical amount)
0.5	2.5	11.0	− 9°	1.4 (4 %)
3.2	2.4	11.0	− 8°	7.9 (21 %)
14.8	1.4	11.2	− 11°	30.0 (80 %)
16.0	1.3	11.2	− 11°	31.7 (85 %)

Fraction (g)	B. p.$_{760}$	n_D^{25}	OC_2H_5 Calc. 57.0 %
1 (9.7)	184°	1.4303	56.2
2 (55.2)	184−185°	1.4306	56.4
3 (4.6)	185−186°	1.4305	56.5
residue (14)			

All fractions show a negative Beilstein test for halogens. Yield 69.5 g of diethoxydihydrofuran = 63 %; current efficiency = 74 %.

Diethoxydihydrofuran has previously been prepared by ethoxylation of furan by Clauson-Kaas [6] (*cf.* Clauson-Kaas, Limborg and Fakstorp [7]) (yield 56 %, b. p.$_{12}$ = 71−73°, n_D^{25} = 1.4309) and by Fakstorp, Raleigh and Schniepp [8] (yield 79 %, b. p.$_1$ = 50−53°, n_D^{25} = 1.4310).

Identification of malealdehyde tetraethyl acetal (II). By fractional distillation of the residue from an electrolytic preparation of diethoxydihydrofuran about 7 g of a higher boiling liquid could be isolated. One sample (b. p.$_{11}$ = 112−118°, n_D^{25} = 1.4243, d^{23} = 0.932), which gave a week positive Beilstein test for halogens, was analyzed. The microhydrogenation was performed in 2 ml of ethanol using 36 mg of substance and 30 mg of Adams platinum oxide catalyst.

$C_4H_4(OC_2H_5)_4$ (232.3)	Calc.	C 62.0	H 10.4	OC_2H_5 77.6	$\bar{/}$ 1.00
	Found	» 61.7	» 10.5	» 77.9	» 0.96

84 mg of the sample was left standing for 1 hour at room temperature with 0.25 ml of phenylhydrazine in 12 ml of 32 % aqueous acetic acid. A precipitate of malealdehyde

bis-phenylhydrazone was formed and isolated in the usual way [2]. Yield of crystallized product 47 mg = 49 %; m. p. 169—170° (Kofler stage, corr.); previously [9] found 171°.

$C_{16}H_{16}N_4$ (264.3) Calc. N 21.2
Found » 21.3

The above evidence shows that the higher boiling product is rather pure malealdehyde tetraethyl acetal. Wohl and Mylo [10] previously found b. $p._{11}$ = 112° and d^{23} = 0.926 for this compound.

SUMMARY

Directions are given for the preparation of 2,5-diethoxy-2,5-dihydrofuran (I) by electrolysis. Malealdehyde tetraethyl acetal (II) was isolated as a by-product of the reaction.

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ACTA CHEMICA SCANDINAVICA 6 (1952) 572 – 577

Electrolytic Methoxylation of Some β-Isopropylfurans

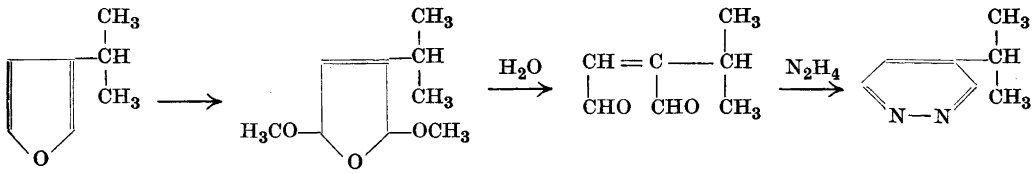
NIELS ELMING

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The methoxylation of furans to 2,5-dialkoxy-2,5-dihydrofurans by the action of an oxidizing agent on a methanolic solution of the furan appears to be a very general reaction. So far furan [1–2], silvan [3], furfuryl alcohol [3], furfuryl acetate [3], furfuryl methyl ether [4], furfural [5], furfural dimethyl acetal [3], furfural diacetate [6], methyl 2-furoate [7] and 2,5-dimethylfuran [8] have been methoxylated. The oxidation is best carried out electrolytically in a methanolic solution of ammonium bromide or sulfuric acid.

The electrolytic method of methoxylation has now been tried on three β-substituted furans, *viz.* β-isopropylfuran, 4-isopropyl-2-furaldehyde dimethyl acetal and methyl 4-isopropyl-2-furoate. All gave good yields of the corresponding dimethoxydihydrofurans, which are new compounds.

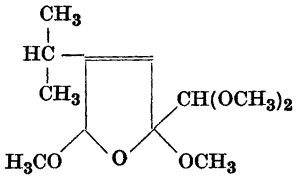
Hydrolysis of dimethoxy-β-isopropyldihydrofuran and addition of an aqueous solution of dinitrophenylhydrazine to the hydrolysate gave a dinitrophenylhydrazone with the analytical values of a monohydrazone of isopropylmalealdehyde. The parent dihydrofuran must therefore be 2,5-dimethoxy-3-isopropyl-2,5-dihydrofuran I. In agreement herewith hydrolysis of dimethoxy-isopropyl-dihydrofuran and addition of hydrazine gave a base with the formula $C_7H_{10}N_2$, which must be 4-isopropylpyridazine II.



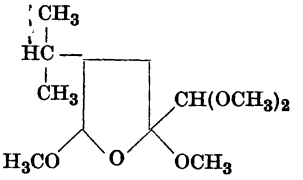
I II

4-Isopropylpyridazine is also a new compound. It was characterized by a picrate and a methiodide. The yield of 4-isopropylpyridazine in the above synthesis was only 19 per cent.

Dimethoxy-4-isopropyl-dihydro-2-furaldehyde dimethyl acetal was hydrogenated catalytically to the corresponding tetrahydrofuran. The structure of these dimethoxy-compounds was not determined, but in view of what is known in general about the oxidation of furans the methoxy-groups in both compounds are probably in the 2,5-positions (formulas III and IV).

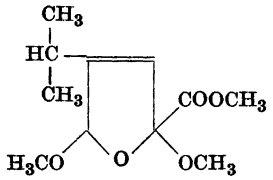


III

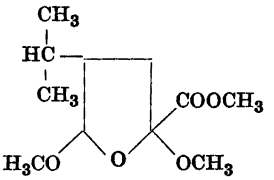


IV

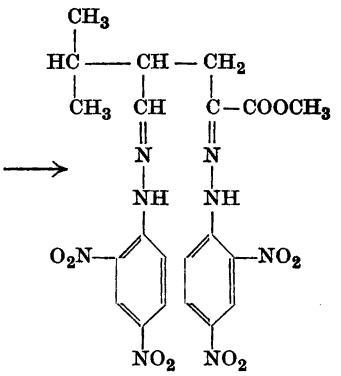
Methyl dimethoxy-4-isopropyl-dihydro-2-furoate was also hydrogenated to the corresponding tetrahydrofuran, which with dinitrophenylhydrazine in methanol formed a bis-dinitrophenylhydrazone with the formula $C_{20}H_{19}O_9N_8$ (OCH_3). This proves that the methoxy-groups in both compounds are in the 2,5-positions (formulas V and VI).



V



VI



The above reactions bring the number of furans, which have been methoxylated to dihydrofurans, up to 13.

EXPERIMENTAL

Microanalyses by F. Limborg, K. Glens and A. Grossmann

2,5-Dimethoxy-3-isopropyl- 2,5-dihydrofuran (I). A mixture of 22.0 g of β-isopropylfuran [9] (0.20 mole), 275 ml of methanol (technical product) and 5.0 g of ammonium bromide was electrolyzed and worked up in the usual way *Cf.* [3].

Hours	Current (ampere)	Potential across the cell during electrolysis (volt)	Temperature in the cell °C	Ampere hours (per cent of theoretical amount)
0.1	2.0	3.4	— 13	0.3 (3 %)
1.2	1.8	3.5	— 16	2.4 (22 %)
3.5	1.7	3.6	— 17	6.5 (60 %)
5.2	1.5	3.6	— 17	9.3 (86 %)
5.8	1.3	3.8	— 16	10.2 (95 %)

Fraction (g)	B. p.$_{15}$	n_D^{25}	OCH_3 Calc. 36.0 %
1 (4.5)	83—84°	1.4385	35.7
2 (3.0)	84—86°	1.4387	35.7
3 (14.2)	84—86°	1.4388	35.9
4 (3.1)	85—86°	1.4388	35.9

All fractions were colourless. Yield (fractions 1—4) 24.8 g of dimethoxy-isopropyl-dihydrofuran = 72 %; current efficiency 76 %. Fraction 3 was further analyzed for carbon and hydrogen.

$C_7H_{10}O(OCH_3)_2$ (172.2) Calc. C 62.8 H 9.4
Found » 62.6 » 9.4

43 mg of dimethoxy-isopropyl-dihydrofuran was boiled with 2 ml of *N*/10 sulfuric acid for one minute. After cooling 50 ml of an 0.2 % solution of dinitrophenylhydrazine in 2 *N* hydrochloric acid was added, the mixture shaken vigorously and left standing for 1.5 hours with occasional shaking. The orange-red hydrazone was isolated in the usual way. Yield 50 mg of crude isopropylmalealdehyde dinitrophenylhydrazone = 65 %. Yield after crystallization from absolute ethanol 25 mg; m. p. 175—177° (Hershberg apparatus corr.). The product was dried (sulfuric acid, 0.05 mm, 20°, 20 hours) and analyzed.

$C_{13}H_{14}O_5N_4$ (306.3) Calc. C 51.0 H 4.6 N 18.3 OCH_3 0
Found » 50.7 » 4.8 » 18.1

In another experiment the crude product was recrystallized twice from absolute ethanol; m. p. 178—180°.

Found C 51.0 H 4.9 N 18.1 OCH_3 0.2

4-Isopropylpyridazine (II). 12.0 g of 2,5-dimethoxy-3-isopropyl-2,5-dihydrofuran (0.07 mole) was stirred at room temperature with 168 ml of *N*/10 sulfuric acid. After stirring for 10 minutes the mixture became homogeneous, but 5—10 minutes later it again became turbid. 100 ml of methanol was added whereby the mixture became almost clear. After cooling to 0° a solution of 10.5 g of 50 % hydrazine hydrate (0.105 mole) in 28 ml of water was added during 15—30 seconds whereby the temperature rose to 7°. The mixture was left standing overnight and was then refluxed for 30 minutes. After cooling 27 ml of 4.8 *N* sulfuric acid was added, the methanol evaporated in vacuum and 5.2 g of sodium hydroxide in 10 ml of water added. The resulting solution was extracted continuously with ether, until no more high-boiling products could be extracted. From the etheral extract 3.62 g of a pale-yellow liquid was isolated by distillation; b. $p._{15}$ = 122—150°; n_D^{25} = 1.5011. 2.56 g of this product was added to 500 ml of a 1 % aqueous solution of picric acid at 50° and the mixture left standing for 2 hours at room temperature with occasional shaking. The resulting picrate of 4-isopropylpyridazine was filtered off and washed with water; yield 3.76 g = 22 % proportionate to dimethoxy-isopropyl-dihydrofuran; m. p. 92—95°.

$C_{13}H_{13}O_7N_5$ (351.3) Calc. C 44.5 H 3.7 N 19.9
Found » 44.7 » 4.1

Another sample of isopropylpyridazine picrate from another experiment was crystallized twice from ethanol; m. p. 95—97°.

Found C 44.9 H 3.9 N 19.8

The free isopropylpyridazine ($C_7H_{10}N_2$ (122.2)) was prepared from 3.74 g of the picrate in the usual way and purified by distillation.

Fraction (g)	B. $p._{14}$	n_D^{25}	C Calc. 68.8 %	H Calc. 8.3 %	N Calc. 22.9 %
1 (0.13)	121°	1.5015	68.7	8.2	22.6
2 (0.98)	121°	1.5025	68.8	8.5	23.2

Yield 1.11 g of isopropylpyridazine = 85 % proportionate to the picrate (19 % proportionate to dimethoxy-isopropyl-dihydrofuran).

The methiodide of 4-isopropylpyridazine was also prepared and crystallized twice from absolute ethanol-ether; m. p. 156—158°.

$C_8H_{13}N_2I$ (264.1) Calc. C 36.4 H 5.0 N 10.6 I 48.1
Found » 36.5 » 4.4 » 10.8 » 48.0

Dimethoxy-4-isopropyl-dihydro-2-furaldehyde dimethyl acetal. 14.7 g of 4-isopropyl-2-furaldehyde dimethyl acetal [9] (0.080 mole), 270 ml of methanol and 5.0 g of ammonium bromide were electrolyzed and the reaction mixture worked up in the usual way *Cf.* [3].

Hours	Current (ampere)	Potential across the cell during electrolysis (volt)	Temperature in the cell °C	Ampere hours (per cent of theoretical amount)
0.5	1.9	3.4	— 18	1.0 (20 %)
1.5	1.8	3.5	— 17	2.8 (65 %)
2.5	1.4	3.8	— 19	4.4 (102 %)

Fraction (g)	B. $p_{0.1}$	n_D^{25}	OCH_3 Calc. 50.4 %
1 (1.1)	62—64°	1.4449	46.6
2 (14.7)	64—65.5°	1.4440	49.6
3 (0.7)	66—67°	1.4446	49.2

All fractions were colourless. Yield (fraction 2) 14.7 g of dimethoxy-isopropyl-dihydro-furaldehyde dimethyl acetal = 75 %; current efficiency 73 %.

$C_8H_{10}O(OCH_3)_4$ (246.3) Calc. C 58.5 H 9.0
Found » 58.7 » 9.2

Dimethoxy-4-isopropyl-tetrahydro-2-furaldehyde dimethyl acetal. 4.06 g of dimethoxy-isopropyl-dihydro-furaldehyde dimethyl acetal and 20 ml of anhydrous methanol were shaken with 0.6 g of Raney nickel under 105—120 atmospheres of hydrogen at 120—130° for 21 hours. The reaction product was isolated as usual. Yield 2.31 g of dimethoxy-4-isopropyl-tetrahydro-2-furaldehyde dimethyl acetal = 56 %; colourless liquid, b. $p_{0.1}$ = 58—60°; n_D^{25} = 1.4349.

$C_8H_{12}O(OCH_3)_4$ (248.3) Calc. C 58.0 H 9.7 OCH_3 50.0
Found » 58.2 » 10.0 » 48.5

Methyl 2,5-dimethoxy-4-isopropyl-2,5-dihydro-2-furoate (V). 12.6 g of methyl 4-isopropyl-2-furoate [9] (0.075 mole), 280 ml of methanol and 1.87 g of concentrated sulfuric acid were electrolyzed and the reaction mixture worked up in the usual way *Cf.* [3] (see p. 577).

Yield 10.5 g of methyl dimethoxy-isopropyl-dihydrofuroate = 61 %; current efficiency 47 %; colourless liquid, b. $p_{0.1}$ = 69—71°; n_D^{25} = 1.4520.

$C_8H_9O_2(OCH_3)_3$ (230.3) Calc. C 57.4 H 7.9 OCH_3 40.4
Found » 57.9 » 7.9 » 39.5

Methyl 2,5-dimethoxy-tetrahydro-2-furoate (VI). 4.20 g of methyl dimethoxy-isopropyl-dihydrofuroate and 20 ml of anhydrous methanol were shaken with 0.7 g of Raney nickel under 100—120 atmospheres of hydrogen at 120—125° for 20 hours. Yield 3.05 g

Hours	Current (ampere)	Potential across the cell during electrolysis (volt)	Temperature in the cell °C	Ampere hours (per cent of theoretical amount)
0.7	1.0	4.0	— 19	0.8 (20 %)
1.5	0.9	4.0	— 19	1.6 (40 %)
2.9	0.8	4.1	— 19	2.8 (70 %)
6.2	0.7	4.2	— 20	5.2 (129 %)

of methyl dimethoxy-isopropyl-tetrahydrofuroate = 72 %; colourless liquid, b. $p._{0.1}$ = 68—70°; n_D^{25} = 1.4401.

$C_8H_{11}O_2(OCH_3)_3$ (232.3) Calc. C 56.9 H 8.7 OCH_3 40.1
Found » 57.1 » 8.9 » 39.1

232 mg of methyl dimethoxy-isopropyl-tetrahydrofuroate in 4 ml of anhydrous methanol was added to a solution of 500 mg of dinitrophenylhydrazine in 7 ml of methanol and 1 ml of concentrated sulfuric acid. After 1.5 hours a few mg of a red precipitate was removed by filtration. The next day a yellow precipitate was filtered off, washed 5 times with methanol and dried. Yield 360 mg of bis-dinitrophenylhydrazone = 66 %. Crystallization from acetone-petroleum ether gave 270 mg; m. p. 178—181°. Recrystallization gave 205 mg; m. p. 179—181°.

$C_{20}H_{19}O_9N_8(OCH_3)$ (546.5) Calc. C 46.2 H 4.1 N 20.5 OCH_3 5.7
Found » 46.5 » 4.5 » 20.2 » 6.0

SUMMARY

Some β-substituted furans have been methoxylated by electrolysis to the corresponding 2,5-dimethoxy-2,5-dihydrofurans.

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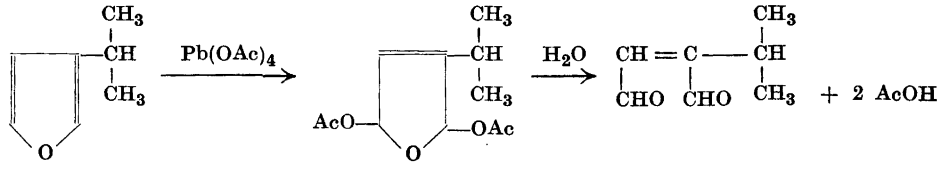
The Acetoxylation of Furans with Lead Tetraacetate; Preparation of 2,5-Diacetoxy-3-isopropyl-2,5-dihydrofuran

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It has been found previously by Clauson-Kaas [1], that furan can be acetoxylated with lead tetraacetate to 2,5-diacetoxy-2,5-dihydrofuran. Later Clauson-Kaas, Limborg and Fakstorp [2] failed to acetoxylate furoic acid and ethyl furoate by this method. The acetoxylation with lead tetraacetate of some other α-substituted furans, *viz.* silvan, furfuryl acetate, furfural diacetate and 2-acetylfuran, has now been attempted but also without success, as no pure compounds could be isolated.

β-Isopropylfuran was, contrary to the α-substituted furans, acetoxylated by lead tetraacetate. The reaction was somewhat more rapid than the reaction between furan and lead tetraacetate. The resulting diacetoxy-isopropyl-dihydrofuran gave — after hydrolysis and addition of dinitrophenylhydrazine to the hydrolysate — a dinitrophenylhydrazone with the formula $C_{13}H_{14}O_5N_4$, which was identical with the hydrazone of isopropylmalealdehyde prepared previously from a hydrolysate of 2,5-dimethoxy-3-isopropyl-2,5-dihydrofuran [3]. This reaction proves that the new compound is 2,5-diacetoxy-3-isopropyl-2,5-dihydrofuran I.



I

EXPERIMENTAL

Microanalyses by Franz Limborg and Kirsten Glens

Attempts to acetoxylate some α-substituted furans. The action of lead tetraacetate on silvan, furfuryl acetate, furfural diacetate and 2-acetylfuran was investigated. The

procedure described previously [4] for the acetoxylation of furan was employed. The progress of the reaction was followed by adding samples of the reaction mixture to water. When the lead tetraacetate had disappeared the reaction mixture was a clear, homogeneous yellow or light-brown solution. In spite of several attempts at different reaction temperatures it was in no case found possible to isolate any pure reaction products.

2,5-Diacetoxy-3-isopropyl-2,5-dihydrofuran (I). 4.4 g of β-isopropylfuran [5] (0.040 mole) was added to a suspension of 17.7 g of lead tetraacetate (0.040 mole) in 100 ml of glacial acetic acid. The mixture was kept at 55—60° for 35 minutes and the reaction product isolated in the usual way [4]. The final distillation gave a fore-run of 0.6 g (b. $p_{\cdot 0.1}$ = 65—82°) and 4.9 g of crude diacetoxy-isopropyl-dihydrofuran = 54 %; b. $p_{\cdot 0.1}$ = 84—90°; n_D^{25} = 1.4550.

4.6 g of the crude product was redistilled. After a fore-run of 0.7 g (b. $p_{\cdot 0.1}$ = 84—88°, n_D^{25} = 1.4547) the main fraction was collected; yield 3.3 g of a pale-yellow liquid; b. $p_{\cdot 0.1}$ = 88—91°; n_D^{25} = 1.4550.

$C_7H_{10}O_3(CH_3CO)_2$ (228.2)	Calc.	C	57.9	H	7.1	CH_3CO	37.7
	Found	»	58.3	»	7.0	»	36.3

Mono-dinitrophenylhydrazone of isopropylmalealdehyde. 57 mg of 2,5-diacetoxy-3-isopropyl-2,5-dihydrofuran was boiled with 2 ml of *N*/10 sulfuric acid for one minute. After cooling 50 ml of an 0.2 % solution of dinitrophenylhydrazine in 2 *N* hydrochloric acid was added, the mixture shaken vigorously and then left standing for 1.5 hours with occasional shaking. The orange-red precipitate was filtered off, washed thoroughly with water and dried. Yield 50 mg of isopropylmalealdehyde dinitrophenylhydrazone = 66 %. Crystallization from absolute ethanol gave 25 mg = 33 %; m. p. 175—177° (Hershberg apparatus, corr.); mixed melting point with the dinitrophenylhydrazone prepared from dimethoxy-isopropyl-dihydrofuran [3] 174—176°.

$C_{13}H_{14}O_5N_4$ (306.3)	Calc.	C	51.0	H	4.6	N 18.3	CH_3CO 0
	Found	»	50.8	»	4.6	» 17.9	» 0.3

SUMMARY

Attempts to acetoxylate silvan, furfuryl acetate, furfural diacetate and 2-acetylfuran with lead tetraacetate were unsuccessful. β-Isopropylfuran and lead tetraacetate gave the new 2,5-diacetoxy-3-isopropyl-2,5-dihydrofuran.

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Fungus Pigments

II. * Cortisalin, a New Polyethenoid Pigment

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A few years ago Erdtman [1] reported the isolation of a new pigment, named corticrocin, from the "yellow mycorrhiza" the fungal component of which is considered to be the mycelium of *Corticium croceum* Bres. (= *C. sulphureum* (Fr.) Fr.). Erdtman also showed that corticrocin is dodecahexaene-(1,3,5,7,9, 11)-dicarboxylic acid-(1,12)(VII) [1].

In his paper, Erdtman pointed out that it would be of considerable interest to see if other *Corticium* species, several of which are coloured, also contain corticrocin or related polyethenoid pigments, since this would strengthen the opinion that the fungal component of the "yellow mycorrhiza" is, indeed, *Corticium croceum*. He has recently been able to isolate corticrocin from a sporophore which according to Professor E. Melin, Uppsala, was an immature sporophore of *Corticium croceum* [2]. The mycelium from this sporophore was obviously connected with the "yellow mycorrhiza" the other component of which in this case was *Vaccinium vitis ideae*.

An investigation of the pigment of *Corticium salicinum* Fries has now been carried out. This fungus grows on dead branches of *Salix* species, particularly in wet places such as marshes and the shores of lakes. The red sporophore is rather small, being seldom more than a few centimeters wide and 1—2 millimeters thick. Although the fungus is rather common, much work is required to collect appreciable quantities.

From this fungus we have isolated a crystalline pigment for which we propose the name cortisalin. It is thus in this case the sporophore, not the mycelium as in the case of *Corticium croceum*, which has given the pigment. The reddish mycelium is less readily accessible and has not been investigated, but undoubtedly it contains the same pigment.

* Part I *Acta Chem. Scand.* **5** (1951) 590.

Cortisalin can be obtained from the fungus by extraction with acetone. The extraction of the dried fungus proceeds very slowly, but the pigment is more rapidly extracted from the fresh material. It was therefore found advantageous to add water to the powdered fungus from time to time during the extraction. The addition of water causes a swelling of the fungus material and this appears to facilitate in some way the removal of the pigment. That this is not due to a higher solubility of the pigment in aqueous acetone follows from the fact that the pure pigment is extremely difficultly soluble in acetone and quite insoluble in water.

The pigment precipitates from the acetone extract as a brown powder and after purification, as described in the experimental section, it is obtained as beautiful violet-red needles. The yield has been somewhat varying in different batches of the fungus, the best being 0.6 per cent. A total of about 2.5 g of crystalline cortisalin has been available for this investigation.

Cortisalin does not melt but decomposes gradually above 290°. It is extremely insoluble in all common organic solvents except pyridine, in which it is rather easily soluble particularly on warming. This low solubility has made the work with cortisalin very difficult and time consuming. Cortisalin dissolves in conc. sulphuric acid, giving a very intense indigo-blue solution. When crystals of cortisalin are suspended in dilute sodium hydroxide they immediately turn black, but do not dissolve.

Diazomethane converts cortisalin very slowly into a red methyl derivative, m. p. 255—256° (dec.), which is somewhat more soluble in chloroform but less soluble in pyridine than is cortisalin. This methylcortisalin can be sublimed in a high-vacuum, but with great loss of material. With acetic anhydride cortisalin is converted into an acetate, m. p. 280—282°(dec.).

It has been very difficult to obtain consistent analyses for cortisalin and its derivatives and it appears doubtful if the pigment has ever been obtained in a state of complete purity. Similar difficulties were encountered in the case of corticrocin. What appeared to be the most reliable of these analyses indicated a formula $C_{24}H_{24}O_4$ for cortisalin, and $C_{26}H_{28}O_4$ with two methoxyl groups for the methyl derivative [3]. Cortisalin itself contains no methoxyl groups. As will be shown below, this formulation of cortisalin can no longer be upheld.

When dimethylcortisalin is hydrolysed with alcoholic potassium hydroxide it is slowly converted into an insoluble, dark-yellow, substance. This is evidently a potassium salt, and upon trituration with dilute sulphuric acid it is converted into a dark-red compound, m. p. 280—282°(dec.). This has only about half of the methoxyl content of the original dimethylcortisalin, and this fact points to the presence of a carboxyl group in cortisalin.

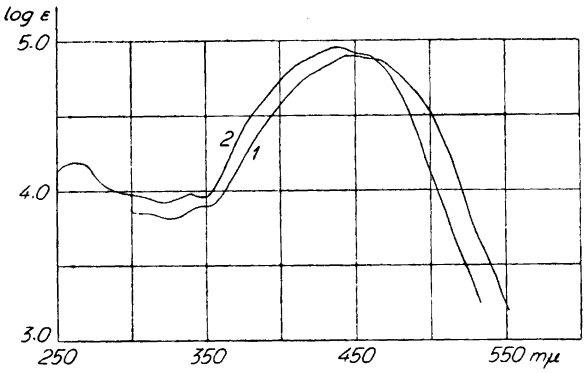
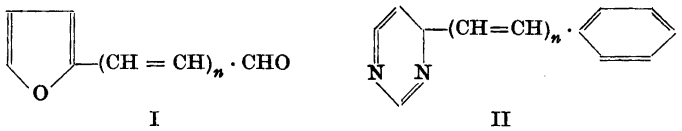


Fig. 1. Absorption spectra of: 1. cortisalin (in pyridine) and 2. dimethylcortisalin (in chloroform).

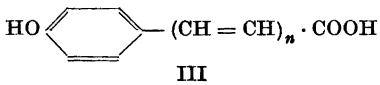
The nature of the second methoxyl group was shown by oxidation of dimethylcortisalin with potassium permanganate in acetone solution, to anisic acid. This shows the presence in cortisalin of a hydroxylated benzene ring connected in the *p*-position to the rest of the molecule.

The absorption spectra of cortisalin and dimethylcortisalin (Fig. 1) bear a striking resemblance to the spectra of polyethenoid compounds. Although the well developed three-peaked maximum of, for example, corticrocin [1] is lacking in these spectra, they resemble the spectra of unsymmetrically substituted polyenes such as (I) [4] and (II) [5].



I

II



III

A similar unsymmetrically substituted polyene structure appears therefore very probable for cortisalin. Taking into account the presence of a carboxyl group and a *p*-hydroxyphenyl group cortisalin might well correspond to the general formula (III). From the position of the maximum it can be judged that n is either 6 or 7 but no definite decision can be made between these two alternatives.

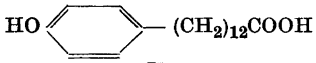
If this view is correct, cortisalin should correspond to either $C_{19}H_{18}O_3$ or $C_{21}H_{20}O_3$, and dimethylcortisalin to $C_{21}H_{22}O_3$ or $C_{23}H_{24}O_3$. The analytical

data are however in poor agreement with either of these formulations. In particular, the methoxyl values of dimethylcortisalin are much too low to correspond with either $C_{21}H_{22}O_3$ or $C_{23}H_{24}O_3$.

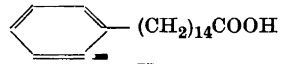
Upon catalytic hydrogenation in ethanol suspension cortisalin is converted into a colourless crystalline compound. This hydrogenation is extremely slow, requiring several days for the hydrogenation of 0.2 g of cortisalin, due to the extremely low solubility of cortisalin. It has therefore not been possible to determine the exact amount of hydrogen consumed in the hydrogenation. The hydrogenation product which is obtained only in moderate yield has m. p. 98—100° and its U. V. absorption spectrum shows maxima at 225 mμ (log $\varepsilon = 3.6$) and 279 mμ (log $\varepsilon = 3.1$). In alkaline solution, these maxima are displaced to 242 mμ (log $\varepsilon = 3.75$) and 297 mμ (log $\varepsilon = 3.2$). This behaviour is almost identical with that of *p*-cresol with maxima at 224 mμ (log $\varepsilon = 3.8$) and 280 mμ (log $\varepsilon = 3.3$) [6]. The displacement of the maxima in alkaline solution is also typical of phenols [7]. The presence of a phenolic group in cortisalin is thus confirmed.

Upon treatment with diazomethane, hydrocortisalin is converted into a methyl ester. That the phenolic hydroxyl group has not entered into reaction is shown by the fact that alkaline hydrolysis regenerates hydrocortisalin, and by the identity of the U. V. absorption spectrum with that of hydrocortisalin in both neutral and alkaline solution. The formation of an ester thus confirms the presence of a carboxyl group in cortisalin. Hydrocortisalin gives a sodium salt that is very sparingly soluble in cold water, and dissolves in warm water giving a soap-like solution. It is thus reminiscent of the long chain fatty acids.

If the above mentioned assumption regarding the structure of cortisalin is correct, hydrocortisalin would be either (IV) or (V)



IV



V

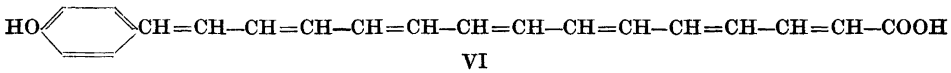
The analytical figures do not however allow any definite decision between these two possibilities.

In view of the indecisive analytical data on all the above-mentioned derivatives of cortisalin, it was decided to synthesise the two hitherto unreported acids (IV) and (V) and compare them with hydrocortisalin.

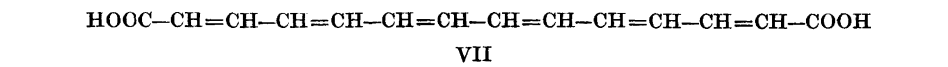
The syntheses of these acids are described in the following paper [8]. 13-*p*-hydroxyphenyltridecanoic acid (IV) has m. p. 94—96° and its methyl ester m. p. 71—72°. Both give definite depressions of m. p. when mixed with hydrocortisalin and its methyl ester respectively. On the other hand 15-*p*-hydroxyphenylpentadecanoic acid (V) has m. p. 99—100° undepressed in admixture

with hydrocortisalin. The identity was further confirmed by comparison of the x-ray diffraction patterns *.

Hydrocortisalin has therefore the structure (V) and there is little doubt that cortisalin must be represented by the structure (VI) in spite of the poor agreement of the analytical data with the theoretical.



VI



VII

Regarding the configuration of the double bonds, nothing definitive can be said, but it seems not unreasonable to expect an all-*trans* arrangement (compare corticrocin [1]).

Cortisalin (VI) thus differs from corticrocin (VII) in having two more carbon atoms in the aliphatic chain, and a *p*-hydroxyphenyl group in the place of the second carboxyl group. The great similarity in structure between corticrocin and cortisalin can be taken as further evidence that the mycelium from which corticrocin was isolated really belonged to a fungus of the genus *Corticium*, and may further indicate that both compounds are derived from a common progenitor in much the same way as crocetin and azafrin may be derived from a C_{40}-carotenoid.

It should be noted that the *p*-hydroxyphenyl group occurs also in atromentin [9], another fungus pigment.

EXPERIMENTAL

Isolation of cortisalin. Of various methods tried for the isolation of the piment, the following was found to be the most satisfactory. The finely ground fruit body of *Corticium salicinum* (222 g) was extracted with acetone in a Soxhlet-apparatus. Initially the extract was dark brown, but later the colour changed to yellow. When no more coloured material was being removed, water was added, after which the extract became yellow again. The extraction was continued until no change in colour occurred on the addition of water. This requires a time of about two weeks. The cortisalin precipitates as a brown powder in the flask of the extraction apparatus. It is advisable to filter off the precipitate from time to time to avoid unnecessary exposure to heat. The precipitate was washed with water and extracted with ether in a Soxhlet apparatus to remove wax-like impurities. The cortisalin remains undissolved as a dark brown powder. This was extracted with hot pyridine which easily dissolves cortisalin leaving a small amount of insoluble impurities. Crude cortisalin (2.6 g) crystallised from the pyridine solution on cooling. This material contains an appreciable amount of brown amorphous impurities and was extracted again with ether and recrystallised from pyridine. In this way 1.25 g of essen-

* The author wishes to express his thanks to Docent E. Stenhagen, Uppsala, for this information.

tially pure cortisalin was obtained as violet-red needles. It has no melting point but decomposes above 290°. From another batch of 270 g of fungus 1.05 g of cortisalin was obtained by a similar procedure. Samples for analysis were purified by repeated recrystallisation from pyridine. (Found: C, 77.0, 77.0, 77.8; H, 6.3, 6.2, 6.4. $C_{21}H_{20}O_3$ requires C, 78.7; H, 6.3 %.)

Dimethylcortisalin. Cortisalin (200 mg) was suspended in ether (200 ml) and an ethereal solution of diazomethane (ca. 2 g) was added. Only a very slight evolution of nitrogen could be observed. The mixture was allowed to stand, with occasional shaking, for two weeks, during which time the red-violet crystals had been transformed into a bright red powder. This was collected and extracted with chloroform in a continuous extraction apparatus. Dimethylcortisalin precipitated from the chloroform extract during the extraction, and was recrystallised from diethyl succinate giving 100 mg of bright red glistening leaflets, m. p. 255—257° (dec.). Samples for analysis were sublimed in vacuum (0.5 mm) and then recrystallised from diethyl succinate. (Found: C, 78.4, 77.8, 78.7; H, 7.0, 6.9, 6.8; OCH_3, 14.2, 14.8, 15.1. $C_{23}H_{24}O_3$ requires C, 79.3; H, 7.0; OCH_3, 17.8 %).

Cortisalin methyl ether. Dimethylcortisalin (15 mg) was suspended in 2 per cent alcoholic potassium hydroxide solution (200 ml), and the mixture was heated on a water-bath for 10 hours, during which time the crystals of dimethylcortisalin changed to a brown microcrystalline powder. The alcohol was removed and water was added. The insoluble brown precipitate was filtered off, extracted with boiling chloroform in order to remove unchanged dimethylcortisalin, then suspended in 2-N sulphuric acid and set aside overnight. The precipitate which was then dark red was filtered off, washed with water and extracted with pyridine. From the pyridine extract, cortisalin methyl ether crystallised, on cooling, as dark red micro needles (4 mg), m. p. 280—282° (dec.). (Found: OCH_3, 8.4; $C_{22}H_{22}O_3$ requires OCH_3, 9.3 %).

Acetylcortisalin. Cortisalin (50 mg) was dissolved in pyridine and acetic anhydride (1 ml) was added. The solution darkened appreciably, and was set aside overnight then poured into water. The precipitate was collected, dried, and extracted with chlorobenzene. The acetylcortisalin separated as yellow-brown needles which were purified by recrystallisation from chlorobenzene-dimethyl succinate. M. p. 280—282° (dec.). (Found: C, 76.2, H, 6.1; $C_{23}H_{22}O_4$ requires C, 76.2, H, 6.1 %).

Oxidation of dimethylcortisalin. Dimethylcortisalin (50 mg) was placed in a folded filter paper suspended in one neck, of a two-necked flask. The same neck was equipped with a reflux condenser. Acetone was placed in the flask which was heated on a water-bath. The refluxing acetone slowly dissolved the dimethylcortisalin. Finely powdered potassium permanganate was added in small portions through the other neck. The rate of addition of the potassium permanganate must be adjusted so that the solution does not contain too large an excess of potassium permanganate or so much dimethylcortisalin that it precipitates. The oxidation was interrupted after two days although part of the dimethylcortisalin still remained in the filter paper. This amounted to 20 mg, 30 mg thus having been dissolved. Water was added and the acetone was distilled off on a water-bath. The solution was then acidified with dilute sulphuric acid and sodium hydrogen sulphite was added to dissolve the manganese dioxide. 2 Mg of unreacted dimethylcortisalin was removed by filtration, and the solution was extracted with ether. The ether solution was extracted with sodium carbonate solution, which was then acidified and again extracted with ether. Evaporation of the ether yielded a white crystalline mass (10 mg). After sublimation and recrystallisation from ether-light petroleum this had m. p. 182—184°, undepressed in admixture with anisic acid.

Hydrogenation of cortisalin. Cortisalin (200 mg) was suspended in absolute alcohol and hydrogenated at 2 atm. pressure, in the presence of a Pt-catalyst. After about one week the cortisalin had dissolved and the solution was colourless. The alcohol was removed in vacuum, and the white solid mass obtained was dissolved by warming with a few ml of 2 *N* sodium hydroxide and the solution was filtered. On cooling, the sodium salt of hydrocortisalin separates as a white amorphous powder. When attempts were made to extract an ether solution of hydrocortisalin with sodium hydroxide the formation of this difficultly soluble sodium salt caused the formation of a very troublesome emulsion. Hydrocortisalin was obtained from the sodium salt by acidification, as a slightly yellow precipitate (140 mg). The yellow colour was removed by filtering an ether solution through a small amount of acid-washed aluminium oxide, and the product was recrystallised from dilute alcohol or ether-light petroleum, m. p. 98—100°. Mixed with synthetic 15-*p*-hydroxyphenylpentadecanoic acid of m. p. 99—100° it melted at 99—100°. (Found: C, 74.7, H, 10.0; $C_{21}H_{34}O_3$ requires C, 75.4, H, 10.3 %).

Methylation of hydrocortisalin. Hydrocortisalin (100 mg) was dissolved in ether and an excess of diazomethane in ether was added. After three hours the evolution of nitrogen had ceased, and the excess of diazomethane was distilled off with part of the ether. The remaining colourless solution was filtered through a small amount of aluminium oxide and evaporated. The crystalline residue was recrystallised from light petroleum, m. p. 76—76.5°. (Found: C, 76.2, H, 10.4, OCH_3, 8.4; $C_{22}H_{36}O_3$ requires C, 75.8, H, 10.4, OCH_3, 8.9 %).

On alkaline hydrolysis, hydrocortisalin m. p. 99—100°, identified by a mixed m. p. determination, was regenerated.

SUMMARY

From the sporophore of *Corticium salicinum* Fries a redviolet pigment has been isolated. This has been termed cortisalin and has been shown to be 15-*p*-hydroxyphenylpentadeca-2,4,6,8,10,12,14-heptenoic acid.

The author wishes to thank all those who have helped in the collection of the fungus material. Part of this investigation has been financially supported by a grant from *Statens Naturvetenskapliga Kommission*, which is gratefully acknowledged.

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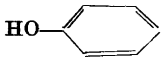
Fungus Pigments

III. * The Synthesis of Two ω-*p*-Hydroxyphenylfatty Acids

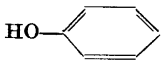
JARL GRIPENBERG

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As outlined in the preceding paper [1] the two acids

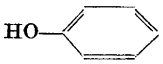


13-*p*-hydroxyphenyltridecanoic acid and

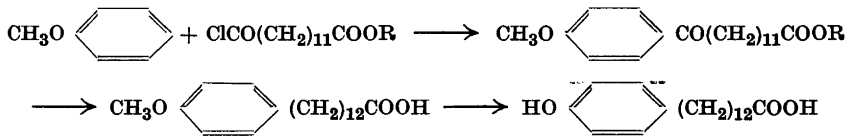


15-*p*-hydroxyphenylpentadecanoic acid were needed for comparison with the hydrogenation product of cortisalin.

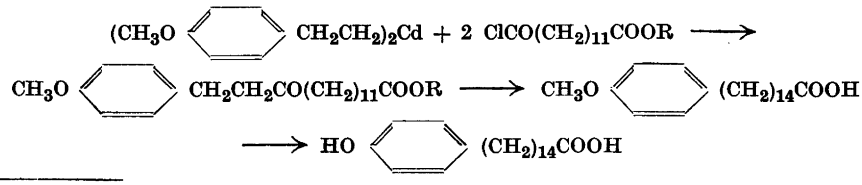
A general method for the synthesis of acids of the type



has been worked out by Papa, Schwenk and Hankin [2], and this method was used for the synthesis of 13-*p*-hydroxyphenyltridecanoic acid which was obtained without difficulty according to the following scheme:



The synthesis of 15-*p*-hydroxyphenylpentadecanoic acid by a similar method would have required tridecandicarboxylic acid-(1,13) and its half ester which are not easily available. Instead the method of Cason [3] was used, the synthesis being carried out as follows:



* Part II. *Acta Chem. Scand.* **6** (1952) 580.

Clemmensen reduction of 15-*p*-methoxyphenyl-13-ketopentadecanoic acid gave, in this case, a product which could not be obtained quite pure. As partial demethylation during the Clemmensen reaction could well account for the impurity obtained, the impure material was subjected to demethylation in the hope that this would give homogenous material. This was however not the case, and the impurities could not be removed by crystallisation. Although repeated crystallisation from different solvents gave a seemingly pure compound of a rather sharp m. p. 98—99° its analysis gave low carbon and hydrogen values. The impurities could be removed by chromatography on alumina from a benzene-ether solution, whereby the 15-*p*-hydroxyphenylpentadecanoic acid was retained on the column, the impurities being eluted. Elution with ether containing a small amount of acetic acid gave the pure 15-*p*-hydroxyphenylpentadecanoic acid with m. p. 99—100°.

EXPERIMENTAL

Methyl 13-p-methoxyphenyl-13-ketotridecanoate. Monomethyl brassylate (8.8 g) was converted into the acid chloride by treatment with thionyl chloride. The acid chloride was dissolved in tetracloroethane (10 ml) and this solution was added to a stirred mixture of aluminium chloride (9 g), anisole (4 g) and tetrachloroethane (20 ml) kept below 0°. Stirring was continued for two hours, then the mixture was allowed to warm up to room temperature, and poured onto ice. The organic layer was separated, and the aqueous layer was extracted with tetrachloroethane. From the combined tetrachloroethane solutions, the solvent and excess of anisole were removed by steam distillation, and the remaining oil which solidified was recrystallised from methanol giving methyl 13-*p*-methoxyphenyl-13-ketotridecanoate (10.9 g, 92 %), m. p. 66—67°. (Found: C, 72.4, H, 9.4; $C_{21}H_{32}O_4$ requires C, 72.4, H, 9.3 %).

The above ester (0.5 g) was saponified by boiling with methanolic sodium hydroxide for three hours. The sodium salt, which crystallised on cooling was collected and dissolved in a small amount of acetic acid, and dilute sulphuric acid was added. The free acid which precipitated was collected and recrystallised, first from acetic acid and then from benzene-light petroleum, m. p. 93—94°. (Found: C, 71.9, H, 9.2; $C_{20}H_{30}O_4$ requires C, 71.8, H, 9.0 %).

13-p-Methoxyphenyltridecanoic acid. I. Methyl 13-*p*-methoxyphenyl-13-ketotridecanoate (5.8 g) was boiled with amalgamated zinc (30 g) hydrochloric acid (2 : 1, 90 ml) and toluene (30 ml). Conc. hydrochloric acid was added from time to time, and an additional amount of amalgamated zinc (15 g) was added after 48 hours. After the mixture had been boiled for 72 hours, unreacted zinc was filtered off and washed with ether. The combined ether and toluene layer was separated and the solvents removed by steam distillation. The remaining oil was saponified by boiling for three hours with methanolic sodium hydroxide. Dimethyl sulphate (1 ml) was added and boiling was continued for another hour. The methanol was distilled off, the sodium salt of 13-*p*-methoxyphenyltridecanoic acid which separated on cooling was collected and converted into the free acid (4.5 g; 87 %) which was recrystallised from light petroleum, m. p. 71—72.5°. (Found: C, 75.0, H, 10.3; $C_{20}H_{32}O_3$ requires C, 75.0, H, 10.1 %).

II. 13-*p*-Methoxyphenyl-13-ketotridecanoic acid (1.7 g) was added to a solution of sodium (1.7 g) in glycol (50 ml) and, after the addition of hydrazine hydrate (2.5 g), the mixture was heated at 175° for 30 hours. The cooled solution was poured into water and acidified, the precipitated acid was taken up in ether, and the ether was removed and the residue was recrystallised from light petroleum, m. p. 70—72°, undepressed in admixture with the acid prepared by the alternative route.

13-p-*Hydroxyphenyltridecanoic acid.* 13-*p*-Methoxyphenyltridecanoic acid (1.4 g) was dissolved in acetic acid (6 ml), hydrobromic acid (50 %; 2 ml) was added, and the mixture was boiled for five hours. On cooling the 13-*p*-hydroxyphenyltridecanoic acid crystallised out and was filtered off. By the addition of water to the mother liquor a further amount of the acid can be obtained. Recrystallised from benzene it has m. p. 94—96°. (Found: C, 74.3, H, 10.2; $C_{19}H_{30}O_3$ requires C, 74.5, H, 9.9 %).

Methyl 13-*p*-hydroxyphenyltridecanoate was prepared by esterification of the acid with methanol and sulphuric acid. After recrystallisation from light petroleum and vacuum distillation it has m. p. 71—72°. (Found: C, 75.0, H, 10.2; $C_{20}H_{32}O_3$ requires C, 75.0, H, 10.1 %).

15-p-*Methoxyphenyl-13-ketopentadecanoic acid.* A Grignard solution was made from 2-*p*-methoxyphenylethyl bromide[4] (9.8 g) and magnesium (1.1 g). Finely powdered cadmium chloride (4.3 g) was added with efficient stirring, and the mixture was boiled for one hour. The ether was then distilled off with the simultaneous addition of benzene. The acid chloride of monomethyl brassylate, prepared from monomethyl brassylate (7.5 g) and thionyl chloride was then added dropwise. The mixture was boiled for 15 minutes, set aside overnight, then poured into very dilute hydrochloric acid. The benzene layer was separated and washed with water, and the benzene was removed by steam distillation. The remaining oil was taken up in ether, and the ether solution was washed with sodium carbonate and evaporated. The oil obtained was saponified by boiling with methanolic sodium hydroxide. On cooling, the sodium salt of 15-*p*-methoxyphenyl-13-ketopentadecanoic acid crystallised out and was filtered off and washed with methanol and ether. It was decomposed with sulphuric acid, and the acid was taken up in ether. Removal of the ether and recrystallisation from dilute acetic acid gave 15-*p*-methoxyphenyl-13-ketopentadecanoic acid, m.p. 88—89° (4.9 g, 47 %). (Found: C, 73.1, H, 9.5; $C_{22}H_{32}O_4$ requires C, 72.9, H, 9.4 %).

15-p-*Methoxyphenylpentadecanoic acid.* 15-*p*-Methoxyphenyl-13-ketopentadecanoic acid (2 g) was boiled with amalgamated zinc (5 g), hydrochloric acid (2 : 1, 30 ml) and toluene (10 ml) for 48 hours. Unreacted zinc was filtered off and the toluene was removed by steam distillation. The remaining oil (1.7 g), which solidified upon cooling, was recrystallised from acetic acid giving a product, m. p. 77—78° which was still somewhat impure. (Found: C, 75.4, H, 10.4; $C_{22}H_{30}O_3$ requires C, 75.8, H, 10.4 %).

15-p-*Hydroxyphenylpentadecanoic acid* Crude 15-*p*-methoxyphenylpentadecanoic acid (1 g) was added to hydrobromic acid (50 %, 2 ml) and acetic acid was added until a clear solution was obtained. The mixture was boiled for one hour. On cooling a small amount of 15-*p*-hydroxyphenylpentadecanoic acid crystallised out and water was added to precipitate the remainder of the acid, which was collected and recrystallised from dilute acetic acid, benzene and dilute alcohol. It was then dissolved in ether and benzene and chromatographed on aluminium oxide. Elution with ether removed crystalline material which was not further investigated, and further elution with ether containing a few drops of acetic acid gave 15-*p*-hydroxyphenylpentadecanoic acid, which after recrystallisation from dilute alcohol had m. p. 99—100° (Found: C, 75.4, H, 10.3; $C_{21}H_{34}O_3$ requires C, 75.4, H, 10.3 %).

SUMMARY

The synthesis of 13-*p*-hydroxyphenyltridecanoic acid and 15-*p*-hydroxyphenylpentadecanoic acid are described.

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Abbau von Kartoffelstärke durch α-Amylasen aus Malz und aus *Bacillus subtilis*. Differenzierung der Abbauprodukte

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Im Jahre 1937 zeigten Blom, Bak und Braae[1], dass absolut β-freie α-Amylase aus Malz und α-Amylase aus *Bacillus subtilis* Kartoffelstärkekleister in derselben Weise angreifen. Der Kleister wird dünnflüssig; das Vermögen, mit Jod Farbenreaktion zu geben, verschwindet bei ca. 32 % TM. Von Anfang an werden reduzierende Spaltprodukte gebildet. Ohne Rücksicht darauf, ob Mono-, Di-, Tri- oder höhere Oligosaccharide gebildet werden, wird das Reduktionsvermögen als Maltose berechnet, da dieser Zucker beim enzymatischen Stärkeabbau eine zentrale Stellung einnimmt. Der Grad des enzymatischen Stärkeabbaus wird in Prozenten der Menge Maltose angegeben, die gebildet werden würde, falls Stärke sich vollständig zu Maltose abbauen liesse. Prozent der theoretisch möglichen Menge Maltose verkürzen wir mit % TM und deuten durch Anhängen von W, B bzw. BR an, dass die Bestimmung nach Willstätters, Bertrands bzw. Blom-Rosteds Kaliumkupferkarbonat [2] Methode ausgeführt ist. Das Reduktionsvermögen ist ein Ausdruck für die Anzahl reduzierender Bruchstücke und gibt keine Aussage über deren Kettenlänge.

Beim Stärkeabbau durch α-Amylasen aus Malz und aus *B. subtilis* wächst das Reduktionsvermögen schnell bis ca. 40 % TM. Der Abbau geht mit ständig abnehmender Geschwindigkeit weiter. Bei Anwendung grosser Enzymmengen und langer Reaktionszeit lässt sich der Abbau auf 90—95 % TM treiben [3] (Tabelle 1).

Es ist bisher noch nicht gelungen, die durch α-Amylase gebildeten Stärkeabbauprodukte präparativ zu trennen. Es werden Mono-, Di-, Tri- und Oligosachharide (Dextrine) gebildet. Glucose, Maltose und Maltotriose sind durch *Saccharomyces carlsbergensis* vergärbar. *Saccharomyces uvarum* vergärt Glucose und Maltose; *Zygosaccharomyces Marxianus* vergärt nur Glucose [4]. Dex-

Tabelle 1. Abbau von Kartoffelstärke durch α-Amylasen.

Kleister 2,00 %, pH = 5,0, t = 20,0°
Reduktionsvermögen, ber. als Maltose.

Zeit	Malz (1940) % TMW. Färbung mit Jod			Subtilis (1950) % TMBR
	1 Teil Enzym	2 Teile Enzym	3 Teile Enzym	
½ Std.	6,5 blau	11,2 blau	16,9 dunkelbraun	
¾		16,3 dunkelbraun	22,3 rotbraun	41,7
1	11,8 blau	21,0 rotbraun	27,3 rot	
1½	16,8 dunkelbraun	27,5 rot	32,3 farblos	
2	20,4 braun	31,3 rot	34,8	54,1
3	27,0 braun	35,2 farblos	37,8	
4	30,7 braun	37,5	40,0	
5	33,4 farblos	39,2	41,8	60,7
6	34,9	40,4	43,4	62,4
1 Tag	46,1	53,8	57,6	75,7
2	52,8	61,2		80,8
3	55,7		70,6	
4		68,3		85,8
5	60,1		76,4	
6		71,9		
7	62,2		78,0	89,0
9	64,6	74,1		
11				91,3
18				95,2

trine werden von keinem der genannten Organismen angegriffen. In der vorliegenden Arbeit sind die Gärungen mit einer Ausnahme mit *S. carlsbergensis* durchgeführt.

Alle unsere Versuche sind mit Kartoffelstärke ausgeführt. Einzelheiten sind im Abschnitt »Methodik» gesammelt. Kartoffelstärkekleister wird durch α-Amylase abgebaut; die Enzymwirkung wird bei verschiedenen Abbaugraden durch Kochen sistiert. Das Reduktionsvermögen sowie die Trockensubstanz wird vor und nach der Gärung bestimmt. Die Resultate solcher Versuchsreihen sind in Tabelle 2 und 3 zusammengestellt.

Absolut β-freie α-Malzamylase und α-Subtilisamylase spalten von Anfang an sowohl vergärbare als unvergärbare Saccharide ab. Die Anzahl unvergärbarer reduzierender Dextrine erreicht einen maximalen Wert nach einem Stärkeabbau von 35—40 % TM. Das Maximum ist breit. Erst nach Erreichung

Tabelle 2. Abbau von Kartoffelstärke durch a-Malzamylase. (1940). Differenzierung der Abbauprodukte durch Gärung mit S. carlsbergensis.

Kleister 2,00 %, pH = 4,8, t = 20,0°

Reduktionsvermögen, ber. als Maltose			Vergoren	
Vor Gärung		Nach Gärung	Scheinbare Maltose	Trocken-substanz
% TMW	% TMB	% TMB	%	%
10,3	8,3	6,2	2,1	1,3
13,0	9,7	7,5	2,2	1,7
—	14,9	12,5	2,4	2,3
20,9	17,7	12,8	4,9	4,0
29,0	26,0	17,9	8,1	7,4
30,3	27,8	19,2	8,6	10,1
—	30,6	19,8	10,8	10,1
36,1	33,3	20,5	12,8	13,0
38,5	35,5	21,4	14,1	15,8
40,3	38,0	21,6	16,4	16,5
46,5	44,1	21,8	22,3	22,4

eines Abbaus, der grösser als ca. 60 % TM ist, nimmt die Anzahl reduzierender Dextrine ab. Der maximale Wert von ca. 21 % TM ist derselbe für beide Amylasen.

Beim Stärkeabbau durch α-Subtilisamylase erreichten Blom, Braae und Bak [5] im Jahre 1938 dasselbe Maximum. Zum gleichen Resultat kam 3 Jahre später Smits van Waesberghe [4] (Tabelle 4). Bemerkenswert ist, dass man mit Pankreasamylase in demselben Abbauintervall von 40—60 % TM gleichfalls ein Maximum unvergärbarer Dextrine hat. Der maximale Wert ist jedoch nur halb so gross, 10,3 % TM [6].

In Tabelle 2 sind die Differenzen des Reduktionsvermögens und der Trockensubstanz vor und nach der Gärung zusammengestellt. Aus dieser Versuchsreihe geht hervor, dass die vergärbaren Saccharide aus Disaccharid und/oder molaren Mengen Mono- und Trisaccharid bestehen. Bei höheren Abbaugraden wird Monosaccharid in grösseren Mengen gebildet; es kann sich hier nur um Glucose handeln [7–9]. Bei Vergärung mit *Z. Marxianus* zeigte Smits van Waesberghe, dass von 33 % TM ab Glucose in zunehmender Menge gebildet wird; bei ca. 80 % TM sind ca. 17 % der Stärke als Glucose vorhanden. Smits van Waesberghe machte es ausserdem wahrscheinlich, dass neben Glucose und Maltose vergärbare reduzierende Trisaccharide gebildet werden. In

Tabelle 3. Abbau von Kartoffelstärke durch α-Subtilisamylase. (1940). Differenzierung der Abbauprodukte durch Gärung mit S. carlsbergensis.

Kleister 2,00 %, pH = 4,6, t = 25,0°.

Reduktionsvermögen, ber. als Maltose			Vergoren	
Vor Gärung		Nach Gärung	Scheinbare Maltose	Trocken-substanz
% TMW	% TMB	% TMB	%	%
4,9	3,5	3,3	0,2	
8,0	5,9	5,6	0,3	
12,2	10,4	8,6	1,8	
16,3	14,0	10,9	3,1	
21,5	19,3	14,9	4,4	
27,4	25,5	17,4	8,1	
31,2	29,6	19,2	10,4	
33,5	31,7	20,0	11,7	
36,5	34,3	19,9	14,4	
39,3	37,2	20,6	16,6	
41,7	40,0	21,3	18,7	
44,5	42,8	21,2	21,6	
46,7	45,0	21,5	23,5	
53,3	52,0	20,5	31,5	
*) 63,6	63,4	20,0	43,4	35,1
*) 69,9	68,5	18,7	49,8	42,0
*) 77,4	76,8	16,8	60,0	51,5
*) 82,0	81,6	16,6	65,0	54,9
*) 85,2	85,1	15,5	69,6	58,2

*) Abbautemperatur = 50,0°

unseren Versuchen mit Pankreasamylase haben wir [10] gezeigt, dass diese Amylase wesentlich grössere Mengen von Trisaccharid bildet als α-Amylase aus Malz und *B. subtilis*. Auf diesem Wege ist es uns gelungen, Trisaccharid präparativ herzustellen.

Zur weiteren Charakterisierung der unvergärbaren reduzierenden Dextrine haben wir nach Sistierung der α-Amylase den Abbau mit β-Gerstenamylase fortgesetzt. Die Versuche sind auf verschiedene Weise durchgeführt. In den Versuchen mit α-Malzamylase — im Jahre 1940 ausgeführt — wurde die β-Amylase zu den unvergorenen Abbauprodukten zugegeben (Tabelle 5). In den Versuchen mit α-Subtilisamylase — im Jahre 1950 ausgeführt — wurde die β-Amylase erst nach Vergärung zugesetzt (Tabelle 6). In beiden Fällen ist das Reduktionsvermögen nach Einwirkung der Enzyme vor und nach der

Tabelle 4. Auszug aus Tabellen von Smits van Waesberghe. Abbau von Kartoffelstärke durch α-Subtilisamylase. (1941). Differenzierung der Abbauprodukte durch Gärung.

Kleister 5 %, pH = 6,3.

Tabelle Nr.	Temperatur während Abbau °C	Reduktionsvermögen, ber. als Maltose: Vor Gärung % TM	Reduktionsvermögen, ber. als Maltose: Nach Gärung *S. cerevisiae* % TM	Scheinbare Maltose vergoren %	Wirkliche Glucose %	Wirkliche Maltose %
42	37	20,5	14,7	5,8	Spur	
45	55	23,2	16,1	7,1	0,4	
45	55	31,0	17,8	13,2	0,5	
42	37	32,4	20,1	12,3	0,2	
43	37	36,5	20,1	16,5	1,6	
57	37	37,0	21,1	15,9	1,6	10,1
43	37	39,9	20,4	19,5	2,4	
57	37	40,7	21,6	19,1	2,6	10,9
45	55	45,6	20,2	25,4	4,2	
42	37	45,8	21,1	24,7	3,6	
57	37	48,9	22,5	26,4	4,6	14,2
43	37	52,4	20,0	32,4	5,6	
40	55	61,3	21,1	40,2	8,3	
40	55	67,6	19,1	48,5	10,6	
40	55	82,1	13,3	68,8	16,9	

Gärung bestimmt. In der letzten Versuchsreihe erreicht man einen höheren Abbau der Stärke, einerseits auf Grund der höheren Reaktionstemperatur, andererseits auf Grund der Entfernung von Reaktionsprodukten, welche den weiteren Abbau der Stärke verlangsamen. β-Amylase vergrössert das Reduktionsvermögen, indem der grösste Teil der unvergärbaren Dextrine zu vergärbaren Spaltprodukten abgebaut wird. Das Reduktionsvermögen der Dextrine, die β-Amylase anzugreifen vermag, beträgt maximal 18 % TM der ursprünglichen Stärke. Dieses Maximum erreicht man bei einem Stärkeabbau mit α-Malzamylase von ca. 35 % TM, mit α-Subtilisamylase von weniger als 42 % TM. Mit anderen Worten bildet α-Amylase unvergärbare Bruchstücke in einer Anzahl von 21 % TM; von den 21 Bruchstücken werden 18 von β-Amylase in vergärbare Spaltprodukte überführt.

Der Quotient — Zunahme des Reduktionsvermögens bei der Einwirkung von β und des Reduktionsvermögens der durch β spaltbaren Bruchstücke — gibt die durchschnittliche Anzahl von Bindungen pro Bruchstück, welche β-Amylase spaltet. Diese Anzahl ist grösser als zwei bei Abbaugraden der Stärke unter 45 % TM, gleich zwei bei weitergehendem Stärkeabbau.

Tabelle 5. Abbau von Kartoffelstärke durch α-Malzamylase. (1940). Differenzierung der Abbauprodukte durch β-Gerstenamylase und durch Gärung mit S. carlsbergensis.

Kleister 3,00 %, pH = 4,8, t = 20,0°.
Reduktionsvermögen, ber. als Maltose, % TMB.

α-Amylase		β-Amylase		Durch β-Amylase spaltbare α-Dextrine	Durch β-Amylase gespaltene Bindungen in α-Dextrinen
Vor Gärung	Nach Gärung	Vor Gärung	Nach Gärung		
31,5	19,4	72,2	2,5	16,9	2,4
35,4	20,6	74,2	2,4	18,2	2,1
36,8	20,4	77,3	2,1	18,3	2,2
44,1	21,8	80,6	3,6	18,2	2,0

Tabelle 6. Abbau von Kartoffelstärke durch α-Subtilisamylase. (1950). Differenzierung der Abbauprodukte durch β-Gerstenamylase und durch Gärung mit S. carlsbergensis.

Kleister 2,00 %, pH = 5,5, t = 40,0°.
Reduktionsvermögen, ber. als Maltose, % TMBR.

α-Amylase		β-Amylase nach α-Amylase und Gärung		Durch β-Amylase spaltbare α-Dextrine	Durch β-Amylase gespaltene Bindungen in α-Dextrinen	Total-abbau β- nach α-Amylase ber.
Vor Gärung	Nach Gärung	Vor Gärung	Nach Gärung			
41,7	22,0	72,6	3,8	18,2	2,8	92,3
54,1	22,4	57,8	5,2	17,2	2,1	89,5
60,7	21,7	51,1	5,5	16,2	1,8	90,1
67,3	19,8	48,2	4,1	15,7	1,8	95,7
70,2	18,1	44,5	4,3	13,8	1,9	96,6

Zwecks Differenzierung der vergärbaren Kohlenhydrate wurden in einem Versuch zwei verschiedene Organismen angewandt. 3 %iger Kleister von unbehandelter Kartoffelstärke wurde zuerst mit α-Subtilisamylase zu 36,0 % TMW, darauf mit β-Gerstenamylase zu 71,9 % TMW abgebaut. Trockensubstanz und Reduktionsvermögen wurden bestimmt vor und nach der Gärung mit *S. uvarum*, bzw. *S. carlsbergensis*.

Durch Bestimmung der Trockensubstanz wurde gefunden, dass *S. uvarum* 49,8 % TM, *S. carlsbergensis* 69,9 % TM vergären; durch Bestimmung des

Reduktionsvermögens, dass *S. uvarum* 53,8 % TMBR, *S. carlsbergensis* 67,4 % TMBR vergären. 30, 1 % TM der Trockensubstanz sind unvergärbare Dextrine.

Nach Smits van Waesberghe vergärt *S. uvarum* nur Glucose und Maltose. Beim Vergleich des Reduktionsvermögens und der Trockensubstanz zeigt sich, dass die von *S. uvarum* vergärbaren Saccharide in unserem Versuch zu mehr als 90 % Maltose gewesen sind. *S. carlsbergensis* vergärt mehr als *S. uvarum*, nämlich 20,1 % TM der Trockensubstanz; hierbei handelt es sich um Trisaccharide, da das Reduktionsvermögen 13,6 % TMBR beträgt.

METHODIK

α-Amylase aus Malz. Ein Malzauszug wird mit Phosphatpuffer auf pH 7,0 eingestellt und 60 Minuten auf 60° C erwärmt. Die vollständige Zerstörung der α-Amylase wird nach den Angaben von Blom, Bak und Braae [1] kontrolliert.

α-Amylase aus B. subtilis. Eine 1 %ige Lösung eines Trockenpräparates von Subtilisamylase wird mit Phosphatpuffer auf pH 7,0 eingestellt und 15 Minuten auf 70° C erwärmt.

β-Amylase aus Gerste. Ein Gerstenauszug wird mit Acetatpuffer auf pH 4,0 eingestellt. Nach 4-stündigem Stehen bei Zimmertemperatur ist jede Spur von α-Amylase zerstört. Zwecks Stabilisierung wird auf pH 5,0 eingestellt.

S. carlsbergensis. Bierhefe wird von gröberen Partikeln durch Sieben, von löslichen Bestandteilen durch wiederholtes Aufschlämmen in Wasser befreit.

S. uvarum. Centraalbureau voor Schimmelcultures, Baarn, Nederland.

Stärke. Kartoffelstärke, superior Qualität.

In den folgenden Versuchen wird genauer Kontakt mit der ursprünglichen Stärkemenge durch Wiegen vor und nach den einzelnen Manipulationen bewahrt.

Stärkeabbau durch α-Amylase. 10,00 g trockene, aschefreie Stärke werden in einem mit zugehörigem Propeller tarierten, weithalsigen 500 ml Duranrundkolben abgewogen und durch Rühren mit einem schnell laufenden Motor in 350 ml Wasser aufgeschlämmt. Ohne das Rühren zu unterbrechen, wird der Kolben in ein siedendes Wasserbad getaucht und nach 15 Minuten in ein Wasserbad von 20° (Versuche mit Malzamylase) oder von 40° (Versuche mit Subtilisamylase) überführt. Nach weiteren 15 Minuten wird das Rühren unterbrochen, Acetatpuffer, Enzymlösung und Toluol zugesetzt und der Inhalt mit Wasser auf 500,0 g gebracht. Das Gemisch wird nun 1 Minute gerührt und endlich der Kolben verschlossen. Nach passender Zeit (Vorversuche!) bei 20°, resp. bei 40° wird gekocht, um die Enzymwirkung zu sistieren und Toluol abzudampfen. Nach Abkühlung wird Wasser bis zum Gewicht wie vor dem Kochen zugesetzt.

Fortzetzung des Abbaus mit β-Amylase. a) Eine passende Menge β-Amylase und einige Tropfen Toluol werden zu der durch α-Malzamylase abgebauten Stärkelösung zugesetzt. Nach 20-stündigem Stehen bei 20° wird die Lösung einige Minuten gekocht, um die Enzymwirkung zu sistieren und Toluol abzudampfen. *b)* Eine passende Menge β-Amylase und einige Tropfen Toluol werden zu der durch α-Subtilisamylase abgebauten und vergorenen Stärkelösung (siehe später) zugesetzt. Nach 20-stündigem Stehen bei 40° wird die Lösung einige Minuten gekocht, um die Enzymwirkung zu sistieren und Toluol abzudampfen.

Gärung mit S. carlsbergensis. 50,0 g einer gekochten Lösung werden in einer 250 ml Flasche mit Patentverschluss abgewogen und mit 10,0 g einer Hefesuspension (entsprechend etwa 200 mg Trockensubstanz) versetzt. Die Gärung ist unter Schütteln bei ca. 32° C in 2 bis 3 Tagen vollendet. Die Hefe wird abfiltriert.

Gärung mit S. uvarum. 100,0 g der gekochten Lösung werden in einem 300 ml Erlenmeyerkolben abgewogen und durch halbstündiges Dampfen in 3 aufeinanderfolgenden Tagen sterilisiert. Der Kolben wird mit *S. uvarum* geimpft und bei ca. 28° inkubiert. Nach 2 bis 3 Wochen ist die Gärung vollendet. Es wird Wasser bis zu 100,0 g zugesetzt und die Hefe abfiltriert.

ZUSAMMENFASSUNG

Der Abbau von Kartoffelstärkekleister durch α-Amylase aus Malz und aus *B. subtilis* verläuft auf dieselbe Weise. Es werden durch Bierhefe vergärbare und unvergärbare, reduzierende Spaltstücke gebildet. Die Anzahl der vergärbaren Spaltstücke steigt während des Abbaus ununterbrochen an. Die Anzahl der unvergärbaren Spaltstücke steigt anfangs ebenfalls an, erreicht aber ein Maximum, um später abzunehmen. Die Anzahl unvergärbarer Spaltstücke ist maximal bei einem Stärkeabbau von 35 % TM bis 60 % TM und beträgt innerhalb dieser Grenzen 21 % TM. 6/7 (~ 18 % TM) der unvergärbaren Spaltstücke lassen sich durch β-Gerstenamylase in vergärbare Spaltstücke zerlegen.

Nach Abbau der Starke durch α-Subtilisamylase bis ca. 40 % TM und weitergehendem Abbau durch β-Amylase aus Gerste bis ca. 70 % TM sind 50 % der Stärke in Disaccharid, 20 % in Trisaccharid und 30 % in unvergärbare Dextrine umgewandelt.

Wir möchten der Leitung der Tuborg-Brauereien für ihr wohlwollendes Entgegenkommen unseren Dank aussprechen.

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Über die Gleichartigkeit von Kartoffelstärken verschiedener Herkunft

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Stärkekleister wird bei der Einwirkung von α-Amylasen dünnflüssig. Diese Reaktion benützt man zur Bestimmung der Aktivität von α-Amylasepräparaten, zum Beispiel in der von Blom und Bak [1] ausgearbeiteten Methode. Das gleiche α-Amylasedauerpräparat gab nach dieser Methode untersucht dieselbe Enzymaktivität bei Verwendung 14 verschiedener Proben von prima Kartoffelstärke. Der mittlere Fehler betrug ± 1 %. Die Stärken stammten aus 6 verschiedenen europäischen Ländern und waren aus Kartoffeln der Ernten 1929 bis 1937 hergestellt.

Stärkekleister wird bei der Einwirkung von α- und β-Amylasen verzuckert. Diese Reaktion benützt man zur Bestimmung der diastatischen Kraft. Hierzu verwendet man Lösungen von löslicher Stärke. Verschiedene Handelspräparate löslicher Stärke geben mit demselben Amylasepräparat verschiedene Werte der Aktivität. Nach dem Kriege war es in Dänemark unmöglich, lösliche Stärke zu beziehen. Anstatt lösliche Stärke selbst herzustellen, welche a priori mit dem soeben genannten Mangel behaftet ist, haben wir mit Erfolg einen anderen Weg eingeschlagen. Wir haben eine Methode zur Bestimmung der diastatischen Kraft unter Verwendung unbehandelter Kartoffelstärke ausgearbeitet [2]. Das gleiche Malzamylasedauerpräparat gab, nach dieser Methode untersucht, dieselbe Enzymaktivität bei Verwendung 18 verschiedener Proben von prima Kartoffelstärke. Die Stärken stammten aus 10 verschiedenen europäischen Ländern und waren aus Kartoffeln der Ernten 1931 bis 1948 hergestellt. Der mittlere Fehler betrug ± 1 % mit unbehandelter Kartoffelstärke, mit löslicher Stärke dagegen ± 5 %.

In der vorhergehenden Arbeit [3] wurde gezeigt, dass beim Abbau von Kleister aus unbehandelter Kartoffelstärke durch α-Amylase aus Malz und aus *Bacillus subtilis* unvergärbare reduzierende Dextrine in einer maximalen

Tabelle 1. Abbau von Kartoffelstärke verschiedener Provenienz durch α-Subtilisamylase. Differenzierung der Abbauprodukte durch β-Gerstenamylase und durch Gärung mit S. carlbergensis. (1950)
Kleister 2,00 %, pH = 5,0, t = 40,0°
Reduktionsvermögen, ber. als Maltose, % TM, nach Blom und Rosted.

Bezeichnung	Provenienz	Ernte	α-Amylase		β-Amylase nach α-Amylase und Gärung		Durch β-Amylase spaltbare α-Dextrine
			Vor Gärung	Nach Gärung	Vor Gärung	Nach Gärung	
S	Norwegen	1947	37,7	20,6	71,7	2,6	18,0
Y	England	1947	37,7	21,0	69,1	2,8	18,2
Q	Frankreich	1947	39,1	21,1	69,9	2,8	18,3
DK	Dänemark	1948	39,2	22,0	69,9	4,1	17,9
E	Dänemark	1937	39,8	21,0	69,1	3,5	17,5
Z	Tschechoslowakei	1947	40,0	21,5	71,6	2,6	18,9
A	Deutschland	1931	40,5	21,8	69,1	3,3	18,5
B	Holland	1933	40,6	21,7	69,6	3,4	18,3
C	Holland	1936	40,8	21,6	70,4	3,0	18,6
Ho	Holland	1947	41,7	22,0	72,6	3,8	18,2
C	Holland	1936	41,9	21,7	69,1	3,1	18,6
I	Schweden	1937	42,8	21,4	69,2	2,6	18,8
A	Deutschland	1931	46,7	22,1	69,2	3,5	18,6
Mittel				21,5		3,2	18,3

Anzahl von 21 % TM* gebildet werden. Smits van Waesberghe[4] kam zu demselben Ergebnis. Bei einer darauf folgenden Behandlung mit β-Gerstenamylase konnten wir zeigen, dass 18 von den 21 Dextrinen in vergärbare Spaltprodukte umgewandelt werden.

Eine Reihe von Proben von prima Kartoffelstärke aus unserer Sammlung wurde wie in der vorhergehenden Arbeit untersucht. Der Stärkekleister wird durch α-Amylase aus *B.subtilis* zu etwa 40 % TM abgebaut und das Reaktionsgemisch darauf mit Bierhefe vergoren. Der Abbau wird nunmehr mit β-Amylase aus Gerste fortgesetzt und das Reaktionsgemisch wiederum mit Bierhefe vergoren. Das Reduktionsvermögen wird nach Einwirkung jeden Enzyms vor und nach der Gärung nach der Kupferkarbonatmethode von Blom und Rosted[5] bestimmt.

Im ganzen wurden 13 Proben von Kartoffelstärke untersucht. Die Stärken stammten aus 8 verschiedenen Ländern und waren aus Kartoffeln der Ernten 1931 bis 1948 hergestellt. In allen Fällen gab die Untersuchung dasselbe Resultat. α-Subtilisamylase bildet unvergärbare Dextrine in einer Anzahl von 21,5%

* % TM = % der theoretisch möglichen Menge Maltose, die gebildet werden würde, falls Stärke sich vollständig zu Maltose abbauen liesse.

TM. 18,3 von diesen werden durch β-Amylase zu vergärbaren Spaltprodukten abgebaut.

Aus der ersten Versuchsreihe über die Verflüssigung, aus der zweiten über die Verzuckerung und aus der dritten über die maximale Anzahl der Dextrine darf man den Schluss ziehen, dass weder Klima, Boden oder Sorte einen Einfluss auf die Konstitution der Kartoffelstärke haben.

ZUSAMMENFASSUNG

Beim Abbau von Kartoffelstärkekleister durch α-Amylase aus *B. subtilis* werden durch Bierhefe vergärbare und unvergärbare Spaltstücke gebildet. Die Anzahl der unvergärbaren Spaltstücke, Dextrine, erreicht ein Maximum. Die Dextrine lassen sich zum grössten Teil durch β-Gerstenamylase zu vergärbaren Spaltstücken abbauen.

Es sind 13 Proben von Kartoffelstärke aus 8 verschiedenen Ländern der Ernten 1931 bis 1948 untersucht, in allen Fällen mit demselben Resultat. α-Subtilisamylase bildet unvergärbare Dextrine in einer Anzahl von 21,5 % TM. 18,3 von diesen werden durch β-Amylase zu vergärbaren Spaltprodukten abgebaut.

Aus früheren Versuchen über die Verflüssigung (14 Proben) und über die Verzuckerung (18 Proben) von Stärkekleister durch Amylasen und aus obigen Versuchen lässt sich schliessen, dass weder Klima, Boden oder Sorte einen Einfluss auf die Konstitution der Kartoffelstärke haben.

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Studies Related to Naturally Occurring Acetylene Compounds

VIII. The synthesis of Methyl *n*-Dec-2-en-4:6:8-triynoate, an Isomer of the Naturally Occurring Dehydromatricaria Ester

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A year ago Kjellrun Stavholt and N. A. Sörensen [1] described in this journal the isolation from European mugwort (*Artemisia vulgaris* L.) of a crystalline methyl ester, $C_{11}H_8O_2$. Catalytic hydrogenation gave methyl caprate, and for spectral reasons these authors regarded formulae I and II as the most probable for this highly unsaturated compound.

I. $H_3C-CH=CH-C\equiv C-C\equiv C-C\equiv C-COOCH_3$

II. $H_3C-C\equiv C-C\equiv C-C\equiv C-CH=CH-COOCH_3$

$H_3C-C\equiv C-C\equiv CH + HC\equiv C-CH=CH-COOCH_3$

In theory both I and II may occur in *cis* and *trans* modifications. With the object of distinguishing between these 4 possibilities, we have carried out the synthesis of the *trans*-modification of II by Glaser coupling of methyl diacetylene, prepared according to Schlubach and Wolf [2], and methyl pent-2-en-4-ynoate prepared according to Heilbron, Jones and Sondheimer [3]. The Glaser coupling gave, as is usual with methyl pent-2-en-4-ynoate, high yields of dimethyl octa-1:7-diene-3:5-diyne-1:8-dicarboxylate, and the asymmetric coupling product, *trans* methyl *n*-dec-2-ene-4:6:8-triynoate, occurred only in traces. The separation of these compounds was achieved by chromatography of the reaction product on alumina. The pure *trans* methyl *n*-dec-2-ene-4:6:8-triynoate was a slightly yellow crystalline substance, m.p. 95.5°, whereas the ester isolated from mugwort root melted at 112–113°. As will be seen from Fig. 1 the U.V. spectra of the synthetic and the naturally occurring methyl *n*-decene-triyn-oates are very closely related, but with definite and characte-

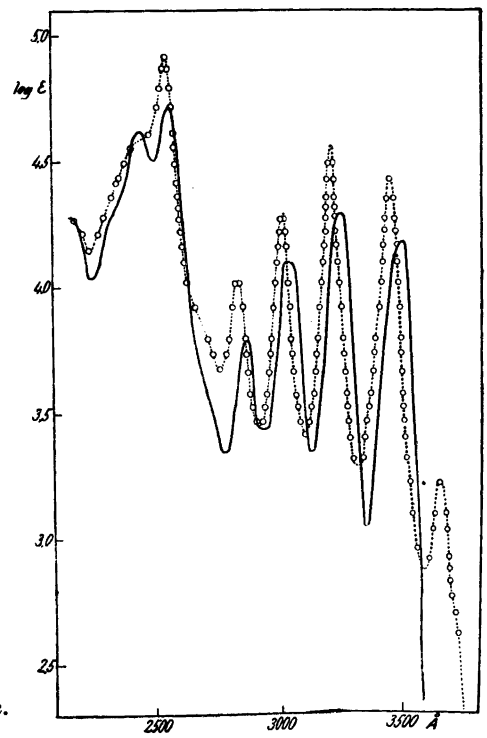


Fig. 1. Ultraviolet absorption of dehydromatricaria ester in hexane.

· · · o · · · *Ester from European mugwort* [1].
———— *Methyl* n-*dec-2-en-4 : 6 : 8-triynoate.*

ristic small differences. The synthetic isomer has a weak absorption band on the long wave side at 3630ÅU, which is lacking in the mugwort ester. This band does not follow acetylenic spacing with respect to the following 4 absorption bands and would seem to belong to another band system.

The second difference is that in the synthetic isomer the four very sharp maxima occur at about 35 ÅU towards shorter wavelengths than for the mugwort ester. This difference is of the same magnitude and of the same sign as between *cis* and *trans* Lachnophyllum ester [4,5].

In ethylenic chromophors the *trans* isomers absorb at longer wavelengths than the *cis*-isomers, and therefore we hesitate to conclude that the mugwort ester is the *cis*-isomer of II.

Furthermore the very strong band group below 2600 ÅU, which shows fine structure in the mugwort ester, has only a shoulder on the short wave side in the synthetic isomer.

The properties of the synthetic isomer make it very probable that the mugwort ester is one of the 2 possible isomers of formula I or the *cis* isomer of II.

EXPERIMENTAL

trans *Methyl* n-*dec-2-ene-4:6:8-triyn-oate.* 3.2 g methyldiacetylene and 5.5 g methyl pent-2-en-4-ynoate was added to a solution of 112 g ammonium chloride and 70 g cuprous chloride in 250 ml water. The suspension was shaken 1 hour in a mechanical shaker, then 1 hour by hand. The reaction mixture showed unusual foaming and gave heavy emulsions when extracted with ether. The ethereal extract, which was solid, was extracted with petroleum ether (b. p. $< 40°$) the residue went into solution in benzene-petroleum ether (6 : 4). Both solutions were adsorbed on slightly activated alumina and eluted with petroleum ether, benzene-petroleum ether mixture, pure benzene and finally ether. The less soluble fraction, which was dissolved in benzenepetroleum ether (see above), gave only dimethyl octa-1,7-dien-3,5-diyne-1,8-dicarboxylate as crystallizing reaction product. The more soluble part of the reaction product — the petroleum ether solution — afforded in the first ether eluate 50 mg of slightly yellow crystals, which after recrystallization from petroleum ether melted at 95.5° C.

$C_{11}H_8O_2$ (172.1)	Calc.	C 76.73	H 4.68				
	Found	» 76.4	» 4.53				

U.V.-spectrum in hexane solution, Fig. 1.

λmax	3630	3440	3210	3012	2832	2556	Å
ν » $\cdot 10^{-12}$	826.4	872.1	934.6	996.0	1059.3		cm^{-1}
$\Delta\nu$ » »		62.5	61.4	63.3			»

Catalytic hydrogenation. 7.2 mg was hydrogenated with PdO_2-catalyst. The activity of the catalyst which was generated immediately before use, was unexpectedly great and caused some uncertainty in the initial reading. Hydrogen consumption (0°, 760 mm) 6.3 ml; calc. 7.1 ml. The hydrogenation product had the characteristic strawberry odour of methyl caprate.

SUMMARY

Glaser coupling of methyl diacetylene with methyl pent-2-en-4-yn-oate, gave the *trans* isomer of methyl *n*-dec-2-en-4:6:8-triynoate (II), m.p. 95. 5° C. This synthetic dehydromatricaria ester is different from the dehydroester found in European mugwort. The U.V.-absorption of the two isomers is closely related and strengthens the supposition that the naturally occurring dehydromatricaria ester is either the *cis*-isomer of II or one of the isomers of I.

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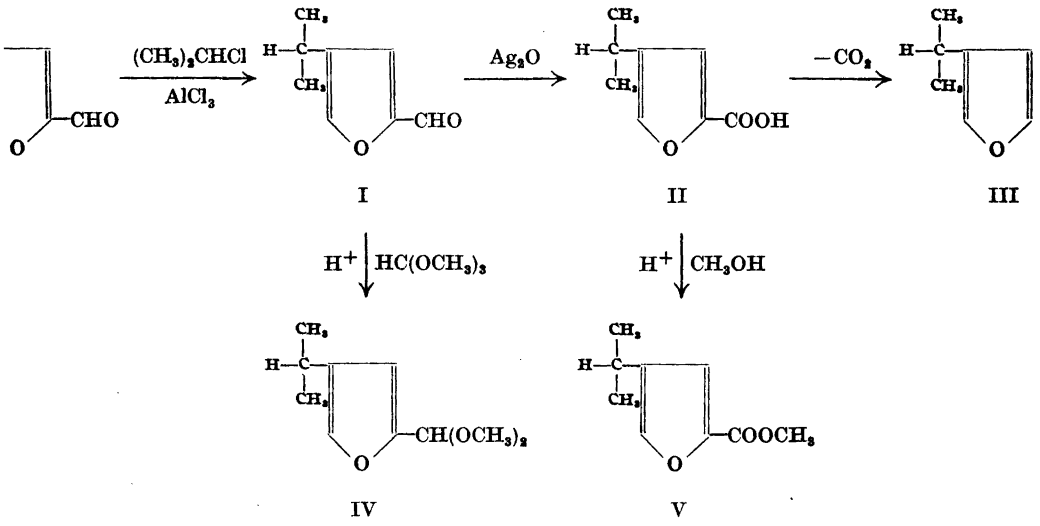
Short Communications

Preparation of Some β-Isopropylfurans

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In the course of an investigation of the alkoxylation of furans, compounds I–V have been prepared by the sequence of reactions shown below:



The directions of Gilman *et al.*[1–3] were – with some modifications – followed for the preparation of I–III, while IV and V, which are new compounds, were prepared by well-known methods. Gilman *et al.* have determined the structure of I–III. The structure of IV and V follows from their synthesis and from analyses.

EXPERIMENTAL (Microanalyses by F. Limborg and K. Glens). *4-Isopropyl-2-furaldehyde (I)* (*cf.* Gilman and Calloway [1]; Gilman, Calloway and Burtner [3]). 242 g of freshly distilled furfural (2.50 moles) and 200 g of isopropyl chloride (2.55 moles) are mixed and added during 1 3/4 hours to a stirred suspension of 405 g of powdered aluminum chloride (3.00 moles) in 1400 ml of carbon disulfide. The reaction flask is cooled occasionally to maintain a gentle reflux. Stirring is continued for 18 hours at room temperature. The mixture is poured on cracked ice with vigorous stirring, and the two resulting layers are separated. The carbon disulfide solution is washed with water, 4 *M* sodium acetate and 0.8 *M* sodium hydrogen carbonate and dried with sodium sulfate. The carbon disulfide is

distilled off and the residue distilled under nitrogen.

Fraction (g)	B. p.$_{16}$	Colour
1 (82)	< 80°	dark
2 (67)	80°—110°	—
3 (34)	110°—145°	—
residue (71)		—

Fraction 2 is redistilled under nitrogen through a 5 cm column packed with glass helices.

Fraction (g)	B. p.$_{13}$	n_D^{25}	Colour
1 (8.0)	55°— 90°		dark
2 (1.9)	90°— 92°	1.5052	light-brown
3 (37.0)	92°— 95°	1.5025	—
4 (4.4)	95°—105°	1.4988	—

Yield (fraction 3) 37.0 g of isopropylfuraldehyde = 11 %. Another distillation through the same column gives 33.0 g = 9.5 %; b. p.$_{13}$ = 92°—95°; n_D^{25} = 1.5032. The colour of this product is also light-brown. Gilman and Calloway [1] obtained a 12 % yield of their product (b. p.$_{21}$ = 101°—103°, n_D^{25} = 1.5041).

4-Isopropyl-2-furaldehyde dimethyl acetal (IV). 18.0 g of isopropylfuraldehyde (0.13 mole), 15.2 g of orthoformic acid trimethyl ester (0.14 mole), 16 ml of anhydrous methanol and 0.3 g of ammonium chloride are mixed and the mixture refluxed for 3 hours. After cooling the ammonium chloride is filtered off and the low boiling products evaporated in vacuum. The residue is taken up in ether, washed twice with water and dried with solid potassium carbonate. The ether is distilled from a water-bath and the residue distilled further through a 5 cm column packed with glass helices. After a fore-run (1.0 g, b. p.$_{13}$ = 93°—97°, n_D^{25} = 1.4522) the main fraction distils at 97°—99°/13 mm. Yield 19.5 g = 82% of a pale-yellow liquid; n_D^{25} = 1.4519.

$C_8H_{10}O(OCH_3)_2$ (184.2)
Calc. OCH_3 33.7 Found OCH_3 33.1

Another distillation through the same column gives 16.7 g = 70 %; pale-yellow liquid, b. p.$_{13}$ = 97°—99°; n_D^{25} = 1.4524.

$C_8H_{10}O(OCH_3)_2$ (184.2)
Calc. C 65.2 H 8.8 OCH_3 33.7
Found » 64.9 » 9.0 » 33.4

4-Isopropyl-2-furoic acid (II). 20.5 g of isopropylfuraldehyde (0.15 mole) in 30 ml of ethanol is poured into a suspension of silver oxide (from 57.0 g of silver nitrate (0.33 mole)) in 150 ml of 50 % ethanol. A solution of 12.2 g of sodium hydroxide (0.30 mole) in 60 ml of water is added dropwise to the stirred suspension during 15 minutes. The temperature is kept at 40°—45° by cooling. Stirring is continued for 30 minutes without cooling whereby the temperature drops to 33°. Finally the mixture is refluxed for 20 minutes. After filtration the ethanol and part of the water are evaporated in vacuum. The residue is washed once with ether, acidified with concentrated hydrochloric acid and extracted 3 times with ether. The etheral extract is washed once with water and dried with sodium sulfate. The ether is evaporated in vacuum. Yield 21.0 g of isopropylfuroic acid = 91 %. 1.00 g of the above product was crystallized from ether-petroleum ether and dried (sulfuric acid, 0.1 mm, 20°, 8 hours). Yield 0.84 g; m. p. 76.0°—77.5° (Hershberg apparatus, corr.); Gilman and Calloway [1] found 76°—77°. $C_8H_{10}O_3$ (154.2) Found neut.equiv. (phenolphtalein indicator) 154.4.

4-Isopropyl-2-furoic acid methyl ester (V). 16.5 g of isopropylfuroic acid, 100 ml of methanol and 9 ml of concentrated sulfuric acid are mixed and refluxed for 4 hours and the ester isolated in the usual manner. The final product is distilled through a 5 cm column packed with glass helices. Yield 13.1 g of isopropylfuroic acid methyl ester = 73 %; colourless liquid, b. p.$_{14}$ = 110°—112°; n_D^{25} = 1.4783.

$C_8H_9O_2(OCH_3)$ (168.2)
Calc. C 64.3 H 7.2 OCH_3 18.5
Found » 64.1 » 7.1 » 17.7

3-Isopropylfuran (III). Six 5.0 g-portions of isopropylfuroic acid are decarboxylated as described by Gilman, Calloway and Burtner [3] (*cf.* also Shephard, Winslow and Johnson [4]). The distillate from the decarboxylation (21 g) is dried with magnesium sulfate and distilled under nitrogen through a 5 cm column packed with glass helices. Yield 16.6 g of isopropylfuran = 78 %; colourless liquid, b. p.$_{760}$ = 111°—113°; n_D^{25} = 1.4344; n_D^{20} = 1.4368. Gilman, Calloway and Burtner [3]

obtained a 56 % yield of their product (b. $p._{760}$ = 111°–112°, n_D^{20} = 1.4369).

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Melting Points of *p*-Nitrobenzyl Esters of Aryl Substituted Propionic Acids

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In connection with other work in progress in this laboratory, a convenient method for identifying carboxylic acids was required. The melting points of the *p*-nitrobenzyl esters prepared according to the method originally proposed by Reid [1] was found to be suitable for this purpose. The method was first applied to acids with similar constitutions and melting points that had been prepared in this laboratory [2]. As the melting points of these *p*-nitrobenzyl esters may have a general interest in connection with the appraisement of Reid's method, they are reproduced in Table 1.

The melting points of the *p*-nitrobenzyl esters are rather evenly spread over a temperature range of 55° and almost all differences between the melting points of the derivatives are sufficiently large for identifaction purposes.

The esters were prepared according to Wild [3].

1. Reid, E. E. *J. Am. Chem. Soc.* **39** (1917) 124; Lyman, J. A. and Reid, E. E. *ibid.* **39** (1917) 701; Lyons, E. and Reid, E. E. *ibid.* **39** (1917) 1727; Bliche, F. F. and Smith, F. D. *ibid.* **51** (1929) 1947.
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Table 1.

Propionic acid		*p*-Nitrobenzyl ester		
Substituents	M.p. ° C	M.p. ° C	Calc. N	Found N
β,β,β,-Triphenyl-	177–178	133–134	3,20	3,26
β,β-Diphenyl-β-*o*-tolyl-	174.5–175.5	110–111	3,10	3.09
β,β-Diphenyl-β-*m*-tolyl-	118–119	106–107	3.10	3.11
β,β-Diphenyl-β-*p*-tolyl-	195–196	120–121	3.10	3.12
β,β-Diphenyl-β-*o*-anisyl-	169–170	115–116	3.00	2.99
β,β-Diphenyl-β-*p*-anisyl-	155–156	83– 84	3.00	2.99
β,β-Diphenyl-	154–155	90– 91	3.88	3.87
β-Phenyl-β-*o*-tolyl-	131–132	83– 84	3.73	3.68
β-Phenyl-β-*p*-tolyl-	145–146	78– 79	3.73	3.77
β-Phenyl-β-*p*-anisyl-	124–125	87– 88	3.58	3.48
β,β-Di-p-anisyl-	139–141	93– 94	3.32	3.29
β-Phenyl-β-*p*-biphenyl-	176–177	110–111	3.20	3.27
β-Phenyl-β-naphthyl-1-	149–150	104–105	3.40	3.30

obtained a 56 % yield of their product (b. $p._{760}$ = 111°–112°, n_D^{20} = 1.4369).

1. Gilman, H., and Calloway, N. O. *J. Am. Chem. Soc.* **55** (1933) 4197.
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β,β-Diphenyl-β-*m*-tolyl-	118–119	106–107	3.10	3.11
β,β-Diphenyl-β-*p*-tolyl-	195–196	120–121	3.10	3.12
β,β-Diphenyl-β-*o*-anisyl-	169–170	115–116	3.00	2.99
β,β-Diphenyl-β-*p*-anisyl-	155–156	83– 84	3.00	2.99
β,β-Diphenyl-	154–155	90– 91	3.88	3.87
β-Phenyl-β-*o*-tolyl-	131–132	83– 84	3.73	3.68
β-Phenyl-β-*p*-tolyl-	145–146	78– 79	3.73	3.77
β-Phenyl-β-*p*-anisyl-	124–125	87– 88	3.58	3.48
β,β-Di-p-anisyl-	139–141	93– 94	3.32	3.29
β-Phenyl-β-*p*-biphenyl-	176–177	110–111	3.20	3.27
β-Phenyl-β-naphthyl-1-	149–150	104–105	3.40	3.30

Microbiological Determinations of Amino Acids in Foodstuffs. III

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The present paper in continuing the work started in 1949 describes the results obtained by the microbiological determinations of amino acids in foodstuffs which are commonly used for nutritional purposes in this country.

Source and preparation of material. The rape meal, meadow fescue and red clover were obtained from the National Animal Experimental Station, Uppsala. The yeast autolysate was produced by Jästbolaget, Stockholm. The rape meal was the rest left after the commercial production of rape oil. Each one of the two plant materials were derived from the third crop. They were harvested 3 weeks after the second crop and before any flowers had developed. The whole plants with an average height of about 20 cm were used. The yeast autolysate was prepared from baker's yeast. All materials were freed from lipids as previously described [1]. The methods of hydrolysis were described in the same paper. Constant moisture values were obtained by drying at 70° *in vacuo* and in the presence of P_2O_5.

Microbiological procedures. Microorganisms, basal media, ranges of standard curves, incubation times and some other details were described in a previous paper [2].

Results and Discussion. In Table 1 the nitrogen values and content of crude protein are recorded in the usual way. Comparing the two plant materials, the red clover should contain more of crude protein. However, when the figures are calculated for 100 g of fresh material the meadow fescue contains 3.8 g and the red clover 3.6 g of crude protein.

To facilitate a comparison with previous data of the amino acid composition in foodstuffs the microbiological values are calculated as described in the first paper of this series [1]. The values are given in Table 2.

With regard to the content of essential amino acids the rape meal compares favourably with the previously analyzed

Table 1. Nitrogen and crude protein content of the foodstuffs. Percentages calculated for ash- and moisture-free material.

Material	N per cent	Crude protein N × 6.25	Ash per cent	Moisture per cent
Rape (*Brassica Napus* L. var. *Oleifera* Metzg.) whole meal	9.10	57.0	26.2	6.2
Yeast autolysate	12.2	76.2	7.4	25.0
Meadow fescue (*Festuca pratensis*) whole plant	3.18	19.7	2.4	79.3
Red clover (*Trifolium pratense*) whole plant	4.52	28.3	1.6	86.2

Table 2.

1. — Values expressed as percentage for ash- and moisture- free material.
2. — Values expressed as percentage in crude protein (total nitrogen in hydrolysate × 6.25).
3. — Amino acid nitrogen in percentage of total nitrogen in hydrolysate.

Amino acid	Rape meal			Yeast autolysate			Meadow fescus			Red clover		
	1	2	3	1	2	3	1	2	3	1	2	3
Alanine	0.9	1.9	1.8	3.6	9.2	8.9	1.0	5.4	5.3	1.0	4.8	4.8
Arginine	3.0	5.6	11.2	1.7	4.3	8.6	0.5	2.7	5.6	0.5	2.1	4.3
Aspartic acid	6.8	9.7	6.5	5.4	13.7	9.0	—	—	—	—	—	—
Cystine	0.9	1.7	1.2	0.6	1.5	1.1	—	—	—	—	—	—
Glutamic acid	9.0	17.1	10.2	4.2	10.7	6.3	—	—	—	—	—	—
Glycine	3.3	6.3	7.3	2.0	5.0	5.8	1.3	5.7	6.7	1.2	5.6	6.5
Histidine	1.4	2.6	4.5	0.8	2.1	3.6	0.3	1.7	2.9	0.4	1.9	3.2
Isoleucine	2.0	3.7	2.4	2.0	4.8	3.2	1.0	4.9	3.2	1.1	4.8	3.2
Leucine	3.0	5.7	3.8	2.7	6.9	4.6	1.6	8.0	5.4	1.8	8.1	5.4
Lysine	1.8	3.5	4.2	2.9	7.4	8.9	1.0	5.2	6.1	0.9	4.1	4.9
Methionine	0.6	1.1	0.7	0.5	1.2	0.7	0.2	0.8	0.5	0.2	0.7	0.4
Phenylalanine	2.1	4.0	2.1	1.5	3.5	1.9	0.9	4.7	2.5	1.1	5.1	2.7
Proline	4.2	8.0	6.1	1.4	3.4	2.6	—	—	—	—	—	—
Serine	4.5	8.6	7.2	2.1	5.3	4.3	—	—	—	—	—	—
Threonine	2.0	3.8	2.8	1.1	2.9	2.1	0.7	3.4	2.5	0.6	2.5	1.9
Tryptophan	0.8	2.0	1.7	0.9	1.3	1.1	0.5	2.5	2.1	0.5	1.8	1.5
Tyrosine	1.2	2.3	1.1	0.9	2.3	1.1	0.5	2.4	1.2	0.7	3.0	1.5
Valine	3.0	5.7	4.3	2.1	5.2	3.9	1.0	5.0	3.7	1.2	5.6	4.2
NH_3-N			12.5			12.0						
Total	50.5	93.3	91.6	36.4	105.3	89.6	10.6	52.4	47.7	11.1	50.1	44.5

lupine meals and bean meal [1,2]. The amino acid composition of the autolyzed baker's yeast closely resembles that found in brewer's yeast. There are no figures available for a comparison with the results obtained in the two plants. Lugg [3] in his recent review only gives some average figures for the bulk proteins prepared from leguminosae and graminae plants. However, with the exception of arginine and methionine our values for the other amino acids fall fairly well within the ranges given by Lugg.

The technical assistance of Mr. S. Eklund, Mr. E. Kristenson and Mrs. I. Kristenson is greatfully acknowledged.

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Sorption Isotherms on Ion Exchangers by emf Titrations

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For the experimental study of ion exchange equilibria the batch equilibrium method has been the one most frequently used. Very recently two methods that permit the calculation of sorption isotherms from leaking curve data have been developed. Duncan and Lister [1] have shown how points on the isotherm can be obtained from sorption curves, the so-called break-through method. Glueckauf [2] and Sillén [3] have given equations that permit calculation of the isotherms from desorption curve data.

Of the above-mentioned methods, the batch equilibrium method is simple but time-consuming. The break-through method gives only one point on the isotherm for every sorption curve measured and is thus rather laborious. The third method mentioned allows the whole isotherm to be calculated from a single desorption curve, but large errors may be introduced if the values of the equilibrium quotient at the ends of the diagram are unknown (*cf.* below.)

By modifying the batch equilibrium method to a titrimetric one, time is saved and the calculations are still very simple.

This method has been applied to the $Ag^+ - H^+$ exchange on Wofatit KS. 1.00 g H^+ saturated resin, 0.5—1.0 mm in diameter, and 200.0 ml 100.0 mC HNO_3 have been titrated with 100.0 mC $AgNO_3$ or 10.00 mC $AgNO_3$—90.00 mC HNO_3 mixture. The whole apparatus was kept in a thermostated room at 25.0 ± 0.3° C. The Ag^+ concentration was measured with the Forsling-Sillén apparatus, as described in an earlier paper [4]. Equilibrium was, in most cases, attained within 30 minutes with the stirring speed used in the experiments.

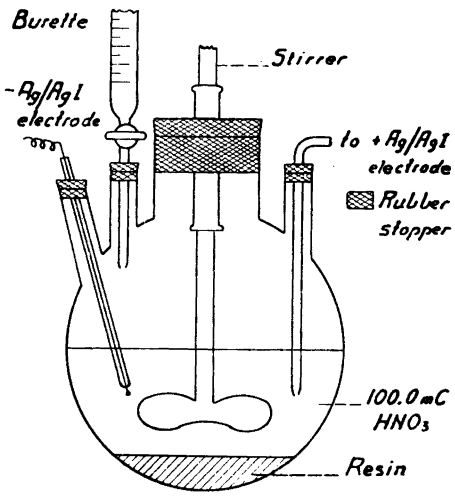


Fig. 1. Schematic figure of the titration apparatus. When the emf was measured the stirrer was stopped.

After the titration the sorption capacity (s_0) of the resin was determined by saturation with H^+, expelling the H^+ with KNO_3 and titration with NaOH. From the assumption of the equivalent exchange and a knowledge of s_0, $[Ag^+]$, $[Ag^+]_{tot}$ and the added amount of HNO_3, the equilibrium quotient ($\varkappa$) was calculated for the reaction:

$$Ag^+ + HR \rightleftharpoons H^+ + AgR \qquad (1)$$

In Fig. 1 is given a picture of the apparatus and Fig. 2 gives a comparison between $\varkappa$ values obtained by titration and by the batch method. $\varkappa$ is plotted against the mole fraction of silver in the resin (β). The batch equilibrium values are taken from Table 7a in ref. 4. The agreement is quite good. The spread of the values obtained by titrations is not larger than for that of the values obtained by

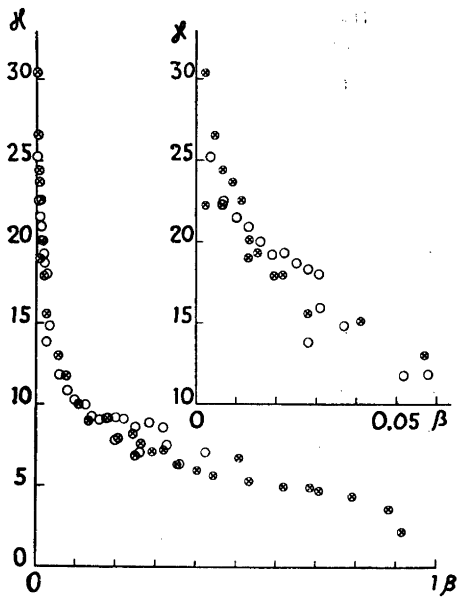


Fig. 2. $\varkappa$ is plotted against β. ⊙ denotes values obtained by titrations. (⊗) denotes values obtained by the batch method.

the batch method. The titration method has proved to be much simpler and faster than the batch equilibrium method.

It may be noted that only about half the β interval has, in this case, been covered by the titration method. By starting from Ag^+ saturated resin and $AgNO_3$ it is possible to cover the whole interval by titrations. However, the amount of H^+ in the solution is, in this case, very uncertain as it is determined as a difference. In this case it would be better to use a H^+ sensitive electrode *e.g.* a glass electrode and change to a Ag/AgI electrode for $\beta \approx 0.5$.

As electrodes sensitive to a number of ions are available, many ion exchange equilibrium studies can be carried out as emf titrations. For instance the $Cu^{2+}-H^+$ exchange can be studied with both a copper amalgam and a glass electrode. The diffusion potentials must be known for the ion pair and the ionic strength in question. They can according to Ekedahl [5-6] be determined rapidly by titrations without resin.

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Preparation of D-Valine by Asymmetric, Enzymatic Hydrolysis of the Isobutyl Ester of DL-Valine

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DL-valine has earlier been resolved by fractional crystallization of the brucine salt of its formyl derivative [1] or of its menthoxyacetyl derivative [2]. Duschinsky and Jeannerat [3] prepared the L-isomer of valine by oxidation of the D-isomer in the racemic form of valine with D-amino acid oxidase from hog kidneys. Because the enzymes in yeast destroy the L-valine and leave the D-form intact, it is possible with this procedure to obtain the latter free from the L-isomer [4]. Price, Gilbert and Greenstein [5] produced the valine isomers by means of asymmetric hydrolysis of chloroacetyl-DL-valine.

A simple method for producing D-valine is described in the present paper. It is based on the asymmetric hydrolysis of the isobutyl ester of DL-valine with an enzyme preparation obtained from pancreas. Complete digestion of the ester results in free L-valine and the isobutyl ester of D-valine.

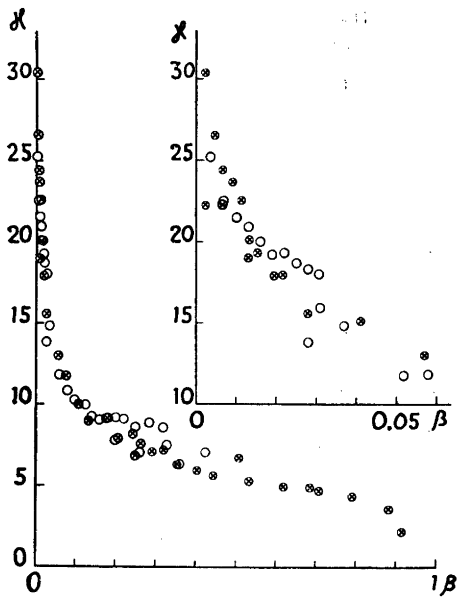


Fig. 2. $\varkappa$ is plotted against β. ⊙ denotes values obtained by titrations. (⊗) denotes values obtained by the batch method.

the batch method. The titration method has proved to be much simpler and faster than the batch equilibrium method.

It may be noted that only about half the β interval has, in this case, been covered by the titration method. By starting from Ag^+ saturated resin and $AgNO_3$ it is possible to cover the whole interval by titrations. However, the amount of H^+ in the solution is, in this case, very uncertain as it is determined as a difference. In this case it would be better to use a H^+ sensitive electrode *e.g.* a glass electrode and change to a Ag/AgI electrode for $\beta \approx 0.5$.

As electrodes sensitive to a number of ions are available, many ion exchange equilibrium studies can be carried out as emf titrations. For instance the $Cu^{2+}-H^+$ exchange can be studied with both a copper amalgam and a glass electrode. The diffusion potentials must be known for the ion pair and the ionic strength in question. They can according to Ekedahl [5-6] be determined rapidly by titrations without resin.

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Preparation of D-Valine by Asymmetric, Enzymatic Hydrolysis of the Isobutyl Ester of DL-Valine

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DL-valine has earlier been resolved by fractional crystallization of the brucine salt of its formyl derivative [1] or of its menthoxyacetyl derivative [2]. Duschinsky and Jeannerat [3] prepared the L-isomer of valine by oxidation of the D-isomer in the racemic form of valine with D-amino acid oxidase from hog kidneys. Because the enzymes in yeast destroy the L-valine and leave the D-form intact, it is possible with this procedure to obtain the latter free from the L-isomer [4]. Price, Gilbert and Greenstein [5] produced the valine isomers by means of asymmetric hydrolysis of chloroacetyl-DL-valine.

A simple method for producing D-valine is described in the present paper. It is based on the asymmetric hydrolysis of the isobutyl ester of DL-valine with an enzyme preparation obtained from pancreas. Complete digestion of the ester results in free L-valine and the isobutyl ester of D-valine.

The latter is isolated by means of its solubility in ether and alcohol. The D-valine is obtained after acid hydrolysis of the ester.

It is not, however, possible to isolate L-valine with this technique. This is because the enzymatic hydrolysis of the isobutyl ester of DL-valine is extremely slow, necessitating large quantities of enzyme. This results in an admixture of other amino acids freed from the enzyme preparation, a fact which makes it difficult to isolate L-valine. Attempts were made to do so with this method, both with the help of the formyl derivative and of the esters after distillation, but it proved impossible to obtain pure L-valine. The reason is presumably to be sought in the difficulty, pointed out by Ehrlich and Wendel [6], in separating isoleucine and valine.

Both the isopropyl ester and the *n*-propyl ester of DL-valine are hydrolyzed asymmetrically with pancreatic enzymes. The isobutyl ester has, however, been found to be more suitable for the preparation of D-valine, since it is more stable in aqueous solution than the *n*-propyl ester and is hydrolyzed more rapidly with the enzyme than the isopropyl ester. Amino acids have earlier been resolved by asymmetric hydrolysis of their esters in order to obtain the isomers of leucine [7], methionine [8,9], phenylalanine [10] and tryptophane [11].

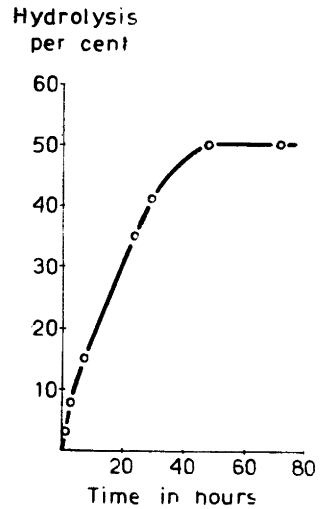


Fig. 1. Asymmetric hydrolysis of the isobutyl ester of DL-*valine with a pancreatic enzyme preparation.*

The experiments were made with 1 ml of the isobutyl ester of DL-valine and 5 ml of a 4 per cent pancreatic enzyme solution. The reaction was carried out at room temperature (22° C). Hydrolysis was followed by formol titration. The values given in the curve were obtained after the hydrolysis which occurs when 1 ml of the isopropyl ester is mixed with 5 ml of distilled water had been substracted from the experimental values. Under these conditions, hydrolysis of the isobutyl ester of DL-valine with water amounted to 4.0 per cent after 24 hours, 7.1 per cent after 48 hours and 9.1 per cent after 72 hours.

Experimental. Isobutyl ester of DL-*valine.* — 500 g of DL-valine are suspended in 2 500 ml of anhydrous isobutyl alcohol containing 10 per cent of anhydrous hydrochloric acid. The mixture is refluxed for three hours, after which a clear solution is obtained. The solution is evaporated *in vacuo* to an oil. This is mixed with 2 500 ml of anhydrous isobutyl ester containing 10 per cent of hydrochloric acid. After evaporation, the treatment with isobutyl alcohol and hydrochloric acid is repeated. The oil thus obtained is then mixed with 1 000 ml of ether and 500 ml of water and the mixture cooled to — 10° C. Concentrated ammonia is added with stirring until the reaction is faintly alkaline to phenolphthalein. The ether layer is separated and the aqueous solution extracted twice more, each time with 1 000 ml of ether. The ether extracts are dried over sodium sulfate. The sodium sulfate is removed and washed with 100 ml of anhydrous ether. The ether extracts are distilled *in vacuo* and the fraction distilling between 73—77° C at 5 mm contains the isobutyl ester of DL-valine; yield 610—650 g (83 to 88 per cent of theory). $C_9H_{19}O_2N$ Calculated, N 8.09%; found, N 8.12%

Enzyme preparation. — The enzymes used in the present investigation were produced as follows. — 180 g of pancreas dry powder according to Willstätter and Waldschmidt-Leitz [12] are shaken for two hours with 1 800 ml of glycerol. The mixture is filtered after the addition of 1 800 ml of water and to the

clear solution two volumes of acetone are added. The precipitate is filtered and the precipitate is washed with 200 ml of acetone and 200 ml of ether. Yield 30—40 g.

The enzyme preparation is dissolved by shaking for 30 minutes in distilled water immediately before use. The rate of hydrolysis is shown in Fig. 1. It is evident from the figure that the D-ester is stable against the pancreatic enzymes for at least 72 hours and that, with the amount of enzymes used, practically all the L-ester is hydrolyzed after 24 hours. The fact that hydrolysis with enzymes is asymmetrical is shown by the method for the preparation of D-valine described in the following. The investigations showed that, under similar experimental conditions, the isobutyl ester of DL-valine is also hydrolyzed to some extent with water.

Preparation of D-*valine.* — 100 g of the isobutyl ester of DL-valine are shaken for 48 hours with 400 ml of a 5 per cent solution of pancreatic enzymes at room temperature. 400 ml of ether and 20 ml of concentrated ammonia are added to the mixture. The ether layer is separated and the aqueous solution extracted twice more, each time with 100 ml of ether. The ether extracts are dried over sodium sulfate and the ether evaporated after the sodium sulfate has been removed. The remaining oil is shaken for 48 hours at room temperature with 100 ml of 5 per cent pancreatic enzyme solution. While stirring 100 ml of ether and 5 ml of concentrated ammonia are added to the digestion mixture. The ether layer is separated and the aqueous solution extracted twice more, each time with 100 ml of ether. The ether extracts are dried over sodium sulfate. The sodium sulfate is removed and the ether is evaporated. The oil of the isobutyl ester of D-valine thus obtained is refluxed for 12 hours with 350 ml of 20 per cent hydrochloric acid. The solution is evaporated *in vacuo* to dryness and for a second time after adding 100 ml of water. The hydrochloride of D-valine thus obtained is dissolved in 200 ml of 96 per cent alcohol. 50 ml of pyridine are added and the mixture is placed overnight in the refrigerator. The precipitate is filtered off and washed with 50 ml of alcohol and 50 ml of ether. The impure D-valine is recrystallized by dissolving in hot water (11 ml per g), decolorized with 0.5 g of norit and the clear solution treated with an equal volume of 95 per cent alcohol. The precipitate is washed with absolute alcohol and ether as described. To obtain an analytically pure sample, the D-valine is again recrystallized and washed as outlined above. The product thus obtained weighs 14—16 g (41 to 47 per cent of theory). Analysis of the D-valine obtained with this method gave the following results:

$C_5H_{11}O_2N$ Calculated, N 11.96; found, N 11.95

$\alpha_D^{23} = -28.2$ to $-28.6°$ ($c = 2$, in 6 N hydrochloric acid).

The figures given in the literature are $-29.0°$ (Fischer [1]), $-27.1°$ (Price, Gilbert and Greenstein [5]) and $-27.6°$ (Ehrlich and Wendel [6]) respectively.

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On the Alleged Complexity of Sodium in Polyhexametaphosphate

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According to Lamm and Malmgren [1] the Na in $K_2Na_4(PO_3)_6$ is bound in complex form. That is, they state that they have dialyzed a solution of the polyhexametaphosphate (PMP) against 0.1 N HCl, and found that only K exchanges with

clear solution two volumes of acetone are added. The precipitate is filtered and the precipitate is washed with 200 ml of acetone and 200 ml of ether. Yield 30—40 g.

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BERNHARD ANDERSEN and KIRSTEN LEHMANN

Laboratory of Zoophysiology, University of Copenhagen, Copenhagen, Denmark

According to Lamm and Malmgren [1] the Na in $K_2Na_4(PO_3)_6$ is bound in complex form. That is, they state that they have dialyzed a solution of the polyhexametaphosphate (PMP) against 0.1 N HCl, and found that only K exchanges with

H. We have not been able to confirm this in the experiments given below.

PMP was prepared according to Tammann [2]: 1 equivalent of KH_2PO_4 was heated for 20 minutes to a glowing red. It made no difference whether it was heated to respectively 650°, 750° or 950° C. The resulting KPO_3 was pulverized and treated with a concentrated NaCl solution which contained 2 equivalents of NaCl. The pulverized material swelled and formed a highly viscous and elastic mass which was dissolved in water and freed from the unreacted remainder of KPO_3 by filtration. The PMP was precipitated twice with alcohol and freed from Cl^- by triturating with 50 % alcohol. The composition of the compound agreed with the formula $K_2Na_4(PO_3)_6$. Na and K were determined in the flame spcetrophotometer and P was determined according to Fiske and Subbarow [4].

The characteristics of PMP described by Tammann have been confirmed by us with the exception of conductance which we have not repeated.

The following tests were made:

1. PMP was dissolved in water and precipitated twice with KCl. Flame spectrometry showed that all the Na was replaced by K.

2. PMP was dissolved in 0.1 *N* HCl and ultrafiltered through cellophane dialyzing tubes [3]. The ultrafiltrate contained Na as well as K (see Table 1).

3. In order to be sure that the presence of Na in the above mentioned ultrafiltrate was not caused by hydrolysis the following experiment was made. A PMP solution was so prepared by the addition of KCl that $C_{Na} \sim C_K$. This solution was ultrafiltered and Na and K were found in the filtrate in equal amounts. The appearance of Na in the filtrate was not due to the fact that the dialyzing tubes were permeable to PMP, as analysis of the filtrate showed that there was absolutely no phosphate present.

Table 1. Flame spectrometry of Na and K in the ultrafiltrate of $K_2Na_4(PO_3)_6$ solution with added HCl and KCl.

Exprt. nr.	KH_2PO_4 heated to	Substance added to PMP solution	Found C_{Na}/C_K in ultrafilt.	C_{Na}/C_K in liq. residue
III	650°	HCl giving $C_H = 0.1$	1.98	2.26
II		KCl giving $C_K \sim C_{Na}$	0.88	0.88
IV			1.15	0.76
V	750°		0.51	0.57
VII			1.09	0.91
VIII	950°		1.06	1.12
IX			0.60	0.96
X			0.70	0.84

According to Lamm and Malmgren it should be expected that (C_{Na}/C_K) filtrate = 0 in all experiments.

4. A known amount of PMP was precipitated with $BaCl_2$. The Ba content of the precipitate was determined as $BaSO_4$, and the content of phosphate was determined colourimetrically. The composition of the precipitate was found to be $Ba(PO_3)_2$. Thus, Ba replaces Na as well as K in the PMP.

The tests performed give no reason for the assumption of complex formation between the Na-ion and the PMP-ion.

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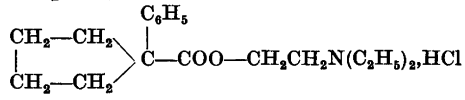
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Antispasmodic Derivatives of Dialkylphenylacetic Acids *

KAI ARNE JENSEN, O. ROSENLUND HANSEN and RUTH HAMMER

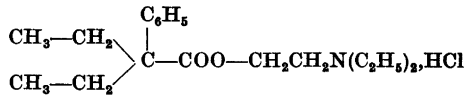
Research Laboratory of A/S Ferrosan, Copenhagen, Denmark

In 1947 Domenjoz *et al.*[1] investigated the spasmolytic activity of diethylaminoethyl 1-phenyl-cyclopentane-1-carboxylate (Parpanit):

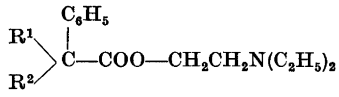


The application of this compound against parkinsonism [2] has made it especially well known.

Although it is known that the dimensions of drug molecules may be of great importance for their pharmacological activity it seemed to us to be doubtful that the cyclopentane ring should be of essential importance, since many similar compounds (Trasentin *etc.*) are known to possess antispasmodic activity. We therefore prepared the closely related compound diethylaminoethyl phenyldiethylacetate:

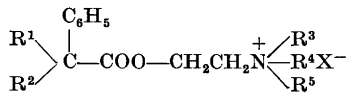


Actually this compound was very active, and other basic esters of dialkylphenylacetic acids of the type:



were similarly very active. The most active of these compounds was the diethylaminoethyl ester of methyl-ethyl-phenylacetic acid, the spasmolytic activity of which was found to be ten times as great as that of Trasentin with the toxicity only half as great. The corresponding quaternary compounds are even more active.

Table 1. Derivatives of dialkylphenylacetic acids:



R^1	R^2	$R^3=R^4$	R^5	X	Formula	M.p. °C	% N calc.	% N found	spasmolyt. act. (Trasentin = 1)
C_2H_5	C_2H_5	CH_3	H	Cl	$C_{16}H_{26}O_2NCl$	126	4.68	4.93 *	1.5
C_2H_5	C_2H_5	C_2H_5	H	Cl	$C_{18}H_{30}O_2NCl$	142	4.28	4.38	2.5
C_2H_5	CH_3	C_2H_5	H	Cl	$C_{17}H_{28}O_2NCl$	127	4.47	4.42	10.0
CH_3	CH_3	C_2H_5	H	Cl	$C_{16}H_{26}O_2NCl$	128	4.68	4.55	1.5
C_2H_5	C_2H_5	C_2H_5	CH_3	I	$C_{19}H_{32}O_2NI$	88	3.23	3.36	10.0
CH_3	C_2H_5	C_2H_5	CH_3	Br	$C_{18}H_{30}O_2NBr$	127	3.77	3.74	15
CH_3	C_2H_5	C_2H_5	C_2H_5	Br	$C_{19}H_{32}O_2NBr$	128	3.63	3.73	16
CH_3	C_2H_5	C_2H_5	CH_3	I	$C_{18}H_{30}O_2NI$	122	3.34	3.24	20
CH_3	CH_3	C_2H_5	C_2H_5	$CH_3C_6H_4SO_3$	$C_{25}H_{37}O_5NS$	66	3.02	3.05	—

* calc. C 64.20 H 8.68
found » 64.34 » 8.64

* Dan. Pat. 72436 and 73667 (1951).

The dialkylphenylacetic acids [3] were converted to the dialkylaminoethyl esters by reaction of their sodium or silver salts with β-dialkylaminoethyl chlorides, or alternatively by reaction of the acid chlorides with the dialkylaminoethanols in pyridine solution, the first method being the most convenient. The hydrochlorides were precipitated by passing dry hydrogen chloride thorugh etheral solutions of the esters. The quaternary compounds were prepared by addition of the alkyl bromides or iodides to ethanolic solutions of the basic esters; after standing of the solutions at 0° for some hours the quaternary salts were precipitated in almost quantitative yields by addition of ether and were recrystallized from acetone or acetone-ether. A toluenesulfonate was prepared by heating a solution of β-diethylamino ethyl dimethylphenylacetate (3 g) and ethyl toluenesulfonate (2.5 g) in benzene (25 ml) for 4 hours at 100°.

The compounds prepared are listed in the accompanying Table 1. The method applied will be illustrated by the following typical example:

Methyldiethyl-(methylethylphenylacetoxy-ethyl)-ammoniumbromide. Methylethyl-phenylacetic acid (56 g) was dissolved in ethanol and neutralized with a concentrated solution of sodium hydroxide. Ethanol and water were removed under reduced pressure and toluene was added to the residue and partly distilled off to remove last traces of water. Then a solution of 56 g of β-chloroethyldiethylamine (30 % excess) in 150 ml of toluene was added and the mixture boiled for 4—5 hours. The toluene was removed under reduced pressure and the residue distilled in vacuo. Yield 81 g (93 %) with b.p. 130—35° at 0.5 mm.

A solution of the ester (75 g) in ethanol (50 ml) was cooled at 0°, 75 ml of methyl bromide was added and the solution left for 24 hours in ice box. The bromide was precipitated by addition of 50 ml of ether, filtered and washed with ether. M. p. 127° after recrystallization from acetone. Yield 85 g (86 %).

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Paper Electrophoresis of Enzymes

Amylase and Trypsin

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A large number of biologically active proteins have been studied by the classical Tiselius electrophoresis technique. Unfortunately this method only gives information on the individual protein components regardless of their biological activity unless isolation of the fractions is carried out, which is difficult in preparations which are not quite simple.

Wieland and Fischer [1] developed an electrophoretic method using filter paper, which has recently been used for the fractionation of the serum proteins by several workers (Durrum [2], Turba and Enenkel [3], Cremer and Tiselius [4]). Paper electrophoresis offers better facilities for the fractionation of biologically active proteins than does the classical procedure, because the activity-mobility can be determined directly by a method specific for the substance of interest without showing simultaneously the inactive fractions.

The present authors have studied the practical application of the paper electrophoresis technique on amylase and trypsin, and it is the intention to extend the investigation to cover other enzymes and active proteins.

METHODS

Electrophoresis. An attempt was made to use the simplest possible electrophoresis system as such a system was likely to be the quickest and cheapest. Thus no refrigeration was used (*cf.* Cremer and Tiselius [4]) because experience showed that it was not indispensable.

The paper used was Swedish Munktell 20 filter paper cut into strips 32 cm × 4.5 cm, and the buffer was barbital, with a pH of 8.6 and an ionic strength of 0.05. At the concentrations used, this buffer has no effect on the enzyme activity. Electrophoresis was at first attempted with the paper pressed between two glass plates by a simple method which has been routinely used in this laboratory for serum proteins and the like. However it was found that the enzyme left behind a long tail towards the starting point, presumably due to adsorption on the glass and this prevented accurate measurements. Subse-

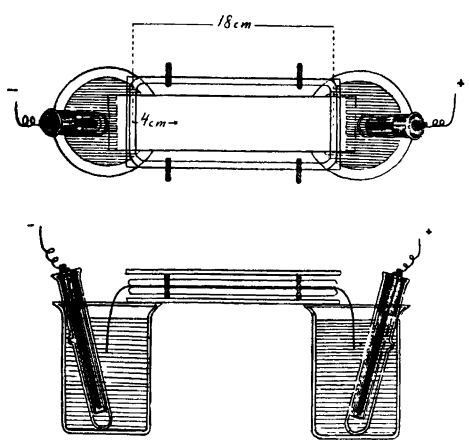


Fig. 1. The principle of the apparatus. Cross and plan sections.

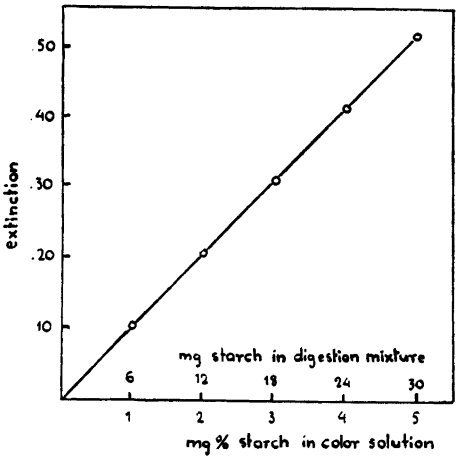


Fig. 2. Standard curve for the determination of amylase.

quently the open pressing device, illustrated in Fig. 1, consisting of a glass lattice closed with glass plates was employed. This arrangement held up to 4 strips at once. After the paper in the lattice had become wet with buffer absorbed from the electrode bowls, 0.02 ml of enzyme solution was pipetted to a point 4 cm from the cathode pressing point. The time of electrophoresis ranged from 3 to 5 hours using a voltage of 250—300 V and a current of 2—4 mA per strip.

Demonstration of enzyme activity in the paper. 1. Amylase. Amylase was determined by a slight modification of the Smith and Roe [5] method based on the photometric measurement of the blue colour of the iodine-starch reaction.

When electrophoresis was completed, the part of paper lying between the points of pressure (18 cm in length) was cut, while wet, into pieces 1 cm wide. These were placed in test tubes each containing 1 ml of 0.05 per cent starch solution and 5 ml of phosphate buffer- NaCl solution (1/50 *M* phosphate buffer of pH 7.2 to which NaCl was added to make it 1/20 *M*) at 37° C. The tubes were shaken to detach the enzyme from the paper and placed in a thermostat at 37° C. One hour later 5 ml aliquots were pipetted into a second series of tubes each containing 1 ml of 1 *N* HCl solution to arrest the enzyme activity. Finally, 1 ml of iodine potassium iodide solution (from a stock solution containing 30 g of potassium iodide and 3 g of iodine in 1 000 ml of distilled water, diluted 1 : 50) was added and the absorption at 620 mμ was measured immediately with a Beckman spectrophotometer. This method was checked by a standard series made from starch solutions of varying concentrations (Fig. 2). It should be borne in mind that the concentrations of both the substrate solution and the iodine solution must be appropriate for the activity of the enzyme solution to be studied.

2. Trypsin. The proteolytic power of this enzyme was determined by Krijgsman's micro method [6], in which casein, the substrate, is precipitated by quinidine, and the resulting turbidity is measured.

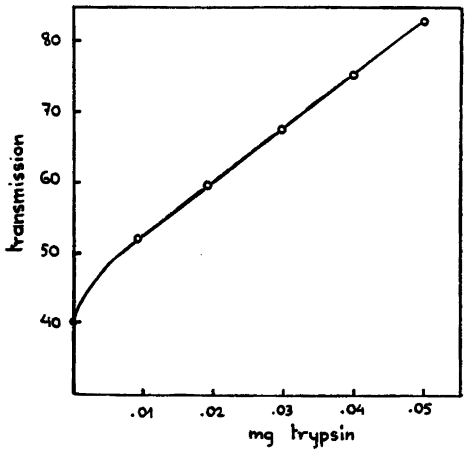


Fig. 3. Standard curve for the determination of trypsin.

In the manner described above, the electrophoresis paper was cut into pieces 1 cm wide, which were placed in test tubes containing 2 ml of 0.125 per cent casein solution (prepared by dissolving 2.5 g of casein in 12 ml of 1 *N* NaOH and adding distilled water to make 2 000 ml), 2.5 ml of M/15 phosphate buffer of pH 8.04, 0.5 cc of physiological saline, and 1 ml of distilled water. The tubes were kept at 37° C for three hours, then 2 ml aliquots were transferred into clean tubes, and 2 ml of *M*/15 phosphate buffer of pH 7.73, 2 ml of distilled water and 2 ml of quinidine precipitant (hot water saturated with quinidine hydrochloride rendered a little acid to litmus with hydrochloric acid) were added. The resulting turbidity was measured after 30 minutes using a stufen-photometer with a S 61 filter. Fig. 3 shows the relation between the turbidity and the enzyme content, and demonstrates that a stufen-photometer can be used for these measurements in place of a nephelometer.

RESULTS AND DISCUSSION

Amylase. The study was carried out on Merck's non-crystalline pancreatic amylase. Tiselius electrophoresis showed that this was a heterogeneous protein mixture with at least three components (Fig. 4). After paper electrophoresis, however, only one of the fractions (the middle one) was found to have amylase activity. On filter paper its mobility was found to be 2.9×10^{-5} $cm^2 \cdot sec^{-1} \cdot volt^{-1}$ (small fluctuations from this value were obtained in dif-



Fig. 4. Tiselius-electrophoresis at pH 8.6 of amylase preparation used. Descending limb.

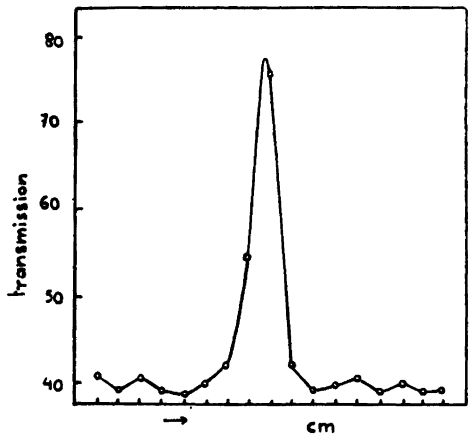


Fig. 5. Electrophoresis of amylase activity on filter paper. pH 8.6, ionic strength 0.05.

ferent experiments) (Fig. 5), a value approximately the same as that obtained by Meyer, Fischer, and Bernfeld[7], who prepared a crystalline amylase from the pancreas of hog, which on Tiselius electrophoresis proved to be uniform, with a mobility of 3.1×10^{-5} cm^2 · sec^{-1} · volt^{-1} at pH 7.9 (ionic strength 0.10) and isoelectric point at pH 5.2—5.6. Bernfeld, Duckert, and Fischer[8] isolated an amylase from human pancreas which was also electrophoretically uniform, with a mobility of 3.75×10^{-5} cm^2 · sec^{-1} · volt^{-1} at pH 10.14.

Trypsin. The preparation used was Merck's non-crystalline pancreatic trypsin. Tiselius electrophoresis revealed two components (Fig. 6). The diagram of activity-mobility obtained by paper electrophoresis was obviously identical with that yielded by Tiselius electrophoresis, and showed that both fractions posessed some proteolytic activity (Fig. 7). The larger (and apparently also most active) fraction was almost iso-electric at pH 8.6, while the smaller had a mobility of 7.0×10^{-5} cm^2 · sec^{-1} · volt^{-1} at this pH.

It is interesting to compare these results with earlier experience on the electrophoresis of proteolytic pancreatic factors. Anderson and Alberty[9] found that the iso-electric points of chymotrypsin were 8.6 and 8.1 at ionic strengths of 0.01 and 0.1 respectively. Approximately the same result was obtained a little later by Kubacki, Brown and Laskowski[10], who found that

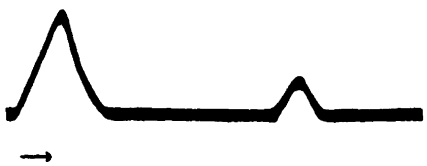


Fig. 6. Tiselius-electrophoresis at pH 8.6 of trypsin preparation used. Descending limb.

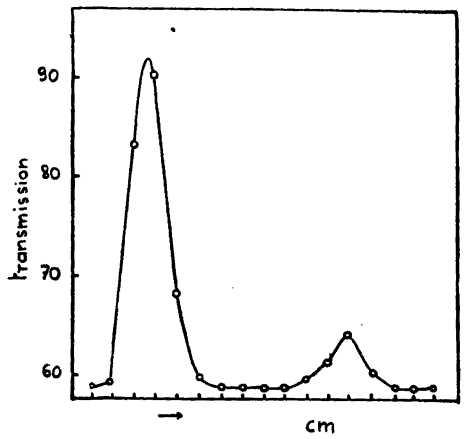


Fig. 7. Electrophoresis of tryptic activity on filter paper. pH 8.6, ionic strength 0.05.

the iso-electric point of chymotrypsin *a* prepared according to Kunitz and Northrop, was 8.3. Brown, Shupe and Laskowski [11] isolated an active protein from bovine pancreas and called it chymotrypsin B because it clearly differed from chymotrypsin *a* in physico-chemical properties. Electrophoretically, the iso-electric point of chymotrypsin B has been determined to be 4.7 (Kubacki, Brown and Laskowski [10]). It is possible that in the preparation examined by the present authors the larger fraction was chymotrypsin *a* and the smaller one chymotrypsin B.

SUMMARY

A technique for the study of enzymes by paper electrophoresis has been described and applied to amylase and trypsin. The amylase activity was found to be electrophoretically uniform, with a mobility of 2.9×10^{-5} $cm^2 \cdot sec^{-1} \cdot volt^{-1}$ at pH 8.6 and ionic strength 0.05. On the other hand, the trypsin activity was, under the same conditions, split up into two components, the larger with a mobility of 0 and the smaller with a mobility of 7.0×10^{-5} $cm^2 \cdot sec^{-1} \cdot volt^{-1}$.

After this investigation was completed, the writers learnt that Wallenfels and Pechmann [12] had used paper electrophoresis for the differentiation of enzymes (amylase, proteinase, lipase, phosphatase) of mould extract. They demonstrated the position of the proteinase using undeveloped cinematographic film as substrate, and the amylase by starch-iodine staining. At 120 V, the proteinase travelled, in 12 hours, at pH 6.8 about 23 mm towards the cathode, and the amylase about 10 mm towards the anode. It seems that the results obtained by the present authors are roughly the same as far as mobility is concerned.

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The Solubility of Hydrogen, Oxygen, and Carbon Monoxide in Some Non-Polar Solvents

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In a previous paper [1] dealing with the solubility of nitrogen in a number of non-polar liquids it was shown that the solubility can be calculated with satisfactory accuracy from the equation:

$$-\log x_2 = -\log x_2^i + \log (\overline{V}_2/V_1) + 0.434\ (1 - \overline{V}_2/V)_1 + \overline{V}_2\ (\delta_1 - \delta_2)^2/4.575\ T \qquad \text{(I)}$$

In this equation x_2 denotes the solubility in mole fraction, x_2^i the "ideal" solubility (see later), $\overline{V}_2$ the partial molal volume of nitrogen in the saturated solution, V_1 the molal volume of the solvent, δ_1 the "solubility parameter" of the solvent defined as the square root of the energy of vapourization per ml ($\delta_1 = (\Delta E_1/V_1)^{1/2}$), δ_2 the "solubility parameter" of the gas (see later) and T the absolute temperature. With regard to the derivation of equation (I) reference should be made to Hildebrand and Scott [2].

When the values $x_2^i = 16 \times 10^{-4}$, $\overline{V}_2 = 53$ ml, and $\delta_2 = 5.2$ are substituted, equation (I) will express the solubility of nitrogen in all the non-polar solvents examined from perfluoro-n-heptane, n-C_7F_{16}, which has a particularly low solubility parameter ($\delta_1 = 5.85$) to carbon disulphide which has a particularly high solubility parameter ($\delta_1 = 10.0$).

It is the aim of the present work to ascertain whether equation (I) with the same accuracy can express the solubility of other gases with low solubility.

The solubility of oxygen was examined because the derived constants together with those previously found for nitrogen makes it possible to calculate the solubility of atmospheric air in non-polar liquids. Hydrogen was chosen as an example of a gas of particularly low solubility. Carbon monoxide was chosen because the author has previously [3,4] determined the solubility of this gas in a considerable number of alcohols.

APPARATUS, PROCEDURE, AND MATERIALS

The apparatus used for the solubility determinations was as that described by Lannung [5] in whose paper an illustration and a description in greater detail may be found. The volume of the gas burette was about 8 ml and that of the flask about 90 ml. The volume of the gas was read with an accuracy of ± 0.005 ml, and the pressure of the gas with an accuracy of ± 0.2 mm Hg. The volume of the solution was in all cases determined with an accuracy exceeding 0.1 per cent. The temperature in the air thermostat was constant within 0.03° C in the case of experiments made at temperatures about 25° C and at temperatures about 50° C it was constant within 0.1° C.

The procedure was as described in the paper by Lannung quoted above. The errors involved in the solubility determinations were in most cases within 1 per cent.

Hydrogen from a cylinder was freed from oxygen by being passed through several glass-spiral washing bottles containing alkaline solution of pyrogallol and was dried by being passed through concentrated sulphuric acid. Following this purification the hydrogen proved by combustion analysis in a gas analysis apparatus as devised by Christiansen [6] to contain 99.5 per cent hydrogen and 0.5 per cent nitrogen.

Oxygen was prepared by heating potassium permanganate placed in a refractory test tube the mouth of which has been drawn out to form a gas siphon. By absorption analysis in alkaline dithionite the composition was found to be: 99.7 per cent oxygen and 0.3 per cent nitrogen.

The carbon monoxide employed was prepared according to a method described in a previous paper [7] and contained 99.5 per cent carbon monoxide and 0.5 per cent nitrogen.

For the solvents used, which were all purified by fractionation in a wire-gauze column, the following physical constants were determined:

n-Heptane (pure grade) from Phillips Pet. Co., boiling point 98.3 – 98.4° C (760 mm) and n_D (25° C) 1.3855.

Benzene ("analytical reagent", Merck), boiling point 80.28° C (760 mm) and melting point 5.43° C.

Carbon disulphide ("analytical reagent", Merck), boiling point 46.21 – 46.26° C (760 mm).

The perfluoro-*n*-heptane used was of the same origin and purity as the substance described in a previous paper [1].

It is necessary to know the vapour pressure of the solvent in order to be able to calculate the solubility of the gas at a partial pressure of 1 at. The vapour pressures at 25° C employed are summarized in Table 1. For the conversion from Bunsen's absorption coefficient (α) to mole fraction the specific gravities of the solvents, also included in Table 1, have been used.

Table 1. Vapour pressures and densities of the solvents at 25° C.

	n-C_7F_{16} [1]	n-C_7H_{16} [8]	C_6H_6 [8]	CS_2 [9]
p mm Hg	80.0	45.7	94.2	357
d 25/4	1.7208	0.6795	0.8737	1.255

EXPERIMENTAL RESULTS

The results of the solubility determinations have been reproduced in Table 2, expressed as Bunsen's absorption coefficient (α), *i.e.* the number of ml of the gas reduced to 0° C and 1 at. which can be dissolved in 1 ml solvent at the temperature concerned when the partial pressure of the gas is 1 at.

Table 2. Solubility of hydrogen, oxygen and carbon monoxide. Experimental values expressed in Bunsen's absorption coefficient (α) at t° C.

Solvent	Hydrogen		Oxygen		Carbon monoxide	
	t	α	t	α	t	α
n-C_7F_{16}	24.80	0.140	25.00	0.548	24.91	0.385
	48.30	0.166	24.96	0.544	24.90	0.385
	24.73	0.141			25.00	0.380
	48.75	0.166				
n-C_7H_{16}					24.88	0.263
					24.93	0.262
C_6H_6					25.03	0.1692
					24.95	0.1691
					24.98	0.1695
CS_2	24.82	0.0606	25.00	0.162	24.90	0.133
	24.86	0.0607	24.82	0.164	24.90	0.132

Table 3. Solubility of carbon monoxide and hydrogen expressed in Bunsen's absorption coefficient at 25° C. Comparison with older measurements.

	Just [10]	Skirrow [11]	Horiuti [12]	Present investigation
CO in C_6H_6	0.156	0.160	0.167	0.169
CO in CS_2	0.0761	0.0879; 0.0761		0.133
H_2 in CS_2	0.0343			0.0607

It appears from Table 3, which includes the solubilities found by other investigators, that for the solubilities of carbon monoxide and hydrogen the

values found are higher than those stated by Just and Skirrow, while the solubility of carbon monoxide in benzene shows good agreement with the result obtained by Horiuti. In a previous paper [1] on the solubility of nitrogen the results also showed good agreement with those of Horiuti's while there were considerable discrepancies between the author's figures and those found by Just.

CORRELATION WITH THEORY

1. *Calculations of the solubility of oxygen and carbon monoxide.* According to Dolezalek [13] and Hildebrand [2] the "ideal solubility" of a gas can be determined on the following basis. Assume the liquefied gas to have a vapour pressure p_2° at. at t° C. As the solubility in an ideal solvent at t° C is equivalent to the concentration to which the liquefied gas must be diluted — under ideal conditions — in order to obtain a partial pressure of the gas equal to 1 at., the ideal solubility expressed in mole fraction x_2^i can be calculated by means of Raoult's law from the equation:

$$x_2^i = 1/p_2^\circ \qquad \text{(II)}$$

For gases which at a temperature of t° C is above their critical temperature a fictitious value of p_2° is obtained by extrapolation of the measured vapour pressures (p_2) of the gas to the temperature t° C. The extrapolation is performed by plotting $\log p_2$ against $1/T$.

It was, however, found through the more detailed investigation of the solubility of nitrogen in non-polar liquids that the value ($p_2^\circ = 1000$ at.) found by extrapolation resulted in such a value of x_2^i (10^{-3}) that the solubilities in the fluorocarbons could not be represented by equation (I). When using, however, a value for x_2^i calculated from:

$$-\log x_2^i = \frac{\Delta H}{4.575}\left(\frac{1}{T_b} - \frac{1}{T}\right) \qquad \text{(III)}$$

where ΔH is the heat of vapourization (1333 cal. per mole, Giauque and Clayton [14]) determined experimentally at the boiling point of nitrogen ($T_b = 77.3$°K), we have, at $T = 298.1$° C, $x_2^i = 16 \times 10^{-4}$. With this value of x_2^i the solubilities could be expressed by equation (I). The same procedure has been used in this paper for the calculation of x_2^i for oxygen and carbon monoxide.

For oxygen x_2^i is found from equation (III) to be equal to 17.6×10^{-4}, ΔH being equal to 1630 cal/mole at $T_b = 90.2$° K according to Clusius and Konnertz [15]. For carbon monoxide x_2^i is found to be equal to 15.5×10^{-4} when substituting $\Delta H = 1444$ cal/mole at $T_b = 81.6$° K according to Clayton and Giauque [16].

In the following a calculation of δ_2 for oxygen and carbon monoxide is performed by means of equation (I). The values of the partial molal volumes of the gases are calculated on the basis of Horiuti's experiments [12] on the expansion displayed by the solvent when a gas is dissolved. The values are only to a slight extent dependent on the solvent, and the value chosen for $\overline{V}_2$ is the average of the values for $\overline{V}_2$ in benzene and carbon tetrachloride. The calculation gives for oxygen $\overline{V}_2 = 46$ ml and for carbon monoxide $\overline{V}_2 = 52$ ml. The values for the solubility parameters of the solvents, δ_1, have been taken from Hildebrand and Scott [2, Appendix 1]. For normal heptane the value 8.1 has been used, *cf.* Hildebrand [17]. The values used will appear from Table 4.

Table 4. Solubility of oxygen and carbon monoxide in mole fraction (x_2) at 25° C. Comparison of experimental and calculated values.

oxygen: $x_2^i = 17.6 \cdot 10^{-4}$; $\overline{V}_2 = 46$ ml; $\delta_2 = 5.7$

carbon monoxide: $x_2^i = 15.5 \cdot 10^{-4}$; $\overline{V}_2 = 52$ ml; $\delta_2 = 5.8$

Solvent	V_1	δ_1	Oxygen				Carbon monoxide			
			$x_2 \cdot 10^4$ exp.	$-\log x_2$ exp.	$-\log x_2$ calc.	Δ	$x_2 \cdot 10^4$ exp.	$-\log x_2$ exp.	$-\log x_2$ calc.	Δ
n-C_7F_{16}	227	5.85	55.3	2.26	2.41	+0.15	38.8	2.41	2.50	+0.09
n-C_6H_{14} a)	132	7.3	19.3	2.71	2.67	−0.04				
n-C_7H_{16}	147	8.1	19.7 a)	2.71	2.74	+0.03	17.3	2.76	2.84	+0.08
c-C_6H_{12} a)	109	8.2	12.3	2.91	2.84	−0.07				
CCl_4 b)	97.1	8.6	12.1	2.92	2.94	+0.02	8.66	3.06	3.04	−0.02
C_6H_6	89.3	9.15	8.16 b)	3.09	3.08	−0.01	6.74	3.17	3.18	+0.01
1,4-dioxane a)	86.1	10.0	5.38	3.27	3.31	−0.04				
CS_2	60.6	10.0	4.42	3.36	3.37	+0.01	3.60	3.44	3.48	+0.04
CS_2 c)							2.06	3.69	3.48	−0.21
$C_6H_5CH_3$ d)	107	8.9					7.98	3.10	3.08	−0.02
$(C_2H_5)_2O$ b)	105	7.4	19.8	2.70	2.74	+0.04	16.9	2.77	2.82	+0.05
$CHCl_3$ d)	80.7	9.3					6.86	3.16	3.24	+0.08
C_6H_5Cl b)	102	9.5	8.62	3.06	3.13	+0.07	6.28	3.20	3.25	+0.05
$C_6H_5NH_2$ d)	91.5	11.2 e)					1.99	3.70	3.86	+0.16
C_5H_5N a)	80.8	10.7	4.58	3.34	3.54	+0.20				
$(CH_3)_2CO$ b)	74.0	9.3 e)	8.46	3.07	3.15	+0.08	7.80	3.11	3.25	+0.14
$C_6H_5NO_2$ d)	103	11.0 e)					3.90	3.41	3.76	+0.35

a) Guerry [18], b) Horiuti [12], c) Just [10], d) Skirrow [11], e) calculated according to the "Hildebrand rule" [2, p. 427].

Calculations have subsequently been performed for oxygen and carbon monoxide by means of equation (I) to find the values of δ_2 which show the closest agreement with the experimental values of the solubilities in the non-polar solvents. For oxygen δ_2 was found to be 5.7 and for carbon monoxide δ_2 was found to be 5.8. The extent to which the constants thus calculated agree with the experimentally determined solubilities will appear from the upper half of Table 4.

It will be seen that the values of the solubilities in perfluoro-*n*-heptane found by calculation are somewhat lower than those determined experimentally — a difference which was also found to exist in the case of nitrogen. It will further be seen that the value found in the present investigation for the solubility of carbon monoxide in carbon disulphide agrees well with the theory.

In the lower half of Table 4 the solubilities of oxygen and carbon monoxide in a number of polar solvents have been summarized. The experimental values have been taken from Skirrow [11] and Horiuti [12]. The calculation has been performed by means of equation (I) which does not allow for polar attraction. It will be seen that in toluene, which among the polar solvents applied is the one with the lowest dielectric constant ($\varepsilon = 2.4$), the solubility can be calculated with the same accuracy as in the non-polar solvents. With regard to the other polar solvents it applies that the experimentally determined solubility is larger than the solubility calculated on the basis of equation (I). In the slightly polar solvents, ether, chloroform and chlorobenzene ($\varepsilon = 4.3$, 5.1, and 5.9, respectively) the difference between the experimental and calculated values for the solubility is small. The difference is somewhat larger in the more polar liquids as aniline, pyridine and acetone ($\varepsilon = 7$, 12, and 21, respectively) and greatest in nitrobenzene which is the most polar solvent among those used ($\varepsilon = 36$). This phenomenon may be explained by assuming the molecules of the solvents to induce moments in the molecules of the solute gas, the consequent increased attraction will result in a higher solubility.

Skirrow [11] has made a series of determinations of the solubility of carbon monoxide in mixtures of benzene and naphtalene and has found that the solubility of carbon monoxide decreases with increasing naphtalene concentration. It will be shown in the following that this decrease in the solubility is in conformity with the theory. For mixtures of benzene and naphtalene molal volumes (V_1) and solubility parameters (δ_1) have been calculated according to the equations [2, p. 201]

$$V_1 = \Phi_n V_n + \Phi_b V_b$$
$$\delta_1 = \Phi_n \delta_n + \Phi_b \delta_b$$

where Φ denotes volume fractions and the subscripts n and b naphtalene and benzene, respectively. For naphtalene the values $V_n = 123$ and $\delta_n = 9.9$ have been used. Table 5 gives the calculated values of V_1 and δ_1 together with the values for the solubility of carbon monoxide calculated on the basis of equation (I). It will be seen that this equation with good approximation represents the variation in the experimental values which, however, must all be assumed to be 3—6 per cent too low, *cf.* Table 3. Skirrow found the solubility of carbon monoxide in pure benzene to be 13 per cent higher than in benzene containing 23.72 mole per cent naphtalene, while when applying equation (I) we find that the solubility should be 10 per cent higher.

Table 5. Calculation of the solubility of carbon monoxide in solutions of naphtalene in benzene at 25° C. Experimental values from Skirrow [11] *expressed in Ostwald's absorption coefficient (λ).*

Mole % naphtalene	V_1	δ_1	λ	$x_2 \cdot 10^4$	
				exp.	calc.
0	89.3	9.15	0.174	6.37	6.55
7.35	92.4	9.22	0.164	6.15	6.35
16.14	96.1	9.31	0.149	5.76	6.14
23.72	99.2	9.38	0.141	5.61	5.94

In two previous papers [3,4] the author has determined the solubility of carbon monoxide in a number of monohydric alcohols. As equation (I) is derived on the condition that the molecules are non-polar and not associated, this equation cannot be used straight away for the calculation of the solubilities of carbon monoxide in alcohols. However, on the basis of the solubility experiments and by means of equation (I) it is possible empirically to determine a solubility parameter for each of the alcohols. The result of such a calculation is given in Table 6, column 5. From the heat of vapourization at the boiling point of these alcohols (column 3) the heat of vapourization at 25° C has been calculated, and from this value $\delta_1 = (\Delta E/V)^{\frac{1}{2}}$ has further been calculated (column 6). For the calculation of the heat of vapourization at 25° C the specific heat difference C_p (liquid) — C_p (vapour) = 0.142 cal/g has been used for all the alcohols. It will be seen from the two last columns of the table that the difference between δ_1, as calculated from the solubility experiments, and δ_1, as calculated from ΔH (25° C) is greatest in the case of the alcohols with the highest dielectric constants. It should be noticed that all the alcohols have nearly the same dipole moment (about 1.8 Debye).

Table 6. Calculation of the solubility parameter (δ_1) for some alcohols.

alcohol	α at 25° C	ΔH at B.p.	V_1 at 25° C	δ_1 at 25° C calculated from the solubility measurement	δ_1 at 25° C calculated from ΔH	$\Delta\delta$	ε
CH_3OH	0.207	8413	40.6	9.85	14.05	4.20	33
C_2H_5OH	0.186	9410	58.7	9.45	12.50	3.05	26
n-C_3H_7OH	0.164	9876	75.2	9.30	11.50	2.20	22
iso-C_3H_7OH	0.177	9574	77.0	9.15	11.10	1.95	26
n-C_4H_9OH	0.153	10460	91.9	9.25	10.85	1.60	18
iso-C_4H_9OH	0.161	10230	92.8	9.15	10.65	1.50	19
sec.C_4H_9OH	0.154	9959	92.2	9.20	10.45	1.25	16
tert.C_4H_9OH	0.175	9667	95.0	9.00	10.10	1.10	11

α = Bunsen's absorption coefficient for carbon monoxide in the alcohols,
ΔH = heat of vapourization, V_1 = molal volume and ε = dielectric constantal.

The solubility parameters for the alcohols given in Table 6, column 5 may in connection with equation (I) be used for the calculation of the solubility of other gases of low solubility in the alcohols concerned. The result of such a calculation has been given in Table 7.

Table 7. Solubility of some gases in methyl and ethyl alcohol at 25° C. Comparison of experimental and calculated values.

Solvent	Hydrogen $-\log x_2$			Nitrogen $-\log x_2$			Oxygen $-\log x_2$		
	exp.	calc.	Δ	exp.	calc.	Δ	exp.	calc.	Δ
CH_3OH	3.80 a)	3.87	+0.07	3.63 a)	3.61	−0.02	3.40 c)	3.33	−0.07
				3.56 c)	3.61	+0.05			
C_2H_5OH	3.69 b)	3.73	+0.04	3.46 a)	3.50	+0.04	3.25 d)	3.12	−0.13

a) Just [10], b) Maxted a. Moon [19], c) Levi [20], d) Timofejew [21].

2. *Solubility calculations for hydrogen.* Gonikberg [22] has on the basis of experiments by Just and Horiuti shown that the solubility of hydrogen in

ether, *m*-xylene, carbon tetrachloride, toluene and benzene can be represented with satisfactory accuracy by the equation,

$$-\log x_2 = -\log x_2^i + \overline{V}_2 (\delta_1 - \delta_2)^2/4.575\ T \qquad \text{(IV)}$$

This equation is obtained from (I) by omitting the correction for the entropy of mixing. For his calculations (20°C) Gonikberg used $x_2^i = 24.6 \times 10^{-4}$ and $\overline{V}_2 = 28.6$ ml (the molal volume of hydrogen at the boiling point 20.4° K). Only in carbon disulphide did the solubility of hydrogen display any considerable deviation from equation (IV) for which reason Gonikberg considered this experiment to have been subject to errors. A redetermination of the solubility of hydrogen actually gave a result which was about 75 per cent higher than that found by Just (Table 3), and this new value shows, as will appear from Table 8, better agreement with the theory.

Table 8. Solubility of hydrogen in mole fraction (x_2) at 25° C. Comparison of experimental and calculated values.

$x_2^i = 5.5 \cdot 10^{-4}$; $\overline{V}_2 = 37$ ml; $\delta_2 = 5.1$

Solvent		V_1	δ_1	$x_2 \cdot 10^4$ exp.	$-\log x_2$ exp.	$-\log x_2$ calc.	Δ
n-C_7F_{16}		227	5.85	14.2	2.85	2.85	0.00
n-C_6H_{14}	a)	130	7.30	6.51	3.19	3.16	− 0.03
n-C_7H_{16}	a)	147	8.10	6.63	3.18	3.23	+ 0.05
c-C_6H_{12}	a)	109	8.20	3.80	3.42	3.34	− 0.08
CCl_4	b)	97.1	8.60	3.26	3.49	3.44	− 0.05
C_6H_6	b)	89.3	9.15	2.62	3.58	3.58	0.00
1,4-dioxane	a)	86.1	10.0	1.93	3.71	3.79	+ 0.08
CS_2		60.6	10.0	1.64	3.79	3.86	+ 0.07
CS_2	c)			0.93	4.03	3.86	− 0.17
m-$C_6H_4(CH_3)_2$	c)	123	8.8	4.12	3.39	3.41	− 0.02
$C_6H_5CH_3$	c)	107	8.9	3.82	3.42	3.47	− 0.05
$(C_2H_5)_2O$	b)	105	7.45	6.25	3.20	3.24	+ 0.04
$CHCl_3$	d)	80.7	9.3	2.20	3.66	3.63	− 0.03
C_6H_5Cl	b)	102	9.5	2.57	3.50	3.62	+ 0.12
$C_6H_5NH_2$	c)	91.5	11.2	1.065	3.97	4.13	+ 0.16
$(CH_3)_2CO$	b)	74.0	9.3	3.02	3.52	3.65	+ 0.13
$C_6H_5NO_2$	c)	103	11.0	1.56	3.81	4.04	+ 0.23

a) Guerry [18], b) Horiuti [12]; the experimental value for the solubility in benzene is with variations which are within 2 per cent in agreement with a value reported by Maxted and Moon [19]. c) Just [10], d) Maxted and Moon [19].

It should be emphasized that the calculation of the ideal solubility of hydrogen is rather inaccurate, the reason being the low boiling point of hydrogen. Hildebrand [2, p. 243] has on the basis of determinations of the vapour pressure of liquid hydrogen (values from *International Critical Tables*) by extrapolation calculated x_2^i to be equal to 8×10^{-4}. When using vapour pressure measurements by Grilly [23], extrapolation provides the value $x_2^i = 25 \times 10^{-4}$, and if x_2^i is calculated on the basis of the heat of vapourization, 219 cal/mole [24] at the boiling point 20.4° K, the result obtained is 65×10^{-4}. As these values for the ideal solubility show such great discrepancies the procedure adopted for hydrogen has been to chose the set of values of x_2^i and δ_2 which on the basis of equation (I) shows the closest agreement with the experimental results. For hydrogen the value $\overline{V}_2 = 37$ ml was calculated on the basis of experiments made by Horiuti [12]. Hence the values $x_2^i = 5.5 \times 10^{-4}$ and $\delta_2 = 5.1$. The accuracy with which the constants determined in this way represent the experiments will appear from the upper half of Table 8. The lower half of Table 8 summarizes a calculation of the solubility of hydrogen in polar liquids. It will be seen that the comments made on the solubility of oxygen and carbon monoxide in polar liquids will also apply to hydrogen.

SUMMARY

1. Determinations of the solubility of hydrogen, oxygen, and carbon monoxide in a number of non-polar liquids have been made. Expressed as Bunsen's absorption coefficient the values found were: *Hydrogen* in perfluoro-*n*-heptane 0.0141 (25° C), and 0.0167 (50° C), in carbon disulphide 0.0607 (25° C); *oxygen* (25° C) in perfluoro-n-heptane 0.546 and in carbon disulphide 0.163; *carbon monoxide* (25° C) in perfluoro-*n*-heptane 0.383, in *n*-heptane 0.263, in benzene 0.169 and in carbon disulphide 0.133.

2. The above data combined with existing data on the solubility in other solvents are used for the determination of the solubility parameters (δ_2) for the gases concerned. It is shown (Tables 4 and 8) that the experimental values agree fairly well with the results obtained from equation (I) when using the following values for δ_2, x_2^i (ideal solubility), and $\overline{V}_2$ (the partial molal volume of the gas):

	$x_2^i \times 10^4$	$\overline{V}_2$	δ_2
hydrogen	5.5	37	5.1
oxygen	17.6	46	5.7
carbon monoxide	15.5	52	5.8

It has further been shown that when equation (I) is used for the calculation of solubility in polar solvents, there will be certain discrepancies between the

calculated and the experimentally determined results. The deviations are greatest for the solvents which have the highest dielectric constants.

3. Skirrow has shown by experiments that the solubility of carbon monoxide in benzene falls off when a substance, as *e.g.* naphtalene, is dissolved in the benzene. It is shown that it is possible on the basis of equation (I) to calculate this reduction in the solubility (Table 5).

4. The solubility parameters for a number of monohydric alcohols are calculated on the basis of experiments on the solubility of carbon monoxide in the alcohols concerned (Table 6). It is shown (Table 7) that the solubilities calculated for hydrogen, nitrogen, and oxygen in methyl alcohol and ethyl alcohol show fairly good agreement with the experimental determinations found in the literature.

The present work has been performed in the Department of Inorganic Chemistry of the Pharmaceutical College of Denmark. I wish to express my gratitude towards the chief of this laboratory, Professor Carl Faurholt for the unfailing interest he has shown my work.

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On the Structure of Nucleic Acids

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During the last few years, X-ray crystal structure determinations have been carried out of a number of decomposition products of the nucleic acids. Although these investigations by no means can be considered complete, the results so far obtained would appear to be important enough to justify a discussion of their contribution to an understanding of nucleic acid structure.

The investigations have been extended to pyrimidines [1,2], purines [3,4] and nucleosides [5,6], and have a bearing on nucleic acid structure from several points of view. Firstly, they give general stereochemical information on the nucleic acids, such as the mutual orientation of sugar and base, and bond angles and bond lengths. Secondly, the study of these crystal structures may provide information regards the forces acting between adjacent nucleotides. Extensive systems of hydrogen bonds are found in all the crystals studied, and such bonds must be expected to occur also in the nucleic acids, probably both within and between the molecules. The occurence of hydrogen bonding in nucleic acids has been suggested earlier from certain anomalies in the titration curves [7]. Thirdly, a comparison of the physical properties (*e.g.* density and refractive indices) of the nucleic acids with those of nucleosides and nucleotides with known structures may render it easier to estimate the structural significance of these properties. Thus the strong negative birefringence of sodium thymonucleate fibres [8] indicates that the pyrimidine and purine rings are far more nearly parallel in this compound than in the crystals of cytidine, which show positive birefringence [5]. A comparison of the densities, which are 1.63—1.65 g/cm^3 for the nucleic acids [9] and in the order of 1.60—1.75 g/cm^3 for the nucleotides and their sodium salts [10], indicates that the nucleotides are packed so as to be roughly equally close in the two cases.

The information obtained regarding the mutual orientation of the components of the nucleotides is of special importance, and will be discussed below.

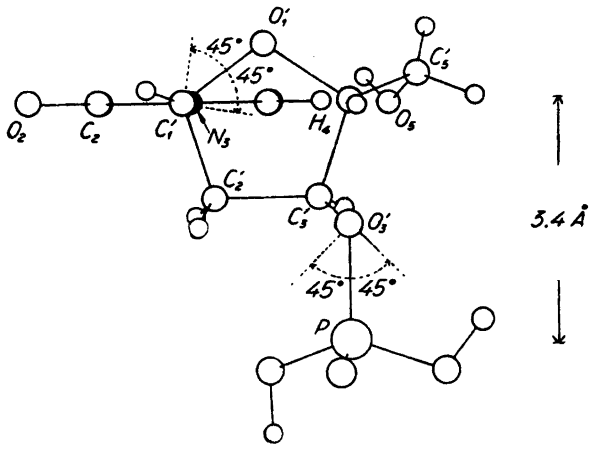


Fig. 1. A pyrimidine nucleotide of the "standard" configuration. The plane of the pyrimidine ring, as well as the bond N_3-C_1', is perpendicular to the plane of the paper.

THE STRUCTURE OF NUCLEOSIDES

In both the two nucleosides studied in some detail, cytidine [5] and 2',3'-*iso*-propylidene 3,5'-*cyclo*adenosine iodide [6], it is found that the bond connecting sugar and base (N-C_1') lies in the plane of the pyrimidine (purine) ring and makes tetrahedral angles with the adjacant ring bonds in the ribose ring. There would seem to be little reason to believe that the other nucleosides are different in this respect, and in the following it will be assumed that these stereochemical features are common to all nucleosides and nucleotides, whether of the ribose or the deoxyribose type [17]. The bond N-C_1' is found to be a single bond, and so rotation about this bond is possible. However, not all relative orientations of the sugar and base are equally feasible, as in many positions the hydrogen atoms at C_2' and C_3' come unfavourable near the atoms O_2 or H_4 of the pyrimidine, and N_3 or H_8 of the purine. The most favourable position would appear to be the one shown in Fig. 1, which is found in the crystal structure of cytidine. Here the pyrimidine is roughly perpendicular to the "plane" of the sugar (the furanose ring is not quite planar [5]) and the bond C_2'-C_3' approximately parallel to the plane of the base. There is, however, a certain range of favourable positions; the ribose ring may be rotated about 45° on either side of the position shown in Fig. 1 without bringing atoms of the ribose ring and the base too close together. For the purine nucleosides one would expect a similar structure, with N_3 approximately in the same position as O_2 in Fig. 1, the six-membered ring thus pointing away from the CH_2OH

group [17]. It should be noted that rotating the pyrimidine or purine 180° about the bond N-C_1' gives a structure which energetically would not appear to differ much from the one shown in Fig. 1.

THE STRUCTURE OF NUCLEOTIDES

No crystal structure determinations of nucleotides have been published, and there is no experimental evidence on the exact orientation of the phosphate group relative to the rest of the molecule. From a consideration of the van der Waals forces, however, it would appear that the positions within the 90° range shown in Fig. 1 probably are the most favourable ones, and that of these, the middle one with the bond P-O_3' roughly perpendicular to the plane of the pyrimidine (purine) is to be preferred. The distance from this plane to the phosphorous atom is about 3.4 Å. The relative position of $(OH)_5'$ and one of the phosphate oxygens may be very favourable to the formation of an intramolecular hydrogen bond.

In the following the structure shown in Fig. 1, and described above, will be called the *standard* nucleotide structure. Supposing that the free 3'-nucleotides have this configuration, the next question that naturally arises is the extent to which the nucleotides maintain this shape when they unite through phospho-ester linkages to form nucleic acids. Clearly the influence of neighbouring nucleotides may modify the mutual orientation of the components within the nucleotides to a certain extent. However, stereochemical considerations and the structure determinations carried out so far are in favour of the *standard* configuration, and until contradictory evidence has appeared, it would seem reasonable to assume that the nucleotides have roughly this shape in the nucleic acids. This point can probably be settled definitely only by a detailed X-ray structure determination of a number of nucleotides, or preferably polynucleotides.

MODELS OF THYMONUCLEIC ACID

Chemical investigations have shown, that in thymonucleic acid the nucleotides are linked together by phospho-ester linkages between hydroxyl groups at C_3' and C_5'. The chain is unbranched, or branched only to a small extent [7]. The molecules have the shape of long stiffish rods of diameter 15—20 Å [12]. The purine and pyrimidine rings are all nearly parallel, and are perpendicular to the long axis of the molecule, as was first pointed out by Signer, Hammarsten and Caspersson [13]. Astbury [9,11] obtained X-ray diagrams of fibres of sodium thymonucleate, the most dominating feature of which was a strong meridional

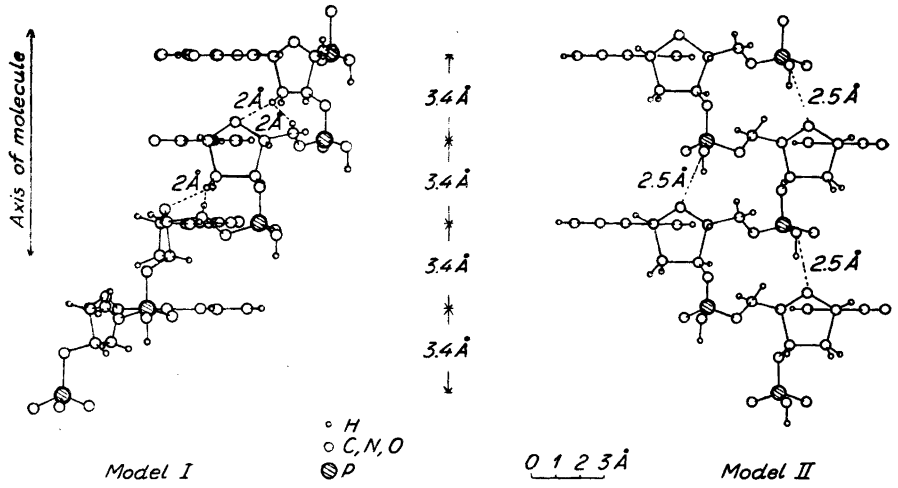


Fig. 2. Two models of thymonucleic acid based on nucleotides of the "standard" configuration. The planes of the purine and pyrimidine rings are perpendicular to the plane of the paper.

spacing of 3.4 Å (more accurately 3.3_4 Å). He also drew attention to the high density of the nucleic acids (1.63—1.65 g/cm^3), which shows that the nucleotides must be packed very closely in the polynucleotide. X-ray diagrams of specimens prepared in a different way show a far less pronounced 3.4 Å meridional reflexion [15].

Astbury [9] has proposed a model of thymonucleic acid, which is in agreement with the data given above. He associated the 3.4 Å spacing with the thickness of the nucleotides, assuming the nucleotides to be flattish and to stand out perpendicularly to the long axis of the molecule. The purine, pyrimidine and sugar rings were all believed to be flat and parallel, so that the structure is not consistent therefore with the results of the X-ray investigations of the nucleosides, which show that the sugar and base are nearly perpendicular to each other.

Two models which retain some of the essential features of the Astbury model, but are based on nucleotides of the *standard* configuration, are shown in Fig. 2. In contrast to the Astbury model, these two modifications of it have the planes of the sugar rings, as well as the bonds P-O_3', roughly parallel to the long axis of the molecule. Normal bond lengths and bond angles are maintained throughout the structure. Most atoms, the phosphorous atoms included, lie in planes 3.4 Å apart, thus explaining the strong 3.4 Å reflexion. It should be emphasized, that there is considerable freedom of rotation about

many of the single bonds in the structure, such as N-C_1', O_3'-C_3' and P-O_3', and considerable deviations from the *standard* configuration are possible within the general structural scheme in Fig. 2. The bonds P-O_3' may for example not be exactly parallel to the long axis of the molecule, and the sugar rings may be slightly twisted. According to Fraser and Fraser [14] a slight reorientation of the phosphate group is necessary to explain the results of their recent infra-red measurements, although the models in Fig. 2 in general fit their data well.

In *model I* the pyrimidine and purine rings are piled in a central column directly on top of each other, 3.4 Å apart, with the ribose rings and the phosphate groups in a spiral enclosing the column. Van der Waals attraction between the flat rings in the column holds the structure together. However, it has not been found possible to place the bases directly on top of each other without bringing some of the other atoms in successive nucleotides too near one another. As indicated in the figure, some of the distances from the hydrogen atoms at C_2' to neighbouring atoms are as short as about 2 Å, which is unfavourable. On the other hand, these steric distortions are not so serious that the model must be rejected, and they are not as great as those involved in the Astbury model.

The relative position of the electronegative groups in model I excludes the direct formation of intra-molecular hydrogen bonds. By means of water molecules, however, such bonding may be assumed to take place between electronegative atoms in successive pyrimidines and purines, as well as to the oxygen atoms of the phosphate groups. Astbury [9] has pointed out that water molecules must play an important part in the stability of crystalline aggregates of sodium thymonucleate.

The spiral in structure I repeats itself after eight nucleotides, the bonds N-C_1' in successive nucleotides making angles of about 45° with one another.

In *model II* the ribose rings and the phosphate groups form a flattish central column, from which the purines and the pyrimidines stand out perpendicularly. Successive bases are pointing outwards in opposite directions, all the bonds N-C_1' being roughly parallel, and there is one base directly above another at a distance of 6.8 Å. There are thus no forces acting between purines and pyrimidines; the whole molecule must be held together by interactions between atoms in the pentose rings and the phosphate groups, which are packed very efficiently in a compact sheet. Only very small steric distortions occur. The oxygen atom of the ribose ring and one of the oxygens of the phosphate group are 2.5—3.0 Å apart, and in the case of the free acid the molecule may possibly be stabilized by hydrogen bonds between these atoms. Hydrogen bonds to the ring oxygen atoms do not occur in crystals of cytidine [5], but appear to exist in crystals of *α*-D-glucose [16]. It will be seen that neighbouring molecules of the structure II

may be closely interleaved, giving a packing which is close enough to account for the high density.

These two models are chosen as examples to illustrate the mode of packing and the possible intra-molecular forces in models of thymonucleic acid based on *standard* shape nucleotides. As emphasized earlier, there is freedom of rotation about the P-O_3' bonds, and structure II may actually be derived from structure I solely by rotation about these bonds. A great number of intermediate structures, as well as combinations of I and II may be derived in this way. However, not all these models are equally feasible; in many of the structures van der Waals repulsive forces will introduce strains, and the molecules will be so irregular in shape that it would appear difficult to pack them closely. This kind of evidence is, of course, by no means conclusive, and on the whole the available evidence about the internal structure of thymonucleic acid does not permit a choice to be made at this stage between the structures I and II, or some of their intermediates.

Astbury observed that the true repeat distance along the fibre axis was much greater than 3.4 Å, probably eight or sixteen times as great, a finding which he associated with the tetra-nucleotide hypothesis favoured at that time. The models I and II may both be made to have repeat units of this magnitude by appropriate choice of the sequence of the nucleotides.

It has been assumed above, that the nucleotides have the *standard* configuration in the nucleic acids, which means that the phosphorous atom falls approximately in the plane of the base in the nucleotide below. By rotation about the bonds N-C_1' and C_3'-O_3' the phosphorous atom may be brought into the plane of its own purine (pyrimidine). Models based on nucleotides of this shape are also possible, and cannot be ruled out in general, but as we have seen above, the available evidence favours the *standard* configuration for the nucleotides.

SUMMARY

The information obtained on the structure of the nucleic acids from crystal structure determinations of nucleosides and related compounds has been discussed. A probable configuration of the nucleotides is suggested, and modifications of the Astbury model of thymonucleic acid proposed. In these new models the pentose rings are roughly parallel to the long axis of the molecule.

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Über die Umsetzung von Formaldehyd mit Allylcarbinol

IV.* Synthese des Pentadien-(2,4)- ol-(1) und des Penten-(2)-diol-(1,5)

SIGURD OLSEN, JACOB GUNDERSEN und ERNST FINSNES

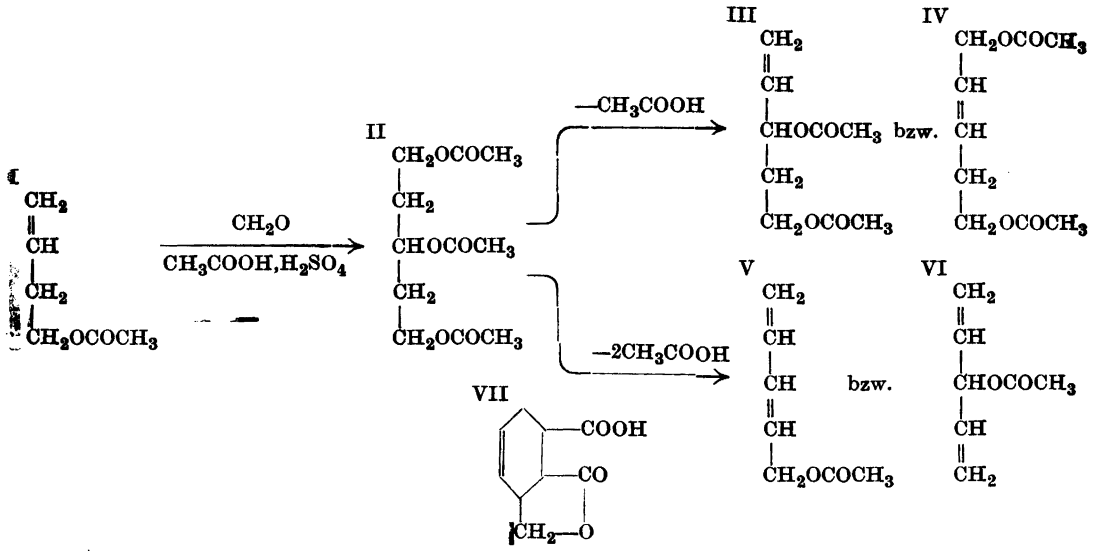
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Im Zusammenhang mit synthetischen Versuchen in der Patulinreihe interessierten wir uns für einen möglichst einfachen Weg zur Herstellung des Pentadien-(2,4)-ol-(1). Die einzige bisher beschriebene Synthese dieser Verbindung gelang Heilbron, Jones, McCombie und Weedon [1]. Nach diesen Autoren wird durch Umsetzuug von Acetylen mit Acrolein bzw. Epichlorhydrin in flüssigem Ammoniak das Penten-(2)-in-(4)-ol-(1) dargestellt und dieses partiell hydriert. — Wir haben uns eines neuen, überraschend einfachen Verfahrens bedient, das uns zudem gleich das erwünschte Acetat des Pentadien-(2,4)-ol-(1) (V) und das Diacetat des von Paul und Tchelitcheff [2] beschriebenen Penten-(2)-diol-(1,5) (IV) in die Hand gab.

Als Ausgangsmaterial verwendeten wir das durch Formaldehydaufbau aus Allylcarbinacetat (I) leicht zugängliche Pentantriol-(1,3,5)-triacetat (II)[3]. Wir gingen dabei von der Erwägung aus, dass bei der Zersetzungsdestillation dieser Verbindung die Essigsäureabspaltung nach Abspaltung von zwei Molekülen zum Stillstand kommt und somit keine wesentliche Bildung ungesättigter Kohlenwasserstoffe erfolgt. Bezüglich der möglichen Reaktionsprodukte kämen dann bei der Abspaltung von *einem* Molekül Essigsäure das Penten-(1)-diol-(3,5)-diacetat (III) und das Penten-(2)-diol-(1,5)-diacetat (IV), bei der Abspaltung von *zwei* Molekülen Essigsäure das Pentadien-(2,4)-ol-(1)-acetat (V) und das Pentadien-(1,4)-ol-(3)-acetat (VI), bzw. deren geometrische Isomere, in Betracht.

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* 12. Mitt. über Formaldehyd-Olefin-Reaktionen.



Der bei einer Temperatur von ca. 500° vorgenommene Crackversuch führte zu einer breiten Fraktion vom $Sdp._{13}$ 55—57°, die aus ziemlich reinem Pentadien -(2,4)-ol-(1)-acetat besteht. Welche Form der geometrischen Isomeren vorliegt, kann gegenwärtig nicht entschieden werden. Die Ausbeute beträgt etwa 30 % der Theorie. Die Jodzahlbestimmung nach Kaufmann ergab nur etwa die Hälfte des berechneten Wertes. Die beiden konjugierten Doppelbindungen liessen sich jedoch durch Hydrierung nachweisen, wobei die berechnete Menge Wasserstoff unter Bildung von n-Amylacetat aufgenommen wurde. Das Pentadien-(2,4)-ol-(1)-acetat gab bei der Umesterung unter gleichzeitiger Polymerisation in schlechter Ausbeute das freie Pentadien-(2,4)-ol-(1), das sich durch Diensynthese mit Maleinsäureanhydrid in das durch Heilbron und Mitarbeiter bekannt gewordene Lacton VII überführen liess. Als Nebenprodukte lieferte der Crackversuch in geringerer Menge einige höhersiedende Fraktionen, in denen bisher nur das Penten-(2)-diol-(1,5)-diacetat (IV) nachgewiesen werden konnte. Die Sicherstellung der Konstitution dieser Verbindung gelang ebenfalls durch Hydrierung, wobei sich das durch Hamonet [4] und Dionneau [5] beschriebene Diacetat des Pentandiol-(1,5) bildete. Das daraus erhaltene freie Glykol wurde in das Bisphenylurethan übergeführt, das in allen Eigenschaften mit dem von Müller und Rölz [6] hergestellten Präparat übereinstimmt und das bei der Mischprobe mit einem aus Tetrahydropyran gewonnenen Bisphenylurethan ohne Depression schmilzt.

EXPERIMENTELLER TEIL

Thermische Spaltung des Pentantriol-(1,3,5)-triacetates

296 g Pentantriol-(1,3,5)-triacetat (aus Allylcarbinacetat [3]) wurden in einer elektrisch heizbaren Quarzkolonne (Länge 80 cm, Durchmesser 3 cm) in der früher für andere Verbindungen beschriebenen Weise [7] in Stickstoffatmosphäre bei ca. 450–500° im Verlaufe von 14 Stunden gespalten, wobei sich die Destillationstemperatur zwischen 110–157° bewegte. In der mit Eis-Kochalz gekühlten Vorlage befanden sich 256 g eines braunen, stechendriechenden Destillates (*A*). Bei der Destillation entstand eine geringe Menge gasförmiger Produkte, die in der Vorlage nicht verdichtet wurden. Das Destillat A wurde zweimal an der Widmerspirale destilliert, wobei man u.a. folgende Fraktionen erhielt:

1. $Sdp._{13}$ 55,5– 57°, ca. 45 g, $D_4^{24} = 0{,}9444$, $n_D^{24} = 1{,}4693$
2. » 106 –117° 10,4 g
3. » 117 –130° 6,4 g
4. Rückstand 21,7 g

Die Fraktion 1. besteht im wesentlichen aus *Pentadien-(2,4)-ol-(1)-acetat* (V); sie wurde in folgender Weise identifiziert:

a) Hydrierung zu n-Amylacetat. 10,4 g der Fraktion 1. wurden in Eisessig mit Platinoxyd im Verlaufe von 5 Stden. hydriert, wobei etwas mehr als die für zwei Doppelbindungen berechnete Menge Wasserstoff aufgenommen wurde. Bei der Aufarbeitung erhielt man 2,7 g einer farblosen Flüssigkeit vom Sdp. 140–149°, $n_D^{26} = 1{,}4036$, $D_4^{23} = 0{,}8840$, welche Konstanten mit den in der Literatur für *n-Amylacetat* aufgegebenen befriedigend übereinstimmen. Auch der durch Umesterung daraus erhaltene *n-Amylalkohol* hatte den richtigen Siedepunkt und lieferte ein *Phenylurethan* vom Schmp. 45,5–46,5°, der mit dem von E. E. Blaise und L. Picard [8] aufgegebenen übereinstimmt. Mischschmelzpunkt mit einem authentischen Präparat unverändert.

$C_{12}H_{17}O_2N$ (207,2) Ber. C 69,54 H 8,26 N 6,76
Gef. » 69,66 » 8,35 » 6,59

b) Umesterung zu Pentadien-(2,4)-ol-(1). 10 g der Fraktion 1. wurden mit methylalkoholischer Salzsäure (0,5 %) umgeestert und bei vermindertem Druck destilliert. Man erhielt 1 g eines farblosen Destillates vom $Sdp._{13}$ 45–54°. Die restliche Flüssigkeit im Kolben polymerisierte. 0,4 g des Destillates wurden nach Heilbron u. Mitarb. mit Maleinsäureanhydrid in Benzol umgesetzt. Man erhielt das *Säurelacton* (VII) in Form farbloser Krystalle, die nach mehrfachem Umkrystallisieren aus Wasser bei 176° schmolzen.

$C_9H_{10}O_4$ (182,2) Ber. C 59,32 H 5,53
Gef. » 58,92 » 5,45

Die Fraktion 3. enthält *Penten-(2)-diol-(1,5)-diacetat* (IV);
Dieses wurde in folgender Weise nachgewiesen:

a) Umesterung zu Penten-(2)-diol-(1,5). Durch Umesterung erhielt man eine farblose Flüssigkeit vom $Sdp._{13}$ 132–136°, J. Z. 218,3 (ber. 248,5).
Bisphenylurethan aus Alkohol Schmp. 159–160,5°

$C_{19}H_{20}O_4N_2$ (342,2) Ber. C 67,04 H 5,92 N 8,23
Gef. » 66,92 » 6,14 » 8,21

b) Hydrierung zu Pentandiol-(1,5)-diacetat. 8 g einer analogen Fraktion aus einem anderen Crackversuch vom $Sdp._7$ 111–112° (V.Z. 575 (ber. 603), J.Z. 124 (ber. 136)) wurden in Eisessig mit Platinoxyd hydriert. Die Destillation ergab 4 g *Pentandiol-(1,5)-*

diacetat vom Sdp.$_7$ 112—113°. Durch Umesterung erhielt man daraus 1,3 g *Pentandiol-(1,5)* vom Sdp.$_9$ 113—114°. A. Franke und F. Lieben[9] geben an für das Diacetat Sdp.$_{28}$ 136—138°, für das freie Glykol Sdp.$_{11}$ 129—130°.

Das aus dem vorstehend beschriebenen Glykol hergestellte *Bisphenylurethan* schmilzt nach dem Umkrystallisieren aus Alkohol bei 172—174°, beim zweiten Schmelzen bei 140—141° und gibt mit dem nachstehend beschriebenen, aus Tetrahydropyran erhaltenen Präparat bei der Mischprobe keine Depression.

Tetrahydropyran (Pentamethylenoxyd)

40,2 g Δ^3-Dihydropyran (dargestellt nach früherer Vorschrift[10]. Neu ermittelte Konstanten: $D_4^{20} = 0{,}9365$, $n_D^{20} = 1{,}4460$, MR = 23,95 (ber. 24,27)) wurden in 80 ml Eisessig mit 1 g Platinoxyd hydriert. Nachdem etwas mehr als die theoretische Menge Wasserstoff aufgenommen worden war, wurde der Katalysator abfiltriert, das Filtrat mit einigen Tropfen Brom-Eisessig-Lösung (zwecks Entfernung evtl. nicht umgesetzen Dihydropyrans!) und Äther versetzt und die Ätherlösung durch Waschen mit gesättigter Sodalösung von Essigsäure befreit. Nach dem Trocknen über Natriumsulfat erhielt man nach Abdestillieren des Äthers 15,1 g einer farblosen Flüssigkeit vom Sdp. 86—87°, $D_4^{22} = 0{,}8851$, $n_D^{22} = 1{,}4178$, MR = 24,51 (ber. 24,74), J.Z.O. Siedepunkt und Dichte stimmen gut überein mit den von G. M. Bennet und W. G. Philip[11] für Tetrahydropyran angegebenen Werten.

5-Brom-pentanol-(1)-acetat

15,1 g Tetrahydropyran wurden unter Rückflusskühlung mit 32 g Acetylbromid und 0,4 g Zinkchlorid 2 Stunden gekocht. Die dunkle Flüssigkeit gab bei der Destillation 30,3 g einer fast farblosen Flüssigkeit vom Sdp.$_7$ 90—95°.

Pentandiol-(1,5)

Eine Mischung von 30,3 g des vorstehend beschriebenen 5-Brom-pentanol-(1)-acetates, 24,6 g wasserfreiem Natriumacetat und 31 g Essigsäureanhydrid kochte man 4 Stden. unter Rückflusskühlung. Das abdestillierte rohe Reaktionsprodukt wurde mit den gleichen Mengen Natriumacetat und Essigsäureanhydrid gekocht. Bei der Vakuumdestillation erhielt man 23,9 g Destillat vom Sdp.$_{10}$ 105—108°, das im wesentlichen aus *Pentandiol-(1,5)-diacetat* besteht. Durch Umesterung erhielt man daraus 3 g *Pentandiol-(1,5)* vom Sdp.$_{10}$ 127—128°.

Bisphenylurethan aus Alkohol Schmp. 172—174° (beim zweiten Schmelzen Schmp. 140—141°

$C_{19}H_{22}O_4N_2$ (344,2) Ber. N 8,18
Gef. » 8,13

ZUSAMMENFASSUNG

Bei der thermischen Spaltung von Pentantriol-(1,3,5)-triacetat entstehen Pentadien-(2,4)-ol-(1)-acetat und Penten-(2)-diol-(1,5)-diacetat. — Δ^3-Dihydropyran wurde zu Tetrahydropyran hydriert und dieses nach Ringöffnung mit Acetylbromid in das Pentamethylenglykol übergeführt.

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The Reaction between Acetylacetone and Citraconic Anhydride *

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As previously described by the author [1] acetylacetone and maleic anhydride formed an adduct when heated together or when the mixture was left for a long time at room temperature. The original object of the investigation was to find out if a dienol of acetylacetone existed containing conjugated carbon-to-carbon double bonds. The reaction which took place did not, however, proceed as expected according to a simple Diels-Alder scheme, but it was found that the adduct was built up of one molecule of acetylacetone and two molecules of maleic anhydride. The explanation of the course of the reaction arrived at was that in a first step two molecules of maleic anhydride by way of a substitution addition formed a double-molecule which, due to an enolization, acted as a dien and to which the mono-enol of acetylacetone was added in a second step.

It has now been found that acetylacetone forms an adduct also with citraconic anhydride. In this case, however, each molecule of the diketone reacts with one molecule only of the acid anhydride. The fact that the new adduct is a monocarboxylic acid indicates that neither in this case has a simple Diels-Alder reaction taken place. Evidently one of the carbonyl groups of the citraconic anhydride or the anhydride bond itself must be involved in the formation of the adduct. Before discussing the structural formula of the adduct and the mode of its formation it will be necessary to describe briefly the reactions of the adduct.

The excellently crystallizing adduct was only slightly soluble in cold water but dissolved readily on boiling. It did not reduce silver nitrate in the presence of ammonia. The iodoform reaction was positive, and the adduct gave with bromine-water a monobromo derivative. With dinitrophenylhydrazine and semicarbazid it gave crystalline monoderivatives. An aqueous solution of the

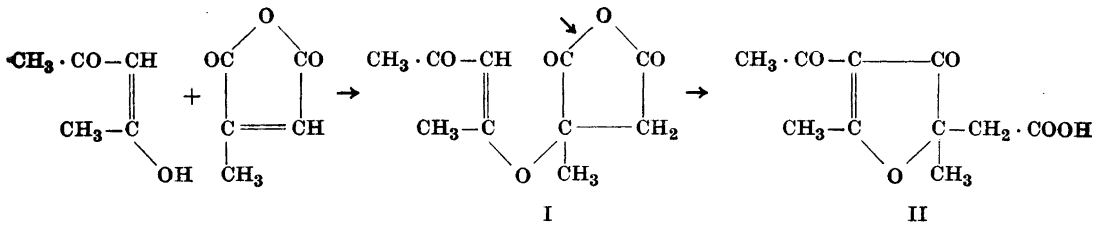
* Presented before the Section of Organic Chemistry at the XII. International Congress of Pure and Applied Chemistry in New York, 1951.

adduct did not give any colour with ferric chloride but under certain conditions the adduct showed a pronounced tendency of enolizing which will be evident from the following. On dissolving the adduct in an excess of sodium carbonate the solution remained colourless and after it had been acidified no colour appeared after the addition of ferric chloride. If, however, the adduct was dissolved in more than one equivalent of alkali hydroxide the solution became strongly yellow. When this solution was acidified the yellow colour disappeared and the solution now gave a deep red-violet colour with ferric chloride. Leaving the acidified solution at room temperature the strength of the colour reaction decreased and disappeared nearly completely in the course of a few days. At the same time most of the unchanged adduct crystallized from the solution. It is quite remarkable that although a keto-form evidently was the stable form in acid medium the conversion of the enol-form into the ketoform proceeded so slowly. On dissolving the adduct in more than two equivalents of alkali a spontaneous degradation took place which will be discussed below.

Another example of the enolizing tendency of the adduct was found in the case of the ester. By means of diazomethane a methyl ester with one methoxyl group was obtained as a colourless crystalline substance. A solution of the ester in ethanol turned yellow on the addition of sodium ethoxide but the colour disappeared again on acidifying. On the other hand a solution of the methyl ester in ether containing a little methanol turned permanent yellow when treated with an excess of diazomethane. From this solution an orange-yellow syrup was obtained which did not crystallize and which obviously contained the methyl ether of the enol-form of the ester. The same enol-ether was formed to a certain degree also in an acid medium, viz. when hydrogen chloride was added to a solution of the methyl ester or of the adduct itself in methanol. Such a solution rapidly took on a yellow, later an orange, colour, and after removing the hydrogen chloride and the methanol an orange syrup was obtained, being a mixture of ordinary colourless ester and the coloured methyl ether of the enol-form from which a part of the former separated as crystals on standing.

The adduct was exceedingly stable in acid medium. It could be boiled with dilute sulphuric acid for half an hour without giving any degradation products. It could even be recovered unchanged after having been dissolved in concentrated sulphuric acid.

By the oxidation of the adduct with potassium permanganate acetic acid, pyruvic acid, and citramalic acid (α-hydroxy-α-methylsuccinic acid) were isolated. Considering this result and the facts given above the following scheme is suggested for the formation of the adduct:

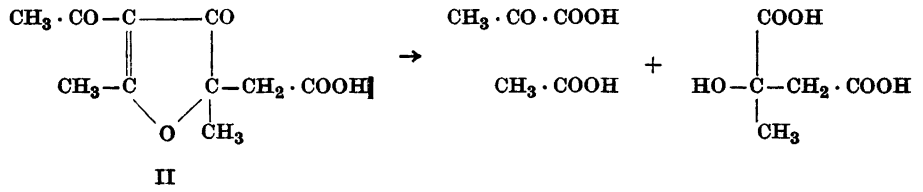


I II

In a first step a substitution addition takes place, the acetylacetone reacting in its mono-enol-form. In the intermediate product (I) which has not as yet been isolated the second mobile hydrogen atom then attacks the anhydride bond as indicated by the arrow. This leads to the adduct (II) which can accordingly be designated as 4-acetyl-2,5-dimethyl-3-keto-2,3-dihydrofuryl-2-acetic acid.

It should be remarked that the sequence of the two steps of the reaction may be the reverse of that given i the scheme, but this will make no difference in the formula for the adduct. At the present time it has not been possible to make a decision between the two alternatives.

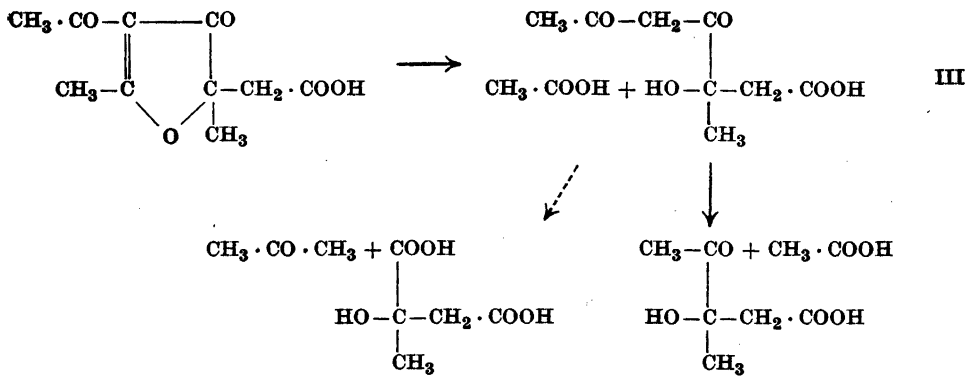
The result of the oxidation mentioned above is in full agreement with formula II:



II

The degradation of the adduct by means of alkali could be carried out either at room temperature or by heating on the steam-bath. At room temperature the degradation was followed by titration showing that the requirement of alkali per mole adduct increased to nearly three equivalents. The two main products of degradation were acetic acid and β-hydroxy-β-methyl-γ-ketovaleric acid, and the result of the titration was therefore in agreement with the following scheme according to which each mole of the adduct gave two mole of acetic acid and one mole of the keto-acid.

As acetone was found to be a minor product of degradation it is reasonable to suggest that the intermediate product III is degraded partly as indicated by the dotted arrow. It has, however, not been possible to isolate citramalic acid from its mixture with the keto-acid which is an oil. The keto-acid gave a



dinitrophenylhydrazone and a semicarbazone and it could be transformed into a methyl ester. The constitution of the keto-acid given above is in agreement with the fact that the acid was reduced to β-methylvaleric acid and that it on treatment with sodium hypobromite gave bromoform and citramalic acid.

In a preliminary report upon this investigation [2] a somewhat different formula for the adduct was suggested. This was based upon the assumption that the keto-acid was a derivative of α-methylvaleric acid, and it was only afterwards proved that the product was in fact derived from β-methylvaleric acid.

Although the formula for the adduct contains a double bond between carbon atoms neither the adduct itself nor its methyl ester when dissolved in non-polar solvents reacted with bromine. This must obviously be due to steric hindrance. On the other hand catalytically activated hydrogen was readily added, but no attempts were made of investigating the oily product from the hydrogenation.

It has also been found that propionylacetone formed a similar adduct with citraconic anhydride as acetylacetone. The adduct could, however, in this case not be obtained in crystalline state but a crystalline dinitrophenylhydrazone was isolated and analyzed.

EXPERIMENTAL

(Melting points not corrected.)

Adduct from acetylacetone and citraconic anhydride

Acetylacetone (224 g = 2 mole) and citraconic anhydride (200 g = 2 mole) were mixed and kept in a closed glass vessel at 95—100° for one month when the mixture took on a dark brown colour. After cooling to room temperature and rubbing with a glass rod the adduct crystallized and was separated from the mother liquor on a glass-sinter filter. The crystals weighed 110 g. The mother liquor after being heated in the same way for

another month gave 95 g more of the adduct. The overall yield was therefore 47.5 % of theory. The crude adduct was washed with cold ethyl acetate and then recrystallized twice from ethyl acetate when it was obtained quite colourless and had m.p. 130°. The same m.p. was found after recrystalization from water.

0.2229 g required 10.51 ml $N/10$ NaOH

$C_{10}H_{12}O_5$	Calc.	C 56.60	H 5.70	M 212.2
	Found	» 56.73, 56.77	» 5.49, 5.58	» 212.1

Dinitrophenylhydrazone: Adding to the aqueous solution of the adduct a solution of 2,4-dinitrophenylhydrazine in 2N hydrochloric acid the hydrazone at first separated amorphous but rapidly turned into red crystals. Recrystallized from ethanol, m.p. 203°.

$C_{16}H_{16}O_8N_4$ (392.3)	Calc.	C 48.98	H 4.11	N 14.28
	Found	» 49.29, 48.98	» 4.18, 4.08	» 14.27, 14.13

Semicarbazone prepared in the usual way and recrystallized from water had m.p. 194°.

$C_{11}H_{15}O_5N_3$ (269.3)	Calc.	C 49.06	H 5.62	N 15.61
	Found	» 49.06	» 5.56	» 15.16

Reaction with bromine: To a little of the adduct dissolved in water bromine-water was added. The colour of the bromine disappeared istantaneously and an oil separated which crystallized in a few minutes. M.p. 95°.

$C_{10}H_{11}O_5Br$ Calc. Br 27.45 Found 27.65

The adduct in acid medium: A little of the adduct was dissolved in conc. sulphuric acid giving a yellow solution. After 15 minutes a part of the solution was poured on ice when the adduct separated as crystals with m.p. 130°. The rest of the solution was left for two days and also then the adduct was recovered unchanged. In another experiment the adduct (2 g) was boiled for half an hour with 2N sulphuric acid (25 ml) and a few ml distilled off. The distillate contained no acid and gave no reaction on acetone with o-nitrobenzaldehyde. From the rest of the solution which gave no colour with ferric chloride 1.75 g of the adduct crystallized on cooling, m.p. 128°.

Methyl ester of adduct

The pulverized adduct dissolved easily in an ethereal solution of diazomethane. On distilling off the ether a colourless liquid remained which crystallized in the course of a couple of months. After recrystallization from petroleum ether the ester had m.p. 55°.

$C_{11}H_{14}O_5$ (226.2)	Calc.	C 58.40	H 6.24	CH_3O 13.70
	Found	» 58.58, 58.69	» 6.14, 6.24	» 15.04

0.2085 g in 17.42 g benzene, Δ 0.267° M 224

As already stated above the ester with an excess of diazomethane gave the methyl ether of an enol-form. The high content found for methoxyl may therefore be due to a contamination of the ester by a little of the ether.

In an attempt to prepare the ester in the usual way the following observations were made. In a solution of 1.3 g adduct in 15 ml methanol a current of dry hydrogen chloride was led until the weight had increased with 4 g. Shortly after starting the current the solution turned yellow and soon became orange (the same colours appeared when starting with a methanolic solution of the ester prepared by means of diazomethane). Next day the solution was placed in a vacuum desiccator above calcium chloride and potassium hydroxide when a dark orange syrup was obtained. In a couple of weeks a little crystalline material had been formed in the syrup. It was pressed on a porous plate and then recrystallized from petroleum ether. M.p. 55°.

Oxidation of the adduct

A solution of the adduct (10 g) in a little more than one equivalent of sodium carbonate and diluted to 300 ml was vigorously stirred and a 5 per cent solution of potassium permanganate (350 ml) added in the course of two and a half hour, keeping the temperature at about 0°. After coagulating the manganese dioxide by heating gently and shaking the filtered colourless solution was concentrated on the steam-bath to 100 ml. Sulphuric acid (40 ml 5 *N*) was added and nearly two third of the solution distilled off. The distillate needed for its neutralization 41.4 ml *N* NaOH, corresponding to 2.48 g acetic acid (one mole per mole adduct would correspond to 2.83 g acetic acid). The neutralized distillate evaporated to dryness gave 3.6 g salt in which acetic acid was identified by well-known methods. The rest of the solution from the distillation (55 ml) was extracted thoroughly with ether giving 3.95 g of a syrup which partly crystallized. The crystalline substance (1.6 g) separated from the syrup on a porous plate was recrystallized twice from ethyl acetate. M.p. 118—120°, alone and after mixing with an authentic sample of citramalic acid.

$C_5H_8O_5$ (148.1)	Calc.	C 40.54	H 5.44	
	Found	» 40.23, 40.18	» 5.59, 5.45	

The presence of pyruvic acid amongst the oxidation products was proved in another experiment. Having oxidized 4.24 g adduct as above and acidified with sulphuric acid a solution of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid was added at about 50°. The orange-yellow precipitate (1.5 g) was recrystallized from ethanol when it had the m.p. 220°, alone or mixed with the hydrazone of pyruvic acid.

$C_9H_8O_6N_4$ (268.2)	Calc.	C 40.31	H 3.01	N 20.89
	Found	» 40.54, 40.37	» 2.92, 3.07	» 21.09, 21.15

Degradation of adduct with alkali

The adduct (4.24 g) was dissolved in 5 equivalents sodium hydroxide diluted to 40 ml and excess of alkali determined by titrating samples of 2 ml of the solution from day to day. The yellow colour of the fresh solution became very faint in the course of one day. The consumation of alkali which just after dissolving the adduct was 1.026 equiv. per mole increased in 1, 2, 3, and 4 days to 2.396, 2.716, 2.782 and 2.816 equiv. respectively. To the rest of the solution (30 ml) 5 *N* sulphuric acid (15 ml) was added and the solution extracted two times with ether, each time with 450 ml. From the dried ethereal solution a yellow syrup was obtained which had a strong smell of acetic acid. It was placed in a vacuum desiccator above potassium hydroxide until the weight remained constant (1.9 g). The syrup would not crystallize. It was readily soluble in water and gave a crystalline orange-coloured dinitrophenylhydrazone which after recrystallization from ethanol had m.p. 175°.

$C_{12}H_{14}O_7N_4$ (326.3)	Calc.	C 44.17	H 4.32	N 17.17
	Found	» 44.18, 44.16	» 4.50, 4.56	» 17.51, 17.45

This correspond to the formula $C_6H_{10}O_4$ for the syrupy degradation product. That the product was a monobasic acid was verified by esterifying a sample with diazomethane and preparing the dinitrophenylhydrazone of the ester. Recrystallized from ethanol, m.p. 132°.

$C_{13}H_{16}O_7N_4$ (340.3)	Calc.	C 45.88	H 4.74	N 16.47
	Found	» 45.95, 45.94	» 4.73, 4.85	» 16.44, 16.51

The composition of the semicarbazone corresponded to the same formula of the degradation product. The semicarbazone showed dimorphism. On crystallization from water a labile modification consisting of thin needles appeared first followed by dense prisms which were stable and into which the needles were transformed on standing. The stable modification had m.p. 204°.

$C_7H_{13}O_4N_3$ (203.2)	Calc.	C 41.38	H 6.45	N 20.68
	Found	» 41.32, 41.40	» 6.30, 6.33	» 20.69, 20.64

The presence of acetone amongst the degradation products was proved in a separate experiment. After the alkaline degradation had taken place the acidified solution was distilled. A part of the distillate on additon of *o*-nitrobenzaldehyde and alkali gave indigo. Another part on addition of dinitrophenylhydrazine in hydrochloric acid gave a crystalline hydrazone which after recrystallization from ethanol had m.p. 127°, alone and mixed with the hydrazone of acetone.

$C_9H_{10}O_4N_4$ (238.2)	Calc.	C 45.38	H 4.23	N 23.52
	Found	» 45.63	» 4.18	» 23.09

Investigation of $C_6H_{10}O_4$

(With stud. real. Svein Ramstad)

Greater quantities of the degradation product $C_6H_{10}O_4$ were prepared in the following way. Batches of 10 g adduct were heated with 125 ml 2 *N* sodium hydroxide for two hours on the steam-bath. After adding 30 ml 5 *N* sulphuric acid and concentrating to two third of the volume a solution containing hydrochloride of semicarbazide (5 g) and potassium acetate (5 g) was added. Each batch gave from 7.9 to 8.2 g semicarbazone.

The semicarbazone was decomposed by heating 34 g with 50 ml water and 100 ml 5 *N* sulphuric acid on the steam-bath till it had dissolved completely. The solution on shaking six times with ether, each time with 600 ml, gave 19.9 g of the degradation product as a nearly colourless syrup.

Reduction: To a solution of the syrup (3.5 g) in ether (400 ml) amalgamated aluminium turnings (30 g) and from time to time small quantities of water were added. The reaction which thus proceeded smoothly was continued for 20 hours. After evaporating the ether sulphuric acid was added and the solution distilled with steam. From the distillate an oil (1.5 g) was obtained which had a pronounced smell of fatty acid and boiled at about 190°. By treatment with phosphorus trichloride it was transformed into an acid chloride which on pouring into conc. ammonia gave a crystalline amide. After recrystallization from ethyl acetate the amide had m.p. 125°, alone and mixed with an authentic sample of the amide of β-methylvaleric acid.

$C_6H_{13}ON$ Calc. N 12.16; Found 11.90, 12.03

Oxidation with hypobromite: To a solution of sodium hydroxide (3.3 g = 8 ¼/100 mole) in water (15 ml) bromine (4.8 g = 3/100 mole) was added while the solution was cooled with ice and shaken vigorously. Under continued cooling and shaking a solution of $C_6H_{10}O_4$ (1.46 g = 1/100 mole) was added. The reaction proceeded rapidly and in a short time the solution became colourless. After adding 10 ml 5 *N* sulphuric acid bromoform was separated in a funnel and the aqueous solution concentrated on the steam-bath. On extracting two times with ether, each time with 500 ml, a viscous syrup (1.2 g) was obtained which crystallized immediately after inoculating with citramalic acid. After recrystallization from ethyl acetate, m.p. 119—120°, alone and mixed with pure citramalic acid.

Adduct from propionylacetone

Propionylacetone (20 ml) and citraconic anhydride (16 ml) were mixed and left for seven weeks at 80°. The brown reaction product was dissolved in sodium carbonate and excess of propionylacetone extracted with ether. The aqueous solution was acidified and extracted with ether when a dark yellow syrup (10 g) was obtained which could not be brought to crystallize. On treating 1 g of the syrup with dinitrophenylhydrazine 1 g of a red crystalline hydrazone separated. Recrystallized from dilute ethanol, m.p. 194°.

$C_{17}H_{18}O_8N_4$ (396.3)	Calc.	C 50.25	H 4.46	N 13.79
	Found	» 50.14	» 4.55	» 14.13

SUMMARY

A crystalline adduct is formed from one molecule of acetylacetone and one molecule of citraconic anhydride. The adduct which is a monocarboxylic acid was found to be a derivative of dihydrofuran. It is suggested that the reaction takes place in two steps but it has as yet not been possible to decide about the sequence of the steps. The adduct is enolized either by the action of alkali or excess of diazomethane or by treatment with methanolic hydrogen chloride. The adduct is remarkably stable against acids but is rapidly degraded in alkaline medium.

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Effect of the Nitrogen Content of Cells on Fermentation by *Aerobacter*

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While studying the dependence of the enzymatic activity of cells on their nitrogen content [3] we also turned our attention to the fermentation processes [1]. De Ley [2] observed with *Aerobacter aerogenes* K_3* that anaerobic fermentation activity rapidly lessened with the decrease in the nitrogen content of the cells and ceased entirely when the nitrogen content was lowered from the normal of about 13 % to approximately 8 % of dry matter. Respiration as well as aerobic acid formation is then still strong. Thus, by lowering the N-content of cells it is possible to change their metabolism in a very remarkable way. Our experiments with the same bacterial strain have also shown that fermentation is retarded when the nitrogen content of cells is lowered, though not as much as in the experiments of De Ley. However, a bacterial mass containing 9.5 % nitrogen had, in our experiments, a fermentation velocity (calculated per unit N of the cells) of only half that of the normal-N cellmass containing 13 % nitrogen (*cf.* below). Virtanen and Aejmelaeus [3] found with *Torula utilis*, too, that the rate of fermentation was lowered by 50 % when the N-content of the cells fell from 9.8 % to 5 % of dry matter. By adding β-alanine to the aerated *Torula* suspension in sugar solution it was possible to lower the N-content to 3.7 %. This low-N Torula still fermented glucose very slowly (activity about 20 % of the normal). Respiration and catalase activity of *Torula* increased considerably up to a nitrogen content of 4.5–5 %. Virtanen and De Ley [4] have dealt with the dependence of the enzymatic activity of cells on their N-content.

* The strain used by Virtanen and De Ley, taken from the laboratory collections labelled as *Escherichia coli* K_3 proved to be of aerogenes type.

In the present paper we shall record results of the experiments which were made in order to examine to what extent the N-content of cells affects the fermentation products and hence the fermentation mechanism. These results are still in some respects incomplete but even as such they indicate the distinct effect of the N-content of cells on the fermentation products of glucose in *Aerobacter*.

EXPERIMENTAL

Preparation of bacterial material. *Aerobacter aerogenes* strain K_3 was cultured parallel in two nutrient solutions, the one containing 8 g $(NH_4)_2SO_4$, the other 0.1 g $(NH_4)_2SO_4$. The composition was otherwise the same, *i.e.:* 25 g. K_2HPO_4, 10 g NaCl, 0.1 $MgSO_4$, and 25 g saccharose in 5 litres of tap water. The cultures were kept at 33° C for 65 hours and the acidity of the culture, to which was added bromothylmolblue as an indicator, was maintained at pH about 6.5 by adding, when necessary, 4 *N* NaOH-solution during the growth. The growth was far better on the greater amount of ammonium sulphate than on the smaller. The bacteria were separated by centrifugation and washed with sterile water. The bacterial mass was suspended in 50 ml of sterile water as homogeneously as possible and for each fermentation experiment was used either 10 ml suspension of the normal-N bacteria or 30 ml of the low-N bacteria. The N-content of each suspension was determined.

Fermentation experiment. Fermentation solution: 50 ml of m/8 phosphate buffer (pH 6.1) with 250 mg glucose. 0.2 ml of 1 % water solution of bromcresolpurple was added. The pH was maintained throughout the fermentation at about 6.0—6.2 by adding alkali as required. The fermentation bottle had a wide mouth closed with a rubber stopper. The stopper was furnished with two glass tubes, the one serving as outlet for the evolved gases which were collected in a mercury sealed gas burette, the other as inlet for the 0.1 *N* NaOH used for neutralisation of the solution. Before the start of fermentation oxygen was removed from the system by a current of nitrogen. Temperature of the thermostat was 37° C. The bottles were shaken mechanically during the fermentation, gas readings being made at intervals of half an hour.

In two experiments sugar was estimated according to Bertrand at intervals of 2 hours. The determinations were made from bottles, which were not connected with the gas burette.

Analytical methods. The fermentation solution was centrifuged and analyzed for acetic acid and formic acid according to Virtanen and Pulkki [5], for formic acid separately according to Riesser [6], for lactic acid according to the principle of Führt-Charnass [7] and for alcohol according to Sémichon and Flanzy [8]. Succinic acid was determined according to Tasman and Smith [9]. With solutions containing lactic acid in amounts corresponding to our fermentation solutions, the method gave in check analyses values which were about 8 % too high. The values obtained were therefore corrected respectively. The total volume of gas evolved was determined volumetrically but CO_2 and H_2 were not determined separately.

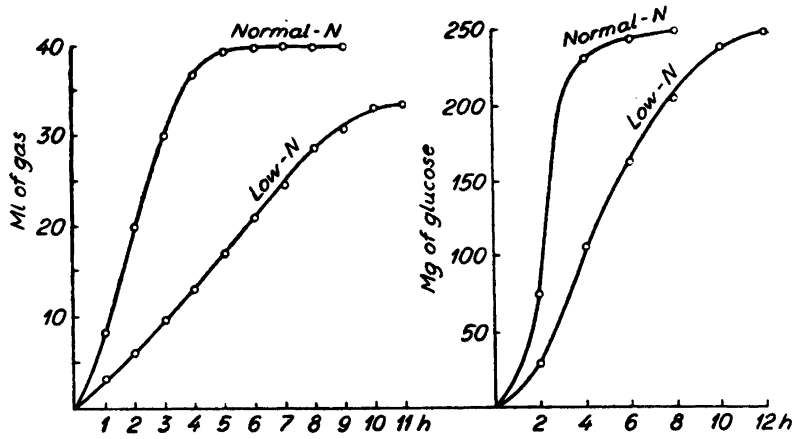


Fig. 1. Fermentation of 250 mg glucose in parallel experiments by normal-N and low-N masses of Aerobacter aerogenes K_3. Gas formation (left) and consumption of sugar (right).

RESULTS AND DISCUSSION

Fermentation velocity was followed in experiment 6 by gas evolution and decrease of sugar (Fig. 1). The low-N bacterial mass used in this experiment and the normal-N bacterial mass contained respectively 9.5 % and 13.1 % N based on dry matter. Judged both by gas evolution and sugar consumption, the rate of fermentation with normal-N mass was about twice as high as with the low-N mass, when calculated per unit nitrogen of the bacterial suspensions used. On the other hand, in experiment 5, in which the low-N bacterial mass contained still 10.5 % N of dry matter, the fermentation velocity was not considerably lower than with normal-N mass.

The fermentation products of normal-N and low-N bacterial masses are shown in Table 1.

Table. 1. Fermentation products of normal-N and low-N bacterial masses in per cent of fermented glucose

Expt. No.	N % of dry bacteria		Formic acid		Acetic acid		Lactic acid		Succinic acid		Ethanol	
	Norm.-N	Low-N	Norm.-N	Low-N	Norm.-N	Low-N	Norm.-N	Low-N	Norm.-N	Low-N	Norm.-N	Low-N
2	13.0	9.3	4.5	5.4	2.4	17.5	42	22	29	35	11	8
3	12.3	9.4	6.5	5.0	4.0	10.8	39	21	22	40	—	—
4	12.8	9.4	6.5	5.5	1.5	13.9	40	21	29	40	12	15
5	13.1	9.5	5.6	4.6	2.7	10.5	42	21	28	38	12	11
6	13.2	10.5	6.0	6.5	2.0	6.6	42	29	26	31	11	14

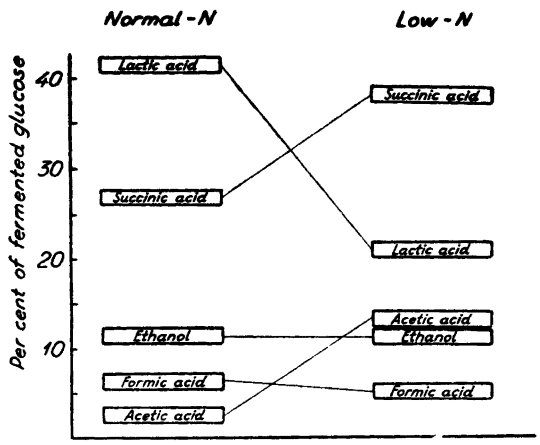


Fig. 2. Graphical illustration of the mutual proportions of different fermentation products with normal-N (avg. 12.8 %) and low-N (avg. 9.4 %) bacterial masses. Experiments 2, 3, 4, and 6 are included.

The results show that *Aerobacter* masses, which contain about 9.3—9.5 % N of dry matter, form from glucose only about half as much lactic acid as do the normal-N *Aerobacter*. These same low-N masses formed 3—9 times more acetic acid than did the normal-N masses, and about 30 % more succinic acid. In the production of formic acid and ethanol no systematic differences were noted. Results from experiment 5, in which the low-N bacterial mass contained more nitrogen, will be discussed below.

Graphical illustration in Fig. 2 shows the differences in the fermentation products formed from glucose by normal-N and low-N bacterial masses.

Since all the conditions in the parallel experiments with low-N and normal-N bacterial masses were as similar as possible, the differences in the fermentation products are probably due to differences in the enzyme system of these bacterial masses. Because of the small amount of lactic acid formed by low-N bacteria it would seem likely that the activity of lacticodehydrogenase has lessened with the decrease in the protein content of cells and therefore the reduction of pyruvic acid to lactic acid is retarded. This, again, would explain the increase in the decomposition of pyruvic acid via another route and the increase in the acetic acid and succinic acid.

It is interesting to note that Tikka [10] in this laboratory found as early as in the 1930's that fermentation products of *E. coli* essentially depend on the acidity of the fermentation solution. At about pH 6.3 the production of lactic acid was about 40 % and that of acetic acid about 5 %, whereas at pH 7.1

the amount of each was about 20 %. The quantitative differences observed by us between the fermentation products of normal-N and low-N *Aerobacters* are very much alike although the pH of both fermentation solutions was kept as constant as possible, *i.e.* 6—6.2. Lowering of the N-content of cells thus affects the enzyme system functioning in the fermentation perhaps in the same way as does the change of pH from acid to alkaline in the fermentation solutions of normal-N bacteria.

Additional evidence that retardation of fermentation and change in the mutual relations of the fermentation products are in fact due to the decrease in the N-content of cells, is provided by the results obtained with low-N *Aerogenes* bacteria of different N-content. Expt. 5 in Table 1 represents fermentation products of a bacterial mass, which contained 10.5 % N of dry matter. The fermentation velocity with this mass was nearly the same as with normal-N masses and the fermentation products differed much less from those obtained with normal-N masses than from those obtained with masses containing 9.3—9.5 % N.

The carbon content of the analyzed fermentation products expressed as per cent of the carbon in glucose is given in Table 2.

Table 2. Carbon content of the analyzed fermentation products in per cent of carbon in glucose acc. to Expt. 6

	C in per cent of C in glucose	
	Normal-N bacteria	Low-N bacteria
Formic acid	3.6	3.0
Acetic acid	2.7	10.5
Lactic acid	41.8	21.0
Succinic acid	28.3	37.0
Ethanol	16.4	14.3
	92.8	85.8

The carbon dioxide content of the gas evolved was not determined, hence, the carbon thus removed is unknown. However, it is evident that normal-N bacteria have not formed considerable amounts of other fermentation products except those mentioned above. On the other hand, low-N bacteria have formed other products in quite appreciable quantities, perhaps acetylmethylcarbinol and butyleneglycol. However, they were not determined and hence, no fermentation balance can be presented as a result of the analyses.

SUMMARY

Low-N *Aerobacter aerogenes* ferments glucose at a noticeably slower rate than onrmal-N bacteria. If the N-content of the cells is lowered from normal, by about

13 %, to 9.3—9.5 % of dry matter the fermentation velocity decreases by about half. Lowering of the N-content to 10.5 % affects but slightly the velocity.

The mutual proportions of the fermentation products are greatly changed by the decrease in the N-content. The cells containing 9.3—9.5 % N produce only about half as much lactic acid as do the normal-N cells, but much more acetic acid and succinic acid. No systematic differences were observed in the amounts of ethanol and formic acid. Low-N bacteria probably form significant amounts of other fermentation products, too, which have not been determined. The results are discussed.

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Inhibition of Oxidative Nitrogen Fixation in Ultrasonic Field by Volatile Substances

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The cause of the oxidation reactions in the ultrasonic field is still a disputed problem. However, it is general to consider cavitation a precondition for the reactions. In this connection attention is drawn to the action of substances inhibiting oxidation reactions. According to Bresler [1] the oxidation reactions are generally inhibited by the effect of substances with a high vapour pressure (of the order of 1 atm.). The inhibition could then be explained by assuming that the substances with a high vapour pressure are distilled from the aqueous solution to the cavitation bubble causing therein so high a gas pressure that electric discharges cannot take place within the bubble. In this way he explains *e. g.* the inhibitory effect of ammonia and ether on the oxidation phenomena.

In the present work a study is made on the inhibitory effect of different volatile substances on nitrogen fixation in the ultrasonic field. The aim of the work is to throw light on the manner of effect of these inhibitors.

METHODS

Ultrasonic vibrations were produced as described previously [2]. Since the earlier studies had revealed that in a strongly acid solution (pH 1–2) chiefly nitrate is formed and in a slightly acid or alkaline solution chiefly nitrite, the experiments were made at about pH 6.5. However, when volatile fatty acids were used the pH was lower (pH 3–6), depending on the amount and dissociation of fatty acid. Nitrogen fixation in our experimental conditions could be followed by nitrite determinations [3] although some nitrate-N was formed. In higher concentrations of fatty acid nitrite determination no longer gives a correct picture of the N-fixation but this has no essential significance since the low concentrations of inhibitors are the most important.

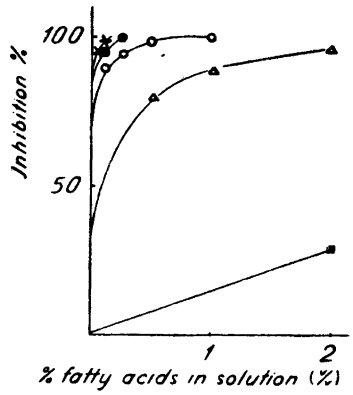


Fig. 1. Inhibitory effect of volatile fatty acids on nitrogen fixation.

□ *Acetic acid* △ *Propionic acid*
○ *Butyric acid* ● *I s o-valeric acid*
× *Caproic acid*

EXPERIMENTS AND RESULTS

100 ml of the test solution were used in the experiments and free access of air was allowed to the test cylinder. The temperature of the solution was maintained at about 20°C by means of cold water passing through the solution in a coil-shaped tube. The inhibition was calculated as per cent of nitrite nitrogen in the solution without added inhibitors. The reaction time was 10 min, except in experiments with methylaniline and aniline, when it was 5 min. Figures 1—5 show the inhibitory effect on N-fixation by volatile fatty acids (1), alcohols (2), aldehydes (3), aniline and methylaniline (4), and benzene, toluene and xylene (5).

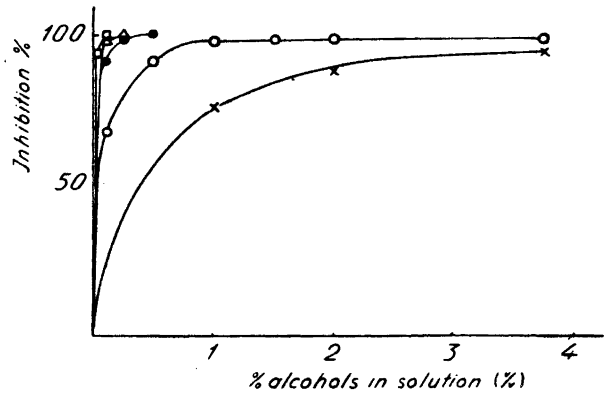


Fig. 2. Inhibitory effect of different alcohols.

× *Methylalcohol* ○ *Ethylalcohol* ● *Propylalcohol*
△ n-*Butyl alcohol* □ *I s o-amylalcohol*

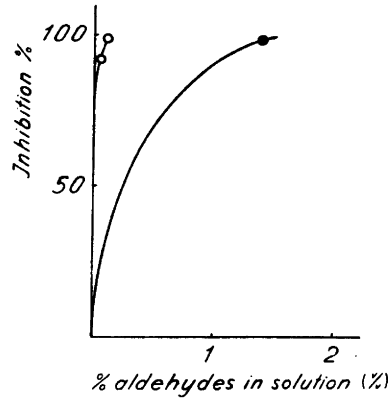


Fig. 3. Inhibitory effect of aldehydes.

○ *Acetaldehyde* ● *Formaldehyde*

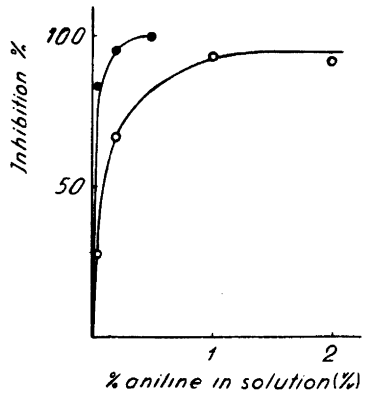


Fig. 4. Inhibitory effect of aniline and methylaniline.

● *Methylaniline* ○ *Aniline*

With respect to the inhibitory effect of fatty acids it is noteworthy that in the presence of propionic acid nitrite was formed within the first 5 min but afterwards it decreased, disappearing entirely after 10 min (Fig. 6). An abundance of hydrogen peroxide was then present in the solution.

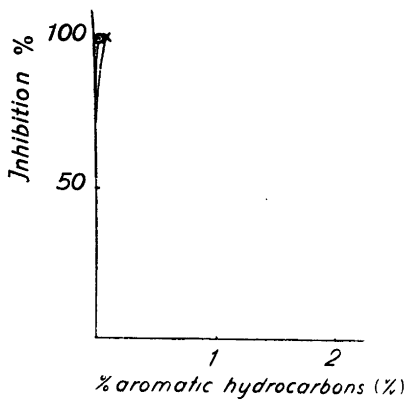


Fig. 5. Inhibitory effect of benzene, toluene, and xylene.

○ *Benzene*
• *Toluene*
× *Xylene*

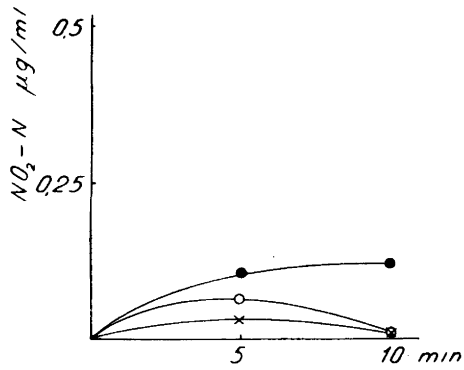


Fig. 6. Formation and disappearance of nitrite at different concentrations of propionic acid.

○ *0.5 % (v/v) propionic acid*
● *1 % (v/v) propionic acid*
× *2 % (v/v) propionic acid*

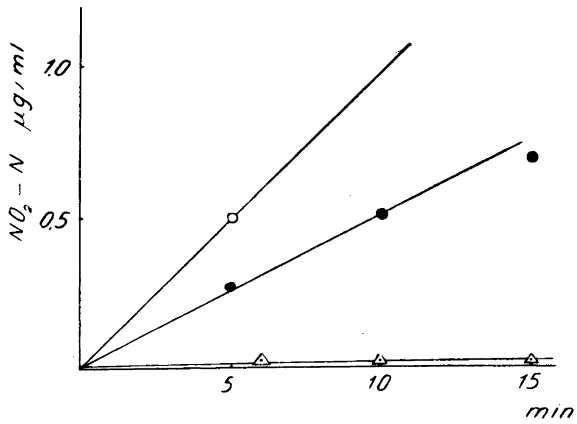


Fig. 7. Nitrite formation in solution of i s o-valeric acid at pH abt. 5 and 11.5, in butyl alcohol at pH abt. 6.5 and 10.

○ *distilled water*
● *0.25 % (v/v) i s o-valeric acid neutralized to pH 11.5*
△ *0.25 % (v/v) butyl alcohol, pH 10*
• *0.25 % (v/v) i s o-valeric acid, not neutralized*

If the fatty acids are neutralized they lose a great part of their inhibitory effect. Fig. 7 shows nitrite formation in 0.25 % solution of *iso*-valeric acid which was neutralized with NaOH to pH 11.5. It can be seen from the same figure that the formation of nitrite was completely inhibited in a corresponding solution of *iso*-valeric acid which was not neutralized. Butyl alcohol completely prevented nitrite formation in a solution made alkaline with NaOH as well as in a pure water solution without NaOH.

DISCUSSION

The results reported on the inhibitory effect of volatile substances on the nitrogen fixation in the ultrasonic field show that the inhibition grows with the increase in the volatility which in the homologous series takes place up to a certain limit with the lengthening of the carbon chain.

The volatilities with steam of the examined substances from dilute solutions are presented in Tables 1 and 2.

In vacuo, at a pressure of about 15 mm the distillation of acetic, propionic, and butyric acids is somewhat lower than at ordinary pressure, but still of the same order. The distillation constant of propionic acid from dilute water solution is *in vacuo* about 1, hence, vapour removed from 1 % solution of propionic acid contains 1 % propionic acid and 99 % water.

Table 1. Distillation constants of some volatile fatty acids (dilute water solutions).

Fatty acids	Concentration %	Distillation constant *k*	Half-distillation value %
Acetic acid [4]	0.09	0.657	36.6
	0.51	0.657	36.6
	1.90	0.660	36.7
Propionic acid [4]	0.07	1.235	57.5
	0.13	1.239	57.6
	1.32	1.270	58.5
Butyric acid [4]	0.05	1.92	73.5
	0.16	1.96	74.4
	0.88	2.00	75.0
	2.29	2.02	75.3
n-Valeric acid [4]		2.50	82.4
iso-Valeric acid [5]		3.27	89.6
Caproic acid [5]		3.08	88.2

Table 2. Distillation constants of some alcohols, aldehydes and anilines (dilute water solutions).

Alcohols	Concentration %	Distillation constant *k*	Half-distillation value %
Methylalcohol [4]	0.05	8.9	99.8
Ethylalcohol [4]	0.10	12.9	~100
Aldehydes			
Formaldehyde [4]	0.09	2.6	83.0
Acetaldehyde [4]	0.04	>40	~100
Anilines			
Aniline [4]	0.05	5.5	98
Methylaniline [4]	0.03	~16	~100

In the light of our results the conclusion that the inhibitory effect of the volatile substances is due to their distillation to the cavitation bubbles does not explain the phenomenon. If we examine our results obtained with different fatty acids we can note that propionic acid in a 0.5 % solution will inhibit nitrogen fixation by as much as 80 %, yet the steam evaporating from such a solution *in vacuo* contains only about 0.5 % propionic acid (Table 1). In 0.1 % butyric

acid solution the inhibition is 90 %, the steam containing only 0.14 % butyric acid. The vapour pressure of propionic acid and butyric acid is so small that we do not consider their inhibitory effect explicable by Bresler's idea that a high gas pressure inside the cavitation bubble prevents electric discharges therein.

On the other hand, the effect of the examined substances on the surface tension seems to deserve serious consideration as an explanation for the observed inhibition. The inhibitory effects of the substances used have been the greater, the more they have lowered the surface tension of the solution. The probable dependence of the energetic state of cavitation on the surface tension may be the cause of the inhibition of the oxidative N-fixation in our experiments.

In fatty acid solution made alkaline (experiment with *iso*-valeric acid on p. 664) inhibition of nitrogen fixation is slight. This can be explained by assuming that the alkali salts of fatty acids lower the surface tension less than do the corresponding free acids [6]. It can also be assumed that lowering of the surface tension takes place at a slower rate in the alkali salt solution than in the corresponding solutions of free acids. The duration of the cavitation bubble in the US field is of the order of 10^{-6} sec. The time being so brief, the surface tension is dynamic yet decreased in low-molecular fatty acid solutions and corresponding alcohol solutions. In soap solutions (observations with Na-oleate solution [6]) decrease in the dynamic surface tension is slower, and therefore, no change occurs in 10^{-6} sec.

We have not yet examined to what extent the volatile substances possibly are oxidized by the effect of ultrasonics. The observation made by us that nitrite is at first more abundantly formed at low propionic acid concentrations and that it is greatly decreased with prolonged reaction time, suggests participation of propionic acid in some way in the oxidation reaction in the ultrasonic field. Prudhomme and Grabar [7] have found that the benzene ring is oxidized in the ultrasonic field, hence, the strong inhibitory effect of aromatic hydrocarbons on nitrogen fixation could possibly be due to this (competition between nitrogen and aromatic hydrocarbons).

Breuning [8] has reported that nitrite is formed in an ultrasonic field, which is brought about with so small energies that cavitation is considered impossible. Accordingly, formation of nitrite would not be associated with the reactions taking place in the cavitation bubble but would proceed along quite different lines. There is, however, no confirmation to this observation.

SUMMARY

Inhibition of nitrogen fixation in the ultrasonic field by certain organic volatile substances of homologous series has been examined. The organic substances belonged to aliphatic fatty acids, aldehydes, alcohols, aromatic hydrocarbons, and amines which were used in 0.05—2 % solution. The reaction took place in water solutions at pH about 6.5, in fatty acid solutions at pH 3—6.

On the basis of the results the cause of inhibition is discussed. The most likely reason suggested is the influence of the volatile substances on the surface activity and the resultant energetic changes in the cavitation. Distillation of substances into the cavitation bubbles has not been regarded as sufficient explanation.

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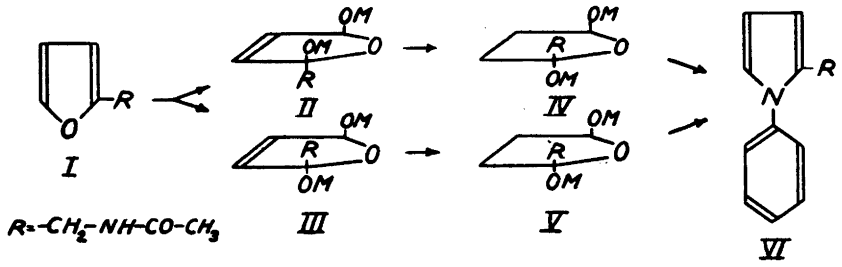
Preparation of *Cis* and *Trans* 2,5-Dimethoxy-2-(acetamidomethyl)-2,5-dihydrofuran, of *Cis* and *Trans* 2,5-Dimethoxy-2-(acetamidomethyl)-tetrahydrofuran and of 1-Phenyl-2-(acetamidomethyl)-pyrrole

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A new electrolytic method for the methoxylation of furans has been described previously [1–4]. This has now been used to methoxylate 2-(acetamidomethyl)-furan (N-furfurylacetamide) (I) to the corresponding dimethoxydihydrofuran. The yield of distilled, analytically pure dimethoxy-2-(acetamidomethyl)-dihydrofuran was as high as 96 per cent. The product was partially crystalline and could be separated by crystallization from ether into about equal parts of a crystalline isomer (melting point 104°) and another isomer, which it was not possible to bring to crystallization. Both isomers were hydrogenated catalytically to the corresponding tetrahydrofurans. Crystalline dimethoxy-2-(acetamidomethyl)-dihydrofuran gave a crystalline dimethoxy-2-(acetamidomethyl)-tetrahydrofuran while the liquid isomer gave a liquid tetrahydrofuran.

When heated with aniline in acetic acid, both tetrahydrofurans gave a high yield of a compound with the formula $C_{11}H_{11}N_2(COCH_3)$. This compound, which could be distilled in vacuum and then crystallized from ether (melting point 88°), gave a positive Ehrlich reaction (for pyrroles with a free α-position), and must therefore be 1-phenyl-2-(acetamidomethyl)-pyrrole (VI). It follows that the tetrahydrofurans are *cis* and *trans* 2,5-dimethoxy-2-(acetamidomethyl)-tetrahydrofuran (formulas IV and V) and that the dihydrofurans are *cis* and *trans* 2,5-dimethoxy-2-(acetamidomethyl)-2,5-dihydrofuran (II and III). Compounds I—VI are all new.



The simple transformation of a 2,5-dimethoxytetrahydrofuran into a pyrrole probably proceeds through the corresponding 1,4-dicarbonyl compound, which then enters a Paal-Knorr pyrrole synthesis with the amine.

EXPERIMENTAL

Microanalyses by Kirsten Glens and Ernst Boss

2-(Acetamidomethyl)-furan (I). 75.0 g of acetic anhydride (technical product) in 75 ml of dry ether was added to a cooled (— 20°), stirred solution of 60.0 g of furfurylamine [5,6] in 100 ml of ether and the resulting amide isolated by distillation. Yield 82.0 g of 2-(acetamidomethyl)-furan = 95%; colourless liquid, b.p.$_9$ = 146—148°; n_D^{25} = 1.4998.

$C_5H_6ON(COCH_3)$ (139.2) Calc. C 60.4 H 6.5 N 10.1 $COCH_3$ 30.9
Found » 60.7 » 6.5 » 10.1 » 29.0

Cis *and* trans *2,5-dimethoxy-2-(acetamidomethyl)-2,5-dihydrofuran (II and III).* 5.00 g of ammonium bromide (0.051 mole) was dissolved in 260 ml of methanol (technical product) and 41.7 g of 2-(acetamidomethyl)-furan (0.30 mole) and the mixture electrolyzed with the set-up used previously for the electrolytic methoxylation of furan [1].

After electrolysis the liquid in the cell was colourless in the upper part of the cell and yellow near the bottom. The liquid was poured into a solution of sodium methoxide

Table 1.

Hours	Current (ampere)	Potential across the cell during electrolysis (volt)	Temperature in the cell °C	Ampere hours (per cent of theoretical amount)
0.5	3.3	4.5	— 11	1.7 (11 %)
2.2	3.0	4.8	— 13	7.3 (45 %)
5.3	2.3	5.1	— 12	16.3 (101 %)
6.0	2.0	5.2	— 12	17.7 (110 %)

(1.20 g of sodium (0.052 mole) in 20 ml of methanol) and the methanol and the ammonia evaporated in vacuum. The residue was distilled further in vacuum without filtering off the precipitate of sodium bromide. Yield 57.8 g of 2,5-dimethoxy-2-(acetamidomethyl)-2,5-dihydrofuran = 96 %; colourless, partially crystalline product, $b.p._{0.6} = 119-134°$.

$C_5H_6ON(COCH_3)(OCH_3)_2$ (201.2) Calc. C 53.7 H 7.5 N 7.0 $COCH_3$ 21.4 OCH_3 30.9
Found » 53.6 » 7.8 » 6.5 » 21.1 » 30.5

Crystallization from 100 ml of ether gave 23.8 g of white crystals, m.p. 99—101° (Hershberg apparatus, corr.). Recrystallization gave a product melting at 104—105° (another crystallization did not change the m.p.).

Found C 53.8 H 7.8 N 6.7 $COCH_3$ 21.2 OCH_3 31.0

The mother liquid from the crystals was distilled in vacuum. Yield 31.6 g of a colourless, very viscous liquid, $b.p._{0.1} = 119-124°$ (no fore-run and no residue).

Found C 54.0 H 7.7 N 7.2 $COCH_3$ 21.1 OCH_3 30.1

Cis *and* trans *2,5-dimethoxy-2-(acetamidomethyl)-tetrahydrofuran (IV and V).* 20.0 g of crystalline 2,5-dimethoxy-2-(acetamidomethyl)-2,5-dihydrofuran and 80 ml of methanol were shaken with 5 g of Raney nickel under 100 atmospheres of hydrogen for 4 hours at room temperature. Yield 19.6 g of crystalline 2,5-dimethoxy-2-(acetamidomethyl)-tetrahydrofuran = 97 %; colourless product, $b.p._{0.1} = 117-124°$; m.p. 53—54°. M.p. after crystallization from ether 58—60° (further crystallization did not change the m.p.).

$C_5H_8ON(COCH_3)(OCH_3)_2$ (203.2) Calc. C 53.2 H 8.4 N 6.9 $COCH_3$ 21.2 OCH_3 30.5
Found. » 53.0 » 8.6 » 6.8 » 20.9 » 30.9

20.0 g of liquid, 2,5-dimethoxy-2-(acetamidomethyl)-2,5-dihydrofuran gave in the same way 19.4 g of liquid 2,5-dimethoxy-2-(acetamidomethyl)-tetrahydrofuran = 96 %; colourless, very viscous oil, $b.p._{0.1} = 111-117°$.

Found C 53.0 H 8.4 N 6.9 $COCH_3$ 21.4 OCH_3 30.2

1-Phenyl-2-(acetamidomethyl)-pyrrole (VI). 2.03 g of crystalline 2,5-dimethoxy-2-(acetamidomethyl)-tetrahydrofuran (0.01 mole), 0.93 g of aniline (0.01 mole) and 3.0 ml of glacial acetic acid were refluxed for 30 minutes and the resulting clear, reddish-brown solution distilled. Yield 1.88 g of 1-phenyl-2-(acetamidomethyl)-pyrrole = 88 %; colourless product, $b.p._{0.4} = 166-171°$; m.p. 85—89°; Ehrlich reaction for pyrroles with a free α-position positive.

$C_{11}H_{11}N_2(COCH_3)$ (214.3) Calc. C 72.9 H 6.6 N 13.1 $COCH_3$ 20.1
Found » 72.4 » 6.7 » 13.3 » 19.8

Crystallization from ether gave 1.50 g; m.p. 88—91° (further crystallizations did not change the m.p.).

Found C 72.6 H 6.7 N 13.3 $COCH_3$ 19.1

2.03 g of liquid 2,5-dimethoxy-2-(acetamidomethyl)-tetrahydrofuran gave in the same way 2.08 g of 1-phenyl-2-(acetamidomethyl)-pyrrole = 97 %; $b.p._{0.1} = 159-166°$; m.p. 84—88°; Ehrlich reaction positive. Crystallization from ether gave 1.40 g; m.p. and mixed m.p. with the above sample 88—90°.

Found C 73.0 H 6.5 N 13.2 $COCH_3$ 19.1

SUMMARY

2-(Acetamidomethyl)-furan (I) has been methoxylated by electrolysis to a mixture of *cis* and *trans* 2,5-dimethoxy-2-(acetamidomethyl)-2,5-dihydrofuran (II and III). Catalytic hydrogenation of the dihydrofurans gave the

corresponding *cis* and *trans* tetrahydrofurans IV and V. Both tetrahydrofurans gave 1-phenyl-2-(acetamidomethyl)-pyrrole with aniline in acetic acid. All the above compounds are new.

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Gasreibung und Adsorption

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Im Gegensatz zu der bekannten Annahme, dass bei der laminaren Strömung durch enge Rohre die gebildete Adsorptionsschicht vollkommen starr und für den Reibungsvorgang ohne Bedeutung sei, wurde vor längerer Zeit — im Anschlusse an schon vorher entwickelte Vorstellungen [1] — die Meinung aufgestellt, dass diese Wandschicht einen je nach Umständen mehr oder weniger in Betracht kommenden Beitrag zum Reibungseffect liefere [2]. Das damals in der Litteratur vorliegende experimentelle Material liess einen unzweifelhaften Zusammenhang der inneren Reibung von Gasgemischen mit der Adsorption der Componenten erkennen.

Da die Reibung stets in Glascapillaren bestimmt worden war, die Adsorption dagegen an anderen Materialien (meist Kohle) so wurde später [3] die Adsorption von Kohlendioxyd und Wasserstoff auch an Glas untersucht, die sich als analog zu der an Kohle erwies und also den oben betonten Zusammenhang bestätigte.

Es war demnach zu erwarten, dass die Beteiligung der Wandschicht am Reibungsvorgang um so deutlicher zu Tage treten sollte, je dicker die Adsorptionsschicht im Verhältnis zum Radius des durchströmten Rohres ist. Eine darauf gerichtete, im Jahre 1932 abgeschlossene Untersuchung von Alfred Freund [4] bestätigte auch diesen Schluss.

Später sind auch andere Forscher zu dieser Auffassung gekommen (vgl. Litteratur unter 5). Da diesen zwar die oben zuerst erwähnten Mitteilungen wohl bekannt sein konnten, nicht aber die bisher unveröffentlicht gebliebene Untersuchung von Freund, so soll deren Inhalt im Folgenden auszugsweise wiedergegeben werden.

Freund bestimmte die Strömungszeit einiger Gase unter gleichen äusseren Bedingungen einerseits durch eine lange Glascapillare, andrerseits durch eine Jenaer Glasfrittenplatte von bekannter mittlerer Porenweite. Die capillaren

Räume in einer solchen sind zwar viel kürzer als das Rohr, aber auch sehr viel enger, so dass die Wandschicht in den Poren der Fritte im Verhältnis zu ihren Radien viel dicker war als in dem Rohr.

VERSUCHSANORDNUNG

Diese bestand aus den Vorrichtungen für Reinigung, Mischung und Analyse der Gase, einschliesslich einer Hilfsanordnung für Zufügung bekannter kleiner Wassermengen, ferner den Apparaten für Druckregulirung und Messung von Volumen und Strömungszeit sowie den im Thermostaten gehaltenen Strömungsrohren nebst Hilfsteilen *.

a. Versuchsmaterial. Untersucht wurden Wasserstoff, Stickstoff und deren Gemische, sowohl hochtrocken wie mit kleinen Wasserdampfzusätzen, sowie Sauerstoff mit kleinem Gehalte an Stickstoff. Die geplante Ausdehnung auf andere Gase wurde durch äussere Umstände verhindert.

Die aus Bomben entnommenen Gase wurden nach bekannten Methoden ** gereinigt und die Gemische sowohl vor wie nach dem Durchtritt durch die Messanordnung analysirt. Jedes Gas wurde in so grosser Menge vorbereitet, dass die Strömungsrohre vor dem Versuche 10 bis 20 Stunden unter Ausheizen damit durchspült werden konnten.

Zwecks Beladung mit Wasserdampf wurde in einem kleinen Glasgefässe Wasser mittels einer Platinspirale erhitzt und der Dampf dem Gase zugeführt. Dieses passirte sodann einen genau auf 0°, 6° oder 18° gehaltenen Condensator.

b. Die Druckregulirung war sehr gründlich durchgearbeitet und geprüft und nicht nur über längere Zeiträume völlig zuverlässig sondern auch sehr genau. Dies war nötig, weil die Strömungszeit, welche zwischen 5 und 15 Minuten lag, auf weniger als 0,1 % gesichert sein sollte. Differenzen der Einzelmessungen von 0,01 % bei Wiederholung des Versuches kamen nur vereinzelt vor.

Von allen Einzelheiten dieses Teils der Versuchsanordnung sei nur die Feinregulirung für den treibenden Druck vor dem Messrohr kurz erwähnt. Sie bestand aus einem horizontalen Huygensmanometer [6] mit tief rot gefärbter Flüssigkeit (Tetralin), deren Meniscus einerseits mittels Glasscala abgelesen wurde, andrerseits den Weg eines schmalen Lichtstrahles vor einer Photozelle unterbrach oder freigab. Die Zelle steuerte direct ein Elektronenrelais und durch dieses ein die weitere Apparatur betätigendes mechanisches Relais. Diese Anordnung sprach völlig zuverlässig auf 1 bis $2 \cdot 10^{-3}$ mm Hg an, was mehr als ausreichend war.

Der Druck p_1 vor dem Strömungsrohr betrug rund 108, der dahinter, p_2, rund 20 g/cm^2. Der mittlere treibende Ueberdruck, $108{,}25 - 19{,}85 = 88{,}40$ g/cm^2, war auf etwa 0,02 % genau definirt.

c. Zur Volumenbestimmung des durchgeströmten Gases diente eine mit zwei Marken versehene und genau ausgemessene Glaskugel (etwa 0,6 Liter), die sich neben den Strömungsrohren im stets auf 20,0° ± 0,005 gehaltenen Thermostaten befand und aus der das Füllwasser durch das Gas verdrängt wurde. Beim Passiren dieser beiden Marken wurde eine Stoppuhr mittels der fein arbeitenden elektrischen Auslösungsvorrichtung nach Lux [7], welche direct auf die Unruhe der Uhr wirkt, ein- oder ausgeschaltet. Damit konnte

* Auführlicshe Angaben über alle Einzelheiten enthält die Dissertation.

** Entfernung von oxydablen Verunreinigungen durch alkalische Permanganatlösung, von CO_2 durch Kali, von O_2 durch glühendes Kupfer, von Wasser durch $CaCl_2$ und P_2O_5.

die Zeit auf 0,2 sec. genau gemessen werden, d.h. bei einer mittleren Dauer von 10 Minuten auf 0,03 %.

d. Die Strömungsrohre. In jedem Versuche wurde eine genau ausgemessene Glascapillare von 17,50 cm Länge und einem mittleren Durchmesser von 0,02778 cm mit einer Jenaer Glasfrittenplatte verglichen. Deren Durchmesser betrug 2 cm, ihre Dicke 0,20 cm und die mittlere Porenweite — nach Angabe der Firma Schott u. Gen. — $1{,}5 \cdot 10^{-4}$ cm. Eine solche Platte stellt zwar kein Bündel gleichweiter parallel geschalteter Capillarrohre dar, doch erfolgt die Strömung in diesen engen Poren trotz ihrer Kürze so langsam, dass Turbulenzgefahr noch weniger besteht als bei dem Glasrohr.

Immerhin sind die Poren noch so weit, dass die unten mitzuteilenden Effecte keinesfalls aus einer Querschnittsverminderung durch die Dicke einer starren ruhenden Wandschicht erklärt werden können. Die Dimensionen beider Teile waren so gewählt, dass die Durchlaufszeiten ungefähr gleich lang waren. Vor jedem Versuche wurden Platte und Rohr über Nacht mit dem Versuchsgase gespült und scharf getrocknet; die Platte wurde dabei durch einen kleinen übergeschobenen elektrischen Ofen auf 380° erhitzt.

e. Die Berechnung der Ergebnisse konnte natürlich nur für das Capillarrohr quantitativ durchgeführt werden, für den Hauptzweck der Untersuchung ist aber nur das Verhältnis der Strömungszeiten erforderlich. Hierfür braucht auch die Hagenbachsche Correctur nicht angebracht zu werden, doch wurde diese für das Rohr berechnet (s.u.).

ERGEBNISSE

Die Mittelwerte aller Einzelversuche sind in der Tabelle 1 zusammengestellt. Die erste Spalte enthält die Gaszusammensetzung nach Volumenprocent, wobei alle Gase als ideal angenommen sind, die zweite unter R den im Capillarrohr gefundenen Reibungscoefficienten in c.g.s.-Einheiten (unter Weglassung des gemeinsamen Factors 10^{-7}), die dritte unter M die zugehörigen Glasmembranwerte. Diese sind aus den gefundenen Durchflusszeiten so berechnet, als wäre das Gas durch das Rohr geströmt, statt durch die Membran, d.h. die Werte unter M verhalten sich zu denen unter R wie die gefundenen Durchflusszeiten.

Absolutwerte. Obwohl die Untersuchung nicht auf absolute Bestimmung gerichtet war, sind doch die Resultate so genau, dass eine solche Berechnung vorgenommen werden kann.

Der Mittelwert des im Rohr bestimmten Reibungscoefficienten von *Stickstoff* beträgt $\eta = 1791 \cdot 10^{-7}$. Die Hagenbachsche Correctur berechnet sich zu $-17 \cdot 10^{-7}$, wird sie angebracht, so ergibt sich 1773. Sollte unser »atmosphärischer« Stickstoff noch Argon enthalten haben — was für den Hauptzweck der Untersuchung belanglos ist — und schätzen wir dessen Menge auf 1 %, so wäre nochmals um — 0,3 % zu corrigieren und als Endwert für reinen Stickstoff 1768 anzunehmen.

Für *Wasserstoff* wurde 908,4 gefunden, nach Anbringung der hier rund 0,4 % ausmachenden Hagenbachcorrectur 904,8.

Tabelle 1.

Nr	% N_2	% H_2	% O_2	% H_2O	R	M
1	100	—	—	—	1798,9	1647,4
2	99,4	—	—	0,60	1793,2	1629,6
3	100	—	—	—	—	1620,3
4	96,9	3,13	—	—	1786,1	1605,6
5	96,3	3,11	—	0,60	1783,8	1600,3
6	96,0	3,10	—	0,92	1778,6	1596,0
7	100	—	—	—	1789,0	1623,8
8	81,6	18,4	—	—	1744,7	1498,9
9	81,1	18,3	—	0,60	1741,6	1492,6
10	80,9	18,2	—	0,91	1742,2	1489,5
11	80,0	18,0	—	2,01	1733,2	1481,6
12	100	—	—	—	1788,8	1617,6
13	47,9	52,1	—	—	1607,2	1221,5
14	47,7	51,7	—	0,60	1604,4	1214,2
15	47,5	51,6	—	0,92	1603,0	1214,6
16	47,0	51,0	—	2,04	1635,6	1239,9
17	100	—	—	—	1791,1	1616,3
18	20,4	79,6	—	—	1336,4	918,0
19	20,3	79,1	—	0,61	1338,6	914,4
20	20,2	78,9	—	0,93	1339,8	915,5
21	19,9	78,0	—	2.04	1341,9	916,4
22	100	—	—	—	1794,7	616,8
23	2,20	97,8	—	—	989,2	671,3
24	2,18	97,2	—	0,61	1001,2	671,5
25	2,18	96,9	—	0,93	1005,6	670,6
26	2,16	95,8	—	2,06	1020,4	678,6
27	100	—	—	—	1796,0	1618,9
28	—	100	—	—	910,9	625,5
29	—	99,4	—	0,61	923,0	626,4
30	—	99,1	—	0,93	930,2	629,5
31	—	97,9	—	2,06	951,2	638,5
32	100	—	—	—	—	1620,9
33	3,70	—	96,3	—	2065,4	1815,6
34	3,68	—	95,7	0.60	—	1804,5

Das Gemisch mit 96,3 % Sauerstoff und 3,7 % Stickstoff ergab 2062,0, corrigiert für kinetische Energie 2043,5, woraus nach der Mischungsregel für reinen *Sauerstoff* folgt 2054.

Für Wasserdampf lässt sich wegen der Kleinheit der zugefügten Mengen die Extrapolation nicht vornehmen.

Einfluss der Wandschicht. Um die mit beiden Strömungsanordnungen gefundenen Reibungswerte auf einander zu beziehen, brauchen wir einen Reductionsfactor, der dann in allen Fällen richtig ist, wenn die mit der Membran gemessenen Zeiten *nicht* durch die Mitwirkung der Wandschicht verändert worden sein können, d.h. wenn keine praktisch in Betracht kommende Adsorption vorliegt.

Dieser Fall ist nahezu vollkommen realisirt bei trockenem Wasserstoff. Für diesen finden wir in Tabelle 1 die Werte $M = 625{,}7$ und $R = 908{,}4$. Legen wir diese Zahl zu Grunde und multipliciren alle Rohrwerte mit dem Factor $625{,}7/908{,}4 = 0{,}688_8$, so ergeben sich diejenigen Coefficienten A, welche mit der Membran ohne Mitwirkung einer Adsorptionsschicht gefunden worden wären. Wenn dann $M > [A = \text{R}.\, 0{,}688_8]$, so hat die Wandschicht einen grösseren Reibungscoefficienten als der innere Teil der Gasmasse, und umgekehrt.

Die in dieser Weise reducirten Coefficienten der Combination $N_2 - H_2$ stehen in Tabelle 2. Sie sind, mit einer unbeträchtlichen Ausnahme, stets kleiner als M, also muss die Reibung in der Wandschicht erheblich grösser sein als im inneren Teile. Dies ist zu erwarten, denn der Stickstoff, der einen grösseren Coefficienten hat als das innere wasserstoffhaltige Gemisch, ist in der Wandschicht angereichert. Die Differenz $M - A$ nimmt aber gegen das Stickstoffende hin immermehr zu und erreicht ihren grössten Wert gerade für reinen Stickstoff.

Querschnittsverminderung durch eine starre ruhende Wandschicht vermag — wie bereits angedeutet — diese Reibungserhöhungen nicht zu erklären. Die Dicke einer solchen Schicht müsste im vorliegenden Falle bis in die Grössen-

Tabelle 2.

% H_2	R	M	A
0	1791,0	1619,5	1234
3,13	1787,2 *	1604,8	1231
18,4	1746,0	1494,9	1202
52,1	1609,2	1223,0	1108
79,6	1336,2	919,8	921
97,8	987,2	671,7	680
100	908,4	625,7	625,7

* Interpolirt.

ordnung 10^{-6} cm reichen. Da dies wohl als ausgeschlossen gelten darf, müssen retardirende Kräfte angenommen werden, welche der Strömungsrichtung entgegen wirken und sich von der Wand an mit abnehmender Stärke bis in die »innere frei strömende» Säule geltend machen. Das radiale Gefälle der Strömungsgeschwindigkeit, welches ohne Mitwirkung dieser schon vor langer Zeit [Vgl. 1,2] postulierten Attractionskräfte parabolisch verlaufen würde, erhält dadurch eine andere Form.

Wenn also, wie dies neuerdings geschehen ist [Vgl. 5] die gaskinetische Theorie der Reibung eine Zerlegung in einen inneren (dreidimensionalen) Strömungsvorgang und einen (nahezu zweidimensionalen) Wandeffect vornimmt, so müssen jene Attractionskräfte mit in Betracht gezogen werden.

Bei den feuchten Gasen liegen die Verhältnisse etwas weniger einfach. Bilden wir für die Serie des stickstoff-freien feuchten Wasserstoffs wie oben die Differenzen $M-A$, so sind diese sehr klein. Hier besteht die Wandschicht zwar fast nur aus Wasser, von dem das innere Gemisch nur einen geringen Bruchteil enthält. Aber die Reibungscoefficienten von Wasser und Wasserstoff liegen einander sehr nahe, so dass man tatsächlich bei Mitwirkung der Wandschicht keine erhebliche Veränderung des Gesamteffectes erwarten darf.

Gehen wir dagegen zu den Gemischen mit hohem Stickstoffgehalt über (Nr 4—6, 8—11, 13—16), so werden jene Differenzen sehr gross und zwar positiv; der ausser dem Wasser in der Wandschicht enthaltene Stickstoff, dessen Reibungscoefficient ja auch hier grösser ist als der des inneren Gemisches, erhöht also den gesamten Effect. Auch dies ist zu erwarten. Dasselbe Bild zeigt der eine Versuch mit feuchtem wasserstoff-freiem Stickstoff (Nr 2).

Bei kleinem Stickstoffgehalt (Nr 18—21, 23—26) liegen jedenfalls complicirtere Verhältnisse vor, auf deren Ausdeutung hier vorläufig verzichtet werden muss.

Eine quantitative Discussion würde natürlich Kenntnis von Dicke und Zusammensetzung der Wandschicht voraussetzen und muss also unterbleiben. Als sichergestelltes Ergebnis aber finden wir die Bestätigung des in den Einleitungsworten angeführten Satzes, *dass bei dem Reibungsvorgange eine Mitwirkung der Wandschicht auftritt*, die ältere Vorstellung einer starren ruhenden Wandschicht also aufgegeben werden muss.

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4. Diese Abhandlung wurde von Herrn A. Freund 1932 zwecks Erwerbung des Doktorgrades der Philosophischen Fakultät zu Leipzig eingereicht und von dieser genehmigt. Äussere Umstände haben ihre sofortige Drucklegung sowie die geplante Erweiterung des Versuchsmaterials verhindert.
5. S. die letzterschienene Veröffentlichung über dieses Problem von W. Mervyn Jones, *Trans. Faraday Soc.* **47** (1951) 381 und die dort citirten Abhandlungen seit 1935.
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Effect of Ultraviolet Light on Absorption Spectra of Carbohydrates

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Kwiecinski *et al.*[1] showed that different carbohydrates after repeated recrystallisations have no specific absorption of ultraviolet light. Marchlewski and Urbánczyk [2] found, however, that hexoses and pentoses treated with alkali got an absorptionmaximum at 270 millimicrons. Starch boiled in alkali gets a band near 280 millimicrons shifting to 255 on acidifying [3].

During investigations of the effect of ultraviolet light upon the biological activity of acid mucopolysaccharides, Balazs and Laurent [4] found that the ultraviolet light absorption spectra of hyaluronic acid and heparin were changed after irradiation and a strong maximum developed at 265 millimicrons. Alkali increased the effect of irradiation.

In the present work we have tried to study the effect of ultraviolet light upon spectra of carbohydrates. That irradiation changes spectra of several other substances is well known. For references see Ellinger [5].

METHODS

For irradiation a mercury lamp with a known emission spectrum was used. Of the energy given off 50 % was localized on the wavelengths under 280 millimicrons. The solutions were irradiated in 0.1 cm thick quartz cuvettes, covered to prevent evaporation. During the irradiation the temperature in the solution rose from 20° C to 52° C in the first 20 minutes; after this time it remained constant. The increase in temperature proved to have a certain influence on the irradiation effect if compared with experiments carried out at constant temperature (20° C). The difference was, however, a relatively small one; therefore the remaining experiments were done without cooling. The adjustment of pH was effected with NaOH and HCl. The solutions were

left to stand some time at 4° C before measuring. All spectra were measured in a Beckman quartz spectrophotometer against a blank of irradiated solvent; we had found, namely, that also NaOH (used for pH-adjustment) showed increased extinction after irradiation, without any absorption maximum, however. The results are expressed as extinction E = -log transmittance.

EXPERIMENTS AND RESULTS

After irradiation of some polysaccharides (dextrin, gum arabic, hyaluronic acid, pectin, chondroitin sulphuric acid) a characteristical change of spectrum was obtained. An absorption maximum appeared at 265 millimicrons, and already a relatively short time of irradiation caused a considerable increase in extinction values. The reasons might be the following:

a. part of the great molecule is broken down and the rise of extinction is due to successively accumulated products, possibly after further action of light.
b. an intramolecular rearrangement, induced by the supplied energy, causes the spectral changes.

That ultraviolet light really causes a depolymerization is certain [4, 6]. After dialysis of an irradiated solution a great deal of the extinction increment disappears, but not all. This suggests a participation of both mentioned points.

We have investigated the spectra of glucose, galactose, mannose, fructose, glucosamine and galacturonic acid. All these substances give after irradiation rise to characteristical absorption with maximum at 265 millimicrons, like the polysaccharides. Here, too, the extinction increases quickly with prolonged irradiation time.

All our preparations had a high degree of purity (commercial pro analysi products, made by different manufacturers and in several different ways) and after computation of the possible extinction values it is obvious that the light absorption cannot be attributed to impurities. Compare, however, Kwiecinski *et al* [1].

The shape and values of the absorption curves received are in the following figures presented as a function of the time of irradiation (Fig. 1), the concentration of the irradiated substance (Fig. 2), and the pH of the solution (Fig. 3).

The influence of acidity can be shortly described in stating that

a. all investigated preparations showed higher light absorption, both irradiated and not irradiated, on rising pH.
b. the irradiation became more effective, as the alkalinity increased.

If a solution first is irradiated in alkaline milieu, and later acidified a shift in the maximum from 265 millimicrons towards 245 occurs and the E maximum

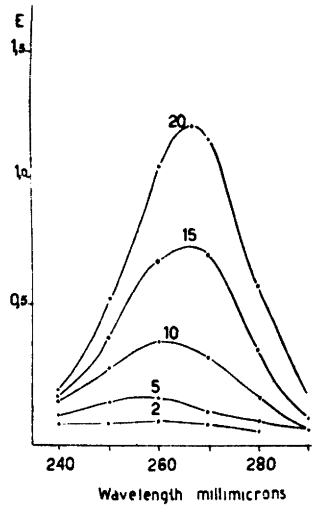


Fig. 1. 50 mg % glucose solution irradiated for 0, 2, 5, 10, 15 and 20 min. pH 9.7.

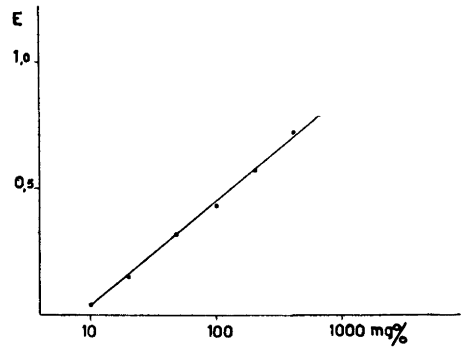


Fig. 2. Glucose in different concentrations irradiated for 10 min. pH 11. Abscissa: glucose concentration (logarithmic scale). Ordinate: Extinction at 265 millimicrons.

sinks considerably. In this connection it is interesting to note that ascorbic acid in weak alkaline solution has a maximum at 265. It shifts on acidifying to 245 [7].

The above is valid for all the monosaccharides tested by us. In addition to that the following holds good for galacturonic acid: after irradiation in

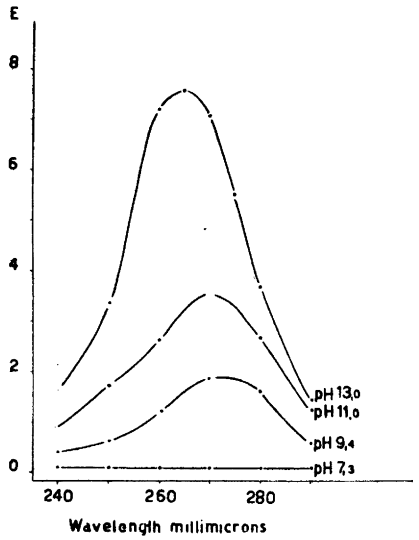


Fig. 3. 50 mg % glucose solution irradiated 30 min. pH 7.3, 9.4, 11.0 and 13.0. Absorption spectra determined without change of pH. E = value read on the photometer × dilution after irradiation.

strongly acid solution (spectrum measured also in acid solution) a maximum near 245 millimicrons. Glucosamine irradiated in acid and measured in alkaline solution gives a maximum near 295 millimicrons.

The postirradiation effect, if any was unsignificant. Experiments carried out in different saline solutions gave somewhat different results, which could be explained, in part, by the absorption of the salts themselves.

No provable CO_2 developed during irradiation.

Changes in absorption spectrum similiar to those just described could also be observed after boiling the sugar solution. Some monosaccharides were treated for 30 minutes in boiling water bath, pH 13. A great rise in extinction and a weak maximum near 265 appeared. This effect was, however, much less than after equal time of irradiation.

The changes in absorption spectra of proteins after ultraviolet irradiation have been observed by several authors. For references see McLaren [8]. Recently an attempt was made to explain those changes in chemical terms as modifications of the aromatic groups [9]. Considering, however, that a great deal of other substances are affected in the same way by ultraviolet irradiation, their interpretation must appear unsatisfactory.

SUMMARY

Ultraviolet irradiation upon alkaline solutions of carbohydrates changes their absorption spectra and forms a maximum at 265 millimicrons. Maximum shifts to 245 millimicrons on acidifying. Spectra for galacturonic acid and glucosamine are changed after irradiation in acid solutions too.

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On the Determination of C-Methyl Groups in Aliphatic Long Chain Compounds

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The determination of methyl groups linked to carbon is often an important matter in connexion with structure determinations. The method originally described by Kuhn and Roth [1] and later modified by Wiesenberger [2] is satisfactory for most types of compounds. However, with aliphatic long chain compounds which have often to be analyzed in this laboratory difficulties are encountered with the method as originally described. The modification described by Ginger [3] has been found to give reasonably good results for saturated fatty acids and alcohols containing up to about 20 carbon atoms. In Ginger's method, the sample is dissolved in sulphuric acid, the solution cooled to room temperature, and the chromic acid solution added drop-wise until the oxidation begins. The solution is then cooled in ice-water and the bulk of the chromic acid oxidation solution added. The mixture is then refluxed for 90 minutes, and the acetic acid distilled off and titrated. When we used this procedure it was found to be essential that most of the sample was oxidized already on the drop-wise addition of the chromic acid solution. If during the later refluxing an appreciable amount of unchanged sample was present, fatty material crept up into the condenser and was thus withdrawn from further oxidation. Long chain hydrocarbons could not be analyzed using this procedure, because, if at all dissolved, they were immediately precipitated from the sulphuric acid solution when the chromic acid was added, and were therefore only attacked to a small extent. It was evident, therefore, that the oxidation conditions had to be changed if such compounds were to be analyzed. In particular, it appeared necessary to increase the degree of dispersion for compounds not soluble in sulphuric acid. The samples with oxidation solution were therefore sealed into glass tubes and the tubes shaken at an elevated temperature for a suitable time. After cooling, the tubes were opened, the contents washed out and the acetic acid formed distilled off and titrated.

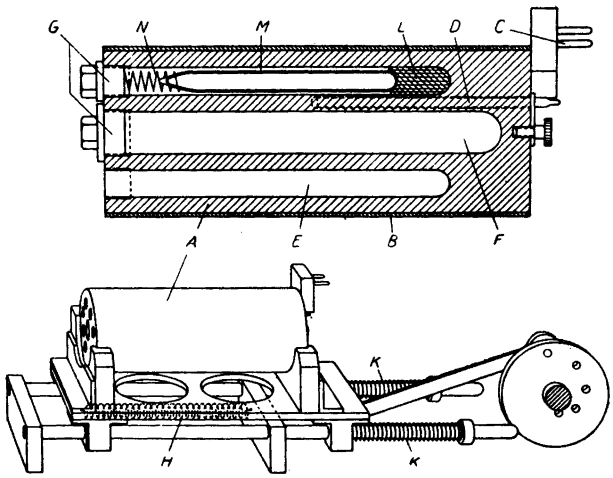


Fig. 1. Mechanical shaker. A = aluminum block, B = heater with asbestos insulation, C = plug connection for heater, D = iron-constantan thermocouple, E, F = bores for bombs, G = retaining plugs, L = cotton wool, N = spring keeping bomb M in position, H and K = springs.

To begin with we used the dilute oxidation solution used by Kuhn and Roth[1], and shook the sample at moderate speed at 120° C (procedure 1). The bombs were shaken for different lengths of time in order to determine the suitable reaction time. The results are shown in the diagrams (Figs. 4—13). With normal chain fatty acids with up to 20 carbon atoms reproducible diagrams were obtained, but with very long chain acids the reproducibility was not satisfactory. With long chain hydrocarbons such as *n*-hentriacontane (Fig. 13) the decomposition was incomplete even after a very long time of shaking. 12-Ketohentriacontane (Fig. 12) on the other hand decomposed very quickly, probably both because of higher solubility in concentrated sulphuric acid and because the carbon chain is readily broken off at the place of the keto group. The shape of the curve after the decomposition shows that the acetic acid formed is attacked only to a negligible degree by the oxidation solution. Analyses 1—6 in Table 1 were carried out following this procedure.

As very long chain hydrocarbons could not be satisfactorily analyzed in the manner just described we tried stronger oxidation solutions, higher temperatures, and more violent shaking in order to obtain a better decomposition of the samples. Stronger oxidation solution and higher temperatures increased, however, not only the rate of formation of acetic acid, but also the rate of its decomposition (Fig. 14). Only the higher rate of shaking had an entirely good

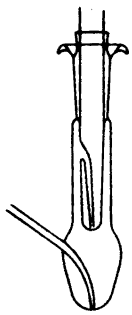


Fig. 2. Distillation flask according to Wiesenberger with sealed-in gas inlet tube.

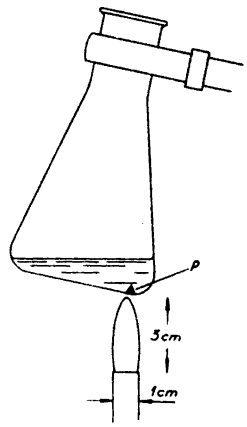


Fig. 3. Boiling the distilled acetic acid prior to titration. P = platinum tetraeder.

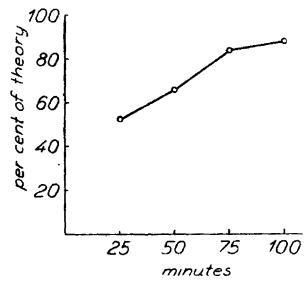


Fig. 4. Di-n-heptylacetic acid, procedure 1.

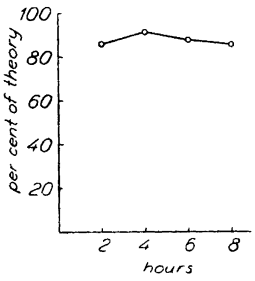


Fig. 5. Methyl 6-methyl-1,11-hendecandioate, procedure 1.

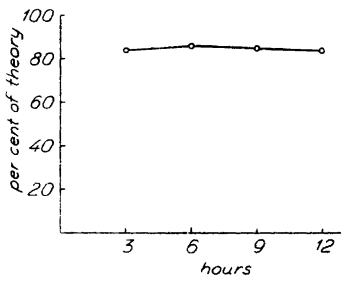


Fig. 6. 16-Methylheptadecanoic acid, procedure 1.

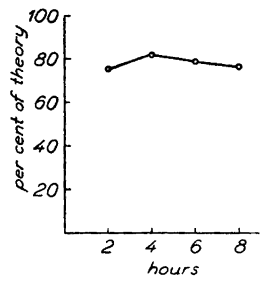


Fig. 7. Methyl 15-methylheptadecanoate, procedure 1.

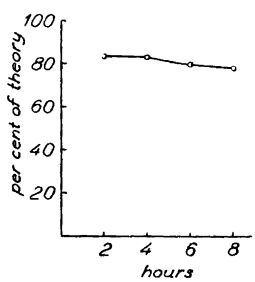


Fig. 8. Methyl 14-methyloctadecanoate, procedure 1.

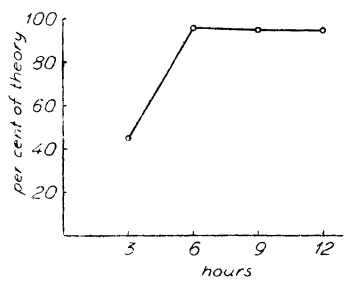


Fig. 9. 33-Methyltetratriacontanoic acid, procedure 1.

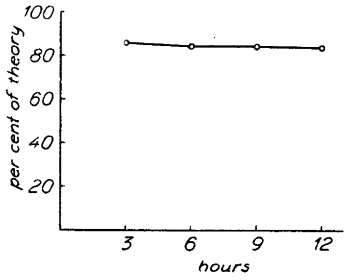


Fig. 10. n-*Eicosanoic acid, procedure 1.*

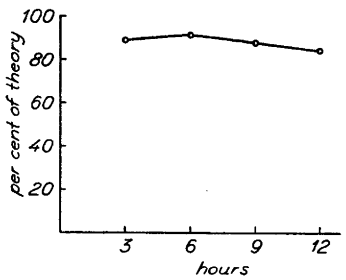


Fig. 11. n-*Hexacosanoic acid, procedure 1.*

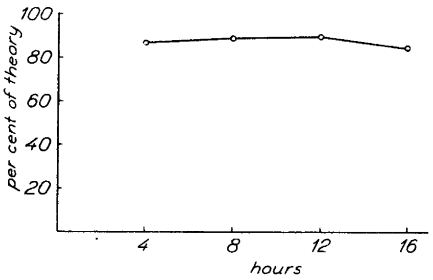


Fig. 12. 12-Ketohentricontane, procedure 1.

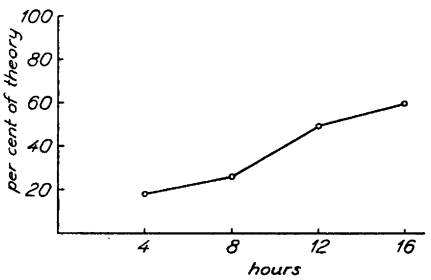


Fig. 13. n-*Hentriacontane, procedure 1.*

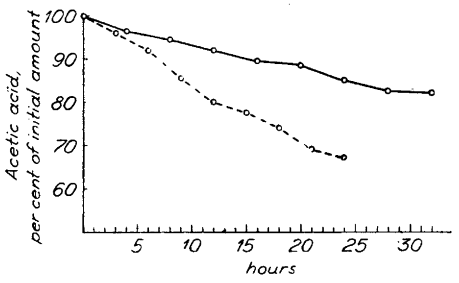


Fig. 14. Rate of destruction of acetic acid. Procedure 1 = full drawn line, procedure 2 = dashed line.

influence on the reaction, as the dispersion of the sample was increased, leading to an increase in the reaction surface. However, with the apparatus used it was not possible to increase the rate of shaking above the figures given for procedure 2 in the experimental part. It is possible that some special method of dispersion of the samples, *e.g.* ultrasound, would give still better results than we have obtained. The best results were obtained using the figures for solution, temperature and shaking given for procedure 2. After four hours shaking following this procedure all organic substance was destroyed and no

*Table 1. Analyses.**

Nr	Substance	CH_3 groups present	CH_3 groups possible to determine as acetic acid	$CH_3-(C)$ % Found	% Calcd	% of Theory	CH_3 groups found
1	*n*-Dodecanoic acid	1	1	6.27	7.52	83.4	0.83
2	—"—			6.25	7.52	83.2	0.83
3	—"—			6.26	7.52	83.3	0.83
4	Methyl 6-methyl-1,11-hendecanedioate			4.69	5.32	80.6	0.81
5	Ethyl azelate	2	2	11.98	12.30	97.4	1.95
6	—"—			11.98	12.30	97.4	1.95
7	*n*-Tetratriacontane	2	2	4.63	6.28	73.7	1.47
8	—"—			4.74	6.28	75.5	1.51
9	—"—			5.02	6.28	79.9	1.60
10	—"—			5.02	6.28	79.9	1.60
11	*n*-Nonatriacontane	2	2	4.12	5.48	75.2	1.54
12	—"—			4.08	5.48	74.5	1.49
13	*n*-Tetratetracontane	2	2	3.68	4.85	75.9	1.52
14	*n*-Hexacosanoic acid	1	1	2.84	3.79	74.9	0.75
15	—"—			2.84	3.79	74.9	0.75
16	14-Methylheptadecanoic acid	2	2	7.57	11.12	68.1	1.36
17	—"—			7.62	11.12	68.5	1.37
18	DL-9-Methyloctadecanoic acid	2	2	7.31	10.07	72.6	1.45
19	—"—			7.07	10.07	70.2	1.40
20	DL-4-Methylpentacosanoic acid	2	2	5.21	7.58	68.8	1.38
21	—"—			5.27	7.58	69.5	1.39
22	DL-5-Methylhexacosanoic acid	2	2	5.05	7.32	69.0	1.38
23	33-Methyltetratriacontanoic acid	2	1	2.40	2.87	83.6	0.84
24	—"—			2.38	2.87	82.9	0.83
25	—"—			2.06	2.87	69.1	0.69
26	Di-*n*-heptylacetic acid	2	2	7.92	10.00	79.2	1.58
27	—"—			8.12	10.00	81.2	1.62
28	DL-α-Methyl-α-ethylsuccinic acid	2	2	13.13	18.77	70.0	1.40
29	—"—			13.18	18.77	70.2	1.40
30	Ethyl β,β-dimethylglutarate	2	1	14.92	20.85	71.6	0.72
31	—"—			15.22	20.85	73.0	0.73

* In analysis 5 the time of shaking was 20 minutes, in analysis 6 one hour, and in all other analyses 4 hours.

Nr	Substance	CH_3 groups present	CH_3 groups possible to determine as acetic acid	$CH_3-(C)$ % Found	% Calcd	% of Theory	CH_3 groups found
32	α-Methyl-ω-phenoxipropyl-acetic acid	1	1	5.11	7.22	70.8	0.71
33	—"—			5.10	7.22	70.6	0.71
34	Methyl-*n*-dodecylacetic acid	2	2	9.08	12.40	73.2	1.46
35	—"—			9.13	12.40	73.6	1.47
36	Methyl hexadecane-1,16-dicarboxylate	0	—	0.06	0	—	0.01
37	—"—			0.06	0	—	0.01
38	Octatriacontane-1,38-dicarboxylic acid	0	—	0.29	0	—	0.12
39	—"—			0.14	0	—	0.06
40	—"—			0.36	0	—	0.15
41	—"—			0.38	0	—	0.16
42	9,30-Diketooctatriacontane-1,38-dicarboxylic acid	0	—	0.52	0	—	0.23
43	—"—			0.52	0	—	0.23
44	Mycocerosic acid *	not known	not known	9.98			3.33
45	—"—			9.98			3.33

* The sample of mycocerosic acid was kindly given by Professor R. J. Anderson, Yale University. All other compounds analyzed have been synthesized in this laboratory.

"wax" crept over during the distillation, as had occurred generally with the other procedures tried and after a shorter time of shaking. Even with this procedure the earlier part of the yield-time curves obtained with samples with very long chains were not well reproducible, but the results after the four hours of shaking of the samples were. Analyses 7—44 were carried out following this procedure.

EXPERIMENTAL

Weighing of the samples.

The samples are weighed into the Pyrex glass bombs, which have an inner diameter of 8—9 mm, an outer diameter of about 12 mm, and a length of 120—150 mm, using the weighing tube of Lieb and Krainick [4]. Liquid samples are weighed into small glass thimbles and thrown into the bomb. The weight of the samples was 15—30 mg in procedure 1 and 8—12 mg in procedure 2.

Oxidation of the samples.

5 ml of oxidation solution are pipetted into the bombs containing the samples, and the bombs are sealed off. In procedure 1 the oxidation solution is 4 normal with respect to chromic acid, in procedure 2 it is 5 normal. In both procedures 50 ml of concentrated sulphuric acid is mixed with 200 ml of the chromic acid solution. For the shaking a mechanical shaker (Fig. 1) is used. An aluminum block with openings for eight bombs and provided with a winding for electrical heating and a thermocouple for automatic temperature control is shaken along its long axis. In procedure 1 the temperature is 120° C, the shaking proceeds at a rate of 74 periods/min. and the length of the stroke is 10 cm. In procedure 2 the temperature is 135° C, and the rate of shaking 150 periods/min., the stroke being 10 cm.

Distillation of the acetic acid.

The bombs are carefully opened in the usual manner and the contents are washed over into the distillation apparatus. To begin with the apparatus of Kuhn and Roth [1] was used. Later the apparatus of Wiesenberger [2] was preferred as it was time-saving and gave more accurate results. It was necessary, however, to seal a side tube into the distillation flask (Fig. 2) and to lead a very feeble nitrogen stream through it, as this proved to be the only way to avoid bumping in our solutions. In all other respects the directions for the distillation given by Wiesenberger were followed. The distillation was found to require some experience in order to distil over the acetic acid quantitatively without getting any sulphuric acid into the distillate.

Titration of the acetic acid.

Pregl and Soltys [5], Friedrich and Rapoport [6], Kuhn and Roth [1], and Wiesenberger [2] report good results when the solution is boiled for a short time before the titration. Hurka [7,8], however, states that the results obtained are low as the boiling causes loss of acetic acid. It is somewhat surprising that none of the above authors says anything about the size of the flame used for the boiling. When the solution was boiled strongly with a large flame large losses occurred already after boiling for 4—5 seconds, in good agreement with the results of Hurka. When we boiled gently with a small flame only negligible losses occurred even after a boiling time of 20 seconds. In the present work a boiling time of 7 seconds was used. The flame was an ordinary Bunsen flame with a height of 3 cm. The flask was held somewhat inclined and a platinum tetraeder was used to avoid bumping (Fig. 3). After the boiling, the flask was immediately immersed in cold water and a small watch glass was placed upon

it. After standing in the water for exactly two minutes one drop of 1 % phenolphtalein solution was added and the solution quickly titrated to the first pink tinge remaining for 5 seconds. Blank titrations were carried out in order to compensate errors.

SUMMARY

The determination of C-methyl groups in aliphatic compounds of high molecular weight by the Kuhn-Roth method has been studied. By improvement in the oxidation conditions, including the use of a stronger oxidation solution, a higher temperature and dispersion of the sample by mechanical shaking, it has been found possible to get satisfactory results for aliphatic compounds of very high molecular weight. The results of the analysis of a large number of normal and branched chain compounds having up to 44 carbon atoms in the molecule are given.

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The Chemistry of the Natural Order *Cupressales* *

VIII. Heartwood Constituents of *Chamaecyparis nootkatensis* — Carvacrol, Nootkatin, and Chamic Acid

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Extensive studies on the extractable heartwood constituents of the natural order *Pinales*, with particular reference to the genus *Pinus*, which have been carried out in recent years in this laboratory [1] have yielded information of considerable interest, not only from the purely chemical view-point, but also in connection with the biological relationship between different species and genera. There seems every indication that similar investigations on other plant orders will be equally profitable, and the order *Cupressales* has been chosen for reasons which should perhaps be briefly outlined.

The order *Cupressales* is divided into two families, the rather heterogeneous *Taxodiaceae* which has only 15 species, and the more uniform *Cupressaceae*, about 150 in number, divided into 11 genera many of which are monotypic or have only a few species. No systematic study of a large genus has been carried out, but as has been pointed out previously [2,3], it is known that the heartwood constituents in the family *Cupressaceae* are frequently of terpenoid type, and certain compounds have been isolated from more than one genus. The genera *Chamaecyparis*, *Thuja*, and *Thujopsis* have all yielded compounds (α-, β-, & γ-thujaplicins and thujic acid) which although not terpenes are of the C_{10}type and are obviously closely related to the terpenes. This fact is particularly interesting, and despite the fact that two *Chamaecyparis* species, *C. obtusa*

* In view of our widening interest in the order *Cupressales*, the general title of this series of papers has been changed and the publications under the general title "Antibiotic Substances from the Heartwood of *Thuja plicata* (D. Don)" will be regarded as Pts. I–VII of this series. Part VII *Acta Chem. Scand.* 5 (1951) 995.

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Sieb. and Zucc. and *C. formosensis* Matsum. both of East Asian origin, have been investigated in some detail [4], *C. nootkatensis* (Lamb) Spach occupies a somewhat isolated position in the genus and consequently was selected for our purposes.

Chamaecyparis nootkatensis (yellow cedar, Alaska cedar, yellow cypress) is a moderately large tree which grows on the Pacific Coast of Canada and elsewhere. The heartwood possesses a yellowish colour, is relatively light, hard, and close-grained, and, because of its resistance to decay and its insect repellant properties, is in considerable demand in Canada for use in furniture manufacture. It is also used extensively for battery separators.

The study of the constituents of the heartwood of yellow cedar was commenced in this laboratory some time ago, but, as some of the original collaborators are no longer engaged in this work, and as a number of interesting and significant findings have been made, it appears desirable to give a brief survey of our results to date.

The only recorded investigation on the steam-volatile constituents of *C. nootkatensis* was carried out by Clark and Lucas [5] as far back as 1926. Although these workers isolated both the wood and leaf oils they confined their detailed study to the latter, identifying α-pinene and limonene, and obtaining evidence, not entirely satisfactory, for the presence of β-pinene, sabinene and *p*-cymene.

We have limited our work to the heartwood components obtainable by steam distillation of the finely divided wood. The steam distillation proceeds in two fairly distinct stages: the initial product is a pale yellow oil which distills rapidly, but if the distillation is continued this oil is followed by further material which distills much more slowly and crystallises out in the form of colourless plates in the condensers and well-cooled distillate. This compound has been obtained pure and has been shown to be a hitherto unknown tropolone for which we have proposed the name *nootkatin* [6]. It is rather surprising that Clark and Lucas do not appear to have noticed the presence of any solid material in the crude distillate but it is possible that they did not continue the distillation sufficiently long or, more likely, that they used metal equipment. They record that the wood oil they obtained was deep red in colour, and this is probably a significant observation as we have noticed that the oil becomes deep red-brown during the course of working up. This is almost certainly due to the formation of small amounts of the ferric salt of nootkatin (*cf.* the ferric salt of tropolone [7–9]). Further, if iron or copper apparatus is used for the distillation, the yield of nootkatin is very small or nil, and in some cases we observed the formation of the green crystalline copper complex of nootkatin in the apparatus. Nootkatin can also be obtained by extraction of the wood with

acetone or alcohol, but no nootkatin was removed by prolonged extraction with cold, dilute sodium hydroxide solution.

The ultra-violet [6] and infra-red [10] absorption spectra of nootkatin indicate that it is a tropolone, and the chemical evidence is in accord with this conclusion. Thus nootkatin forms metal complexes and a monomethyl ether, exhibits no ketonic properties, and, on complete hydrogenation, gives an α-glycol. It has the formula $C_{15}H_{20}O_2$, and thus contains one double bond in addition to those present in the cycloheptatrienolone entity. As mentioned in previous communications [6,10] evidence has been obtained that the extra-nuclear double bond is not conjugated with the ring system, and that it forms part of an isopropylidene structure. Recent work, details of which will be published shortly, has indicated that nootkatin contains two side-chains, one isopropyl and the other 3-methylbut-2-enyl, and it thus bears the same relationship to the sesquiterpenes as α- [11–13], β-[12,14,15] and γ-thujaplicins [2,12] do to the monoterpenes.

Nootkatin is obtained during the normal working up procedure in the phenolic fraction of the oil, and from the same fraction we have isolated significant amounts of carvacrol. This is of interest since, although carvacrol has been obtained from a number of other plant sources, it appears to be a rare constituent of the heartwoods of conifers, the only recorded instance of its isolation being from the wood of *Tetraclinis articulata* [16].

The neutral components of the wood oil have not been investigated in detail as yet, but a preliminary study of the acidic fraction has resulted in the isolation of a new liquid acid for which we propose the name *chamic acid*, and indicated the presence of at least one other acidic substance. Chamic acid, $C_{10}H_{14}O_2$, possesses an unusually high optical rotation (+ 257.5° in MeOH) and probably contains two rings and one double bond. Studies on its structure are proceeding and will be reported at a later date, but it is interesting to note the occurrence of an acid containing a seven-membered ring, thujic acid [17], with the thujaplicins in *Thuja plicata* (western red cedar), and this fact invites the speculation that chamic acid and thujic acid may be related.

As mentioned above, the heartwood of *C. nootkatensis* possesses considerable resistance to insect attack and Clark and Lewis [5] stated that "the wood apparently contains in the oil some valuable preservative constituent". Docent E. Rennerfelt has tested nootkatin and chamic acid for fungicidic properties and has found that they are active. Even the neutral material which constitutes the main fraction of the steam-volatile products of the wood possesses some activity.

EXPERIMENTAL

Extraction of heartwood constituents. The wood which has been used for this investigation has, for the most part, come from a single tree grown near Vancouver, Canada. A smaller sample of wood from a tree grown near Stockholm was obtained and this was found to give a significantly greater yield of nootkatin, the only component which was investigated in detail.

a. *Steam distillation.* The wood was disintegrated in a Wiley mill and steam distilled in batches of ca. 2 kg using a chromium-plated vessel and glass condensers. It is essential that no copper or iron apparatus should be employed if it is desired to isolate nootkatin in good yield. The first portion of the distillate contained an appreciable quantity of oil, but this was later replaced by nootkatin which separated in crystalline form in the condensers and the cold distillate. The distillation was continued until the condensate was clear by which time 120—150 l had been collected. This was saturated with salt and extracted twice with ether and the ether extracts united and evaporated. The resulting dark red-brown oil (ca. 55 g, 2.75 %) was taken up in ether (ca. 1 l) and extracted with saturated sodium carbonate solution (3 × ½ volume), then with *N* NaOH (6—10 × ½ volume) and washed well with water until no more material was extracted (3—4 × ½ volume). Acidification of the sodium bicarbonate solution with hydrochloric acid gave an oil which was recovered by ether extraction (2 × equal volume). Acidification of the sodium hydroxide solution plus washings gave a mixture of oil and crystalline material which was also recovered by ether extraction. The yields of acidic, phenolic, and neutral material varied somewhat from batch to batch but were approximately 3.5 g, 11 g and 40 g (0.18, 0.55, 2.0 %) respectively.

b. *Acetone extraction.* Finely divided wood (3.7 kg) was continuously extracted (Soxhlet) with ether (3 l) until the extract was no longer coloured (48 hrs). The material obtained by evaporation of the ether (102.5 g, 2.8 %) has not been investigated. The extracted wood was air-dried and then extracted in the same way with acetone, until extraction was complete (48 hrs). The dark red acetone solution was concentrated to small volume, and the light brown "membrane substances" (6 g) precipitated by the addition of excess ether, removed by filtration, and washed well with ether. The dark red ethereal solution was extracted successively with saturated sodium bicarbonate solution (3 × equal volume), *N* NaOH (3 × equal volume), and water (2 × equal volume), then dried (Na_2SO_4) and evaporated to give a crude neutral fraction which was distilled with steam. The distillate, which contained oil, was saturated with salt and extracted with ether (2 x) and the ether extracts combined, dried (Na_2SO_4) and evaporated, giving the neutral material as a red-brown oil (9.1 g, 0.25 %).

The acidic fraction (2 g) recovered by acidification (HCl) and ether extraction (2 x) of the sodium bicarbonate solution was steam distilled and worked up similarly, giving 0.55 g (0.015 %) of purified material. Steam distillation of the crude phenolic fraction obtained from the sodium hydroxide extracts and the aqueous washings gave a distillate which deposited crystalline material on cooling and which was worked up as before, giving a semi-solid dark brown product (2 g, 0.05 %) from which nootkatin (1 g, 0.03 %) was isolated by distillation *in vacuo*.

c. *Alcohol extraction.* A sample of Swedish-grown wood (3 kg) was extracted as above using alcohol as solvent in place of acetone, and omitting the preliminary extraction with ether. The extract was worked up as before, yielding 2.8 g (0.09 %) of nootkatin.

Attempted extraction of nootkatin by means of sodium hydroxide. Cold dilute aqueous sodium hydroxide (24 l, 1 *N*) was allowed to percolate through finely powdered wood (830 g, Swedish origin) the percolate being run into excess of dilute hydrochloric acid. The wood was then washed with water (9 l) and the total percolate and washings worked up in the usual way. No nootkatin could be recovered from the extract, but steam distillation of the extracted wood led to the isolation of nootkatin (0.9 g) in about the expected yield.

Isolation of nootkatin. The dark brown viscous phenolic fractions slowly deposited crystalline material which was collected by filtration, washed with a little ether and petroleum ether (b. p. 40—60°) and recrystallised several times from ligroin giving pure *nootkatin* as colourless plates, m. p. 95°, $[\alpha]_D^{20} \pm 0$ (c = 5 in $CHCl_3$).

Light absorption (in *n*-heptane)[6]:

Maxima,	238,	315 *,	324,	357,	373 * mμ.
log ε,	4.49,	3.90,	3.94,	3.79,	3.64

$C_{15}H_{20}O_2$ (232.31)	Calc.	C 77.55	H 8.68	C-Me 17.64,		Mol. Wt.	232.3
	Found	» 77.6	» 8.76	» »	10.93, 10.94	» » [6]	248.6

Distillation of the phenolic mother liquors *in vacuo* gave a low-boiling fraction (mainly carvacrol), followed by nootkatin which crystallised on cooling and was purified by crystallisation from ligroin as before. A higher-boiling fraction which distilled as a yellowish oil has not yet been investigated.

Nootkatin is almost insoluble in water and cold ligroin, but readily soluble in dilute sodium hydroxide solution and nonhydrocarbon solvents. With a limited amount of alcoholic ferric chloride it gives an intense red colour — with an excess an intense green colour, which is visible even in very dilute solution and serves as a sensitive test (*cf* tropolone [7,9]).

The *copper complex of nootkatin* obtained by shaking a chloroform solution of nootkatin with aqueous cupric acetate and evaporating the resulting dark green chloroform solution, crystallised from chloroform-ether as pale green needles, m. p. 234—5° (decomp.). It is insoluble in water and ether and sparingly soluble in acetone and alcohol, but readily soluble in chloroform and benzene.

Nootkatin methyl ether. Nootkatin (0.1 g) was dissolved in ether (2 ml) and a solution of pure diazomethane (1.2 moles) in ether (3.5 ml) was added. Nitrogen was evolved slowly, and the mixture was set aside in the refrigator overnight. Evaporation of the ether gave an oil which was distilled twice *in vacuo*, giving *nootkatin methyl ether* as a pale yellow oil, n_D^{20} 1.5840, which gives no colouration with ferric chloride.

$C_{16}H_{22}O_2$ (346.34)	Calc.	OCH_3	12.6
	Found	»	11.9

This product is probably a mixture of isomers.

Decahydronootkatin. Nootkatin (1.0 g) in glacial acetic acid (25 ml) was hydrogenated at room temperature and pressure in the presence of Adams' catalyst. Four moles of hydrogen were absorbed rapidly; thereafter the rate of absorption decreased markedly. The uptake of hydrogen ceased when a total of five moles had been absorbed. The catalyst was filtered off and the solvent removed *in vacuo*. The resulting oil was dissolved in ether (75 ml), and the ethereal solution was washed with 2*N* NaOH (2 × 10 ml) and water (2 × 10 ml), dried (Na_2SO_4) and evaporated. The resultant oil was distilled *in*

* Point of inflection.

vacuo, giving *decahydronootkatin* as a colourless oil, n_D^{20} 1.4820, which could not be induced to solidify, and probably consists of a mixture of isomers.

$C_{15}H_{30}O_2$ (242.39)	Calc.	C	74.32	H	12.48
	Found	»	75.64	»	11.95

Isolation of carvacrol. The phenolic mother liquor from which the crystalline nootkatin had been removed (9 g) was dissolved in chloroform (70 ml) and the solution shaken with an excess of aqueous cupric acetate. The resulting green chloroform solution was washed with water (3 × 50 ml) until the washings were colourless, dried (Na_2SO_4), concentrated to small volume, and diluted with ether (100 ml). The green crystalline copper complex of nootkatin which separated slowly (0.85 g) was collected after one hour, and the ether solution was extracted with saturated sodium bicarbonate solution (2 × 25 ml), washed with water (2 × 25 ml) and extracted with 2*N* NaOH (3 × 25 ml) and then with water (2 × 25 ml). The sodium hydroxide and aqueous extracts were combined and acidified (HCl) and the resulting red-brown oil that separated was extracted with ether (3 × 25 ml). The ether extract was washed with water (2 × 10 ml), dried (Na_2SO_4) and evaporated, and the resulting dark-brown oil (3 g) was distilled.

The first fraction (1 g) had b. p. ca. 115°/15 mm, n_D^{20} 1.5289, and consisted mainly of *carvacrol* identified by conversion to the *phenylurethane*, m. p. 135—6° either alone or in admixture with an authentic sample (Analysis: Found C, 75.89; H, 6.97: $C_{17}H_{19}O_2$ requires C, 75.81; H, 7.11 %) and by conversion to *2-methyl-5-isopropylphenoxyacetic acid* m. p. 149—50°, undepressed by an authentic specimen. The remainder of the material which distilled was a pale yellow oil b. p. ca. 170—80°/20 mm, n_D^{20} 1.5340, which has not been further investigated.

Isolation of chamic acid. The viscous, brown coloured acidic fraction obtained from the steam distillation of the wood was taken up in acetone and an excess of cyclohexylamine in acetone was added. The white crystalline material which separated immediately was collected and recrystallised from acetone-methanol, giving the pure *cyclohexylamine salt of chamic acid*, m. p. 165—166°, $[\alpha]_D^{20}$ + 164.5° (c = 2.9 in MeOH).

$C_{16}H_{27}O_2N$ (265.38)	Calc.	C	72.41	H	10.25	N	5.28
	Found	»	72.79	»	10.19	»	5.47

The cyclohexylamine salt is moderately soluble in cold water, and acetone, but readily soluble in ethanol and methanol.

Treatment of an aqueous suspension with dilute hydrochloric acid liberated an oil which was shaken out with ether (3 × ½ volume). The ether was washed with a little water, dried (Na_2SO_4) and evaporated giving an oil which was distilled *in vacuo* giving pure *chamic acid* as a colourless oil, b. p. 142°/8 mm, n_D^{20} 1.4998, D_D^{20} 1.0632, $[\alpha]_D^{20}$ + 257.5° (c = 1.4 in MeOH), $[M\alpha]_D$ 45.6.

$C_{10}H_{14}O_2$ (166.21)	Calc.	C	72.26	H	8.49	Equiv.	166	one C—Me	16.27
	Found	»	71.82	»	8.49	»	165	» »	6.68

The p-*bromophenacyl ester*, prepared in the usual way formed colourless leaflets from aqueous ethanol, and melted at 74.5—75°.

$C_{18}H_{19}O_3$ Br (363.75)	Calc.	C	59.51	H	5.27
	Found	»	59.53	»	5.47

The S-*benzylthiuronium salt*, crystallised from ethanol forming colourless plates, m.p. 153—4°.

$C_{18}H_{24}O_2N_2S$ (332.45)	Calc.	S	9.64
	Found	»	9.70

The mother liquors from which the cyclohexylamine salt of chamic acid had been isolated contain a further acid (or acids) which is being studied and will be reported on in a subsequent communication.

SUMMARY

A preliminary study of the steam-volatile constituents of the heartwood of *Chamaecyparis nootkatensis* has resulted in the isolation of carvacrol, the "sesquiterpene type" tropolone nootkatin, $C_{15}H_{20}O_2$, and a new acid, $C_{10}H_{14}O_2$, for which the name chamic acid is proposed.

We are indebted to Dr. W. W. Moyer, Camas, Washington, U.S.A., for providing us with a large supply of Alaska yellow cedar grown in British Columbia, Canada, and to Professor R. Florin, Stockholm, for a supply of Swedish grown (Stockholm) material.

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Bindungstypen in der Stärke

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Untersuchungen über den enzymatischen Abbau der Stärke durch absolut α-freie β-Gerstenamylase und durch absolut β-freie α-Malzamylase führten uns[1] im Jahre 1936 zu der Ansicht, dass Stärke, respektive Stärkeabbauprodukte, Bindungen enthalten, welche

vorzugsweise von α-Amylase,
vorzugsweise von β-Amylase,
sowohl von α- als von β-Amylase,
weder von α- noch von β-Amylase,
ausschliesslich von Maltase

gespalten werden. Unsere Ansicht liess sich nicht mit der damals üblichen Auffassung, dass Stärke nur *einen* Bindungstypus — nämlich 1α4-Bindungen — enthält, in Einklang bringen. Selbst die heute übliche Auffassung, dass Stärke *zwei* Bindungstypen — nämlich neben 1α4-Bindungen noch 1α6-Bindungen — enthält, reicht nicht aus, um die Diskrepanz zu eliminieren. Diese Unstimmigkeit hat uns veranlasst, die Arbeit über die Bindungstypen in der Stärke fortzusetzen.

In der vorliegenden Abhandlung haben wir das Problem auf andere Weise angefasst, und zwar haben wir durch gleichzeitige Bestimmung des Drehungs- und des Reduktionsvermögens den Abbau verfolgt, um eventuelle Zusammenhänge zwischen diesen Eigenschaften und dem Aufbau der Stärke zu finden.

Für Vergleiche von Drehwerten ist es vorteilhaft nicht die spezifische Drehung $[\alpha]$, die auf die Gewichtseinheit des zu untersuchenden Stoffes bezogen ist, sondern die molekulare Drehung [M], die sich auf gleiche Molekülzahlen bezieht, zu verwenden. Die Molrotation [M] ist die Drehung einer Lösung von 1 Mol in 100 ml im 1 dm-Rohr gemessen. Zwischen Molrotation und spezifischer Drehung besteht folgende Beziehung:

$$[\mathrm{M}] = \frac{[\alpha] \cdot M}{100} = \frac{\alpha \cdot M}{c \cdot l}$$

Tabelle 1. Molrotation von Glucose, Maltose und Isomaltose im Gleichgewichtszustand.

	M	$[\alpha]_D$	$[M]_D$
Glucose	180,2	52,7	95
Maltosehydrat	360,3	130,4	470
Isomaltose [2]	342,3	120	411

Aus Stärkeabbauprodukten hat man nur die in Tabelle 1 angeführten drei Zuckerarten in kristallinischem Zustande isoliert.

Wir haben uns die Frage gestellt: welche Drehungsänderungen sind mit der Spaltung einer Glucosidbindung in der Stärke oder in Stärkeabbauprodukten verbunden, und zwar von Gleichgewichtszustand zu Gleichgewichtszustand gemessen.

Das einfachste Stärkeabbauprodukt, welches eine Glucosidbindung enthält, ist das Disaccharid Maltose. 360 mg Maltosehydrat, 1 mmol Maltose entsprechend, werden in Wasser gelöst; durch kurzes Erwärmen wird die Mutarotation aufgehoben. Nach Abkühlung auf 20,0° wird auf 100,0 ml verdünnt. Im 1 dm-Rohr, Natrium-Licht, ist die Drehung dieser Lösung 0,470°. Auf genau dieselbe Weise wird eine Lösung von 2 mmol Glucose bereitet. Diese Lösung zeigt eine Drehung von 0,190°. Bei Spaltung von 1 mmol Maltose entstehen 2 mmol Glucose. Bei dieser Reaktion ändert sich die Drehung von 0,470° bis 0,190° = 0,280°. Für 1 Mol ist die Änderung 280°. Diese Änderung bezeichnen wir mit $[Sp]_D$.

Stärke ist ein Polysaccharid ohne Reduktionsvermögen ausschliesslich aus Glucoseresten aufgebaut. Die Hydrolyse verläuft nach der Formel:

$$(C_6H_{10}O_5)_x + x\,H_2O \rightarrow x\,C_6H_{12}O_6$$

woraus sich berechnen lässt, dass aus 162 mg Stärke 180 mg Glucose gebildet werden. 180 mg Glucose sind 1 mmol. Die spezifische Drehung der Stärke ist 202° [3]; eine Lösung von 162 mg Stärke in 100,0 ml würde im 1 dm-Rohr eine Drehung von $\frac{202 \cdot 162}{100} = 0{,}327°$ zeigen. 180 mg Glucose in 100,0 ml Wasser gelöst zeigen im 1 dm-Rohr eine Drehung von 0,095°. Bei der totalen Hydrolyse ändert sich die Drehung also um 0,232°. Bei dieser Reaktion ist 1 mmol reduzierender Zucker gebildet worden — in diesem Falle Glucose —, es ist mit anderen Worten 1 mmol Bindungen gespalten worden. Für die Spaltung von 1 Mol Bindungen ist die Drehungsänderung $[Sp]_D = 232°$.

In diesen beiden Beispielen haben wir die Anzahl gespaltener Bindungen berechnen können, da wir wussten, wieviele Bindungen vorhanden waren,

und wir sie alle spalteten. Bei partiellem Abbau ist es dagegen notwendig die Anzahl gespaltener Bindungen zu bestimmen. Die Anzahl gespaltener Bindungen ist gleich der Anzahl gebildeter Moleküle reduzierender Saccharide. Wir sehen von der Anwesenheit von Bindungen des Trehalosetypus ab, welche bei Spaltung einer Bindung zur Bildung zweier Moleküle reduzierender Saccharide Anlass geben.

Die Anzahl reduzierender Saccharide wird durch das Reduktionsvermögen bestimmt. Voraussetzung ist, dass ein jedes Stärkeabbauprodukt, unabhängig von der Kettenlänge, dasselbe molare Reduktionsvermögen hat wie Glucose und Maltose. In der vorliegenden Arbeit haben wir zwei Methoden zur Bestimmung des Reduktionsvermögens angewandt, nämlich die Jodmethode von Willstätter-Schudel [4] und die Kaliumkupferkarbonatmethode von Blom-Rosted [5]. Die Jodmethode hat einen stöchiometrischen Verlauf:

$$R-CHO + I_2 + 3\ OH^- \rightarrow R-COO^- + 2\ I^- + 2\ H_2O$$

Kaliumkupferkarbonat hat dasselbe molare Oxydationsvermögen gegenüber Maltose wie gegenüber Glucose.

Alle unsere Bestimmungen der optischen Drehung sind nach Ablauf der Mutarotation und bei 20° mit Na-Licht, D-Linie, ausgeführt. Die angegebenen Werte sind also Gleichgewichtsdrehungen.

Um die von uns gestellte Frage zu beantworten, haben wir eine Reihe von Versuchen über die Hydrolyse von Kartoffelstärke, teils mit Säure, teils mit Enzym durchgeführt.

Ein 3 %iger Kleister, 0,08 *N* mit Hinblick auf Salzsäure, wird in verschlossenen Kolben bei 100° hydrolysiert. In passenden Intervallen wird ein Kolben aus dem kochenden Wasserbad genommen und auf 20° abgekühlt. Drehungs- und Reduktionsvermögen werden sofort bestimmt, um eventuelle Fehler durch Retrogradation möglichst zu eliminieren. Die Lösungen sind ohne Filtrieren verwendbar.

Die Resultate solcher Versuche sind in Tabelle 2 und 3 zusammengestellt. Die Änderung der Drehung per mmol gespaltener Bindungen ergibt sich durch Division der Differenzen zweier Werte von α_{korr} und den zugehörigen Werten von R.

Beispiel: Tabelle 2. Hydrolysegrad 36,0 %—44,3 %

$$\frac{4{,}788 - 4{,}431}{8{,}27 - 6{,}71} = 0{,}229$$

$[Sp]_D$, die Änderung der Drehung per Mol gespaltener Bindungen, ist also 229°.

Tabelle 2. Säurehydrolyse von Kartoffelstärke. Kleister 3,00 %, pH ca. 1, t = 100°. Reduktion: Kaliumkupferkarbonatmethode. Drehung: 1 dm-Rohr.

Versuchsreihe	Reduktion: Hydrolysegrad %	Reduktion: mmol Zucker in 100,0 g Lösung R	Drehung korr. für Dichte α_{korr}	$[Sp]_D$ von Punkt zu Punkt	$[Sp]_D$ vom Nullpunkt zu Punkt
	0	0	6,068		
				166	
63	12,6	2,35	5,678		166
				—	
64	14,9	2,79	5,600		168
				187	
64	23,1	4,31	5,316		174
				199	
63	28,1	5,25	5,129		179
				—	
64	29,8	5,57	5,039		185
				220	
64	36,0	6,71	4,788		191
				229	
65	44,3	8,27	4,431		198
				260	
63	52,6	9,81	4,030		208
				—	
65	54,4	10,15	3,945		209
				273	
65	65,7	12,26	3,369		220
				279	
65	76,5	14,28	2,805		229
				264	
65	85,9	16,02	2,345		232
				284	
65	88,8	16,57	2,189		234

Tabelle 3. Säurehydrolyse von Kartoffelstärke. Kleister 3,00 %, pH ca. 1, t = 100°. Reduktion: Jodmethode. Drehung: 1 dm-Rohr.

Versuchsreihe	Reduktion: Hydrolysegrad %	Reduktion: mmol Zucker in 100,0 g Lösung R	Drehung korr. für Dichte α_{korr}	$[Sp]_D$ von Punkt zu Punkt	$[Sp]_D$ vom Nullpunkt zu Punkt
	0	0	6,068		
				156	
67	10,9	2,04	5,749		156
				172	
67	24,0	4,48	5,329		165
				226	
67	36,2	6,75	4,815		186
				243	
67	42,6	7,96	4,521		194
				230	
67	53,4	9,97	4,059		202
				273	
67	69,0	12,88	3,264		218
				278	
68	73,7	13,76	3,019		222
				267	
67	76,0	14,19	2,904		223
				287	
68	81,4	15,19	2,617		227
				274	
68	85,7	16,00	2,395		230
				283	
68	89,3	16,66	2,208		232
				261	
68	92,6	17,28	2,046		233

Diese Berechnung haben wir teils von Punkt zu Punkt, teils vom Nullpunkt zu jedem Einzelpunkt ausgeführt.

Im Nullpunkt hat R den Wert 0. $[\alpha]_D$ der von uns verwendeten Kartoffelstärke ist als 200,6° bestimmt, und aus diesem Wert ist α_{korr} für den Nullpunkt berechnet.

Die mit beiden Methoden zur Bestimmung des Reduktionsvermögens erhaltenen Resultate stimmen überein, so dass die beiden Tabellen gemeinsam diskutiert werden können.

Das Zahlenmaterial zeigt, dass $[Sp]_D$ nicht konstant ist. Von Punkt zu Punkt berechnet ist $[Sp]_D$ anfangs ca. 160° und steigt während des Abbaus, um bei einem Abbau von ca. 60 % seinen maximalen Wert ca. 280° zu erreichen.

Vom Nullpunkt berechnet steigt $[Sp]_D$ bis ca. 230°. Wie oben berechnet ist der durchschnittliche Wert von $[Sp]_D$ bei Spaltung aller Bindungen der Stärke 232°. Diesen Wert erreicht man bei einem Abbau von ca. 90 %.

Das Ergebnis, dass $[Sp]_D$ nicht konstant ist, kann nur dadurch gedeutet werden, dass mehr als ein Bindungstypus gespalten wird.

$[Sp]_D$ ist für die Glucosidbindung der Maltose 280°; auf gleiche Weise findet man für die Glucosidbindung der Isomaltose $[Sp]_D = 221°$. Sowohl 280° als 221° sind grösser als die Werte, welche wir zu Beginn der Säurehydrolyse finden. Es muss also ein dritter Bindungstypus in der Stärke sein, und dieser unbekannte dritte Bindungstypus wird zuerst hydrolysiert. Nach Hydrolyse von ca. 60 % aller Bindungen erreicht $[Sp]_D$ denselben Wert wie bei der Spaltung von Maltose. Es ist als ob hier nur Maltose oder Bindungen vom selben Typus wie in der Maltose gespalten werden.

Beim Abbau von Stärke durch α-Amylasen werden Mono-, Di-, Tri- und höhere Oligosaccharide gebildet. Beim Abbau durch β-Amylase entsteht nur Maltose. Mit β-Amylase sind die Verhältnisse also weit übersichtlicher, und wir haben deshalb vorgezogen den enzymatischen Abbau der Stärke durch β-Amylase zu untersuchen. Beim Abbau von Kartoffelstärkekleister durch β-Amylase besteht aber die Schwierigkeit, dass wohl das Reduktionsvermögen bestimmt werden kann, Polarisationsmessungen aber nicht möglich sind. Um klare Lösungen zu erhalten wird der Stärkeabbau nach Sistierung der β-Amylase mit Pankreasamylase fortgesetzt. In Intervallen werden das Reduktions- und das Drehungsvermögen bestimmt, um aus diesen Werten durch Extrapolation das Drehungsvermögen vor Zusatz der Pankreasamylase zu berechnen.

Die Resultate dieser Versuche sind in Tabelle 4 zusammengestellt.

Beim Abbau der Stärke durch β-Amylase zu 40—50 % TM hat $[Sp]_D$ vom Nullpunkt berechnet einen Wert von ca. 187°, welcher kleiner ist als 280° und 221°. Der Abbau mit β-Amylase zeigt wie die Säurehydrolyse, dass Stärke

Tabelle 4. Abbau von Kartoffelstärke durch β-Amylase. Fortsetzung des Abbaus durch Pankreasamylase. Kleister 3,00 %, pH = 6,8, t = 40°. Reduktion: Jodmethode. Drehung: 1 dm-Rohr.

Versuchsreihe	Pankreasamylase nach β-Amylase				β-Amylase			
	Reduktion		Drehung korr. für Dichte	Neigung der Pankreas Geraden	Reduktion		Drehung berechnet	
	Abbaugrad % TM	mmol Zucker in 100,0 g Lösung R	α_{korr}	$[Sp]_D$	Abbaugrad % TM	mmol Zucker in 100,0 g Lösung R	α_{korr}	$[Sp]_D$
70	56,8	5,30	5,121					
	64,2	6,00	5,017	151	41,2	3,85	5,343	189
	68,4	6,39	4,956					
71	55,8	5,21	5,140					
	61,4	5,73	5,073	141	45,2	4,22	5,281	186
	66,5	6,20	5,000					
74	57,8	5,39	5,098					
	59,8	5,58	5,069					
	62,5	5,83	5,042	133	51,4	4,80	5,177	186
	65,3	6,10	5,007					
	68,5	6,39	4,962					

% TM: Prozent der Menge Maltose, die gebildet werden würde, falls Stärke sich total zu Maltose abbauen liesse.

einen Bindungstypus enthalten muss, welcher verschieden ist sowohl von der Bindung in der Maltose als von derjenigen in der Isomaltose.

Es ist nun naheliegend, sich die Frage zu stellen, wie dieser dritte Bindungstypus beschaffen ist.

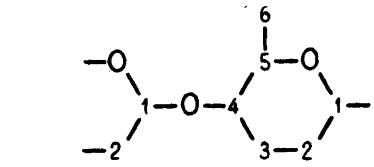
Aus dem hohen Drehungsvermögen lässt sich schliessen, dass β-Bindungen in grösserer Anzahl nicht am Aufbau der Stärke beteiligt sein können [6].

Bei der totalen Hydrolyse der Stärke entsteht ausschliesslich Glucose.

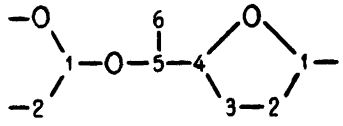
Die Hydrolysenprodukte totalmethylierter Stärke sind [7–10]

85—90 %	2,3,6-Trimethylglucose,
ca. 5 %	2,3,4,6-Tetramethylglucose,
ca. 5 %	2,3-Dimethylglucose.

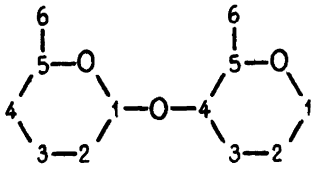
Die nicht methylierten OH-Gruppen der Stärke sind vor Methylierung durch Bindungen geschützt. Diese Bindungen sind teils Glucosidbindungen zwischen



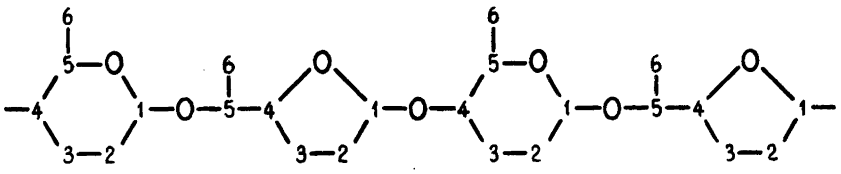
1-4-Brücke und 1-5-Ring



1-5-Brücke und 1-4-Ring



Maltose



Kette von alternierenden Pyranose- und Furanosereste

zwei Glucoseresten, teils Ringbindungen innerhalb desselben Glucoserestes. Der Ring kann entweder zwischen OH-Gruppen an C_1 und C_5 oder zwischen OH-Gruppen an C_1 und C_4 geschlossen sein. Im ersten Falle handelt es sich um einen Pyranoserest, im zweiten Falle um einen Furanoserest.

2,3,4,6-Tetramethylglucose kann nur aus endständigen Pyranoseresten herrühren. 2,3,6-Trimethylglucose stammt aus unverzweigten, nicht-endständigen Kettengliedern. 2,3-Dimethylglucose stammt aus verzweigten, mittleren Kettengliedern und unterscheidet sich nur von 2,3,6-Trimethylglucose dadurch, dass die OH-Gruppe an C_6 an der Isomaltosebindung beteiligt gewesen ist.

Die Bildung von 2,3,6-Trimethylglucose besagt, dass die OH-Gruppen an C_1, C_4 und C_5 in der Stärke sich an Bindungen beteiligen. Eine Glucosidbindung geht von C_1 aus. Hat der andere Glucoserest einen Pyranosering, kann die Bindung nur an C_4 anknüpfen, und hat der andere Glucoserest einen Furanosering, kann die Bindung nur an C_5 anknüpfen.

Es gibt nur diese beiden Möglichkeiten:

1—5-Ring und 1—4-Brücke,
1—4-Ring und 1—5-Brücke.

Beim Abbau der Stärke mit β-Amylase wird ausschliesslich Maltose gebildet, und zwar in einer Menge von ca. 56 % [1, 11], entsprechend einer Spaltung von 28 % aller Bindungen der Stärke. Maltose enthält eine 1—4-Brücke. Lassen wir eine Bildung von Maltose durch sekundäre Synthese ausser Betracht, folgt, dass Stärke 1—4-Brücken und infolge dessen Pyranosereste enthalten muss. β-Amylase bildet ein Disaccharid, spaltet also nur jede zweite Bindung. Es ist deshalb wahrscheinlich, dass ein Unterschied besteht zwischen der Bindung, die gespalten wird, und der Bindung, die ungespalten hinterlassen wird, mit anderen Worten, dass zwei Bindungstypen alternieren. Da es nur zwei Möglichkeiten gibt, muss β-Amylase eine 1—5-Bindung spalten und die 1—4-Bindung intakt lassen. Eine 1—5-Bindung kann nur an einem Furanoserest anknüpfen, weshalb in den Ketten, die β-Amylase angreift, Pyranose- und Furanosereste alternieren müssen.

Die Konstitution des Doppelgliedes, das zwischen zwei 1—5-Brücken liegt, unterscheidet sich von der der Maltose dadurch, dass der Glucosylrest einen Furanosering enthält. Da Ringänderungen in Glucosiden nicht freiwillig verlaufen [12], müssen enzymatisch bedingte Reaktionen vor sich gehen, welche sich in summa in einer Umlagerung des Furanoseringes in einen Pyranosering zu erkennen geben. β-Amylase muss sowohl die 1—5-Brücke als den 1—4-Ring öffnen, also zwei Acetalbindungen an benachbarten C-Atomen hydrolysieren. Nach Freiwerden der OH-Gruppe an C_5 stabilisiert sich das Molekül unter Bildung eines Pyranoseringes zu Maltose.

Man kennt sowohl Pyrano- als Furanoglucoside (Tabelle 5). In allen Fällen ist die Molrotation der Furanoside ca. 100° niedriger als die der Pyranoside. Eine Umlagerung eines Furanosids in das entsprechende Pyranosid würde mit einem Ansteigen der Molrotation um ca. 100° verbunden sein. Eine Umlagerung eines Furanoseringes in einen Pyranosering würde den Wert für $[Sp]_D$ der Spaltung einer α-Glucosidbindung um ca. 100° vermindern, ohne Rücksicht darauf ob Spaltung und Umlagerung am selben Glucoserest oder an

Tabelle 5. Molrotation von Glucosiden.

	M	Pyranosid		Furanosid		Differenz
		$[\alpha]_D$	$[M]_D$	$[\alpha]_D$	$[M]_D$	$[M]_D$
α.Methylglucosid	194,2	158,9 [13])	309	118 [14])	229	80
β.Methylglucosid	194,2	−34,2 [13])	−66	−77 [14])	−150	84
α.Äthylglucosid	208,2	152,0 [15])	316	101 [14])	210	106
β.Äthylglucosid	208,2	−36,7 [15])	−76	−86 [14])	−179	103

zwei verschiedenen Glucoseresten stattfinden. Für Maltose ist $[Sp]_D = 280°$, für Isomaltose 221°, also wesentlich höher als die Werte, die wir anfangs bei der Säurehydrolyse finden oder beim Abbau der Stärke durch β-Amylase beobachten. Dieser Befund harmoniert also mit unserer Anschauung über die Anwesenheit von Furanoseresten in der Stärke.

Hypothesen, dass Stärke aus alternierenden Pyranose- und Furanoseresten aufgebaut ist, sind früher aufgestellt worden, zuerst von Kuhn [16], später von Sutra [17]. Hanes [18] hat sich eingehend mit der enzymatischen Seite dieser Frage beschäftigt; er kam zu dem Schluss, dass Stärke nach der Haworth'schen Formel, also ausschliesslich aus Pyranoseresten aufgebaut sein muss. Auf Grund von reaktionsstatischen und reaktionskinetischen Messungen war auch Freudenberg [19, 20] zu dem Ergebnis gekommen, dass Stärke nur oder fast nur α-Pyranosebindungen enthält. Wären in der Stärke auch Furanosebindungen, müssten diese nach seiner Meinung mit grösserer Geschwindigkeit hydrolysiert werden und sich bei kinetischen Messungen verraten. Haworth [21] hatte nämlich gefunden, dass Methyl- und Äthylglucofuranoside 50 bis 200 Mal schneller hydrolysiert werden als die entsprechenden Pyranoside. Andere Untersuchungen [22] zeigen indessen, dass Veränderungen in der Glykosylgruppe wie im Aglykon die Hydrolysegeschwindigkeit stark beeinflussen. Ja, später hat Haworth [23] einen Fall gefunden, wo das Furanosid stabil, das Pyranosid labil ist. Ein mittlerer Glucoserest in der Stärke ist im Verhältnis zu seiner einen Glucosidbindung Glykosylgruppe, im Verhältnis zu seiner anderen Aglykon. Zudem ist er durch Substitution mit Ketten belastet. Die Verhältnisse sind also von denen in den einfachen Alkylglucosiden so verschieden, dass die Annahme einer geringeren Stabilität der Furanosidbindungen in der Stärke nicht unmittelbar einleuchtend ist.

Freudenbergs Versuche [19] wurden mit Lösungen von Stärke in 50 %iger Schwefelsäure ausgeführt. Der einzelne Versuch dauerte 2—3 Wochen und war mit einer Drehungsabnahme von nur 1,1° verbunden. Unsere Versuchsbedingungen sind ungleich milder und die Drehungsänderungen 5 bis 15 Mal grösser.

METHODIK

Stärke: Kartoffelstärke. Superior Qualität.

β-Amylase: Ein Gerstenauszug wird durch Ansäuren (pH 3,8, 0°, 16 Stunden) von jeder Spur von α-Amylase befreit. Durch Fällung mit Tannin wird ein Dauerpräparat hergestellt.

Pankreasamylase: Aus frischem Schweinepankreas wird nach üblichen Methoden ein Dauerpräparat hergestellt.

Bestimmung des Drehungsvermögens: Polarisationsapparat von Schmidt & Haensch, Monochromator, D-Linie, 4 dm-Rohr, 19°—21°.

Bestimmung des Reduktionsvermögens: Eine passende Menge der zu untersuchenden Lösung wird auf der Analysenwage abgewogen, der Überschuss an Salzsäure mit der berechneten Menge 2 N NaOH abgestumpft und evt. Wasser bis zu 10 ml zugesetzt.

Kaliumkupferkarbonatmethode nach Blom-Rosted [5].

Jodmethode nach Willstätter-Schudel: Die in einem 100 ml Stehkolben abgewogene und wie soeben beschrieben vorbereitete Probe wird mit 10,00 ml 0,1 N Jod und 10 ml 0,5 M Na_2CO_3 versetzt. Der Kolben wird verschlossen im Dunkeln aufbewahrt. Nach 40 Minuten wird mit 10 ml 1 M H_2SO_4 angesäuert und der Jodüberschuss sofort mit 0,1 N Thiosulfat zurücktitriert, wobei mindestens 3,5 ml verbraucht werden müssen. Die Jodlösung wird mittels einer automatischen Pipette, die Thiosulfatlösung mittels einer 10 ml, in 0,02 ml eingeteilten, Bürette hinzugesetzt. Beide Apparate sind von P. Haack, Wien geliefert.

Bei Bestimmung des Reduktionsvermögens in Stärkelösungen, welche nur durch β-Amylase abgebaut sind, ist auf Folgendes zu achten. Bei der Zugabe von Jod entsteht eine blauschwarze Flockung, welche bei der Rücktitration des Jodes nur langsam in Lösung geht. Die Thiosulfatlösung soll langsam zugesetzt, und kurz vor dem Umschlag soll nach Zugabe jeden Tröpfchens sehr kräftig geschüttelt werden.

Stärkekleister: 15,00 g trockene, aschenfreie Stärke werden in einem mit zugehörigem Propeller tarierten, weithalsigen 500 ml Duranrundkolben abgewogen und durch Rühren mit einem schnell laufenden Motor in 400 ml Wasser aufgeschlämmt. Ohne das Rühren zu unterbrechen, wird der Kolben in ein siedendes Wasserbad getaucht und nach 15 Minuten in ein Wasserbad von 20° überführt. Nach weiteren 15 Minuten wird das Rühren unterbrochen. Je nachdem, ob der Kleister für eine Hydrolyse durch Säure oder Amylasen verwendet werden soll, verfährt man wie in den nächsten Abschnitten beschrieben.

Hydrolyse durch Säure: Zum Kleister werden 10,0 ml 4 *N* HCl zugesetzt und der Inhalt des Kolbens auf 500,0 g aufgewogen. pH = 1,1. Nach kurzem Umrühren wird der Stärkekleister auf 5 bis 8 100 ml Stehkolben verteilt. Nach Verschliessen werden alle Kolben in ein grosses siedendes Wasserbad getaucht. In Intervallen wird ein Kolben aus dem Bad genommen und auf 20° abgekühlt. Die Lösung wird nicht filtriert.

Abbau durch Amylasen: Der Kleister wird mit 5,0 ml 0,2 *M* Na_2HPO_4, 5,0 ml 0,2 *M* KH_2PO_4 und ca. 50 mg des β-Amylasenpräparates versetzt und eine Minute gerührt. Der Kolben wird nun verschlossen in ein Wasserbad von 40° getaucht. Nach passender Zeit (1 bis 4 Stunden) wird pH von 6,8 auf ca. 1 durch 10,0 ml 4 *N* HCl verschoben. Bei dieser Acidität wird die β-Amylase schnell zerstört. Nach ca. 10 Minuten wird pH wieder auf 6,8 durch 20,0 ml 2 *N* NaOH zurückgeführt. Eine Probe von ca. 50 g wird zur Bestimmung des Reduktionsvermögens entnommen. Zum Rest werden ca. 10 mg des Pankreaspräparates hinzugesetzt und der Kolben nach kräftigem Schütteln wieder im Wasserbade von 40° angebracht. In passenden kurzen Intervallen werden Proben von ca. 90 g in tarierte Kolben, die ca. 2 g 4 *N* HCl enthalten, gegossen. Dadurch wird pH wieder auf ca. 1 verschoben, um die Wirkung der Pankreasamylase zu sistieren. Nach Abkühlen werden die Lösungen durch 9 cm Filter, worauf ca. 75 mg Kieselgur angebracht sind, filtriert. Nur die letzten 25 ml des Filtrates werden zur Bestimmung von Drehung, Dichte und Reduktion benutzt.

Während des ganzen Versuches wird durch Wiegen vor und nach den einzelnen Manipulationen genauer Kontakt mit der ursprünglichen Stärkemenge bewahrt.

Zwecks Bestimmung der Blindwerte werden vollständige Parallelversuche ohne Stärke durchgeführt.

Nach der Methode der kleinsten Quadrate wird die Neigung, $[Sp]_D$, der Geraden durch die Pankreaspunkte und daraus das Drehungsvermögen, welches dem Reduktionsvermögen nach Sistierung der β-Amylase entspricht, berechnet.

ZUSAMMENFASSUNG

Die Hydrolyse von Kartoffelstärkekleister durch Salzsäure (0,08 *N*, 100°) wird durch gleichzeitige Messungen von Drehungs- und Reduktionsvermögen verfolgt. Die Resultate lassen sich nur dadurch erklären, dass Stärke ausser den bekannten Bindungen vom 1—4- und 1—6-Typus noch Bindungen von einem dritten Typus enthält. Dieses Ergebnis lässt sich mit der Bildung von 85—90% 2,3,6-Trimethylglucose bei der Säurehydrolyse methylierter Stärke in Einklang bringen durch die Annahme der Anwesenheit von Glucofuranoseresten, welche wiederum Bindungen vom 1—5-Typus bedingen.

Der Abbau von Kartoffelstärkekleister durch β-Gerstenamylase ist auf ähnliche Weise untersucht. Aus diesen Versuchen und dem Befund, dass β-Amylase ausschliesslich ein Disaccharid und zwar Maltose bildet, muss der Schluss gezogen werden, dass in dem Teil der Stärke, welcher von β-Amylase angegriffen wird, Pyranose- und Furanosereste alternieren. β-Amylase spaltet die 1—5-Bindung zwischen einem Pyranose- und einem Furanoserest und öffnet den Furanosering, worauf das Zwischenprodukt sich zu Maltose stabilisiert.

Die [Verfasser wünschen der Leitung der Tuborg-Brauereien] ihren Dank auszusprechen.

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X-ray Investigation of Cu-Ce Alloys, Rich in Copper. With a Discussion of the Interatomic Distances in the A_5B Structure Type

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The phase diagram of the system Cu-Ce has been determined by Hanamann[1]. He concluded from a thermal investigation of Cu-Ce alloys that four intermediate compounds exist, namely Cu_6Ce, Cu_4Ce, Cu_2Ce and CuCe. In the present paper, an X-ray investigation of alloys with cerium contents of 25 atomic percent will be reported.

The copper used in the investigation was obtained from Boliden Mining Co. and had a purity of 99.99 %. The cerium was less pure. A preliminary spectroscopic investigation showed that the chief impurities were Fe and Mg, and smaller amounts of Mn and Ca. The chemical analysis gave the following values: Fe: 0.9 %; Mg; 1.2 %; Ca, Mn < 0.1 %. Another sample of Ce used in the alloys from which single crystals of Cu_6Ce and Cu_4Ce were obtained, showed a considerably lower Mg content of 0.5 %.

The analysis of Ce was carried out in the following way: Ce was precipitated as oxalate, and re-precipitated twice. The Fe was precipitated with $NH_3 + NH_4Cl$, the precipitation dissolved and precipitated again, and determined as Fe_2O_3. Mg was determined as phosphate in the filtrate. 1.27 % Mg was obtained in this way. To avoid some of the reprecipitations, another method was also tried and with satisfactory results. With this latter method the solution containing Ce, Fe, and Mg was passed through an ion exchange column with Amberlite IR 120. Sufficient ion exchanger was used so that 95 % of Ce was absorbed. A qualitative analysis of the absorbed Ce showed no traces of Feand Mg. The remaining Ce was precipitated together with Fe with $NH_3 + NH_4Cl$, and Mg was determined in the filtrate as phosphate. 1.10 % Mg was found by this method, which was also used for the second sample of Ce.

The alloys were prepared by melting together Cu and Ce in evacuated silica tubes. The molten alloys were shaken vigourously. They were then rapidly quenched and the ingots annealed for 1—2 weeks. Filings were taken,

avoiding the surface layer of the ingots, which contained some oxide. The filings were then annealed again.

The Cu Phase

The cube edge of the Cu used in the investigation was $a = 3.615 \pm 0.002$ Å. (Cr-K radiation. $K_{\alpha_1} = 2.28962$ Å, powder photographs in focussing cameras) agreeing satisfactory with the precision value for pure Cu of $a = 3.6152$ Å [2]. Powder photographs of alloys in the two phase region Cu + Cu_6Ce, annealed at 770°, showed a Cu-phase with — within the limits of the errors in the determination — the same cell dimensions. Assuming Vegard's law to be valid, we can estimate that the solubility of Ce in solid Cu is < 0.5 atomic % at this temperature.

The Cu_6Ce Phase

It was found to be very difficult to attain equilibrium for alloys with 10—20 atomic % Ce. In spite of prolonged melting-time and prolonged annealing (several weeks sometimes proved insufficient), the x-ray examination showed for some alloys the presence of three phases: Cu and two intermediate phases. In other cases, different part of an ingot gave widely different powder photographs. Thus the composition of the first intermetallic phase in the system could not be deduced from the powder photographs. Single crystals of the phase could, however, be picked out under the microscope from an ingot with 17 atomic % Ce. Two samples of about 10 mg were analysed, and the following values obtained:

Observed weight %	Cu: 72.2	Ce: 27.1
Calculated for Cu_6Ce:	73.2	26.8

Cu was determined by electrolysis. As there was some indication in the literature[3] that Ce might disturb the Cu electrolysis, some control experiments were carried out. Samples with known amounts of Cu and 50 respectively 75 % Ce were electrolyzed. However, the added Cu amount was always completely recovered. Ce was precipitated after the electrolyses as oxalate and weighed as oxide.

Because of the difficulty in obtaining homogeneous samples, it was difficult to obtain a reliable value for the density. However, determinations on several samples indicated a value of 8.0. Preliminary cell dimensions from rotation and Weissenberg photographs were refined from powder photographs (Cr-K). The following orthorhombic unit cell was obtained:

$$a = 8.08_5 \text{ Å} \qquad b = 5.09_7 \text{ Å} \qquad c = 10.17_2 \text{ Å}$$

The reflexions in the range $\sin^2\Theta = 0.07-0.50$ are listed in Table 1.

Table 1. Powder photographs of Cu_6Ce Comparison between observed and calculated $\sin^2\Theta$ values. Cr-K radiation, β-reflexions omitted.

hkl	$10^4\sin^2\Theta_{calc}$	$10^4\sin^2\Theta_{obs}$	I_{obs}
202	1310	1304	w
211	1435	1430	vvw
013	1646	1647	v st
113	1850	1851	st
020	2020	2016	vvst
104	2230	2236	vvst
302	2314	2305	m
121	2348	2353	m
311	2438	2439	vvst
022	2527	2530	st
122	2728	2739	w
114	2735		
312	2819	2827	st
220	2823		
204	2832		
221	2948	2941	m
303	2950		
401	3338	3326	vst
105	3371	3372	vst
015	3675	3687	vw
304	3835	3823	vw
321	3953	3963	w
223	3964		
205	3973		
124	4250	4251	w
322	4334	4324	vw
314	4340		
106	4766	4772	vvw
224	4852	4837	vvw
413	4857		
421	5358	5368	w
206	5368		

Assuming 4 formula units Cu_6Ce in the unit cell, a density of 8.27 would be expected, in plausible agreement with the observed value of 8.0.

The reflexions in the Weissenberg photographs (Cu$-K$) registering *hk*0, *hk*1, *h*0*l*, and *h*1*l* reflexions indicated that the space group is $D_{2h}^{16}-Pnma$, provided that the structure is centro-symmetric. With this space group the four Ce atoms must be placed in 4(*c*):$x\frac{1}{4}z$, because in the other four-fold positions the atoms are placed only $\frac{b}{2} = 2.55$ Å.

apart, which is improbably short for a Ce—Ce distance ($r_{Ce} = 1.8$ Å). Because of the mirror-plane perpendicular to b, the atoms in the general positions will be $\frac{b}{2} = 2.55$ Å, this implies that the y parameter must be close to 0 for atoms in the general position. For the atoms with $y = 0$ (thus for those in the general position and in 4(a) and 4(b)) the structure factor of the reflexions $hk0$ is equal to zero, when k is odd. The Weissenberg photograph $hk0$ shows, however, that some of the reflexions $h10$ are in fact stronger than those with k even. This indicates that not only the Ce atoms but also some of the Cu atoms must be placed in 4(c). Consideration of the intensities of the reflexions $0k0$ indicates that eight Cu atoms are placed either in 4(a) + 4(b), or in 8(d) with $y \sim 0$, and four Ce atoms + sixteen Cu atoms in the position 4(c) with $y = \frac{1}{4}$. The structure determination thus involves the determination of five or six x parameters and an equal number of z parameters, but attempts to solve the problem have so far been unsuccessful.

The Cu_4Ce Phase

Single crystals of this phase were easily obtained from alloys with 20—25 atomic % Ce. The hexagonal unit cell determined from powder photographs (Cr-K radiation) has the dimensions $a = 5.150 \pm 0.002$ and $c = 4.102 \pm 0.002$ Å. There were no systematic extinctions in the Weissenberg photographs.

It was easily found that the observed intensities corresponded to a structure of the same type as Zn_5Ca[4], and it was therefore inferred that the formula of this Cu-Ce phase was Cu_5Ce. However, at this stage of the investigation a paper appeared by Heumann [5] on the composition of this phase. Heumann concludes from a microscopical investigation of alloys that the composition of the phase is Cu_4Ce and he assumes that the unit cell, for which he gives the dimensions $a = 5.131$ kX $c = 4.124$ kX, contains 1.2 formula units, thus six atoms. This indicates that 0.2 Ce atoms are placed in one or both of the Cu positions (see below) together with 4.8 Cu atoms. Because of the large difference in atomic radii ($r_{Cu} = 1.25$, $r_{Ce} = 1.8$ Å) this seemed rather improbable but further investigation confirmed Heumann's results.

Because of the difficulty in reaching equilibrium for alloys containing this phase, too, the analysis was carried out in microscale on selected single crystals by the same method as for Cu_6Ce. The following values were obtained:

Observed weight %:	Cu: 64.2	Ce: 35.5
Calculated for Cu_4Ce:	64.47	35.53

As for Cu_6Ce, a reliable determination of the density was difficult, but determinations on different samples placed the value at about 8.0. With 1.2 Cu_4Ce in the unit cell the calculated density would be 8.36 and with 1 Cu_4Ce in the cell 6.95. Obviously the six atomic positions in the cell are completely or very near completely filled with atoms. The arrangement of the atoms is:

Table 2. Weissenberg photographs of Cu_4Ce. Comparison between $|F_{obs}|$ and $|F_{calc}|$. Cu-K radiation.

$h\,k\,l$	$\lvert F_{obs}\rvert$	$\lvert F_{calc}\rvert$	$h\,k\,l$	$\lvert F_{obs}\rvert$	$\lvert F_{calc}\rvert$
001	25	26	111	83	109
002	182	147	112	50	58
003	33	20	113	116	80
004	166	102	114	83	43
005	30	15	115	91	61
100	7	1	220	66	114
200	75	88	221	17	19
300	45	55	222	66	105
400	50	62	223	17	16
500	13	2	224	116	86
101	43	49	330	17	41
102	0	2	331	33	65
103	66	35	332	33	38
104	7	3	210	0	3
105	35	27	211	18	39
201	31	39	212	0	3
202	70	83	213	17	31
203	36	27	214	15	2
204	75	57	310	0	4
301	106	87	311	0	33
302	45	49	312	0	3
303	99	73	313	13	29
304	73	41	314	8	3
401	15	26	410	26	44
402	35	57	411	55	70
403	13	23	412	20	41
501	18	28	413	83	61
502	10	3	510	0	3
110	50	70	511	31	26

Space group D_{6h}^1

Ce in 1 (*a*): 000 0.2 Ce + 4.8 Cu in 2 (*c*): $\frac{1}{3}\frac{2}{3}0$; $\frac{2}{3}\frac{1}{3}0$ + 3 (*g*): $\frac{1}{2}0\frac{1}{2}$; $0\frac{1}{2}\frac{1}{2}$; $\frac{1}{2}\frac{1}{2}\frac{1}{2}$.

The Ce atoms may obviously be placed either in 2(*c*) with only Cu atoms in 3(*g*), or in 3(*g*) with only Cu atoms in 2(*c*), or in both positions. The effect on the intensities is however too slight to enable a conclusion to be drawn as to which of these three arrangements is correct. Table 2 gives the observed and calculated *F*-values, assuming the Ce atoms to be statistically distributed among the Cu atoms.

The interatomic distances are:

(Cu_1 denotes an atom in 2 (*c*), Cu_2 an atom in 3 (*g*).)

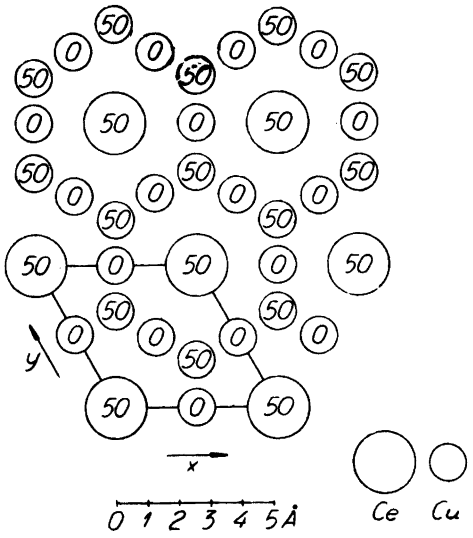


Fig. 1. Cu_4Ce. Projection on the xy plane of the structure. The figures denote the height of the atoms in percentages of c.

Ce— 6 Cu_1	= 2.973 Å ± 0.002;	Cu_1—3 Ce	= 2.973 Å ± 0.002;
Ce—12 Cu_2	= 3.292 » »	Cu_1—3 Cu_1	= 2.973 » »
Ce— 2 Ce	= 4.102 » »	Cu_1—6 Cu_2	= 2.533 » »
		Cu_2—4 Ce	= 3.292 ± 0.002 Å
		Cu_2—4 Cu_1	= 2.575 Å
		Cu_2—4 Cu_2	= 2.533 »

As will be seen, the coordination number of the Ce atoms is twenty (Fig. 1).

The Relation between Bond Number and Atomic Radii for the A/B Structure Type

It is of some interest to discuss the relation between bond number and radius recently put forward by Pauling [6] for the A_5B structure type, partly because of the high coordination number of the B atoms and the large contraction in A_1—B distances, and partly because of the astonishing deviation from the ideal formula for some phases belonging to this structure type.

The equation used by Pauling is: $R(1)-R(n) = 0.300 \log n$. $R(1)$ and $R(n)$ are the radii corresponding to the bond numbers 1 and n, respectively. The bond number n is defined as the number of shared electron pairs in the bond.

The following table gives the cell dimensions of all hitherto investigated A/B phases. The original values are probably all expressed in kX units and have been transformed into Å units.

	a	c	
Co_5Ce	4.955 Å	4.055 Å	Heumann [5]
Co_5Th	4.950	4.039	»
Ni_5Ca	4.960	3.948	Nowotny [7]
Ni_5La	4.962	4.008	»
Ni_5Ce	4.874	4.004	»
Ni_5Pr	4.948	3.973	Fülling [8]
Ni_5Cd	4.91	3.99	Endter, Klemm [9]
Ni_5Th	4.921	3.990	Heumann [5]
Cu_5Ca	5.107	4.073	Nowotny [7]
$(Cu_{4.8}La_{0.2})La$	5.169	4.116	»
$(Cu_{4.8}Ce_{0.2})Ce$	5.150	4.102	this paper
Zn_5Ca	5.382	4.250	Nowotny[7]
Zn_5La	5.427	4.225	»
$Zn_5(Th_{0.6}Zn_{0.4})$	5.247	4.451	» [10]

Somewhat different values have been given for Cu_5Ca and Zn_5Ca by Haucke [4] with $a = 5.092$ Å, $c = 4.086$ Å, and $a = 5.416$ Å, $c = 4.191$ Å, respectively.

From the values in the above table, the interatomic distances in the A_5B phases have been calculated. These distances have been compared with distances calculated from Pauling's equation assuming the values of the metallic valency V, which are shown in Table 3. The radii of the metals used in the calculations are taken from Pauling [6] and transformed into Å units.

The following table gives the B—6 A distances in the A_5B phases compared with those calculated in this way. As will be seen, differences ranging from 0.5 to 0.1 Å are obtained.

The small differences in electronegativity for the A and B metal — of the order 0—1.2 units — makes it rather improbable that there is an appreciable amount of ionic character in the bonds, as was assumed by Dehlinger, Nowotny *et. al.* (see (13) and (14)) in several alloys, especially those containing alkali atoms. In fact, the direction of the electron transfer, which probably takes place in these phases (from the A atoms to the B atoms, see below) will decrease these differences in electronegativity and thus the amount of ionic character in the bonds still more.

The effect of an electron gain or loss on the two kinds of atoms was now considered. Pauling assumed that the gain of an electron for a calcium atom results in an decrease in radius (because of dsp hybridization) slightly less than the difference r(1)Ca—r(1)Sc[6]. If we follow this suggestion, the following radii would be expected:

Table 3.

$V_A \rightarrow$	Co_5 5.78	Ni_5 5.78	Cu_5 5.44	Zn_5 4.44
V_B ↓				
Ca 2		2.864 Å 3.349 − 0.485	2.949 3.352 − 0.403	3.107 3.411 − 0.304
La 3		2.866 3.160 − 0.295	2.984 3.181 * − 0.197	3.133 3.258 − 0.125
Ce 3.2	2.860 3.095 − 0.234	2.814 3.087 − 0.273	2.973 3.116 * − 0.143	
Pr 3.1		2.857 3.108 − 0.251		
Gd 3		2.835 3.076 − 0.241		
Th 4	2.858 3.043 − 0.185	2.841 3.039 − 0.198		3.039 3.104 * − 0.075

		+ 1 electron	+ 2 electrons
r(1)Ca:	1.739 Å	1.46 Å	1.34 Å
r(1)La:	1.693 »	1.44 »	
r(1)Th:	1.655 »	1.50 »	

The effect of the electron loss on the A metals is small and — as it probably amounts only to 0.02—0.04 Å — has been neglected (see below).

A—B distances corresponding to the addition of one and two electrons to the B metals were now calculated from these radii and the new values for valencies and bond numbers. Thus we obtain for Ni_5Ca:

+ 1 electron $n_1 = 0.305$; Ca—6 Ni$_1$ = 1.46 + 1.15—0.6 log n_1 = 2.94Å
+ 2 » $n_1 = 0.410$; Ca—6 Ni$_1$ = 1.34 + 1.15—0.6 log n_1 = 2.74 Å

* Calculated for the ideal composition A_5B. See p. 715.

As will be seen from Table 3, the actual value observed is 2.863 Å. Interpolation between the values for + 1 and + 2 electrons gives a gain of 1.4 electrons.

The electron gain were calculated in the same manner for the Ca, La, and Th phases. The results are collected in the table p. 12.

Attempts were made to find whether a relation exists between the differences in electronegativity and the contractions. As the electronegativity for Cu is probably higher than both that for Ni and that for Zn [11, 12] whereas the contractions steadily decrease in the series Ni—Cu—Zn (with the same B partner), there seems to be at least no simple relation between the electronegativities and the contractions (see the table below). It must, however, be remembered that the electronegativity values are very approximate.

It seemed possible that the relative size of the atoms $\frac{r_B}{r_A}$ was of importance for the magnitude of the electron gain of the B atoms. The quotients $\frac{r_B}{r_A}$, calculated with the radii for 18 coordination for the B atom and 12 coordination for the A atom, and also the differences in electronegativity $n_A - n_B$ have been collected in the table below together with the values of the electron gain g of the B atoms:

		Ni	Cu	Zn
Ca	g	1.4	1	0.7
	r_B/r_A	1.625	1.584	1.466
	$n_A - n_B$	0.8	1.2	0.2
La	g	0.9	0.6	0.4
	r_B/r_A	1.589	1.549	1.433
	$n_A - n_B$	0.6	1.0	0
Th	g	0.9		0
	r_B/r_A	1.558	1.519	1.406
	$n_A - n_B$	0.6	1.0	0

As will be seen, the values of the electron gains and those of the relative size of the atoms decrease in the same way for the listed compounds. Thus it seems, that the relative size of the atoms in the A_5B alloy is the dominating factor in determining the amount of electron transfer, though of course the

difference in electronegativity may also be of importance. An investigation of the contractions of the interatomic distances in the Laves' phases of the C 15 structure type shows, that for them this latter factor (the difference in electronegativity) is the most important, though the influence of the relative size is obvious also in that case (Byström and Kierkegaard to be published).

It was first assumed that this electron gain was proportional to the difference in valency. We can then calculate the gains from the value in Ni_5Ca from the formula $g = 1.4 \frac{V_A - V_B}{V_{Ni} - V_{Ca}}$. The following values are obtained:

	Ni	Cu	Zn
Ca	1.4	1.3	0.9
La	1.0	0.9	0.5
Th	0.7		

As will be seen, the agreement between the two sets of values may indicate a relation, s suggested. However, the further study of the Laves' phases indicated no such relation, and the agreement between the two sets of g values is probably accidental.

The gain of 0.5—1.4 electrons by the B atoms corresponds to a mean loss of 0.1—0.3 electrons by the A atoms. As is apparent from a comparison between the radii of the transition metals, this loss has a comparatively small influence on the interatomic distances: an increase in the A—A distances of some few hundredths of an Å unit would be expected. The increase actually found varied between — 0.02 and 0.10 Å with a mean value of 0.05 Å, the smallest increase being found for the Zn alloys with also the least amount of electron transition. It must, however, be observed that the cell dimension of the A_5B phases may not have the four figure accuracy assumed in the calculations of the distances. A comparison of Nowotny's and Haucke's values (see p. 1000) shows differences as large as 0.06 Å. A detailed discussion of the differences in observed and calculated A—A distances is therefore hardly possible without a careful re-determination of the cell dimensions.

The suggested electron transfer in the A_5B phases may offer an explanation of the deviation from the ideal formula observed for three of the alloys. As we have seen, the electron gain of the B atoms leads to a partial equalisation of the metal valencies, and also of the metal radii. Thus the difference between the "normal" metal radii of Cu and Ce is about 0.55 Å but the electron transfer diminishes this difference to about 0.2 Å. The increase in the A radii, which probably results from the occurence of Ce (and La) atoms in Cu positions, was not taken into account in the calculations of the A—B and A—A distances. Assuming Vegard's law to be valid, it amounts to about 0.02 Å and may be an explanation of the relatively large differences between observed and calculated A—A distances for these two alloys (0.08—0.09 Å).

For the Zn—Th phase a solution of A atoms in the B atoms is found (Nowotny [10]), leading to the formula $Zn_5(Th_{0.6}Zn_{0.4})$. This gives an almost complete equilization of the metal valencies of the A and B atoms, the values being 4.44 and 4.17 respectively. The quotient $R(18)_A'R(12)_B$ has a low value and hence no or a very small contraction of the A—B distances is to be expected. The apparent $R(1)$ radius for the B atoms, derived from the observed A—B distances, is 1.59 Å. The value expected from Vegard's law (0.6 R_{Th} + 0.4 R_{Zn}) is 1.49, but this deviation is by no means unacceptable.

The assumption of an electron transfer from the A atoms to the B atoms seems to lead to an explanation of the contraction of the A—B distances and elongation of the A—A distances in the A_5B phases. It is, of course, of interest to investigate whether contractions found in alloys of other structural types can be explained in a similar way. In a following paper the contractions on the Laves' phases of the C 15 structure type will be discussed.

SUMMARY

The cell dimensions and symmetry of two intermediate Cu—Ce phases, Cu_6Ce and Cu_4Ce, have been determined. Cu_4Ce belongs to the A_5B structure type with a cell content of 1.2 Cu_4Ce.

The interatomic distances in the A_5B structure type are discussed. It is shown that the amount of the contractions of the A—B distances is due to the relative size of the atoms and probably also to the difference in electronegativity. An electron transfer from the A toms to the B atoms is shown to agree with the observed distances.

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A New Method of Measuring Small Refractive Index Differences

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A modification of the Rayleigh interference refractometer, characterized by the production of an optical image of the light source slit in one dimension only and an optical image of the cell in the other dimension, was described by Calvet[1–3] and by Philpot and Cook[4]. The new instrument and its application to diffusion and electrophoresis has since been the object of a number of investigations[5–13] which show that it is an extremely valuable tool in physico-chemical measurements. The present paper deals with still another application, the measurement of small refractive index differences.

The measurement of such differences, *e.g.* that between a solution and the solvent, is a frequently performed operation in chemical laboratories as an accurate and convenient way of determining the concentration of the solution. A direct measurement of the refractive index difference is superior to two separate measurements of absolute refractive indices for several reasons. First, the time required is less, and the accidental error is smaller in one measurement than in two, Second, a number of systematic errors are automatically eliminated in the direct procedure.

The differential refractometers that have been described in the literature are, in general, based on the measurement of the angular deflection of light in a double-prismatic cell, or on interferometric measurement of optical thickness in white light. While the interferometric method is superior in accuracy, the result is sometimes obscured by the fact that the optical path length compensator does not have the same colour dispersion as the solutions under investigation. In addition, the result obtained does not refer to any specified wave-length and is thus a little undefined. With coloured solutions, the white light interferometric method often fails altogether.

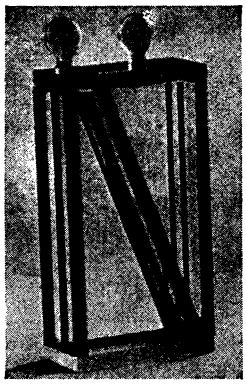


Fig. 1. The double-prismatic cell.

The present differential refractometer makes use of a double-prismatic cell, but in conjunction with a Rayleigh-Calvet-Philpot interference refractometer operating with monochromatic light. In this way, the high accuracy characteristic of the interferometric method is guaranteed at the same time as the above-mentioned difficulties have been removed.

CELL CONSTRUCTION

The cell used is shown in Fig. 1. It has a parallelepipedic form with the outside dimensions of 31 × 57 × 14 mm. The smaller vertical walls as well as the diagonal partition wall are each made of one piece of high-precision optical glass. Consequently the two side walls are composed of triangular pieces cemented between the afore-mentioned walls. All three optical walls extend a distance outside one of the side walls. When the cell is traversed by light, this wall will subdivide the aperture defined by the cell into two portions, both having exactly equal thicknesses of glass in the light paths. In the roof piece of the cell, there are two small conical holes with glass stoppers, for filling and emptying the cell. Since this cell is used in a water-bath, the two prismatic chambers to the right are not covered. When it is immersed into the water, the latter will automatically fill these spaces. For a cell to be used in air, equality between the optical path lengths inside and outside the cell would have to be guaranteed by other means.

The two halves of the cell are filled with the two solutions to be compared. The smaller chamber, with the tip of the triangle towards the bottom, is suitably used for the solution, which is generally not available in quantity. Its total volume is 4.5 ml, but measurements can be carried out with any smaller volume. Reduction of the volume by a factor of 4 will only reduce the accuracy to 1/2 since it is the height of the liquid column that defines the accuracy.



Fig. 2. Rayleigh interferogram obtained with distilled water and a one per cent sugar solution in the two chambers of the double-prismatic cell. The number of fringes per unit length in a direction parallel with the black line is proportional to the refractive index difference.

THEORETICAL

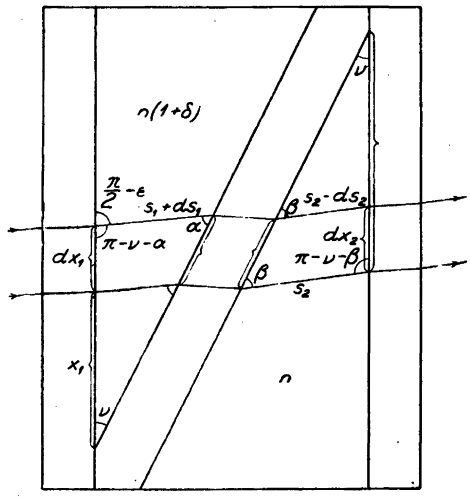
It is easy to realize that the optical thickness of the cell along a horizontal line will be a linear function of the height coordinate, while the optical thickness of the comparison cell is independent of this coordinate. When the cell is put in place into the optical system of the Rayleigh-Calvet-Philpot interferometer, therefore, the resulting interferogram will contain a number of equidistant fringes which make a certain angle with the cell axis and which are more closely spaced along this axis, the greater the refractive index difference in the cell. Fig. 2 shows such an interferogram obtained with distilled water and a one per cent sugar solution in the cell, and with the modification of the interferometer described in reference [12]. The vertical black line is the optical image of a vertically suspended thread which is superposed on the interferogram and serves as a reference line.

In an exact theory of such a cell as this, one has to take into account the fact that the light does not travers it in a horizontal direction, and that the optical path length has to be computed along the actual course of the ray. Fig. 3 shows a drawing of the cell and two mutually parallel rays of light traversing it. The notation used is evident directly from the figure. The difference in optical path length between these two rays is:

$$\mathrm{d}s = n(1 + \delta)\ \mathrm{d}s_1 - n\ \mathrm{d}s_2 \tag{1}$$

but the relations:

$$\frac{\mathrm{d}s_1}{s_1} = \frac{\mathrm{d}x_1}{x_1} \text{ and } \frac{\mathrm{d}s_2}{s_2} = \frac{\mathrm{d}x_2}{x_2} \tag{2}$$



give:

$$ds = n\ (1 + \delta)\ \frac{s_1}{x_1}\ dx_1 - n\ \frac{s_2}{x_2}\ dx_2 \tag{3}$$

According to the sine theorem, we have:

$$\frac{s_1}{x_1} = \frac{\sin v}{\sin \alpha} \text{ and } \frac{s_2}{x_2} = \frac{\sin v}{\sin \beta} \tag{4}$$

$$\frac{dx_1}{dl} = \frac{\sin \alpha}{\sin\ (v + \alpha)} \text{ and } \frac{dl}{dx_2} = \frac{\sin\ (v + \beta)}{\sin \beta} \tag{5}$$

This gives the equation:

$$\frac{ds}{dx_2} = n\ (1 + \delta)\ \frac{\sin v \sin (v + \beta)}{\sin \beta \sin (v + \alpha)} - n\ \frac{\sin v}{\sin \beta} \tag{6}$$

Since x_2 and $x_2 + dx_2$ are the exit coordinates, they are directly related to the coordinates on the photographic plate by the magnification factor; the cell and the plate being corresponding image planes. Equation (6), therefore, gives after division by the cell magnification directly the fringe density along the vertical line on the photographic plate.

With the aid of the relation:

$$\frac{\pi}{2} - \varepsilon = \alpha + v \tag{7}$$

equation (6) can also be written in the form:

$$\frac{1}{n}\frac{ds}{dx_2} = (1+\delta)\frac{\sin v \sin (v+\beta)}{\sin \beta \cos \varepsilon} - \frac{\sin v}{\sin \beta} \tag{8}$$

We will now regard the right-hand side of this equation as a function of the two independent variables δ and ε and derive the second-degree approximation of this function according to the formula:

$$F(\delta, \varepsilon) = F(0,0) + \delta \frac{\partial F(0,0)}{\partial \delta} + \varepsilon \frac{\partial F(0,0)}{\partial \varepsilon} +$$

$$+ \frac{1}{2}\left[\delta^2 \frac{\partial^2 F(0,0)}{\partial \delta^2} + 2\delta\varepsilon \frac{\partial^2 F(0,0)}{\partial \delta \partial \varepsilon} + \varepsilon^2 \frac{\partial^2 F(0,0)}{\partial \varepsilon^2}\right] \tag{9}$$

It is then to be remembered that β is also a function of these two variables according to Snell's law:

$$(1+\delta) \sin (v+\varepsilon) = \cos \beta \tag{10}$$

Its partial derivatives are:

$$\frac{\partial \beta}{\partial \delta} = -\frac{\sin (v+\varepsilon)}{\sin \beta} \tag{11}$$

$$\frac{\partial \beta}{\partial \varepsilon} = -\frac{(1+\delta) \cos (v+\varepsilon)}{\sin \beta} \tag{12}$$

For $\delta = \varepsilon = 0$ they assume the values $-\tan v$ and -1, respectively, since β is then $= \pi/2 - v$.

The partial differentiation of the function $F(\delta,\varepsilon)$ gives the result:

$$\frac{\partial F(0,0)}{\partial \delta} = \tan v \qquad \frac{\partial F(0,0)}{\partial \varepsilon} = 0 \tag{13}$$

$$\frac{\partial^2 F(0,0)}{\partial \delta^2} = \tan^3 v \cdot \qquad \frac{\partial^2 F(0,0)}{\partial \delta \partial \varepsilon} = 0 \qquad \frac{\partial^2 F(0,0)}{\partial \varepsilon^2} = 0 \tag{14}$$

The second-degree approximation of $F(\delta,\varepsilon)$ can thus be written:

$$\frac{ds}{dx_2} = n\delta \tan v + n \frac{\delta^2}{2} \tan^3 v \tag{15}$$

From the disappearance of ε in this expression we conclude that the fringe displacement is independent of the angle of incidence. This means that a vertically extended light source slit or grating can be used without obscuring the result. Compare in this respect reference [8].

The δ^2 term in (15) allows us to compute the deviation of the fringe density from the linear dependence on δ expressed by the simple formula:

$$\frac{ds}{dx_2} = n\ \delta\ \tan\ v \tag{16}$$

If we require the second term not to exceed 10^{-6} in refractive index, we get for this cell, with $\tan v = 0.4$, the condition:

$$\delta < 0.00485 \text{ and } \Delta n = n\ \delta < 0.00647 \tag{17}$$

for water solutions. We should thus expect an exactly linear dependence of the fringe density on the refractive index difference up to $\Delta n = 650 \cdot 10^{-5}$, which corresponds to about 4.5 per cent sugar and 3.5 per cent protein solution. Consequently the simple equation (16) can be used in the majority of practically occurring cases.

If greater refractive index differences than (17) have to be measured, the cell with $\tan v = 0.4$ is unsuitable, not only due to the non-linear dependence, but also because the fringe density becomes too high for ordinary photographic material to resolve. The useful refractive index range of the instrument, however, can be increased at will by constructing other cells with smaller values of $\tan v$.

The accuracy to be expected from this new method and instrument can easily be computed. If the availabie height (the height of the cell, or the height of the liquid column if the cell is not completely filled) is h cm, the effective thickness of the cell becomes $h \tan v$, and the difference in optical thickness between top and bottom of the cell $h \tan v\ \Delta n$ cm $= h\ \Delta n \tan v/\lambda$ wavelengths. According to experience gained in the references [5–13], it is possible under favourable conditions to localize a fringe to within 1/50 of the separation between fringes. We thus get the following expression for the ultimate limit of accuracy of this method:

$$\Delta n = \frac{\lambda}{50\ h\ \tan\ v} \tag{18}$$

With the numerical figures of the actual cell, $h = 4.5$ cm and $\tan v = 0.4$, and with $\lambda = 5461 \cdot 10^{-8}$ (the green mercury line), we get $\Delta n = 0.6 \cdot 10^{-6}$.

EXPERIMENTAL

In order to test the performance of the cell and the accuracy obtainable by this method, measurements were carried out on a number of sugar solutions in distilled water of exactly known concentrations. A stock solution was pre-

pared by dissolving 12.5070 g of sucrose (Merck's analytical reagent) in distilled water in a 250 ml calibrated volumetric flask. This gave a concentration of 5.0201 g per 100 ml at 25°. A series of lower concentrations were then prepared by diluting this stock solution, using calibrated pipettes and volumetric flasks. The resulting concentrations are given in Table 1, the first column. Accurate refractive index data for sugar solutions have been presented by Gosting and Morris [14]. Column 2, Table 1, gives the refractive index increments corresponding to the concentrations in column 1, calculated by interpolation from Gosting's and Morris' values.

Table 1.

Sugar concentration g/100 ml	Refractive increment · 10^6	Fringes / cm observed	Fringes / cm calculated	Fringes / cm discrepancy
0.0000	0.0	0.60	0.56	+0.04
0.1000	143.0	4.01	4.03	−0.02
0.2007	287.1	7.48	7.53	−0.05
0.3006	430.1	11.01	11.01	0.00
0.5014	717.4	18.00	17.99	+0.01
1.0028	1434.7	35.43	35.41	+0.02
2.0056	2869.0	70.26	70.26	0.00
3.0094	4304.2	105.13	105.13	0.00
4.0123	5737.7	139.63	139.95	−0.32
5.0201	7178.3	174.62	174.96	−0.34

One of the chambers of the double-prismatic cell was filled with the sugar solution, the other with distilled water. The cell was then put into the water-bath, the temperature of which was kept at 25.0°. After a time period for temperature equilibration, the interferogram was photographed. One photograph was also taken with distilled water in both chambers of the cell. Green mercury light ($\lambda = 5461$ Å) was used.

After developing, the plates were measured in a comparator. They were aligned on the comparator table with the aid of the reference line seen in Fig. 2, and the distance between two fringes, one in each end of the cell, was measured. Finally the number of fringes per cm was calculated. These figures are given in column 3, Table 1.

The figures in columns 2 and 3, Table 1, were then treated by the method of least squares, and the resulting linear relationship between fringe density and refractive index increment was:

$$\frac{ds}{dX} = 0.024296\,\Delta n + 0.556 \qquad (19)$$

From this equation, one should expect the fringe densities given in column 4, Table 1. The discrepancies between these and the observed values, column 3, are given in column 5.

DISCUSSION

The experiments described above show a good agreement with theoretical expectations. In addition, they give an accurate calibration of this particular cell. The constant term in equation (19) indicates a slight optical imperfection of the cell or a slight maladjustment of the optical system, and has to be added to the observed fringe density in every measurement.

The last column in Table 1 shows that the method is capable of an accuracy in the readings of some hundredths of a fringe in the lower concentration range. With the dimensions of this particular cell, this corresponds to one unit in the sixth decimal place in the refractive index. Naturally this accuracy does not persist at higher concentrations simply because the resolving power of the comparator sets a limit at high fringe densities.

The method should, in the first place, be useful for those laboratories where an electrophoresis or a diffusion apparatus with an astigmatic optical system is available. The only thing to be supplied is then the double-prismatic cell itself. That is also the way in which this investigation has been carried out. Should the apparatus not be equipped with facilities for Rayleigh interference observation, but only for *Schlieren*-optical measurements, the cell can still be used on the basis of measuring the angular deflection in the cell. However, some reduction fo the accuracy is then to be expected.

SUMMARY

A method for measuring small refractive index increments based on interferometric measurement of the optical thickness of a double-prismatic cell in monochromatic light has been described. It is capable of a precision of $1 \cdot 10^{-6}$ refractive index units, and the fringe density has been shown to be proportional to the refractive index difference within a wide range, the limit of which has been given mathematically. The method is supposed to be of value for rapid and accurate measurements of concentrations of solutions, especially in laboratories where electrophoresis or diffusion apparatus with astigmatic optical systems are available.

This investigation is part of a research program for the development of improved methods of optical analysis of stationary and flowing liquids, which program is generously supported by the Swedish Technical Research Council. Laboratory facilities and additional financial aid have been given by *LKB-Produkter Fabriksaktiebolag*, Stockholm, which is also gratefully acknowledged.

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Utilization of Ion Exchangers in Analytical Chemistry

XIX. Separation of Aldehydes and Ketones from Organic Acids

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In a previous paper[1] it has been shown that aldehydes and ketones are taken up quantitatively in a column containing an anion exchanger in the bisulphite form. The adsorption occurs under the formation of α-oxysulphonic acids. It was shown that in this way aldehydes can be separated from alcohols; the alcohol passes into the filtrate and may be easily washed out with water, while the aldehyde is retained in the column.

The aim of the present work is to study the separation of aldehydes and ketones from organic acids by means of anion exchangers. The anion exchanger must be in such a form that the resin retains acids but does not react with aldehydes and ketones. Investigations have been performed with anion exchangers in free base form, in chloride form, and in bicarbonate form. The compounds investigated were formaldehyde, acetaldehyde, furfural, benzaldehyde, salicylaldehyde, vanillin, crotonic aldehyde, glyoxal, acetone, and methyl-ethyl-ketone.

A. ANION EXCHANGERS

Experiments have been performed with anion exchangers of strongly basic type (Amberlite IRA-400 and Dowex 2, cross-linking 4 %) as well as with resins of weakly basic type (Amberlite IR-4B and Amberlite IR-45). A particle size of 0.12–0.30 mm in air dried condition has been used for all resins except for Dowex 2, where the particle size was 0.12–0.25 mm.

Anion exchangers of the strongly basic type have been used in the free base form as well as in the chloride and the bicarbonate form. The ion exchanger was transformed into the hydroxyl form by passing 400 ml 1 *M* sodium hydroxide solution through the column and subsequent washing with water (about 500 ml) until the effluent was neutral against phenolphthalein.

The anion exchangers of the weakly basic type were treated with 400 ml 1 *M* sodium carbonate solution and carefully washed with water.

In the experiments with anion exchangers (Amberlite IRA-400) in the chloride form the resin was saturated with chloride ions by percolating 400 ml 1 *M* sodium chloride solution followed by 200 ml 0.1 *M* hydrochloric acid through the resin bed. Subsequently the resin was washed with water until chloride could not be detected in the effluent.

The transformation into the bicarbonate form was performed by passing 400 ml 1 *M* sodium bicarbonate solution through the column (Amberlite IRA-400) and subsequent rinsing with water.

B. EXPERIMENTAL METHOD

Most experiments have been performed with aldehydes and ketones without the presence of acids in order to study the behaviour of the carbonyl compounds. Ordinary column operation has been used in these experiments. The diameter and height of the column was 9.8 and 140 mm respectively.

50 ml of a solution containing 4.1 meg aldehyde or ketone were allowed to pass through the filter layer. (When benzaldehyde and salicylaldehyde were used in water solution the amounts of these aldehydes were smaller because of the low solubility of these aldehydes.) After the passage of the solution, the ion exchanger was washed with water to give a total effluent volume of 100 ml. The amount of aldehyde resp. ketone in this solution was determined. The resin was then washed with further 300 ml of water and the aldehyde resp. ketone in this filtrate was determined. The flow-rate was about 2 ml/min. In the experiments with the anion exchanger in the bicarbonate form with aldehydes in the presence of acids a modified method was used, which is described in Section F.

C. ANALYTICAL METHODS

The determinations of aldehydes and ketones were in most cases made spectrophotometrically in ultra-violet light with Beckman spectrophotometer. The method cannot be adopted for glyoxal and formaldehyde, which do not show any absorption in ultra-violet. Glyoxal was determined colorimetrically according to Ariyama [2] and formaldehyde according to the chromotropic acid method [3,4]. As a control, colorimetric determinations were also used for furfural [5], crotonic aldehyde [6], and acetaldehyde [7].

D. EXPERIMENTS WITH ANION EXCHANGERS IN THE FREE BASE FORM

The simplest method would be to perform the separation by means of an anion exchanger in free base form. In this case the acids would be adsorbed and the aldehydes and ketones obtained in water solution free from ions, which could interfere with their determination. The results from adsorption experiments with aldehydes and ketones on an anion exchanger of strongly basic type (Amberlite IRA-400) are given in Table 1.

As the table shows the ketones have been recovered quantitatively in the filtrate. Of formaldehyde, furfural, and benzaldehyde 10—20 per cent have not been found in the effluent, probably due to a polymerization at the passage

Table 1.

	Added		Per cent of added aldehyde or ketone in the effluent	
	g	meg.	at 100 ml effluent	at 400 ml effluent
Formaldehyde	0.123	4.1	50.0	79.4
Acetaldehyde	0.181	4.1	See below.	
Furfural	0.394	4.1	74.0	85.5
Benzaldehyde in water	0.13	1.2	62.4	88.4
Benzaldehyde in 25 % alcohol	0.435	4.1	66.2	88.8
Salicylaldehyde in water	0.234	1.9	0.02	0.025
Salicylaldehyde in 25 % alcohol	0.50	4.1	0.015	0.02
Vanillin	0.62	4.1	0.010	0.015
Crotonic aldehyde	0.29	4.1	26.9	37.7
Glyoxal	0.12	4.1	<0.05	<0.20
Acetone	0.238	4.1	97.2	100.8
Methyl-ethyl-ketone	0.295	4.1	96.1	100.3

through the ion exchanger, which in hydroxyl form behaves as a strongly alkaline solution.

On passing solutions of crotonic aldehyde and acetaldehyde through the resin bed a considerable polymerization of the aldehydes seems to occur. In both cases the ion exchanger was strongly darkened after passage of the aldehyde solutions. Only 37 per cent of the ingoing amount of crotonic aldehyde was found in the effluent. For acetaldehyde an examination of the effluent in an ultra-violet spectrophotometer showed that the absorption of the filtrate was ten times stronger than that of the original solution. This may be explained by the known tendency of acetaldehyde to form cyclic compounds; such compounds have generally considerably stronger absorption in ultra-violet than the aliphatic compounds.

Salicylaldehyde and vanillin are taken up quantitatively by the resin in free base form. These two aldehydes contain a phenolic group, which may explain the quantitative retention, as this resin type is known to adsorb phenols [8].

Also glyoxal is retained quantitatively by the ion exchanger. It is known that in alkaline medium glyoxal undergoes Cannizzaro's reaction even at room temperature, under formation of glycollic acid, according to the formula [9]:

$$OHC-CHO + H_2O \rightarrow HOH_2C-COOH$$

If this reaction occurs when glyoxal comes in contact with the resin the glycollic acid should be adsorbed in the column. The following experiment shows that this explanation holds true.

1 g glyoxal in water solution was percolated through a layer of Amberlite IRA-400 (diam. 20 mm, height 180 mm), saturated with hydroxyl ions. After washing with water 200 ml 1 *M* $NaHCO_3$ solution was percolated through the resin layer; in the filtrate glyoxal could not be detected. The sodium ions were removed by passing the solution through a cation exchanger (Amberlite IR-120) saturated with hydrogen ions; during the filtration, part of the carbonic acid was removed; the rest of the carbonic acid was driven off by boiling the solution under reflux cooling Afterwards the solution was percolated through a carboxylic cation exchanger (Amberlite IRC-50), saturated with barium ions, in order to transform the acid to the barium salt. After rinsing with water the filtrate was evaporated to a small volume and the barium salt precipitated in 95 per cent ethanol. The yield was 96 per cent of the calculated value. The salt was analysed and the following values were obtained.

	Found	Calculated for barium glycolate
C:	16.85	16.71
H:	2.24	2.12
Ba:	47.95	47.79

As seen from the above various complications occur if aldehydes are percolated through a strongly basic anion exchanger, saturated with hydroxyl ions. Such ion exchangers are therefore unsuitable to separate aldehydes from organic acids. On the other hand they can be used for separation of ketones from organic acids.

The complications with the aldehydes may be ascribed to the strongly basic groups of Amberlite IRA-400. It was therefore of interest to study the possibility of obtaining better results if anion exchangers of a weakly basic type were used. Therefore experiments were performed with the weakly basic ion exchangers Amberlite IR-4B and Amberlite IR-45. 4.1 meg. of furfural in water solution were percolated through these anion exchangers as before. In the filtrate from IR-B only 60 per cent of the added amount furfural was recovered. In the filtrate from IR-45 only traces of furfural could be detected. This ion exchanger, which before the experiments was light yellow in colour, darkened after the passage of the furfural solution.

While the anion exchangers of strongly basic type contain quaternary ammonium groups those of weakly basic type contain amino groups, *e. g.* $-NH_2$ and $-NHR$. It may be concluded that furfural reacts with amino groups of the latter type of resin under the formation of condensation products of the same type as the Schiff's bases.

E. EXPERIMENTS WITH ANION EXCHANGERS IN THE CHOLORIDE FORM

The behaviour of solutions containing aldehydes and ketones on passing through a column containing an anion exchanger (strongly basic type) in the chloride form has also been investigated. The experimental method was the same as described above. The results are presented in Table 2.

Table 2.

	Added		Per cent of added aldehyde or ketone in the effluent	
	g	meg	at 100 ml effluent	at 400 ml effluent
Formaldehyde	0.123	4.1	73.0	99.2
Acetaldehyde	0.181	4.1	90.0	100.9
Furfural	0.394	4.1	80.4	101.0
Benzaldehyde in water	0.13	1.2	19.3	95.7
Benzaldehyde in 25% alcohol	0.435	4.1	23.0	96.0
Salicylaldehyde in water	0.234	1.9	25.6	89.9
Salicylaldehyde in 25 % alcohol	0.50	4.1	24.2	90.0
Vanillin	0.62	4.1	21.8	70.3
Crotonic aldehyde	0.29	4.1	98.2	98.5
Glyoxal	0.12	4.1	79.6	82.5
Acetone	0.238	4.1	97.0	99.2
Methyl-ethyl-ketone	0.295	4.1	96.7	100.4

As seen from the table formaldehyde, acetaldehyde, furfural, crotonic aldehyde, and the ketones have been recovered quantitatively or almost quantitatively in the filtrate whereas glyoxal and the aldehydes of higher molecular weight — salicylaldehyde, benzaldehyde, and vanillin — are much more difficult to wash out. It may be pointed out that in the experiments with benzaldehyde, salicylaldehyde, and vanillin the aldehyde concentration in the effluent reaches a maximum at an effluent volume of about 150 ml.

Most aldehydes and the ketones are thus only slightly adsorbed on an anion exchanger in the chloride form. An ion exchanger in this form therefore can be used to separate organic acids from most aldehydes and ketones. A disadvantage is, however, that the aldehyde and ketone solution as well as the solution which after regeneration contains the acids also contains chloride ions.

F. EXPERIMENTS WITH ANION EXCHANGERS IN THE BICARBONATE FORM

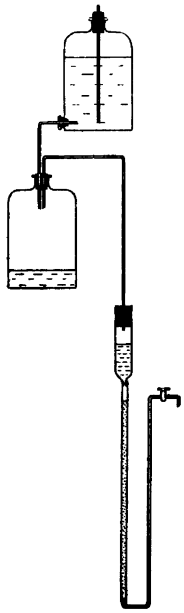


Fig. 1.

In preliminary experiments furfural solutions were percolated through layers of anion exchange resins (strongly basic type), saturated with carbonate or bicarbonate ions. It was shown that no adsorption occured in the resin layers. In the following experiments an ion exchanger in bicarbonate form has been chosen as the pH is lower in the resin phase and therefore less risk of decomposition of the aldehydes would be at hand. The experimental method was the same as before. Only the flow-rate was increased to 8 ml/min. The results are given in Table 3. The apparatus presented in Fig. 1 was used in these experiments.

Table 3.

	Added		Per cent of added aldehyde or ketone in the effluent	
	g	meg	at 100 ml effluent	at 200 ml effluent
Formaldehyde	0.123	4.1	91.0	100.2
Acetaldehyde	0.181	4.1	100.0	100.0
Furfural	0.394	4.1	99.5	100.1
Benzaldehyde	0.13	1.2	60	100.6
Salicylaldehyde	0.234	1.9	<0.05	<0.1
Vanillin	0.62	4.1	<0.05	<0.1
Crotonic aldehyde	0.29	4.1	98.0	98.7
Glyoxal	0.12	4.1	99.0	99.4
Acetone	0.238	4.1	98.6	99.0
Methyl-ethyl-ketone	0.295	4.1	99.0	99.2

From the table it is seen that all aldehydes and ketones, with the exception of salicylaldehyde and vanillin, are quantitatively or almost quantitatively recovered in a filtrate volume of 200 ml. In the experiments with salicylaldehyde and vanillin carbon dioxide was evolved when the solutions were brought into contact with the resin. This means, that a reaction occurs between the bicarbonate ions and the phenolic group of these aldehydes. The aldehydes

are adsorbed due to their acidic character. If an acid was present in the aldehyde solution, it could be expected that part of the aldehyde should pass into the filtrate. This proved to be the case. On addition of 0.4 g acetic acid to the solution of the salicylaldehyde, 30 per cent of the aldehyde was recovered in 400 ml filtrate.

The experiments presented in Table 3 have been performed with Amberlite IRA-400. Other experiments showed that Dowex 2 (4 % cross-linking) behaved in the same manner.

From these experiments it may be concluded that a separation of aldehydes and ketones can be conveniently performed by using anion exchangers in the bicarbonate form. The aldehydes and ketones are determined in the effluent and the acids may be eluted with sodium carbonate solution. Experiments have been performed with solutions of formic acid and acetic acid containing formaldehyde and furfural.

It may be pointed out that it is not appropriate to percolate the acid solution directly through the column as the carbon dioxide evolved causes channeling in the resin bed and may even prevent the passage of the solution. Therefore the solution containing the carbonyl compound and the acid is first shaken in a beaker for ½—1 minute with an anion exchanger in the bicarbonate form. The solution should not be shaken longer time than 1—2 minutes as for prolonged time of contact the aldehyde is difficult to wash out. From the beaker the solution and the resin are poured into an ion exchange column, containing a short layer, about 3 cm in height, of the anion exchanger. This resin layer takes up the last traces of the acid. In order to increase the flow-rate, the same method as before may be used (Fig. 1). The resin layer is subsequently washed with water to an effluent volume of 200 ml. In this filtrate the aldehydes are determined.

The acids, adsorbed on the ion exchanger, may afterwards be eluted by 100 ml 0.1 *M* sodium carbonate solution and subsequent washing with water. The eluate was shaken in a beaker with the cation exchanger Amberlite IR-120 in the hydrogen form in order to liberate the acids. To complete the reaction the solution and the resin were poured into a column in which there was a shallow layer of the same resin. After rinsing the column with water the effluent was boiled for ten minutes under reflux cooling in order to remove carbon dioxide. Afterwards the solution was titrated with 0.1 *N* NaOH against phenolphthalein.

The results presented in Table 4 show that the aldehydes can be quantitatively separated from the acids by means of an anion exchanger in the bicarbonate form. The acids may be eluted with carbonate solution and determined quantitatively.

Table 4.

Added		Found	
g acid	g aldehyde	g acid	g aldehyde
Formic acid	*Formaldehyde*		
0.138	0.280	0.138	0.277
0.173	0.277	0.172	0.277
0.173	0.197	0.174	0.197
Acetic acid	*Formaldehyde*		
0.388	0.200	0.384	0.199
Acetic acid	*Furfural*		
0.310	0.400	0.310	0.398
0.312	0.400	0.311	0.401

In some preliminary experiments an attempt was made to elute the acids with 0.1 *M* and 0.2 *M* sodium hydroxide solution and sodium bicarbonate solution. However, it was found that it was easier to obtain a quantitative elution by means of carbonate solution especially for formic acid.

SUMMARY

Experiments have been performed in order to investigate the possibility of separating aldehydes and ketones from organic acids by adsorbing the latter on anion exchangers. The experiments show that in many cases a quantitative separation cannot be obtained by means of anion exchangers in the free base form (strongly as well as weakly basic type) as the aldehydes react with the exchanger.

A quantitative separation can be performed with anion exchangers (strongly basic type) in the bicarbonate form, the acids being retained by the resin whereas the aldehydes and ketones remain in solution. The adsorbed acids are eluted with sodium carbonate solution. The acids are liberated by treating the solution with a cation exchanger (sulphonic acid type) in the hydrogen form.

The method cannot be used for solutions containing salicylaldehyde and vanillin which are adsorbed due to their phenolic hydroxyl groups.

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Utilization of Ion Exchangers in Analytical Chemistry

XX. Separation of Ketones from Alcohols

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The aim of the present work is to study the possibility of separating ketones from alcohols with the aid of an anion exchanger saturated with bisulphite. Experiments have been performed with methanol and ethanol in the presence of acetone and methyl-ethyl-ketone.

In a previous paper it was shown that aldehydes and ketones may be quantitatively retained on passing through a column containing an anion exchanger in the bisulphite form. The adsorption is due to a formation of α-oxysulphonic acids. The aldehydes are very difficult to wash out because the aldehyde-bisulphitecompounds are rather stable. Under the same conditions the alcohols are easily washed out and for that reason the aldehydes and the alcohols may be easily separated from one another by anion exchangers saturated with bisulphite [1].

For the ketones the corresponding α-oxysulphonic acids are less stable. On that account it is comparatively easy to wash out the ketones with water from an anion exchanger in the bisulphite form. Therefore, if a separation should be possible, a higher ion exchange bed must be used to achieve a separation between alcohols and ketones than for the separation of aldehydes from alcohols.

Apparatus: The ion exchanger used was Amberlite IRA-400 (particle size 0.12–0.30 mm in air-dry condition). The ion exchange columns were of ordinary type; the height of the resin layer was 550 mm and the diameter 9.8 mm. The ion exchanger was saturated with about 500 ml 1 *M* $NaHSO_3$ solution, after which it was washed with water until bisulphite could not be detected in the effluent. Because of the great filter resistance of the ion exchange resin, pressure was used in order to facilitate the passage of the solutions and the washing water through the column.

DETERMINATION OF ETHANOL AND METHANOL IN THE PRESENCE OF ACETONE

In these experiments 50 ml of a solution containing 0.5 g acetone and varying amounts of methanol or ethanol were passed through the column. Afterwards the filter layer was washed with water to give a total effluent volume of 100 ml. Washing was then continued for a further 100 ml water. Under adsorption and washing the flowrate was about 1.5—2 ml/min. In the filtrates the alcohols were determined pycnometrically. The extremely sensitive salicylaldehyde-method ([2]) was used to test for the presence of acetone. The results are presented in Table 1.

As the table shows the alcohol is quantitatively recovered in the two filtrates. In these filtrates less than 0.2 per cent of the ingoing amount of acetone is found. A quantitative separation of acetone from alcohols has accordingly been achieved.

DETERMINATION OF ETHANOL IN THE PRESENCE OF METHYL-ETHYL-KETONE

In these experiments the procedure was altered in order to simplify the alcohol determination. It could be assumed that, during the passage of a mixture of alcohol and water through an ion exchange layer, the water originally present in the resin bed should be easily displaced. As no preferential adsorption between alcohol and water might be expected the concentration of the effluent should, after a short time, be the same as that of the ingoing solution.

In preliminary experiments with 2 per cent and 19 per cent ethanol in water passed through the ion exchanger and collected in fractions, it was shown that, already after 60 ml had passed, the concentration of the effluent was the same as that of the ingoing solution and that on prolonged filtration the concentration was — within the limits of experimental errors — the same (Fig. 1).

After these preliminary experiments 100 ml of water solutions with varying amounts of ethanol and 0.61 g methyl-ethyl-ketone were prepared. The solutions were passed through the layer of Amberlite IRA-400, in the bisulphite form. The first 70 ml of the effluent were discarded and then a volume, suitable for pycnometric alcohol determination, was taken. As in this case the ketone concentration in the original solution was low, the alcohol concentration of the ingoing solution may be considered the same as that of the effluent The results presented in Table 2 show that good agreement has been obtained.

The presence of methyl-ethyl-ketone in the effluent was tested by direct spectrophotometric determination in ultra-violet light (Beckman spectro-

photometer) and by the method suggested by Thomson [2]. By none of the methods could methyl-ethyl-ketone be detected.

The procedure applied in this section is somewhat simpler than that adopted in the previous section but may, as already pointed out, be used only if the ketone concentration is low. The accuracy for the two modifications is about the same.

Table 1.

Added g			Found g			
Ethanol	Methanol	Acetone	Filtrate No.	Ethanol	Methanol	Acetone
0.50	—	0.50	1	0.48	—	< 0.0005
			2	*0.02*	—	< 0.0005
			1 + 2	0.50		
0.45	—	0.50	1	0.45	—	< 0.0005
			2	*0.00*	—	< 0.0005
			1 + 2	0.45		
2.00	—	0.50	1	1.97	—	< 0.0005
			2	*0.04*	—	< 0.0005
			1 + 2	2.01		
5.08	—	0.50	1	5.05	—	< 0.0005
			2	*0.03*	—	< 0.0005
			1 + 2	5.08		
—	0.50	0.50	1	—	0.50	< 0.0005
			2		*0.00*	< 0.0005
			1 + 2		0.50	
—	2.00	0.50	1	—	1.93	< 0.0005
			2	—	*0.06*	< 0.0005
			1 + 2		1.99	
—	5.20	0.50	1	—	5.08	< 0.0005
			2	—	*0.10*	< 0.0005
			1 + 2		5.18	

Table 2.

Added g/100 ml		Found g/100 ml	
ethanol	methyl-ethyl-ketone	ethanol	methyl-ethyl-ketone
0.52	0.61	0.52	< 0.003
2.06	0.61	2.05	< 0.003
5.06	0.61	5.08	< 0.003

ELUTION OF THE KETONES

As previously shown [1] the ketones may be recovered from the column already by washing with water. In the present paper higher columns have been used and therefore large amounts of water would be required if washing was performed at room temperature. However, the equilibrium

$$R_2CO + HSO_3^- \rightleftharpoons R_2C(OH)SO_3^-$$

is known to be displaced to the left for an increased temperature. Therefore it must be expected that the elution may be considerably facilitated when working at elevated temperature. This was confirmed by experiments.

In the experiments given in the present paper the elution was performed by means of water at 75° C. In order to maintain this temperature a jacketed column was used. The outlet tube was cooled by ice water in order to prevent ketone losses.

For a comparison an experiment has also been made in which elution was performed by means of a solution 0.15 *M* in respect to $NaHCO_3$ and 0.075 *M* in respect to Na_2CO_3. This solution had in the previous investigations ([1]) proved satisfactory for elution of furfural.

The flow rate was kept constant in all experiments at 2 ml/min. The filtrate was taken up in fractions of 25 ml. Acetone was determined according to Thomson [2] and with *o*-nitrobenzaldehyde ([3]). When eluted with water the acetone was also determined spectrophotometrically in ultra-violet light (Beckman spectrophotometer). The latter method was also used for methyl-ethyl-ketone. Results obtained from the various analytical methods for the determination of acetone showed good mutual agreement. The results are presented in Fig. 2.

As seen from the figure methyl-ethyl-ketone is more easy to wash out with hot water than acetone. The methyl-ethyl-ketone-bisulphite compound is

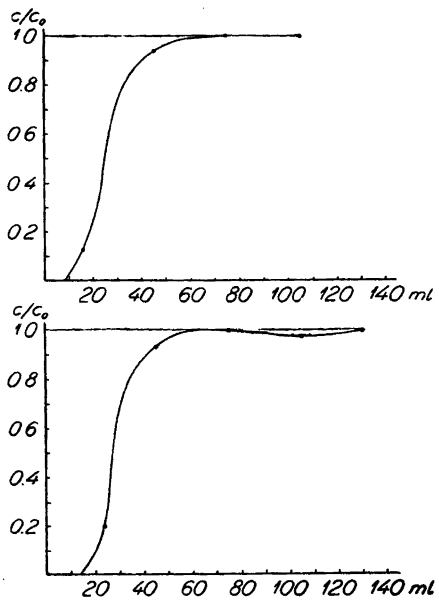


Fig. 1. Displacement of water from an anion exchange column by means of aqueous alcohol solutions.

c = *alcohol conc. in the effluent*
c_0 = *alcohol conc. in the influent*
A. 2 % alcohol
B. 19 % alcohol

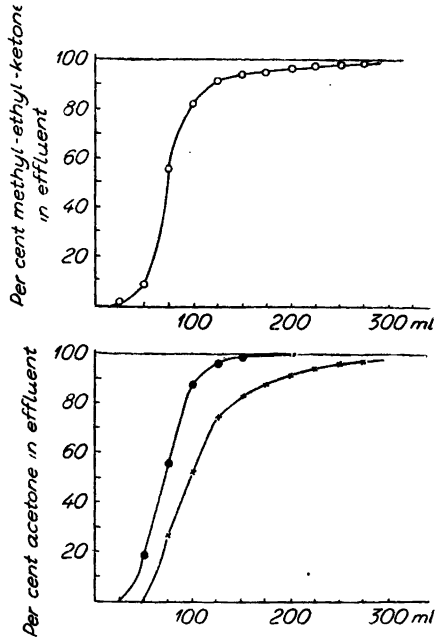


Fig. 2. Integral elution curves for acetone and methyl-ethyl-ketone.

● *acetone; eluant: 0.075 M* Na_2CO_3 + *0.15 M* $NaHCO_3$
× *acetone; eluant: water 75° C*
○ *methyl-ethyl-ketone; eluant: water 75° C*

evidently less stable than the acetone-bisulphite compound. This is in accordance with the behaviour of the aldehyde-bisulphite compounds, the stability of which is diminished with increasing molecular weight of the aldehydes.

With 300 ml of hot water about 99 per cent of the acetone have been eluted, whereas 99 per cent of methyl-ethyl-ketone is washed out already with 200 ml. The last traces of both acetone and methyl-ethyl-ketone are, however, difficult to wash out as the figure shows.

With the carbonate-bicarbonate solution acetone is quantitatively eluted already at about 200 ml. In this case there are no "tails" of the elution curves.

SUMMARY

Ketones can be separated quantitatively from alcohols by adsorbing the former in a column filled with an anion exchange resin in the bisulphite form.

Alcohol may be determined pycnometrically in the effluent and the ketones subsequently eluted, e.g. by means of hot water.

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Constituents of Pine Heartwood

XXVIII.* Investigation of Four additional *Pinus* species by Paper Partition Chromatography

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Part XXV of this series[1] described the results of an investigation, by paper chromatographic methods, of forty-eight *Pinus* species, representing twelve of the thirteen groups into which the genus is divided in Shaw's system[2]. The group which was not represented was *Gerardianæ* (subgenus *Haploxylon*, subsection *Paracembra*), consisting of two species, *P. Bungeana* Zucc. from China, and *P. Gerardiana* Wall. from the Himalayas. We have now obtained heartwood samples of both these species, and of two other species not previously investigated, *P. longifolia* Roxb. from the Himalayas and *P. formosana* Hayata from Formosa. These four species have been investigated by the methods described in Part XXV[1] and the results are summarised in Table 1.

The two species from the group *Gerardianae* contain a combination of heartwood phenols not found in any other pines hitherto investigated. In contrast to all the other pines from the subgenus *Haploxylon* they do not contain any flavones (chrysin or tectochrysin), nor any dibenzyls (dihydropinosylvin or its monomethyl ether). They contain the four most common heartwood phenols, pinosylvin and its monomethyl ether, pinocembrin and pinobanksin together with pinostrobin and strobobanksin. The last-mentioned substance is a flavanone containing a methylphloroglucinol nucleus[3], and phenols of this type (strobopinin, cryptostrobin, strobochrysin and strobobanksin) have hitherto only been found in pines belonging to the group *Strobi*[4]. As judged from the intensity of the spots on the chromatogram, the content of strobobanksin is rather high. In addition, strobopinin and probably also cryptostrobin have been found in *P. Bungeana* but not in *P. Gerardiana*. Strobo-

* XXVII. *Svensk Papperstidn.* **54** (1951) 275.

Table 1. Heartwood constituents of four P i n u s species.

Symbols and abbreviations, see Part XXV [1].

Species	Pinosylvin	PSM	DHPS	DHPSM	Pinocembrin	Chrysin	Pinostrobin	Tectochrysin	Pinobanksin	Strobobanksin	Strobopinin	Cryptostrobin
A. Haploxylon												
Subsection *Cembra*, group *Strobi:*												
P. formosana Hayata	+	+	?	+	+	+	+	−	+	+	−	−
Subsection *Paracembra*, group *Gerardianae:*												
P. Bungeana Zucc.	+	+	−	−	+	−	+	−	+	+	+	?
P. Gerardiana Wall.	+	+	−	−	+	−	+	−	+	+	−	−
B. Diploxylon												
Subsection *Parapinaster*, group *Longifoliae*												
P. longifolia Roxb.	+	−	−	−	+	−	−	−	+	−	−	−

None of the unknown fluorescent compounds D—G (see Part XXV [1]) have been found in these species.

chrysin can not be satisfactorily identified on paper chromatograms by the method used here [1].

Thus, the pines belonging to the group *Gerardianae* seem to be unable to hydrogenate stilbenes to dibenzyls or to dehydrogenate flavanones to flavones. These two reactions, probably connected in some way, are characteristic of the other pines of the subgenus *Haploxylon* [4]. On the other hand, the group *Gerardianae* is distinguished from the subgenus *Diploxylon* by the occurrence of pinostrobin and C-methylflavanones. From a chemical standpoint, therefore, it would be justified to regard *Gerardianae* as a third subgenus of the genus *Pinus*. It may be mentioned here that two samples of *P. Gerardiana* and one of *P. Bungeana* have been investigated.

Of the other two pine species investigated, *P. formosana* is very closely related to *P. parviflora*, which has been previously studied [1]. *P. formosana* contains pinostrobin and strobobanksin, which have not been found in *P. parviflora*, but the latter species, however, contains two other C-methylflavanones, strobopinin and cryptostrobin.

Finally, the composition of the heartwood extract from *P. longifolia* has one striking feature — the complete absence of pinosylvin monomethyl ether. *P. canariensis*, the pine which is most closely related to *P. longifolia*, contains that compound, as do all the other *Diploxylon* pines hitherto investigated[1].

EXPERIMENTAL

The methods used for the analysis of the heartwood extracts were those described in Part XXV[1].

SUMMARY

The heartwoods of *Pinus Bungeana* Zucc. and *Pinus Gerardiana* Wall. contain stilbenes and flavanones, including C-methylflavanones, but none of the flavones or dibenzyls which have been found in all other pines from the subgenus *Haploxylon*.

The heartwood constituents of *Pinus formosana* Hayata and *Pinus longifolia* Roxb. are those which could be expected from a taxonomic standpoint, expect that *P. longifolia* does not contain any pinosylvin monomethyl ether.

We are indebted to Dr. W. G. Campbell, Princes Risborough, England, Dr. K. A. Chowdhury, Dehra Dun, India, and Dr. R. W. Hess, New Haven, Conn., USA, for providing wood samples.

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Studies on the Hydrolysis of Metal Ions

II. The Hydrolysis of the Mercury(II) Ion Hg^{2+}

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It has been known for a long time that solutions of mercury(II) salts in water have an acid reaction; this is especially strong if the anion is perchlorate or nitrate, neither of which forms strong complexes with Hg^{2+}. A possible mechanism is that the ion $Hg(H_2O)_n^{2+}$ acts as an aquo acid, splitting off one or more protons. Omitting the co-ordinated water molecules we may write the formulas for the formation of the first two products as follows:

$$Hg^{2+} + H_2O \rightleftharpoons HgOH^+ + H^+ \qquad q_1=[HgOH^+][H^+][Hg^{2+}]^{-1} = k_{a1} \quad (1)$$

$$Hg^{2+} + 2H_2O \rightleftharpoons Hg(OH)_2 + 2H^+ \qquad q_2=[Hg(OH)_2][H^+]^2[Hg^{2+}]^{-1} = k_{a1}k_{a2} \quad (2)$$

where k_{a1} and k_{a2} would be the first and second acid constants of the acid $Hg(H_2O)_n^{2+}$.

There are few data recorded in literature on these equilibria, and they do not allow of reliable separate estimations of k_{a1} and k_{a2}, as will be seen below. Garrett and Hirschler [1] measured the solubility of HgO in water at 25° C and, by combining it with the previously determined solubility product $[Hg^{2+}][OH^-]^2$, calculated the "basic dissociation constant of $Hg(OH)_2$" as $1.8 \cdot 10^{-22} = 10^{-21.74}$. From this value and $pK_W = 14.00$ one may calculate $pk_{a1} + pk_{a2} = 6.26 = pq_2$.

From two pH measurements at 21.5° C, J. Bjerrum [2] obtained the values 2.68 and 2.88 for pk_{a1}, neglecting the second dissociation step. He estimated the most probable value to be $pk_{a1} = 2.8$ and concluded that $pk_{a2} = 3.5$. From Ley's conductivity measurements [3] on mercury(II) perchlorate solutions at 25° C, Bjerrum estimated pk_{a1} to be of the order of magnitude 2.4, in reasonable agreement with his own measurements.

Garrett and Howell [4] measured the solubility of HgO at 25° C in nitric acid at various concentrations and estimated the equilibrium constant for $Hg(OH)_2 \rightleftharpoons HgOH^+ + OH^-$ to be $7.1 \cdot 10^{-12} = 10^{-11.15}$ and for $Hg(OH)_2 \rightleftharpoons Hg^{2+} + 2OH^-$, $2.2 \cdot 10^{-23} = 10^{-22.66}$, which by the way differs from [1]. With $pK_w = 14.00$ these values would give $pk_{a1} = 2.49$, $pk_{a2} = 2.85$.

On the other hand Hayek [5] concluded from the great solubility of HgO in strong mercury(II) perchlorate solutions (of the order of magnitude 2—4 C) and from the existence of a solid salt $Hg_3O_2(ClO_4)_2$ that these solutions contain polynuclear ions $Hg(HgO)_n^{2+}$.

If this were so, there would be a strong analogy with bismuth solutions; the hydrolysis of Bi^{3+} has been shown by Granér and Sillén [6] not to proceed by mononuclear reactions analogous to equation (1) and (2) but rather by the formation of polynuclear complexes such as $Bi_nO_{n-1}^{(n+2)+}$. Other ions studied in this laboratory — thorium [7], cerium(IV) [7], and iron(III) [8] — have also proved to hydrolyze with the formation of polynuclear complexes. It thus seemed worth while investigating whether the mercury(II) ion behaves in the same way.

Let us state immediately that our results can be fully explained by the mechanism given in (1) and (2), although we find that Hg^{2+} differs from most other acids by having $k_{a2} > k_{a1}$. There was no evidence of polynuclear mercury ions at the low total concentrations studied by us, although they may have existed at the very high concentrations discussed by Hayek [5].

Another reason for studying the hydrolysis of Hg^{2+} was the fact that a large amount of work had recently been devoted to studying the complexes of Hg^{2+} with Cl^-, Br^- and I^- [9]. In all these measurements the equilibria had been studied at 25° C for solutions with $[H^+] = 10$ mC and ionic strength as close to 0.5 C as possible, achieved by adding suitable amounts of $NaClO_4$. The use of 10 mC H^+ was intended to keep the fraction of Hg^{II} present in the form of $HgOH^+$ etc. small and constant. It seemed of interest to know what this fraction had actually been. This was one reason for now choosing the same ionic strength as in the investigation of the mercury-halide complexes.

As will be seen in the following, for calculating the hydrolysis equilibria of Hg^{2+} it is necessary to know the magnitude of the hydrolysis of the mercury(I) ion, Hg_2^{2+}, and *vice versa* a great part of the apparent acidity of the Hg_2^{2+} ion is really due to the acidity of small amounts of Hg^{2+} present in the solution. The investigations of Hg^{2+} and Hg_2^{2+} were for this reason carried out in close co-operation. The studies on mercury(I) ions were started by Forsling even before we took up the Hg^{2+} studies, and the results will be published as Part III [10] of this series.

CHOICE OF METHOD AND EXPERIMENTAL CONDITIONS

It was decided to study the hydrolysis equilibria of Hg^{2+} at 25.0° C with $[ClO_4^-] = 500$ mC and no other anions present. As usual, by keeping the ionic strength and thus the activity factors practically constant, we could use concentrations instead of activities in the formulas for emfs and equilibrium constants. The particular choice of 0.5 C ClO_4^- facilitated a comparison with previous measurements on mercury-halide complexes.

From the analysis of our solutions we knew the amounts of Hg^{II} and hydrogen ions added per litre, $a = [Hg^{II}]_{tot}$ and H (the hydrogen ion concentration if mercury had not hydrolyzed). For studying the equilibria we thought it desirable to measure the actual concentrations both of free Hg^{2+} ions and of hydrogen ions.

For measuring $[Hg^{2+}]$ a Hg electrode could not be used, since it would have reacted thus $Hg^{2+} + Hg(l) \rightleftharpoons Hg_2^{2+}$; the equilibrium constant $[Hg_2^{2+}]\,[Hg^{2+}]^{-1}$ is about 130 [11]. However, $[Hg^{2+}]$ could be determined by adding Hg_2^{2+} and measuring the redox potential of the solution. Advantage was taken of the fact, proved in Part III [10], that Hg_2^{2+} is a much weaker acid than Hg^{2+}.

For measuring the hydrogen ion concentration, neither a hydrogen electrode nor a quinhydrone electrode could be used, since in both cases metallic mercury would be obtained by reduction. It was thus necessary to use glass electrodes which, although capricious, gave sufficiently accurate results.

Types of cells used. — As in previous work, the reference calomel electrode was of the following type:

$$CE \,\|\, = Hg,\ Hg_2Cl_2 \,|\, 4\ C\ NaCl \,|\, 0.5\ C\ NaClO_4 \,| \qquad (3)$$

The two types of cells used in this work may be written schematically

$$- CE \,\|\, Hg^{2+},\ Hg_2^{2+},\ H^+ \,|\, \text{glass electrode} + \qquad \text{(cell of type 1)}$$

$$- CE \,\|\, Hg^{2+},\ Hg_2^{2+},\ H^+ \,|\, Pt + \qquad \text{(cell of type 2)}$$

We shall write for convenience introducing the new function φ

$$[Hg^{II}]_{tot} = a;\ [H^+] = h;\ [Hg^{2+}] = a\ (1 + \varphi)^{-1} \qquad (4)$$

For cells of type 1 the emf E_1 will be

$$E_1 = E_{01} + E_j^* + 59.16\ \log\ h \qquad (5)$$

In (5) E_{01} is a constant *, whereas E_j^* is a function of h and comprises the

* The constants E_{01} etc. in this series are not identical with E_{10} etc. in the papers on mercury-halide complexes; however, $E_{02} \approx E_{20}$ and E_{03} (in part III) $\approx E_{10}$, the differences being the corrections for E_j and hydrolysis at $h = 10$ mC.

diffusion potential, the asymmetry potential of the glass electrode, and its deviations from the ideal slope. The sum ($E_{01} + E_j^*$) was determined as a function of h by an acid-base titration immediately preceding each titration with mercury solutions. There was no need to determine E_{01} and E_j^* separately.

For cells of type 2 we have

$$E_2 = E_{02} + E_j + 59.16 \log [Hg^{2+}] - 29.58 \log [Hg_2^{2+}] \quad (6)$$

In this equation, E_{02} is a constant (see footnote p. 749), which is at first unknown, and E_j a function of h. From the work of Biedermann and Sillén[12] we took a preliminary equation $E_j = -0.0972\ h$ mV, if h is expressed in mC.

Using (4) we may transform (6) into the following equation, where the left member contains only known quantities:

$$E_2 + 0.0972\ h - 59.16 \log a + 29.58 \log [Hg_2^{2+}] = E_{02} - 59.16 \log (1 + \varphi) \quad (7)$$

Denoting by E_2' and φ' the values of E_2 and φ for a fixed value $h = h'$ we find from (7), provided a and $[Hg_2^{2+}]$ are kept constant.

$$59.16 \log \left[(1 + \varphi) - \log\ (1 + \varphi')\right] = E_2' - E_2 + 0.0972\ (h' - h). \quad (8)$$

EXPERIMENTAL

Reagents. — Solutions of mercury(I) perchlorate and mercury(II) perchlorate were prepared and analyzed as described previously[13]. The total perchlorate content was, however, determined by running a known amount of the perchlorate solution through an ion exchanger in its hydrogen form, and titrating for H^+ with NaOH. For some reason, the results sometimes differed by almost as much as 0.5 % from those obtained with the earlier method, electrolyzing away the mercury and then titrating with NaOH. As a check a third method was used, namely binding the mercury by adding excess of sodium bromide, and titrating the excess H^+ with NaOH. The perchlorate content was calculated from the $[H^+]$ obtained in this way and the Hg values obtained by electrolysis. This value proved to agree within 0.05 % with the $[ClO_4^-]$ values obtained by the ion exchange method. For this reason the ion exchange values were considered reliable.

The perchloric acid and sodium perchlorate were also prepared and analysed as in previous work[13].

A stock solution of 50 % NaOH was kept in a paraffinated bottle. The NaOH solutions prepared from it by dilution were analysed by titration with HCl, which had been standardized against a weighed amount of HgO and excess of KI.

The cell was made up of a calomel electrode, a salt bridge, and a titration vessel. The first two were of a new and convenient design, and will be described in detail by Forsling, Hietanen and Sillén in part III[10]. The cell was kept in a paraffin oil thermostat at 25.0 ± 0.1° C. The solutions were kept free from air by bubbling nitrogen, which was taken from a cylinder, made free from oxygen by Meyer's and Ronge's method[14], saturated with water vapor by bubbling through 0.5 C $NaClO_4$ in the thermostat, and rinsed from droplets in a cotton-wool filter. The whole work was carried out in a thermostat room of approximately 25° C.

The glass electrode was a commercial shielded one, of Radiometer's make. The platinum electrode was of the type shown in Fig. 3, ref. 13. The emf:s for cells of type 1 were measured with a valve potentiometer (type Radiometer pHM3), those for cells of type 2 with a Jensen compensator type 10A, and a Multiflex galvanometer.

Procedure. — Our calculations are based on seven titrations, the data on which are given in Table 1. Three solutions were prepared for each titration, and are denoted as S_1, S_2 and T. The solutions were made to contain 500 mC ClO_4^-; for each solution Table 1 gives the concentrations of the other ions present, except for Na^+ which was the balance. The concentrations given for S_2 are the analytical ones, calculated as if no hydrolysis had occurred. The last columns of Table 1 give the order and amounts in which the solutions were added; the principle was the same in all titrations, so we shall only discuss titration 1.

Table 1. Survey of titrations.

No.	Symbol	a mC	S_1 mC H^+	T mC OH^-	S_2 mC H^+	S_2 mC Hg^{2+}	S_2 mC Hg_2^{2+}	ml added S_1	T	S_2	S_2 + T
1	△	10.00	10.00	37.00	18.64	30.00	3.00	41	9	25	18 + 36
2	▲	10.00	10.00	39.93	30.31	30.00	15.00	41	9	25	20 + 40
3	◇	3.00	10.00	40.00	4.00	6.00	3.00	41	9	50	13.5 + 13.5
4	■	10.00	20.06	40.00	19.53	20.00	4.00	41	19	60	64 + 64
5	●	3.00	20.04	49.68	10.07	6.00	3.00	60	23.5 *	30	28.5 + 28.5
6	□	1.00	20.04	49.68	33.53	3.00	6.00	60	22	41	25.5 + 51
7	○	1.00	20.04	49.68	33.53	3.00	6.00	60	22	41	25 + 50

The first part of the titration was an acid-base titration, with only the glass electrode inserted in the titration vessel. The vessel originally contained 41.00 ml of the acid solution S_1. The emf of the cell (type 1) was measured, and the alkaline solution T was added in portions and E_1 measured after each new addition. From these measurements, a diagram was made giving $(E_{01} + E_j^*)$ as a function of the hydrogen ion concentration h. This diagram was used for calculating h from E_1 in the latter part of the titration; the plot was usually roughly linear but varied slightly from day to day, the constancy of glass electrodes being what it is.

When 9.00 ml of T had been added, and the total volume was 50.00 ml, mercury ions were introduced into the solution by adding 25.00 ml of S_2. The platinum electrode was introduced, and from now on both the CE-glass electrode emf (E_1) and the CE-platinum electrode emf (E_2) were measured.

In the latter part of the titration, for each new addition of T, half as much of S_2 was also added. In this way the concentrations of mercury(I) and mercury(II) in the solution were kept constant and equal to one third of the concentrations in S_2. In the second half of titration 1, a total of 18 ml S_2 and 36 ml T was added. The experiment was interrupted when a lasting precipitate appeared in the solution.

* In expt. 5, the solution in the titration vessel was removed and replaced by 30.00 ml S_1 after the acid-base titration.

Table 2.

Data from titration no. 6.

Table 2 a. Acid-base titration, Initially 60.00 ml S_1 (20.04 mC H^+). T (49.68 mC OH^-) was added.

T ml	$H = h$ mC	E_1 mV	$E_{01} + E_j^*$ mV
0.00	20.04	+ 1.8	− 75.2
2.00	17.79	− 0.5	− 74.4
5.00	14.68	− 5.2	− 74.2
10.00	10.08	− 14.3	− 73.7
15.00	6.10	− 26.6	− 73.1
20.00	2.61	− 48.5	− 73.1
22.00	1.33	− 66.0	− 73.3

Table 2 b. Mercury titration. Initially 41.00 ml S_2 (33.53 mC H^+, 3.00 mC Hg^{2+}, 6.00 mC Hg_2^{2+}) was added to the last solution from Table 2 a. Then for each addition of T, half as much S_2 was added.

T ml	E_1 mV	h mC	$h-H$ mC	E_2 mV	$\frac{1+\varphi}{1+\varphi'}$	φ	$h^2\varphi$ mC^2	$h\varphi$ mC
0.00	− 9.4	12.26	* 0.20	552.22	1.000	0.0200	2.909	0.241
2.00	− 11.2	11.38	0.13	552.11	1.0074	0.0276	3.493	0.310
10.00	− 18.4	8.47	0.10	552.33	1.0097	0.0300	2.102	0.251
20.00	− 29.1	5.50	0.11	552.22	1.0252	0.0461	1.394	0.275
30.00	− 43.1	3.15	0.20	551.11	1.0797	0.1014	1.006	0.319
32.00	− 46.8	2.73	0.21	550.56	1.1051	0.1273	0.949	0.347
36.00	− 54.6	2.00	0.31	548.73	1.1899	0.2137	0.855	0.427
40.00	− 63.3	1.42	0.51	545.55	1.3493	0.3763	0.759	0.534
41.00	− 65.7	1.29	0.56	544.21	1.4228	0.4512	0.751	0.582
43.00	− 70.6	1.07	0.71	541.55	1.579	0.611	0.699	0.653
44.00	− 73.1	0.96	0.77	539.80	1.691	0.725	0.668	0.696
46.00	− 78.2	0.79	0.95	535.40	2.009	1.049	0.655	0.829
48.00	− 83.7	0.64	1.13	529.57	2.521	1.572	0.644	1.006
49.00	− 86.8	0.57	1.23	526.26	2.869	1.927	0.626	1.098
50.00	− 89.6	0.51	1.33	522.69	3.297	2.364	0.615	1.205
51.00	− 92.6	0.45	1.43	518.99	3.809	2.887	0.584	1.299

* For the first three points, H was used instead of h in the calculations. — To avoid rounding-off errors, an ample number of digits is sometimes given.

In titrations 3—5, the solutions S_2 and T were added in the ratio 1 : 1, making the mercury concentrations in the latter part of the experiment equal to half those in S_2.

After each addition, the emfs attained almost immediately their new values; it was checked that they remained constant for about 10 minutes, before a new addition was made.

Data from titration no 6 are given in Table 2. In this experiment, the linearity of $E_{01} + E_j^*$ was unusually poor for low h; in the calculations we used a straight line extrapolated from higher h values.

FORMULAS FOR MONONUCLEAR MECHANISM

For each point of any of our titrations, the experimental data gave the analytical concentrations of hydrogen ions ($= H$), mercury(II) ions ($= a$), and mercury(I) ions. Moreover, from E_1 and the preceding acid-base titration, the real hydrogen ion concentration h could be found.

From E_2 by means of equation (8) we could get the quantity $(1 + \varphi)(1 + \varphi')^{-1}$, where φ' (and h') are the values for the first point of the latter (mercury) part of the titration. Since h' was generally large, φ' was small, and could be corrected for by successive approximations as described below. Even at the lowest acidities, the correction for the hydrolysis of Hg_2^{2+} was within the limits of experimental error; its approximate magnitude was known from the work of Forsling, Hietanen and Sillén (part III) [10].

In Fig. 1 and 2, the quantities $(h-H)a^{-1}$ and $[Hg^{2+}]a^{-1} = (1 + \varphi)^{-1}$ are given as functions of log h. Within the limits of experimental error, the points for different total mercury(II) concentrations a are seen to fall on the same curve. This is as would be expected with a mononuclear mechanism, as is seen from formulas (9) and (15) below. However, if polynuclear complexes were formed in appreciable amounts, one would expect a relatively stronger hydrolysis, thus a higher curve in Fig. 1 and a lower curve in Fig. 2, for the highest a values.

We thus find it justifiable to assume that the hydrolysis of the Hg^{2+} ion leads chiefly to the mononuclear products $HgOH^+$ and $Hg(OH)_2$. If this is so, we find from (1) and (2)

$$a = [Hg^{II}]_{tot} = [Hg^{2+}] + [HgOH^+] + [Hg(OH)_2] = [Hg^{2+}](1 + q_1h^{1-} + q_2h^{-2}) \tag{9}$$

From (9) and the last equation in (4) we see immediately that

$$\varphi = q_1h^{-1} + q_2h^{-2} \tag{10}$$

By multiplication of (10) by h^2, and h, we obtain the equations

$$h^2\varphi = q_1h + q_2 \tag{11}$$

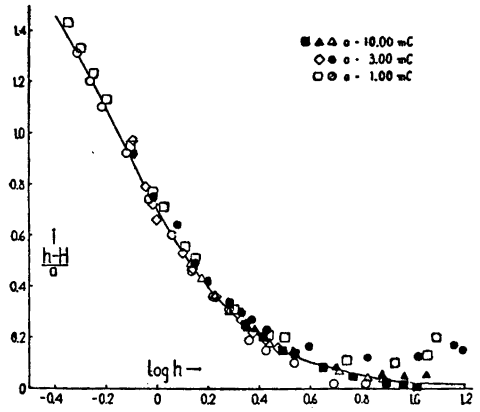


Fig. 1. $(\bar{n}-H)a^{-1}$, *number of protons split off per mercury (II) ion, as a function of* log h *(in mC). Curve calculated with* $q_1 = 0.20$ *mC,* $q_2 = 0.50$ mC^2. *Experimental points: symbols as in Table 1.*

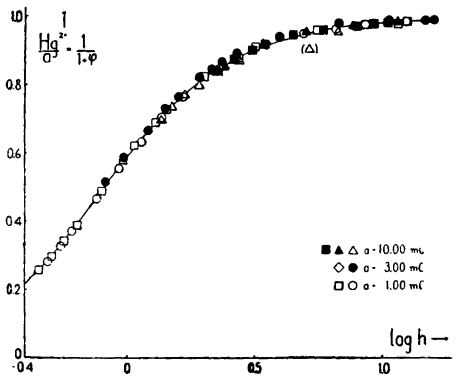


Fig. 2. $(1+\varphi)^{-1}$, *fraction of* Hg^{II} *present as free* Hg^{2+}, *as a function of* log h *(h in mC). Experimental points: symbols as in Table 1. Curve calculated with* $q_1 = 0.20$ *mC,* $q_2 = 0.50$ mC^2.

$$h\varphi = q_1 + q_2 h^{-1} \tag{12}$$

By plotting $h^2\varphi$ as a function of h, and $h\varphi$ as a function of h^{-1} one should thus obtain two straight lines, each of which would give the values for q_1 and q_2. The real hydrogen ion concentration h should be the sum of the analytical one H, and the amount of hydrogen ions set free by the hydrolysis processes (1) and (2):

$$h = H + [HgOH^+] + 2\ [Hg(OH)_2] \tag{13}$$

With (13), (1) and (2) we obtain

$$h-H = [Hg^{2+}]\ (q_1 h^{-1} + 2\ q_2 h^{-2}) \tag{14}$$

and with (14), (4) and (9)

$$(h-H)a^{-1} = (\varphi + q_2 h^{-2})\ (1+\varphi)^{-1} \tag{15}$$

RESULTS

Plots of (11) and (12) were constructed, neglecting φ' at the first point of the titration. From these plots rough values for q_1 and q_2 were obtained. With them an approximate value for φ' was calculated for each titration, by means of which better values for $h^2\varphi$ and $h\varphi$ were obtained. When these were plotted against h and h^{-1}, better values for q_1 and q_2 resulted and so on. By four successive approximations we obtained the values

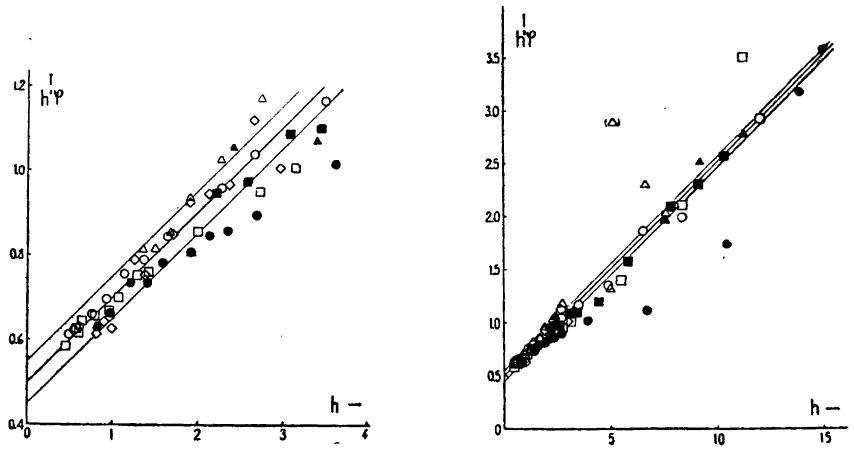


Fig. 3a. $h^2\varphi$ *(mC²) as a function of* h *(mC). Fig 3b. Left part of Fig. 3a on larger scale. Lines calculated for* $q_1 = 0.20$ *mC and* $q_2 = 0.50$ *mC² (thick line), 0.45 or 0.55 mC² (thin lines).*

$$q_1 = 0.20 \pm 0.03 \text{ mC} = (2.0 \pm 0.3) \cdot 10^{-4} \text{ C} = 10^{-3.70 \pm 0.07} \text{ C} = k_{a1} \quad (16)$$

$$q_2 = 0.50 \pm 0.05 \text{ mC}^2 = (5.0 \pm 0.5) \cdot 10^{-7} \text{ C}^2 = 10^{-6.30 \pm 0.05} \text{ C}^2 \quad (17)$$

$$k_{a2} = q_2 q_1^{-1} = 10^{-2.60 \pm 0.09} \text{ C}. \quad (18)$$

In Figs. 1, 2, 3, and 4, the agreement is shown between the experimental points and the curves or lines calculated assuming these values for the constants. In Figs. 3 and 4, thin lines show how much the line would be shifted by the maximum estimated deviation in q_1 or q_2. Most experimental points are seen to be in the neighbourhood of the calculated curves although some deviate especially in the diagrams in Fig. 3 and Fig. 4 where the points are very sensitive to small errors.

The agreement in Fig. 1 is especially encouraging, since the experimental values for $h-H$ and (15), were not used for calculating q_1 and q_2.

DISCUSSION

In comparison with the values estimated by J. Bjerrum [2], $pk_{a1} = 2.8$ and $pk_{a2} = 3.5$, ours are practically reversed. Strangely enough Hg^{2+} with $pk_{a1} = 3.7$ and $pk_{a2} = 2.6$ seems to have a second dissociation constant larger than the first. This behavior Bjerrum could hardly have guessed at; we do not know of any other example previously recorded in literature.

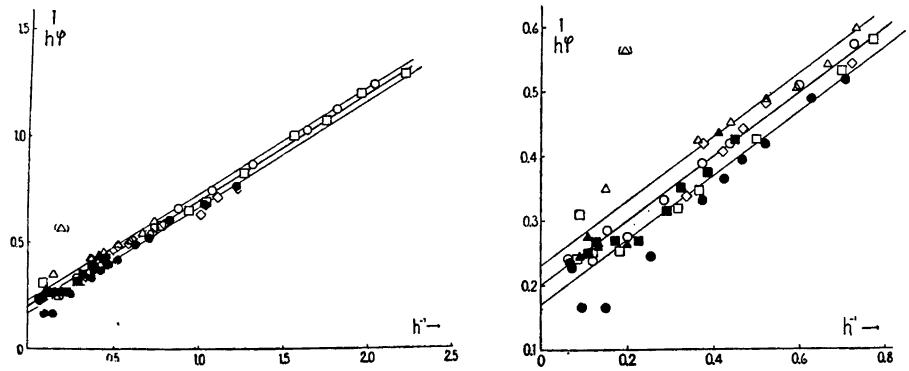


Fig. 4a. hφ (mC) as a function of h^{-1} (mC^{-1}). Fig. 4b. Left part of Fig. 4a on larger scale. Experimental points: symbols as in Table 1. Lines calculated for $q_2 = 0.50\ mC^2$ and $q_1 = 0.20\ mC$ (thick line), 0.17 or 0.23 mC (thin lines).

For a polyprotic acid, say H_2A, the ratio between two subsequent acidity constants is $k_{a1}k_{a2}^{-1} = [HA^-]^2\,[H_2A]^{-1}\,[A^{2-}]^{-1}$. For oxy acids with several OH groups on the same central atom, this ratio is usually of the order of magnitude 10^5. Even for oxalic acid, where the two protons are split off from OH-groups on different C atoms, the ratio is about 1 000. If the ratio were determined by statistical factors only, its value would be 4. The large values ordinarily found are ascribed to the electrostatic forces making it more difficult for the second proton to leave the molecule than it was for the first one.

The corresponding constant for the equilibrium

$$Hg^{2+} + Hg(OH)_2 \rightleftharpoons 2\ HgOH^+$$

$$k_{a1}\,k_{a2}^{-1} = [HgOH^+]^2\,[Hg^{2+}]^{-1}\,[Hg(OH)_2]^{-1} = q_1^2\,q_2^{-1} = 0.08 \pm 0.03 \qquad (19)$$

is extraordinarily low, and one may calculate that the fraction of the total Hg^{II} content present in the form of $HgOH^+$ will never exceed about 12 %. In a diagram (Fig. 5) giving the relative amounts of Hg^{2+}, $HgOH^+$ and $Hg(OH)_2$ at various log h, the range for $HgOH^+$ will thus be rather narrow. An unusually narrow range has also been found for $HgCl^+$; at 25° C and with 0.5 C ClO_4^-, the equilibrium constant for $Hg^{2+} + HgCl_2^+ \rightleftharpoons 2\ HgCl^+$ is 1.80. The corresponding constants for $HgBr^+$ and HgI^+, 5.8 and about 100, exceed the statistical value but are still low [15), 16)].

The third acidity constant of Hg^{2+} is very much lower than the first two; Garrett and Hirschler [1] have estimated $pk_{a3} = 14.85$. This is also in line with the halide complexes [9]; the linear complex HgX_2 is easily formed, but it takes a much higher $[X^-]$ to obtain the higher complexes HgX_3^- and HgX_4^{2-}.

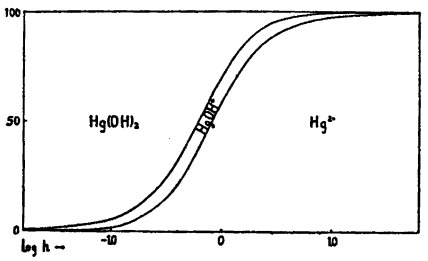


Fig. 5. Distribution of Hg^{II} (in percent) over Hg^{2+}, $HgOH,^{+}$ and $Hg(OH)_2$, as a function of log h (h in mC). Note the narrow range of $HgOH^{+}$.

Later on, Biedermann [17] has found that the ion $TlOH^{2+}$, which is isoelectronic with $HgOH^{+}$, has a low constant of formation from Tl^{3+} and $Tl(OH)_2^{+}$.

A comparison of our data with the solubility of HgO given by Garrett and Howell [4], 0.24 mC, shows that at the end of our titrations the solutions must have been supersaturated with respect to HgO.

SUMMARY

The hydrolysis of the Hg^{2+} ion has been studied at 25.0° C and $[ClO_4^-] = 0.5$ C, using emf methods. In the concentration range studied, the data could be explained by assuming a mononuclear mechanism with the formation of $HgOH^{+}$ and $Hg(OH)_2$. (Equ. (1) and (2)). For the aquo acid $Hg(H_2O)_n^{2+}$, $pk_{a1} = 3.70 \pm 0.07$, and $pk_{a1} + pk_{a2} = 6.30 \pm 0.05$, thus $pk_{a2} = 2.60 \pm 0.09$. For this acid the second dissociation constant is greater than the first, which means that the equilibrium amount of $HgOH^{+}$ is always rather small (Fig. 5).

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Studies on Ionic Solutions in Diethyl Ether

III. Studies on the Soluble Silver-Iodide Complex formed in $LiClO_4$-Ether Solution

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As described in Part II in this series [1,2], Althin, Wåhlin, and Sillén [2] made potentiometric titrations of Ag^+ with Cl^-, Br^- and I^- in $LiClO_4$-ether medium. Using silver electrodes, they obtained curves of the same type as for the corresponding titrations in aqueous medium, and could calculate the solubility products for AgCl and AgBr in $LiClO_4$-ether, just as it is usually done in H_2O. For AgI, however, an unexpected complication arose. The precipitate of AgI first formed was found to dissolve in excess of I^-; even for I^- concentrations as low as 50 mC, the solubility of AgI proved to be considerable (more than 1 mC, as found from the figures below).

It is true that a similar phenomenon is observed in aqueous solutions [3,4]; the I^- excess needed there is, however, larger. For instance, Erber [4] found that 0.82 C HI dissolves 4.3 mC AgI, 1.02 C HI dissolves 9.7 mC AgI, and 8.09 C HI dissolves 5.22 C AgI. There is some uncertainty about the composition of the soluble complex in aqueous solutions. From the predominance of bicovalent silver complexes such $Ag(CN)_2^-$, and $Ag(NH_3)_2^+$, one might expect the formula to be AgI_2^-. However, Bodländer and Eberlein [3], and Erber [4] considered their data to be more consistent with formulas such as $Ag_2I_7^{5-}$ and $Ag_2I_6^{4-}$.

Althin, Wåhlin, and Sillén assumed the simplest formula, AgI_2^-, and from this and their data calculated the complex formation constant for AgI_2^-, and the solubility product for AgI. However, as stated by these authors, the results were only preliminary. With the apparatus and chemicals then used, a slow oxidation of the dissolved iodide ions took place so that the solutions, which were at first almost colorless, were colored light brown at the end of a titration.

It seemed desirable to study these equilibria with an improved apparatus where this source of error was eliminated. This has now been done. Our results corroborate the work of Althin, Wåhlin, and Sillén, as regards the composition of the complex, and the magnitude of the complex constant and the solubility product.

PRINCIPLES

The cells used may be written schematically:

$$-\mathrm{Ag,AgBr}\,|\,\mathrm{S_c(LiBr,LiClO_4)}\,|\,\mathrm{S_b(LiClO_4)}\,|\,\text{equilibrium mixture}\,(\mathrm{LiI,AgClO_4,LiClO_4})\,|\,\mathrm{Ag+} \qquad (1)$$

or

$$-\text{reference electrode}\,\|\,\text{equilibrium mixture}\,([\mathrm{Ag}]_{tot},\,[\mathrm{I}]_{tot})\,|\,\mathrm{Ag}+ \qquad (1\,a)$$

As a reference electrode, the Ag, AgBr electrode was chosen since it proved to keep a stable potential for a longer time than the corresponding electrode with Ag,AgI, and LiI.

In all solutions, diethyl ether was the solvent. In the solution to the right, the amount of $LiClO_4$ added was such as to keep $[ClO_4^-] + X_e = 1$ C, where X_e is the halogenide excess defined by equation (14). The solution to the right was prepared by adding known amounts of one or two solutions (T, or T_1 and T_2) from burettes to an initial amount, usually 50 ml, of a solution S.

We shall now assume — as has been confirmed both by our data and the measurements in parts I and II [1,2] — that because of the large excess of $LiClO_4$ we can neglect the diffusion potential and consider the activity factors of the various ions as constant. The emf of the cell, E, is then the difference between the electrode potential of the right electrode, e, and that of the reference electrode, e_{ref}.

$$E = e - e_{ref} \qquad (2)$$

Moreover, if our assumptions hold true e varies with the concentration of the silver ions according to Nernst's equation (for 18° C)

$$e = e_{0Ag} + 57.77\ \log\ [Ag^+] \qquad (3)$$

By combining (2) and (3) we find

$$E - 57.77\ \log\ [Ag^+] = e_{0Ag} - e_{ref} \qquad (4)$$

In those parts of our titrations where we have an excess of Ag^+ ions, both quantities to the left in equation (4) are known, and so the difference $e_{0Ag} - e_{ref}$ can be calculated. Our data like those in Part II [2] have shown that this expression keeps reasonably constant, as it should according to our assumptions.

When this constant is known, one may calculate also the low $[Ag^+]$ for solutions with excess of I^-. Of course, one must not postulate *a priori* that these very low concentrations are correct. However, just as for aqueous solutions they are supported by the fact that they fit in with a simple and plausible scheme for the equilibria in the solutions.

Let us denote the total analytical concentrations of silver and iodide ions added to the solution by $[Ag]_{tot}$ and $[I]_{tot}$ C (mole l^{-1}). From both these figures should be subtracted $[AgI]_{prec}$, the amount of AgI precipitated, expressed in mole l^{-1}. We shall assume that the solution contains, in addition to Ag^+ and I^-, only one soluble complex, $Ag_xI_y^{(y-x)-}$. (In the following we shall often leave out its charge for the sake of brevity.)

Denoting the equilibrium constant for the complex formation by $\varkappa$ we have

$$[Ag_xI_y] = \varkappa [Ag^+]^x [I^-]^y \tag{5}$$

The content conditions are

$$[I]_{tot} = [I^-] + [AgI]_{prec} + y [Ag_xI_y] \tag{6}$$

$$[Ag]_{tot} = [Ag^+] + [AgI]_{prec} + x [Ag_xI_y] \tag{7}$$

The equations hitherto given should hold true for all points in any of our titrations. We shall now consider the special case of solutions with iodide excess, thus $[I]_{tot} > [Ag]_{tot}$. In such solutions, $[Ag^+]$ may be neglected in comparison with the other quantities in (7).

In a *clear solution*,

$$[AgI]_{prec} = 0$$

$$[Ag_xI_y] = x^{-1} [Ag]_{tot} \tag{8}$$

and

$$[I^-] = [I]_{tot} - yx^{-1} [Ag]_{tot} \tag{9}$$

as is easily seen from (6) and (7). By taking logarithms in (5) and rearranging we find, using (8) and (9)

$$\log \varkappa = \log [Ag]_{tot} - \log x - x \log [Ag^+] - y \log ([I]_{tot} - yx^{-1} [Ag]_{tot}) \tag{10}$$

With the special assumption that the complex is AgI_2^-, (10) takes the form

$$\log \varkappa = \log [Ag]_{tot} - \log [Ag^+] - 2 \log ([I]_{tot} - 2 [Ag]_{tot}) \tag{11}$$

In equation (10), $[I]_{tot}$ and $[Ag]_{tot}$ are known from the analyses and the volumes added, and $[Ag^+]$ is known from the emf measurements. One may thus, for a titration, try various values for x and y, calculate $\varkappa$ by means of (10)

and test which set of x and y gives the best constancy in $\varkappa$. As will be seen below our data point to the composition AgI_2^-.

For a solution with *precipitated AgI present*, we have the additional equilibrium condition

$$[Ag^+][I^-] = k_s \tag{12}$$

From (5) and (12) we find

$$[Ag_xI_y] = \varkappa k_s^x [I^-]^{(y-x)} \tag{13}$$

The excess of iodide ions, X_e is defined by

$$X_e = [I]_{tot} - [Ag]_{tot} \tag{14}$$

We find from (6), (7), (14), and (13)

$$X_e = [I^-] + (y-x)\,[Ag_xI_y] = [I^-] + (y-x)\,\varkappa k_s^x\,[I^-]^{(y-x)} \tag{15}$$

If we had neglected the formation of the soluble complex, set $[I^-] = X_e$, and tried to calculate the solubility product, we would have got an apparent solubility product k_s',

$$k_s' = [Ag^+]X_e = k_s\,(1 + (y-x)\,\varkappa k_s^x\,[I^-]^{(y-x-1)}) \tag{16}$$

The apparent solubility product will thus in general vary with $[I^-]$. However, in the special case where the complex is AgI_2^- we have

$$k_s' = k_s\,(1 + \varkappa k_s) \tag{17}$$

which should be independent of $[I^-]$. In this case the simplest way of treating the data is by first calculating the quantity

$$e'_{0AgI} - e_{ref} = E + 57.77 \log X_e \tag{18}$$

which should be a constant. By combining (4) and (18) we obtain

$$(e'_{0AgI} - e_{ref}) - (e_{0Ag} - e_{ref}) = 57.77 \log k_s' \tag{19}$$

One thus first computes with (18) an average value for $(e'_{0AgI} - e_{ref})$, then the average k_s' with (19) and finally by means of (17) and the $\varkappa$ value determined before, one finds the real k_s.

In this connection we should like to point out two printing errors in Part II. Equation (II, 8) should be $X_e = [I]_{tot} - [Ag]_{tot}$ like (14) here, and (II, 12) should be $\log k_s' = 58.86^{-1}\,(e'_{0AgI} - e_{0Ag})$, in analogy to (19) in this paper.

REAGENTS

Purest available anhydrous *diethyl ether* was refluxed over sodium for 2—4 hours in a nitrogen atmosphere. After discarding the first portion, the ether was distilled directly into a vessel where the solid salt to be dissolved was stored under nitrogen. This was the same vessel where the salt had previously been dried (except for LiI, see below).

In order to exclude daylight, the whole room was blacked out, and only subdued artificial light was used. Moreover the whole apparatus was always wrapped in black paper when ether and LiI were present.

Lithium perchlorate and *lithium bromide* were obtained as described in Part I, *silver perchlorate* as described in Part II.

Lithium iodide (anhydrous) was purchased in Baker's BPC quality. A 25 g package was opened, and the whole contents transferred without delay to a vessel previously filled with nitrogen and connected to a distillation apparatus. By this procedure, the LiI solution was shielded from oxygen and light, so that it remained colorless for several weeks.

The solutions were analysed in the ways described in Parts I—II.

The *nitrogen* used in all preparations and experiments was previously purified and dried by Meyer and Ronge's method [6].

APPARATUS

The apparatus was so constructed that all operations could be carried out in a nitrogen atmosphere, without the admission of even traces of air. The solutions were stored in flasks like that in Fig. 1; a ground stopper with one plain hole and one hole connected to a long glass tube. By turning the stopper, these holes may either be shut, or connected in two different ways to two tubes sealed to the outer side of the neck. Nitrogen may be flushed over the solution in the flask, and the solution may be pressed out by tilting the flask and applying nitrogen pressure; the idea is the same as in an apparatus used in previous work on aqueous solutions (Sillén [5], Fig. 2).

The most suitable stopcock grease in both this and other parts of the apparatus proved to be a mixture of glycerol and starch, heated to 140° C; this grease is insoluble in ether and in the ether solutions used.

The solutions S and T to be used in the titrations were prepared by mixing measured amounts of stock solutions and pure ether in measuring flasks on the top of which was sealed a joint like that in Fig. 1. The stock solutions were added through the apparatus shown in Fig. 2. The central bulb, the "mixer", of volume about 500 ml, was provided with holes for four burettes, with one hole for evacuating and admitting nitrogen, and with a stopcock. The inner part of the stopcock was provided with a little funnel through which the solutions could be directed to either of four different outlets, each of which could be connected to a measuring flask.

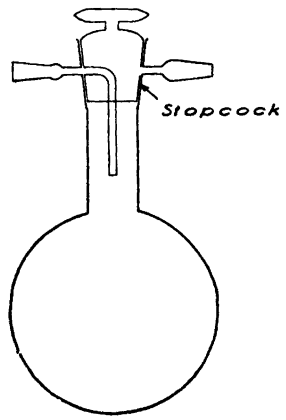


Fig. 1. Flask for storing solutions under nitrogen.

First the calculated volume of LiI, $AgClO_4$, or LiBr solution was added to a certain measuring flask. Then

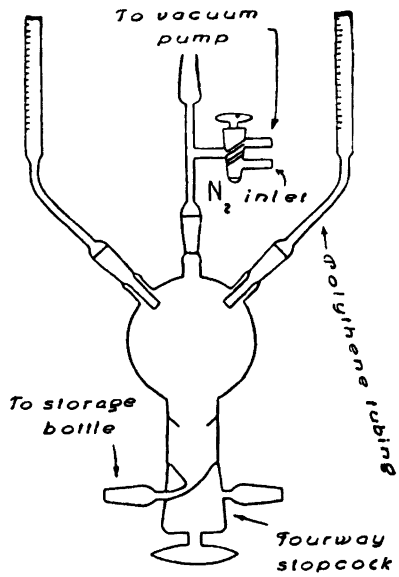


Fig. 2. "Mixer" for preparing solutions of known composition. The central tube goes to the pressure levelling system.

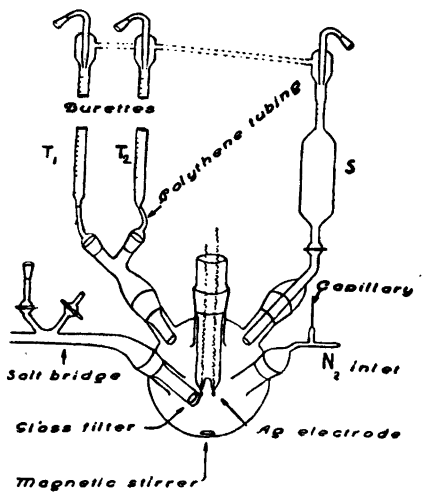


Fig. 3. Main parts of titration apparatus.

we added the calculated volume of $LiClO_4$ solution and finally pure ether until the mark was reached in the measuring bulb. In this way the inorganic salts were washed out of the mixer before the next measuring flask was to be filled.

Instead of with stopcocks the burettes were closed with a short polythene tubing containing a small "glass-bead valve". In this way the apparatus became more flexible and less liable to break than if it had been made entirely of glass: moreover stopcocks were undesirable since ether under pressure has a strong tendency to creep out even through closed and greased stopcocks. In order to level out the pressure the nitrogen in the upper part of the burettes (*cf.* Fig. 3) was connected to the mixing bulb by the central tube in Fig. 2.

By means of this apparatus a number of solutions could be mixed in one procedure, without admitting air.

The titrations were carried out in a complicated apparatus, the principal parts of which are drawn schematically in Fig. 3.

After assembling the cell with its electrodes and burettes, the air was expelled from it by alternatingly evacuating and letting in nitrogen. In order to keep out oxygen and moisture, even in the case of small leakages, a certain overpressure of nitrogen was maintained in it. The nitrogen inlet was provided with a T-tube with a small capillary tube through which a stream of nitrogen continously poured out.

The initial solution S was measured in the vessel to the right of the figure, and added by turning the stopcock. (The pressure was levelled out here as in the burettes.) The solutions T_1 and T_2 to be added in the titration were kept in burettes, closed by polythene tubings with glass-bead valves.

The reference electrode vessel was similar to the titration vessel and always contained 100 mC LiBr, 900 mC $LiClO_4$ (= S_c). The silver and Ag, AgBr electrodes were prepared as described in Part II. The salt bridge was partly made of polythene tubing; it was provided at both ends with sintered glass filters Jena G4, which easily let through gases on evacuation or flushing with nitrogen, but which were rather efficient barriers to the diffusion of the viscous $LiClO_4$-ether solutions. The salt bridge was wide enough to be filled directly without applying a suction, which would have caused the loss of an unknown amount of solution from the titration vessel. With our apparatus the whole cell could be filled with nitrogen and the air expelled by means of a single gas inlet.

The solution in the titration vessel was agitated by means of a magnetic stirrer.

Since it would have been exceedingly difficult to keep this complicated apparatus immersed in a liquid bath, the whole apparatus was kept in a thermostat room, the air temperature of which varied within the limits 18.0 $\pm$ 0.2° C. The variations of the temperature in the solutions was probably still less.

The emf measurements were carried out with a valve potentiometer (Radiometer PHM 3 g).

RESULTS

Table 1 gives a survey of the 5 titrations performed. In titrations 1 and 2 there was Ag^+ in S and I^- in T, in titrations 3—5 S contained I^- and T Ag^+. In the latter case two different T solutions were used, one with about 5 mC Ag^+ (T_1), and one with about 50 mC (T_2). At the beginning of such a titration, the solution T_1 with the lower Ag^+ content was added. After a precipitate of AgI had formed, the Ag^+ content was raised more sharply by adding T_2. A constant E value was usually found within 15 minutes after each addition of T.

Table 2 gives the results of a titration (nr 4) with I^- in S and Ag^+ in T. The table is almost self-explanatory. When 18 ml T_1 had been added, a precipitate of AgI was visible. Altogether 25 ml T_1 was added, and then T_2 was added from the other burette.

Table 1. Survey of the titrations.

Titr. nr.	Ion	S (50 ml) mC	Ion	T_1 mC	Total volume added	T_2 mC	Total volume added
1	Ag^+	15.0	I^-	225.0	75		
2	Ag^+	15.0	I^-	225.0	31		
3	I^-	54.6	Ag^+	15.0	8	50.0	40
4	I^-	50.4	Ag^+	5.0	25	75.6	45
5	I^-	53.5	Ag^+	5.0	20	68.0	75

All solutions in addition contained Li^+ and ClO_4^- so as to make $[ClO_4^-] + X_c = 1C$.

Table 2. Details of titration nr. 4.

ml T_1	ml T_2	Total volume $T_1 + T_2$	$[I^-]_{tot}$ mC	$[Ag^+]_{tot}$ mC	X_e mC	E mV	$e'_{oAgI} - e_{ref}$ mV	log $\varkappa$
2			48.5	0.192	48.3	−285		21.92
3			47.5	0.283	47.2	−274		21.92
4			46.7	0.370	46.3	−266		21.92
5			45.8	0.454	45.3	−259		21.91
6			45.0	0.536	44.5	−253		21.89
7			44.2	0.614	43.6	−248		21.89
8			43.5	0.690	42.8	−241		21.83
9			42.7	0.763	41.9	−237		21.83
10			42.0	0.833	41.2	−235		21.85
11			41.3	0.902	40.4	−234		21.88
12			40.7	0.968	39.7	−232		21.90
13			40.0	1.04	39.0	−230	−311	21.91
14			39.4	1.09	38.3	−229	−311	21.93
15			38.8	1.15	37.6	−228	−310	21.96
16			38.2	1.21	37.0	−224	−307	21.92
17			37.6	1.27	36.3	−222	−305	21.93
18			37.1	1.32	35.8	−221	−305	21.94
19			36.5	1.38	35.1	−227	−311	
25		25	33.6	1.67	31.9	−225	−311	
	5	30	31.5	6.29	25.2	−217	−309	
	10	35	29.7	10.4	19.3	−208	−307	
	15	40	28.0	14.0	14.0	−197	−304	
	20	45	26.5	17.2	9.3	−175	−292	
	25	50	25.2	20.2	5.0	−144	−277	
	30	55	24.0	22.8	1.2	− 74	−243	
					$[Ag^+]$		$e_{0Ag} - e_{ref}$	
	32	57	23.6	23.8	0.2	+865	[+1079]	
	35	60	22.9	25.2	2.3	+892	+1044	
	37	62	22.5	26.1	3.6	+904	+1045	
	39	64	22.1	27.0	4.9	+911	+1044	
	41	66	21.7	27.8	6.1	+917	+1045	
	43	68	21.4	28.6	7.2	+920	+1044	
	45	70	21.0	29.4	8.4	+924	+1044	

Average $e_{oAg} - e_{ref} = + 1044 \pm 1$ mV Average log $\varkappa' = 21.90$

Average $e'_{oAgI} - e_{ref} = - 308 \pm 4$ mV log $\varkappa = 21.89$

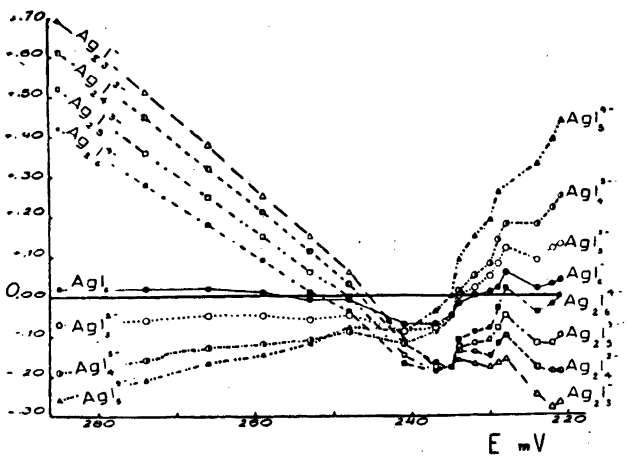


Fig. 4. Testing the constancy of $\varkappa$ for various possible formulas of the soluble complex, using the data of titration 4.

In order to find out the formula of the complex, we tried a number of possible compositions Ag_xI_y of the complex, and calculated log $\varkappa$ by means of equation (10) from the points obtained before a visible precipitate had formed. The results are shown graphically in Fig. 4, where the deviations of log $\varkappa$ from an arbitrary intermediate value are plotted for each set of x and y against E. It is seen immediately that all complexes containing 2 Ag are ruled out since they give a very strong trend in the "constant". A still greater slope would have been obtained by assuming complexes with more than 2 Ag. We thus conclude that the predominant complex formed is mononuclear. Of the mononuclear complexes tried, AgI_4^{3-} and AgI_5^{4-} are completely ruled out, and the constancy is decidedly better for AgI_2^- than for AgI_3^{2-}.

We thus feel justified in assuming that the predominant soluble complex is AgI_2^-, which is made still more plausible by the analogy with other bicovalent silver complexes.

Table 3 gives the mean values of the following quantities from five different titrations: $e_{0Ag} - e_{ref}$ from (4), $e'_{0AgI} - e_{ref}$ from (18), $e_{0Ag} - e'_{0AgI}$, pk'_s from (19), and log $\varkappa$ from (11). For titration 5, two sets of values are listed. The values in parentheses were obtained by using all measured points with Ag^+ excess. However, a comparison with the other titrations made it exceedingly probable that the first few points after the equivalence point had been measured without waiting sufficiently long for the equilibrium. On excluding these values, we obtained the set of figures actually used.

Table 3. Average values for constants from the titrations.

Titr.	$e_{oAg} - e_{ref}$	$e'_{oAgI} - e_{ref}$	$e_{oAg} - e'_{oAgI}$	pk'	$\log \varkappa'$	$\log \varkappa$
1	+1042	−315	+1357	23.49		
2	1041	−315	1356	23.47		
3	1040	−314	1354	23.44	21.86	21.85
4	1044	−308	1352	23.40	21.90	21.89
5	1055	−298	1353	23.42	21.78	21.76
	(1048)		(1346)	(23.30)	(21.66)	(21.64)
		Mean values	1355 ± 3	23.45 ± 0.05	21.85 ± 0.07	21.83 ± 0.07

For titrations 1 and 2 no $\log \varkappa$ values are given for the simple reason that a perfectly clear solution was never obtained in these titrations. There was always AgI present, as was easily understood when the equilibrium constants later on became known. (*Cf.* ref. 24 and Table 1.)

When approximative values for $\varkappa$ and k_s are found, one may calculate at what point AgI would have precipitated in the solutions if equilibrium had always been achieved. For a solution at equilibrium with solid AgI, (13) gives inserting $x = 1$ and $y = 2$

$$[AgI_2^-] = \varkappa k_s [I^-] \tag{20}$$

One may prove from (20), (8), and (9) that in a clear solution where AgI is just about to precipitate

$$\frac{[Ag]_{tot}}{[I]_{tot}} = \frac{\varkappa k_s}{1 + 2\varkappa k_s} \tag{21}$$

Fig. 5 gives corresponding values of $[I]_{tot}$ and $[Ag]_{tot}$ for the points of three titrations, starting with I^- in S. (Titrations nr 3,4, and 5.) The thick line crossing the titration lines corresponds to equation (21), and thus gives the points where AgI would have precipitated at equilibrium. The points, where a precipitate was actually first observed, are marked by arrows. To be strict, one should perhaps exclude all points corresponding to supersaturated solutions (points between the thick line and the arrow) in taking the average value of $\varkappa$. This has been done in the last column, $\log \varkappa$, in Table 3; in the column next to the last, $\log \varkappa'$ was obtained using all values where a precipitate was not observed. For instance in titration 4, all values in the "$\log \varkappa$" column of Table 2 were used for calculating $\log \varkappa'$, but those within brackets were excluded in calculating $\log \varkappa$.

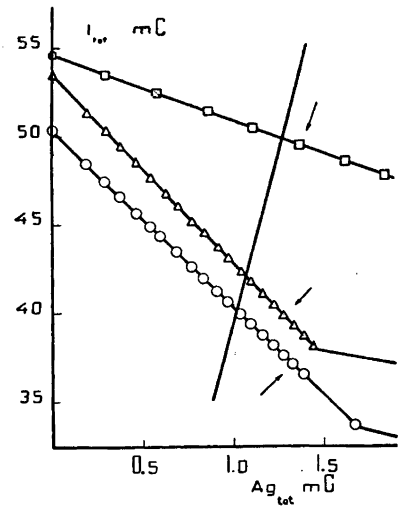


Fig. 5. Plot of $[I]_{tot}$ versus $[Ag]_{tot}$. Lines with marks, points of titrations 3, 4 and 5. To the right of the thick line, solid Ag I should be present at equilibrium. At the arrows, precipitate was actually observed.

Using equation (17) and the mean values in Table 3, we find as our final values:

$$Ag^+ + 2\ I^- \rightleftharpoons AgI_2^- \qquad \varkappa = [AgI_2^-]\,[Ag^+]^{-1}\,[I^-]^{-2}$$

$$\log \varkappa = 21.83 \pm 0.07 \tag{22}$$

$$AgI(s) \rightleftharpoons Ag^+ + I^-;\ k_s = [Ag^+]\,[I^-]\ ;pk_s = -\log k_s = 23.45 \pm 0.05 \tag{23}$$

which gives

$$AgI(s) + I^- \rightleftharpoons AgI_2^-;\ [AgI_2^-][I^-]^{-1} = \varkappa k_s = 10^{-1.62 \pm 0.09} = 0.024 \pm 0.005 \tag{24}$$

These values should be valid under our experimental conditions, in ether solutions containing 1 C $LiClO_4$, at 18° C. They may be compared with the preliminary values in Part II: $pk_s = 22.8$ and $\varkappa = (2 \pm 1) \cdot 10^{22}$ for 23.5° C; the earlier measurements thus gave the right order of magnitude, in spite of the sources of error mentioned above.

SUMMARY

An improved equipment is described for electrometric titrations of ionic solutions in $LiClO_4$-ether medium. The following operations can be carried out in a nitrogen atmosphere, with the exclusion of oxygen and moisture; stock solutions of $LiClO_4$ and various other salts in ether are prepared; ether and stock solutions are mixed in definite proportions to give the concentrations

and ionic strength desired; titrations are carried out, adding one solution from a burette to a measured amount of another; and the emf is measured between an electrode in the titrated solution and a reference electrode also in $LiClO_4$-ether medium.

In $LiClO_4$-ether medium, AgI is soluble in excess of I^-; this occurs at lower I^- than in aqueous solutions. By titrations in 1 C $LiClO_4$-ether, the soluble complex was shown to be very probably AgI_2^-. The equilibrium constants at 18° C in this medium were found to be; $k_s = [Ag^+][I^-] = 10^{-23.45 \pm 0.05} C^2$. $\varkappa = [AgI_2^-]\,[Ag^+]^{-1}[I^-]^{-2} = 10^{-21.83 \pm 0.07}\,C^{-1}$ and $[AgI_2^-]\,[I^-]^{-1} = 0.024 \pm 0.005$.

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We wish to thank Professor Karl Myrbäck for the kind interest he has shown in this work and Professor Arne Ölander for allowing us to work in his institution. We have had valuable discussions with Mrs. Ulla Larsson-Berglund, Mrs. Karin Ekelin, Mr. Bengt Althin, and Mr. Erik Wåhlin.

ERRATA

In Part II, Table 1, a few errors have unfortunately slipped in. The v values from 27.1 on are too high by 0.5, and the chloride concentration in S is 0.0160 C instead of 0.0161. In the calculations, more digits were sometimes used than are given in the Tables. — With the correct values, the value for $e_{0Ag} - e_{0AgCl}$ from this experiment comes out as 846.0 instead of 845.6. This does not change the average mean value for pk_s for AgCl in the solvent.

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On Isolation and Amino Acid Composition of "β-Globulin" Extracted from the Seeds of Barley (*Hordeum vulgare*) *

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Experiments on the fractionation of the nitrogenous components extracted from barley seeds were begun by Osborne in 1895 [1]. This work was continued at the Carlsberg Laboratory in the years 1906–1916 by Schjerning [2] and later on by Bishop in England [3]. Bishop divided the nitrogenous components into four fractions:

1) a leucosin or albumin fraction,
2) an edestin or globulin fraction,
3) a hordein or alcohol soluble fraction, and
4) a glutelin or insoluble fraction.

During the second world war these English results have been critically examined by a group of investigators at "l'École de Brasserie de Nancy" in France under the leadership of Urion [4].

In the last few years Biserte and Scriban [5] from the University of Lille in France have made some amino acid analyses by paper chromatography on different fractions during the brewing process. But they have not tried to analyse the different native proteins in barley.

The present investigation is a continuation of a research program carried out by Quensel [6] at the Physico-chemical Institute in Upsala, Sweden, in the years 1936–1942.

Quensel restricted himself to investigations dealing with the globulins in barleyextracts, and he introduced ultracentrifugation and electrophoresis experiments as purity tests for the different fractions obtained after "salting

* A preliminary report on this work was given on the "7. nordiske kemikermøde, Helsingfors 21–25 august, 1950".

The final paper was presented at the "XIIth International Congress of Pure and Applied Chemistry, New York, September 10 to 13, 1951".



+ limb just before start.

+ limb 287 minutes after start.

Fig. 1. Electrophoresis diagram of β-globulin from barley.

Phosphate buffer, pH 7.05
Ionic strenght: 0.1
Potential gradient:
6.47 volt/cm
Mobility: $u = 3.5$ cm/voltsec.

out" by ammonium sulphate. He succeeded in fractionation of the globulin fraction in four components characterized by different sedimentation velocity and different electrophoretic mobility, and he named them α, β, γ, and δ-globulin after increasing molecular weight.

The molecular weights of these globulins were found to be respectively 26 000, about 100 000, 166 000 and about 300 000. Further progress along this line has been made in Sweden by Säverborn [7], Danielsson [7,8] and Sandegren [9].

EXPERIMENTAL

We have isolated β-globulin from a 1 molar sodium chloride extract of 2 000 g of finely ground barley grains of the type "Kenia" by adding ammonium sulphate to 15/100 of saturation. The precipitate has been purified by four purification cycles, each consisting of a resolution of the precipitate in 1 molar sodium chloride and a new precipitation by 15/100 of saturation with ammonium sulphate. The preparation is then dialysed and after a final precipitation is stored at $-10°$ C.

Quensel never succeeded in the isolation of pure β-globulin from barley. The preparations always were contaminated by α-globulin [6]. We have examined the purity of our preparation by the three following methods: diffusion, electrophoresis and ultracentrifugation.

The diffusion constant is found to be $D_A = 4.8_4$ when calculated by the "area method", and to be $D_M = 4.9_7 \cdot 10^{-7}$ cm^2/sec when calculated by the "second moment method". Identity of the two results would indicate homogenity of the preparation from the point of view of molecular weight.

The electrophoretic mobility is found to be $u = 3.5 \cdot 10^{-5}$ cm^2/volt sec at pH 7.05, and the peak is homogenous through the whole experiment.* See Fig. 1.

Quensel found the mobility $u = 3.6 \cdot 10^{-5}$ at pH 7.02 [6].

The sedimentation constant in 0,2 N NaCl is found to be 6.7 at 24° C.

* Apparatus placed at our disposal in the Carlsberg Laboratory by kind permission of Professor K. Linderstrøm-Lang.

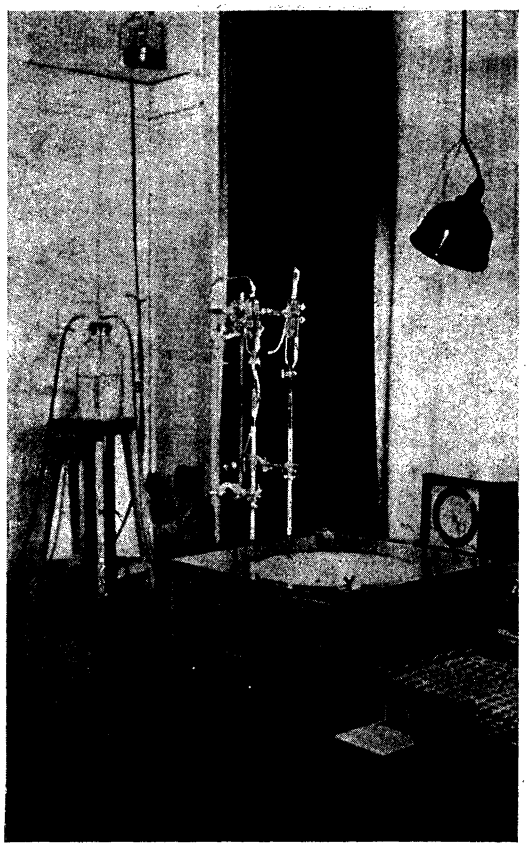


Fig. 2. Set-up for fraction collection from starch columns. A constant air pressure over the reservoirs for the solvents placed on top of the starch columns is maintained through a rubbertube connection to the lower one of the two leveling flasks. Seen to the left in the figure. The pressure corresponds to the difference in level between the two flasks. The fraction collector is constructed in the Carlsberg Laboratory by Professor K. Linderstrøm-Lang and M. Ottesen. For further details, see text.

It seemed to us that we had succeeded in isolating β-globulin from barley seeds in a practically pure state, and we decided to submit this preparation to amino acid analysis. We have chosen the method of Moore and Stein using starch chromatography [10].

The hydrolysis is performed in 6 *N* hydrochloric acid. It is boiled for 24 hours with reflux, and the hydrochloric acid is then removed by evaporation to dryness two or three times. The final residue (consisting of the amino acids) is dissolved in 2 ml of water and Kjeldahl-N determined on an aliquote by a micro method. From this it is calculated how many microliters we have to transfer to the column in order to have about two milligrams of the amino acids. This amount is directly transferred to the surface of the column by a Carlsberg micropipette. The starch column is placed over an automatic fraction collector shown in Fig. 2.

This machine has been constructed in the Chemical Department of the Carlsberg Laboratory. The movement of the circular test tube rack is electromagnetically controlled by an impulse from an electric clock that can be adjusted to give an impulse every five minutes or every multiple of five minutes. In our case it has been adjusted to give an impulse every thirty minutes which means that a new tube is placed under the starch column every half hour.

Fig. 3.

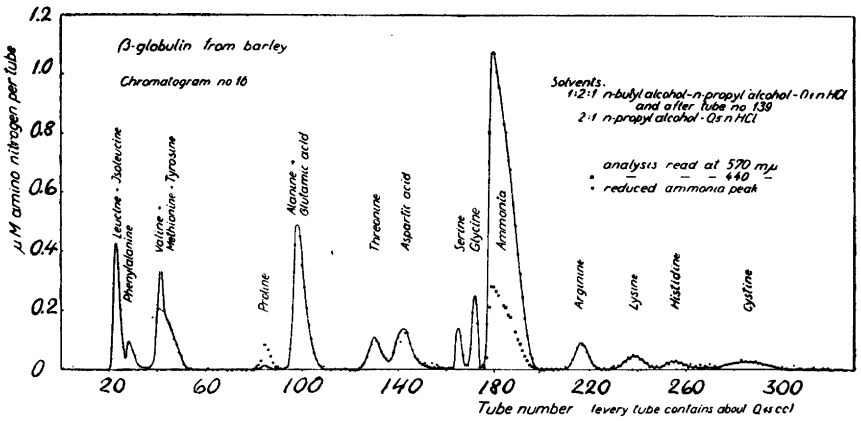


Table 1. Chromatographic analysis of hydrolysates of β-globulin from barley.
Solvents: 1 : 2 : 1 n-butyl alcohol — n-propyl alcohol — 0.1 N HCl and 2 : 1 propyl alcohol — 0.5 N HCl.

Preparation and chromatogram no. / Constituent	N as per cent of Kjeldahl-N						Average	Standard deviation (in relative per cent)
	1st hydrolysate			2nd hydrolysate				
	7	11	16	17	22	23		
Leucine + isoleucine	11.20	10.41	10.45	10.15	9.52	9.06	10.13	± 3
Phenylalanine	2.55	2.68	3.11	2.99	2.37	2.28	2.66	± 5
Valine, methionine and tyrosine	13.60 ca	13.21 ca	14.03 ca	12.45 ca	10.52 ca	13.05 ca	12.81 ca	± 4
Proline	9.91	8.54	11.04	—	12.61	13.34	11.09	± 8
Glutamic acid and alanine	19.09 ca	16.29 ca	21.62 ca	18.85 ca	16.82 ca	17.75 ca	18.40 ca	± 4
Threonine	4.48	3.91	5.29	3.87	4.51	3.62	4.28	± 6
Aspartic acid	6.90	6.02	—	—	6.93	5.74	6.40	± 4
Serine	3.44	—	3.68	—	4.25	3.02	3.60	± 7
Glycine	5.07	—	5.71	—	6.87	4.04	5.42	± 11
Ammonia	17.1 ca	13.71	(13.71)	(13.71)	(13.71)	(13.71)	(13.71)	—
Arginine		5.37	5.26	—	7.31	—	5.98	± 11
Lysine	—	10.40 ca	3.05	—	2.51	—	2.78	± 10
Histidine	—		2.51	—	3.70	—	3.11	± 19
Cystine	—		9.09	—	5.89	—	7.49	± 21
						Sum	107.9 %	± 3 %

The different types of solvent mixtures specified by Moore and Stein [11] are used for separating the amino acids.

We first used *n*-butyl alcohol — *n*-propyl alcohol — 0.1 *N* hydrochloric acid in the proportion 1 : 2 : 1 followed by *n*-propyl alcohol — 0.5 *N* hydrochloric acid in the proportion 2 : 1 when the aspartic acid-peak has appeared. An example of the resulting effluent-concentration-curve is given in Fig. 3.

Fig. 4.

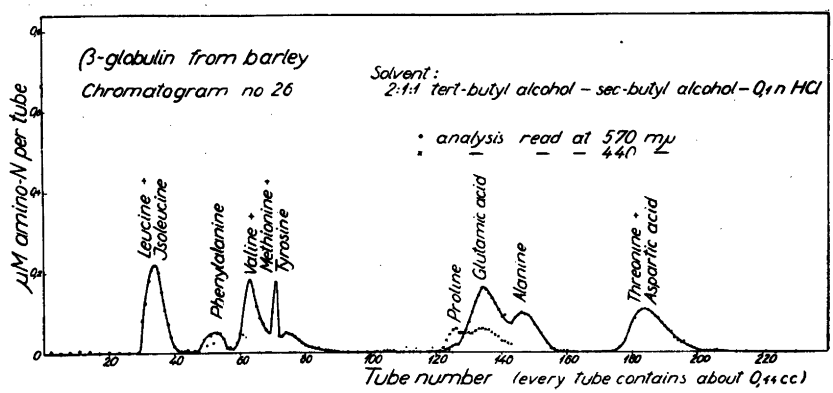


Table 2. Chromatographic analysis of hydrolysates of β-globulin from barley.

Solvent: 2 : 1 : 1 tert.-butyl alcohol — sec.-butyl alcohol — 0.1 N HCl.

Constituent \ Preparation and chromatogram no.	N as per cent of Kjeldahl-N			Average	Standard deviation (in relative %)
	1st hydrolysate	2nd hydrolysate			
	14	26	27		
Glutamic acid	10.46	11.92	12.70	11.69	± 5
Alanine	6.89	7.47	6.95	7.10	± 3

It is in all respects similar to the curve originally obtained by Moore and Stein [11] in experiments with a synthetic mixture containing 17 amino acids and ammonium chloride. The general appearance of the curve and the approximate position of the peaks are the same. There are no peaks in the figure which cannot be ascribed to the common amino acids. No evidence, therefore, has been obtained for the existence in β-globulin hydrolysates from barley of unsuspected amino nitrogen containing constituents. The abnormal height of the ammonia peak has to be ascribed to a content of ammonium sulphate in this hydrolysate. This preparation had not been submitted to dialysis after the last purification cycle. But the location of this abnormal high ammonia peak together with the position of the proline peak (it has a yellow colour) gives a confirmation of the correctness of the names assigned to the peaks in Fig. 3.

As a result of the principle in our fraction collector, that is working on a time basis, the ordinates in our effluent diagrams will not be in concentration units as in the diagrams of Moore and Stein [11] which were obtained by using a fraction collector working on a volume basis. In our diagrams the ordinates state the amount of amino nitrogen per tube.

As proposed by Moore and Stein we have chosen leucine as the reference substance, and in the calculations we have used the colour factors given by them. In Table 1 we have collected the results from 6 diagrams of the type just mentioned.

Fig. 5.

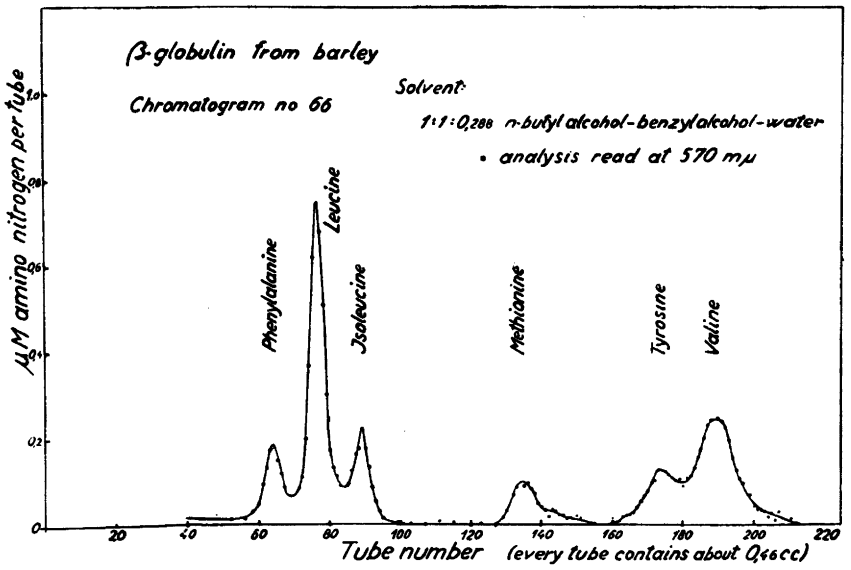


Table 3. Chromatographic analysis of hydrolysates of β-globulin from barley.
Solvent: 1 : 1 : 0.288 n-butyl alcohol — benzylalcohol — water.

Constituent \ Preparation and chromatogram no.	N as per cent of Kjeldahl-N				Standard deviation (in relative %)
	2nd hydrolysate			Average	
	66	67	68		
Phenylalanine	3.34	2.89	3.16	3.13	± 4
Leucine	10.30	10.50	10.10	10.30	± 1
Isoleucine	3.29	4.50	4.16	3.98	± 9
Methionine	2.05	2.08	2.03	2.05	± 0.5
Tyrosine	4.18	4.35	4.72	4.42	± 4
Valine	7.82	8.14	7.49	7.82	± 2

They give the nitrogen content in every amino acid in per cent of the total Kjeldahl-nitrogen in the hydrolysate.

The last four columns give the results from a hydrolysate containing extra ammonia (2. hydrolysate); the first two columns result from a hydrolysate without extra ammonia (1. hydrolysate). In column 7 the average of the results is given, and in the last column the standard deviation in relative per cent for these results is stated.

The data from this type of diagrams have to be combined with results obtained by chromatograms using other solvent mixtures to give a more complete picture of the hydrolysate. This will now be described.

Fig. 6.

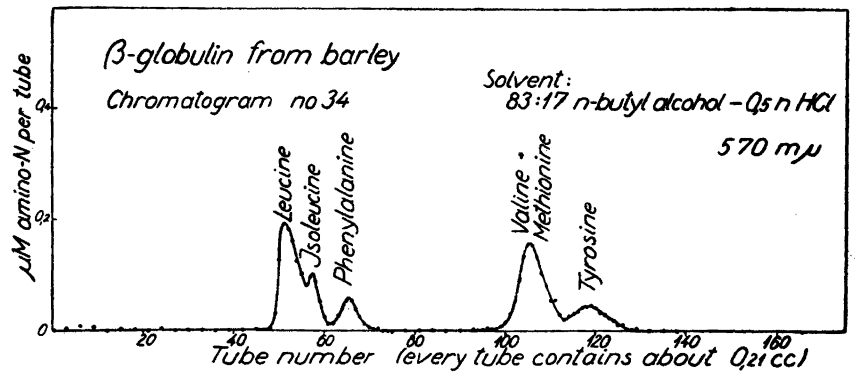


Table 4. Chromatographic analysis of hydrolysates of β-globulin from barley.

Solvent: 83 : 17 n-butyl alcohol — 0.5 *N* HCl.

Constituent \ Preparation and chromatogram no.	N as per cent of Kjeldahl-N				Standard deviation (in relative %)
	1st hydrolysate	2nd hydrolysate		Average	
	35	34	36		
Leucine	8.27	7.70	8.07	8.01	± 2
Isoleucine	2.95	2.08	1.81	2.28	± 25
Phenylalanine	3.04	2.49	2.35	2.63	± 8
Valine and methionine	8.65	8.61	8.72	8.66	± 0.04
Tyrosine	5.16	3.69	4.81	4.55	± 10

Glutamic acid and alanine have been separated by tert-butyl alcohol — sec-butyl alcohol — 0.1 *N* hydrochloric acid in the proportion 2 : 1 : 1 *Cf.* [11].

Fig. 4 shows a diagram of this type. Although the glutamic acid and the alanine peaks are not fully separated in our diagrams, an estimation of the amount of the two components can be made. Of course, the contribution from the proline peak, not separated from the glutamic acid by this solvent has to be subtracted in the calculations.

The results from three chromatograms of this type are collected in Table 2.

This table is arranged as the previous table. In column 4 the mean values are given, and in the last column the standard deviation in relative per cent for these results is shown.

For the separation of the six fast moving amino acids on the 1 : 2 : 1-diagram, that is: leucine, isoleucine, phenylalanine, valine, methionine and tyrosine, Stein and Moore [12]

propose *n*-butyl alcohol — benzyl alcohol — water, mixed in the proportions 1 : 1 : 0.288, as the solvent most able to separate all of them. Fig. 5 gives an example of our result with this solvent. The results from three chromatograms of this type are collected in Table 3.

For the separation of these six amino acids we have also tried to use another solvent proposed by Moore and Stein [12]. It is a mixture of *n*-butyl alcohol — 0.5 *N* hydrochloric acid in the proportions 83 : 17. When used on our hydrolysates it gives a curve as shown in Fig. 6. It has only one drawback: it cannot separate valine and methionine. The results from three chromatograms of this type are collected in Table 4.

When we summarize the results from these four types of solvent mixtures represented by the values in Table 1 to Table 4, we have found the values shown in the first column of Table 5.

As before, the nitrogen content in every amino acid is given in per cent of the total Kjeldahl-nitrogen in the hydrolysate.

The last column gives the standard deviations in relative per cent for these values. For the amino acids leucine, isoleucine, phenylalanine, valine, and methionine the standard deviation seems to be only 3—5 %.

But for the amino acids emerging later, the standard deviation in relative per cent is about ± 10 and slowly increases up to ± 20 % for the last amino acids, mostly because these values are rather small and are only based on two determinations. *

The sum of the nitrogen values in the first column exceeds 100 per cent. This cannot be correct, and it must be due to some systematic incorrectness in the calculations we have made for arriving at these values. Possibly our leucine standard is not quite pure, so that our values will all be too high. Work is proceeding to clear up this question. In column 2 we have the same values as in column 1, but now they are all expressed in per cent of the nitrogen determinated in the *chromatogram*.

In accordance with the estimation of Rees [14] we have assumed a decomposition of threonine and serine during acid hydrolysis for 24 hours. Consequently the values for these two amino acids have been corrected upwards 5 and 10 per cent respectively, and the ammonia value is correspondingly corrected downwards. These corrections have been introduced in the values given in column 3.

The values in column 3 constitute at present our final result of the composition of a hydrolysate from β-globulin from barley. It is expressed as the nitrogen contents of the different amino acids. For facilitating the comparison of these values with tables giving the amino acid composition for proteins in other units, we have also calculated our values as:

a) g amino acid residue per 100 g of protein and
b) g amino acid per 100 g of protein.

These values are given in column 4 and 5 respectively.

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* Recently Moore and Stein [13] have published a method where an ion exchange column (Dowex 50) has been used instead of a starch column and where buffers are used for elution; there the sequence of some of the amino acids is also reversed. This will give much better values for the last (basic) amino acids than found here. Work is in progress to utilize this new technique.

Table 5. The amino acid composition of β-globulin from barley.

Constituent	N as % of Kjeldahl-N	N as % of total chromatographic N		Values in column 3 calculated as:		Standard deviation (in relative %)
		values uncorrected	corrected for assumed destruction	g amino acid residue per 100 g protein	g amino acid per 100 g protein	
	1	2	3	4 z)	5 æ)	6
Leucine	8.01	7.3	7.3	9.5	11.0	± 3
Isoleucine	2.28	2.1	2.1	2.7	3.2	± 3
Phenylalanine	2.80	2.5	2.5	4.2	4.7	± 5
Methionine	2.05	1.9	1.9	2.9	3.2	± 3
Valine	7.82	7.1	7.1	8.1	9.5	± 3
Tyrosine	4.48	4.1	4.1	7.7	8.5	± 9
Proline	11.09	10.1	10.1	11.2	13.3	± 8
Glutamic acid	11.69	10.6	10.6	15.7	17.8	± 5
Alanine	7.10	6.4	6.4	5.2	6.5	± 3
Threonine	4.28	3.9	4.3 x)	5.0	5.9	± 6
Aspartic acid	6.40	5.8	5.8	7.6	8.8	± 4
Serine	3.60	3.3	3.6 x)	3.6	4.3	± 7
Glycine	5.42	4.9	4.9	3.2	4.2	± 11
Amide-NH_3	13.71	12.5	11.8 x)	− 0.14	2.5	—
Arginine	5.98	5.4	5.4	2.4	2.7	± 11
Lysine	2.78	2.5	2.5	1.8	2.1	± 10
Histidine	3.11	2.8	2.8	1.5	1.7	± 19
Cystine + cysteine	7.49	6.8	6.8	8.0	9.4	± 21
Tryptophan y)		destroyed by hydrolysis				
Total	110.09%	100.0%	100.0%	100.16%	119.3%	

x) The average threonine and serine values have been raised by 5 % and 10 %, respectively, in accordance with the estimates of Rees (13) for decomposition of these amino acids during hydrolysis. The value for amide-NH_3 has been correspondingly reduced.

y) A chromatographic estimation of tryptophan in the unhydrolysed protein has been made. We have found 3.8 g per 100 g protein. For further details see text.

z) The values in this column have been found by inserting the corresponding N-values from the 3rd column into the following general expression:

$$\frac{\frac{N_i}{n_i}\,(M_i - M_{H_2O})\;100}{\frac{N_i}{n_i}\,(M_i - M_{H_2O})}$$

where the index i refers to the amino acid in question, M is the molecular weight and n is the number of nitrogen atoms per molecule. The value of amide-NH_3 residue will show a negative figure when calculated in this way, because $(M_{NH_3} - M_{H_2O})$ has a negative value.

æ) The values in this column have been found by inserting the corresponding values from the 4th column (they are called $A_{R,i}$) into the following general expression:

$$\frac{A_{R,i}\;M_i}{M_i - M_{H_2O}}$$

The determination of tryptophan is exclued from this chromatographic analysis, because tryptophan is destroyed in the acid hydrolysis. The Hopkins-Cole reaction for tryptophan is positive on the unhydrolysed protein, so β-globulin from barley contains tryptophan.

An approximate quantitative estimation of tryptophan has been made by the spectrophotometric method for determination of the relative amount of tyrosine and tryptophan in a protein (Goodwin and Morton)[15]. We have found that the molarity of tryptophan amounts to 40 per cent of that of tyrosine. In Table 5, column 6, we have found the tyrosine content to be 8.5 g/100 g protein. It means that the tryptophan content in β-globulin from barley is about 3.8 g/100 g protein.

DISCUSSION

We have been searching the literature for amino acid analyses earlier performed on β-globulin from barley, but have found none. The nearest we have found is the amino acid analysis on the globulin edestin isolated from hemp or flax seeds. A summary of the most reliable of these results is given by Cohn and Edsall[16] in their monograph on amino acids and proteins.

There is a very poor agreement between the results stated therein for the amino acid composition of edestin and the values for β-globulin from barley which we have found, but it has to be remembered that the analyses have been performed in quite different ways.

The differences are still so great that they emphasize the need for amino acid analysis to be performed on every separate globulin fraction isolated from the different seeds. Significant differences have to be expected.

Until now the name edestin often has been uncritically used as the name for the globulin fraction of a seed protein, sometimes without giving the name of the seed. Of course this is very confusing and also reduces the value of the results. The arbitrary use of α, β, γ, and δ as prefixes introduced by Quensel[6] for characterizing the different groups of seed globulins is an improvement, but it is only regarded as a temporary solution of the problem. When more is known about the amino acid composition and other important properties of the proteins it will be justified to revise the names and try to find a nomenclature taking these results into consideration. But at the present moment we have to specify in every single case the fraction we are dealing with.

These investigations constitute the first part of a planned research program comprising a systematic investigation of the different protein components in barley, since possible differences in their structure and composition would be of considerable interest in investigations of the brewing process.

SUMMARY

"β-Globulin" from barley grains has been isolated.

The degree of purity has been established by electrophoresis, diffusion and ultracentrifuge experiments. After hydrolysis both *qualitative* and *quantitative* amino acid determinations have been made by starch chromatography according to the method of Moore and Stein. The following amino acids have been found: leucine, isoleucine, phenylalanine, methionine, valine, tyrosine, proline, glutamic acid, alanine, threonine, aspartic acid, serine, glycine, arginine, lysine, histidine, and cystine together with ammonia. A spectrophotometric estimation of the tryptophan content is also given.

In the analytical part of these investigations I have been assisted by Mrs. Kirsten Nielsen and Miss L. Block-Andersen. My thanks are also due to the chief of the Research Laboratory Dr. Birger Trolle and to the chief of the Carlsberg Laboratory, Professor K. Linderstrøm-Lang for stimulating interest in this work.

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On the Reaction between Methyllithium and Carboxylic Acids

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The aim of this work was to investigate the possibilities of preparing methyl ketones by the action of methyllithium on different carboxylic acids. The methyl ketones have for many reasons been of great interest to prepare, *i.e.* for subsequent oxidative degradation according to Krafft [1]. For the ketone synthesis Krafft [1] used the distillation of the barium salt of the unknown acid together with barium acetate. However, the yields by this method are in general low.

The action of Grignard compounds on carboxylic acids [2] and their derivatives [3] has also been tried. The derivatives of acids having a double bond in β-γ-position have, however, offered some difficulties.

In 1933, Gilman and van Ess [4] showed that on carbonation phenyllithium yielded 70 % benzophenone and no benzoic acid, which is the main product on carbonation of the corresponding magnesium compound. They found that the reason for the high yield of ketone was the higher reactivity of the organolithium compound. If an aryllithium compound was allowed to react with carbon dioxide at temperatures between −50——80°C the following reaction occurred

$$RLi + CO_2 \rightarrow RCOOLi \qquad (I)$$

At a higher temperature (room temperature) another reaction took place. To the lithium salt of I was added one mole of aryllithium, and a dilithium salt of a dihydroxymethane was obtained, which on hydrolysis yielded a ketone in accordance with the following general reaction.

$$RCOOLi + RLi \rightarrow \left[\begin{array}{c} R \quad OLi \\ \diagdown \; / \\ C \\ \diagup \; \diagdown \\ R \quad OLi \end{array}\right] \xrightarrow{H_2O} \begin{array}{c} R \\ \diagdown \\ CO \\ \diagup \\ R \end{array} \qquad (II)$$

Gilman and van Ess [4] (compare Moureu and Delange [5]) also showed that it was possible to prepare mixed ketones in an analogous reaction.

In a paper on the preparation of substances related to vitamine A, Arens and van Dorp [6] describe the preparation of the C_{18}-ketone by the action of methyllithium on β-ionylidene crotonic acid. The reaction was instantaneous and the yield was over 90 %. It may be noticed that no addition to the double bonds occurred in this synthesis. Other authors have obtained similar results [7–10].

An other methyl ketone [11] has been obtained in a yield of 90—95 % by the interaction of methyllithium with lithium cinnamate.

Only in one case, hitherto, has any comparison been made between the use of the free acid or its lithium salt: In the case of benzophenone Gilman and van Ess [4] have made two syntheses. One started with the lithium benzoate, which was allowed to react with one mole of phenyllithium, and was found to give a 70 % yield of ketone and no tertiary alcohol; the second experiment started with benzoic acid and two moles of phenyllithium and yielded 37.2 % of ketone and 14.1 % of triphenylcarbinol.

Two possible explanations are given for the formation of the tertiary alcohol. The first is that benzoic acid is dehydrated by phenyllithium to give benzoic anhydride. This would react with one mole of phenyllithium to give lithium benzoate and "free" benzophenone, which would enter the ordinary reaction of a ketone yielding triphenylcarbinol

$$2\ C_6H_5COOH + 2\ C_6H_5Li \rightarrow (C_6H_5CO)_2O \qquad \text{(III)}$$

$$(C_6H_5CO)_2O + C_6H_5Li \rightarrow (C_6H_5)_2CO + C_6H_5COOLi \qquad \text{(IV)}$$

$$(C_6H_5)_2CO + C_6H_5Li \rightarrow (C_6H_5)_3COLi \rightarrow (C_6H_5)_3COH \qquad \text{(V)}$$

Another explanation is suggested, in which the phenyllithium is supposed to be added directly to the carbonyl bond (without previous formation of lithium salt) yielding an intermediary product (VI), which by phenyllithium is brought over into the alcohol in accordance with the reaction V above.

$$C_6H_5COOH + C_6H_5Li \rightarrow \left[(C_6H_5)_2C\begin{matrix}\diagup OLi \\ \diagdown OH\end{matrix}\right] \rightarrow (C_6H_5)_2CO + LiOH \qquad \text{(VI)}$$

When using methyllithium, however, these side reactions are not likely to occur, because of the markedly lower reactivity of methyllithium as compared with phenyllithium, unless the concentration of methyllithium is strongly increased. Karrer *et al.*[7] have shown that when one mole of β-ionylidene crotonic acid is allowed to react with two moles of methyllithium only ketone is formed. If the number of moles of methyllithium is increased to five, equal quantities of ketone and carbinol are obtained, and by a further increase of methyllithium to ten moles per mole acid, the tertiary alcohol will be formed almost exclusively. In the case of the two higher concentrations the explanations given

by Gilman and van Ess [4] seem very probable. The side reaction of carbinol formation may be avoided by adding the methyllithium solution to the acid, thus avoiding too high concentrations of methyllithium.

To compare the difference in yield when using the free acid or the lithium salt of the acid, experiments have been carried out with cinnamic acid. A series of syntheses was carried out, the results of which are compiled in Table 1.

Table 1. Yield of methyl ketone on refluxing cinnamic acid/lithium cinnamate with methyllithium during different number of hours. (h = time of reflux in hours).

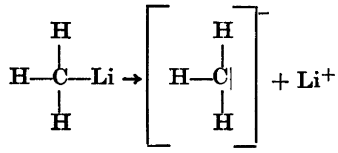
Reactant	*h*	Yield in %
Cinnamic acid	0	77
Lithium cinnamate	2	69
» »	6	79

It appears that slightly higher yields were obtained by using the lithium salt. However, as the lithium salts in some cases are difficult to obtain, all syntheses have been carried out with the free acid.

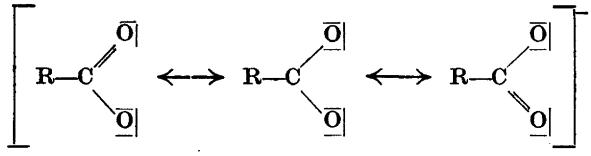
In a series of runs methyllithium was allowed to react with different acids. In Tables 2—5 the yields of methyl ketone have been collated.

The mechanism of the formation of a ketone may be explained in the following way:

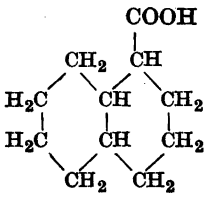
In methyllithium there is, as in the other organometallic compounds, a displacement of electrons towards the carbon atom, with the formation of a carbanion with a free electron pair and a positive lithium ion.



The lithium salt of a carboxylic acid has the following resonance structures



Tables 2—5. Yields of methyl ketones obtained by the reaction of methyllithium with different acids.

Acid	Yield in %
CH_3COOH	0
CH_3CH_2COOH	~ 1
$CH_3(CH_2)_4COOH$	83
$CH_3(CH_2)_{10}COOH$	63
	90 *

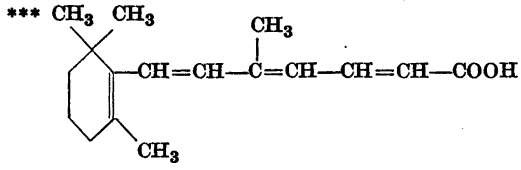
Acid	Yield in %
$H-CH_2COOH$	0
CH_3-CH_2COOH	~ 1
CH_3O-CH_2COOH	16
$C_6H_5-CH_2COOH$	76

Acid	Yield in %
C_6H_5COOH	82
$C_6H_5CH_2COOH$	76
$C_6H_5CH_2CH_2COOH$	100

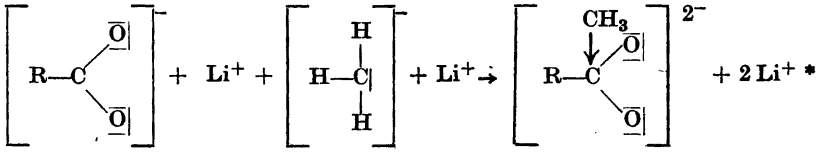
Acid	Yield in %
$C_6H_5-CH_2CH_2COOH$	100
$C_6H_5-CH=CH-COOH$	77
C_{17}-acid ***	> 90**

* According to Mannerskantz, C., private communication.

** According to Arens and van Dorp [6].

*** 

On the addition of methyllithium to the lithium salt of a carboxylic acid, the carbanion with its unshared electron pair adds to the sextet of the carboxy-



* Concerning the degree of polarity of the Li-O bond, little is known.

lic acid anion, with the formation of a new carbon-carbon bond, and the oxygen with one of its unshared electron pairs forms a bond with the lithium atom The dilithium salt of the dihydroxymethane thus obtained is hydrolysed by water to give the methyl ketone.

Of decisive importance for the addition, and thereby for the yield, is the formation of the carbonium ion of the carboxylic acid, which in its turn depends on the nature of the radical R- of the acid RCOOH [12].

In Table 2 some saturated acids have been investigated. It appears that the yield of methyl ketone is satisfactory when the radicals of the acids contain more than 5 or 6 carbon atoms, and that lower acids react incompletely or not at all. The cause of the increasing yield by lengthening the radical may be seen in the electron-repelling effect of the methyl group, an effect which is weakened by inserting methylene groups.

In Table 3 experiments with substituted acetic acids have been compiled. Here the influence of the phenyl and methoxyl groups may be specially noticed. In view of the electron-attracting properties of these groups, it might easily be thought the addition would take place. However, the yield with the methoxyacetic acid seems exceptionally low.

In Table 4 the weakening effect of the methylene group on the electron-attracting effect of the phenyl group may be seen. With hydrocinnamic acid, where a quantitative yield has been obtained, the synthesis has been repeated, and the yield was found to be correct. No tertiary alcohol could be found.

From Table 5 it appears that a certain stabilizing action is exerted by the double bond in the cinnamic acid, but no addition to double bond occurs, not even in such highly conjugated systems as in the C_{17}-acid.

In conclusion it may be said that the preparation of methyl ketones by the action of methyllithium on carboxylic acids generally gives excellent yields, and that no interference with double bonds occurs. As a rule acids having an electron-attracting group seem to give a higher yield of ketone than those containing a electron-repelling group.

EXPERIMENTAL

Preparation of methyllithium

The preparation of methyllithium was carried out in the apparatus shown in Fig. 1, a three-necked flask equipped with a dropping funnel (50 ml), a reflux condenser with a mercury trap, a Hershberg stirrer with a mercury seal and a gas inlet tube.

For the preparation of methyllithium three methods were tried, one using methyl chloride and two with different ratios of methyl iodide/lithium metal. When using methyl chloride [13] the yields varied between 20—50 %.

Using the more easily handled methyl iodide Gilman *et al.*[14] give two different ratios for the concentration of methyl iodide/lithium metal, 1.25/1.0 and 1.0/2.2; both said to yield similar results. However, in the authors experiments the latter ratio has been found to give higher yields, and has therefore been used in this work. (With the ratio 1.25/1.0 the yields were 75—80 %, and with the ratio 1.0/2.2 90—95 % yields were obtained.)

An example of a preparation using the latter ratio is given below:

0.35 mole (2.4 g) of lithium sand, prepared according to Perrine and Rapoport[15], was brought into the reaction vessel with 100 ml of ether *. The air in the apparatus was then expelled with nitrogen gas, previously dried over calcium chloride, phosphorus pentoxide and soda asbestos ("Ascarite"). The Hershberg stirrer was started, and the stream of nitrogen was slowed down. The speed of the gas flow could be noticed at the mercury trap, which also served to give a slight overpressure to exclude moisture, oxygen and carbon dioxide.

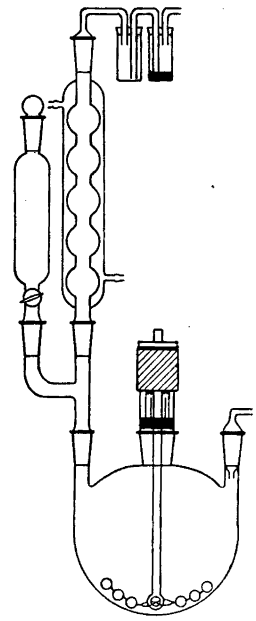


Fig. 1. Apparatus used for the preparation of methyllithium (description see text).

To the lithium metal was added 1 ml of methyl iodide, and the reaction was started by carefully heating on a water bath. When the reaction began the water bath was removed and another 100 ml of ether was added, and 0.16 mole (23 g) of methyl iodide diluted with 75 ml of ether was then introduced at such a rate as to cause gentle reflux. After all the methyl iodide had been added the mixture was refluxed on a water bath for 1 hour, after which time the stirrer was stopped and the solution allowed to cool. The unreacted lithium metal then rose to the surface and a greyish white precipitate of lithium iodide and metallic lithium settled at the bottom of the flask, leaving a weakly opalescent solution between. Samples of the solution were taken and after hydrolysis with water titrated with 0.1 *N* hydrochloric acid. Yield 0.15 mole of methyllithium (230 ml 0.67 *N* methyllithium solution; 96 %).

As there is no exact method of determining the concentration of methyllithium, only small amounts were prepared and were used in the coupling reaction immediately after the preparation.

Preparation of methyl ketones using the free acid

For the coupling reaction between methyllithium and carboxylic acids an apparatus was used consisting of a round-bottomed flask (250 or 100 ml) with a side tube for a gas inlet capillary and equipped with a reflux condenser protected by a sodium hydroxide drying tube. The reaction was carried out in a nitrogen atmosphere, the nitrogen gas being dried over calcium chloride, phosphorus pentoxide and soda asbestos. The coupling was carried out according to the following general scheme:

* The ether has always been dried with sodium wire.

0.03 mole of the acid dissolved in 50 ml of ether was brought into the reaction vessel, and the air was then expelled with a rapid stream of nitrogen. After 2—3 minutes the gas stream was slowed down, just to create sufficient bubbles for stirring the solution during the experiment.

When all air had been expelled 0.06—0.07 mole of an ethereal solution of methyllithium was added through the condenser. A vivid reaction took place, the ether refluxed, and a white precipitate was formed (lithium salt of the acid). After the addition of all the methyllithium the precipitate sometimes dissolved and a weakly opalescent solution was obtained. If necessary, the solution was then refluxed for 10—30 minutes to complete the reaction. After the solution had reached room temperature, water was slowly added. The excess of methyllithium was thus destroyed and lithium hydroxide was formed from the intermediate dilithium salt of the dihydroxymethane.

The alkaline water layer, which contained the lithium salt of unreacted acid, was removed in a separatory funnel, and the ethereal layer washed three times with half its volume of water. The ether solution was then dried over magnesium sulphate, filtered and the ether driven off, first at ordinary pressure and then at 8—10 torr. The ketone was purified if necessary, which however was seldom the case. If needed, derivatives were prepared for characterisation of the ketone.

The acids which have been investigated were coupled with methyllithium in a series of runs. The experiments and the isolation of the methyl ketones were carried out according to the general scheme above. Any deviations from this will be given in the case of the individual acids below.

Acetic acid. 5 g of glacial acetic acid was diluted with 100 ml of ether and the solution was dried over magnesium sulphate, and then filtered.

To 0.023 mole (14 ml of the above solution) of acetic acid was added 15 ml of ether and 0.050 mole of methyl lithium. The solution was boiled under reflux for 1 hour. After hydrolysis with water the ethereal solution was shaken five times, with 20 ml of water each time. The water solutions were mixed and after adjustment of pH to ~ 8, they were distilled. The first fraction, 1 ml, was collected. No aceton could be identified. Yield 0 %.

Propionic acid. 0.027 mole (2.0 g) of propionic acid diluted with 30 ml of ether was allowed to react with 0.058 mole of methyllithium. The isolation was carried out in analogy with that of acetic acid. A 1 ml fraction, which had a faint smell of 2-butanone, was collected. From the 1 ml fraction 20—30 mg of the 2,4-dinitrophenylhydrazone was precipitated. Yield ~ 1 %.

Caproic acid. 0.035 mole of caproic acid in 50 ml of ether was coupled with 0.075 mole of methyllithium. A white precipitate was first formed but dissolved in a few minutes. The solution was boiled under reflux for 5 minutes, after which time it was opalescent. The isolation of the ketone gave 3.3 g of a weakly yellow liquid; b.p. 149°; yield 83 % 2-heptanone.

Lauric acid. To 0.020 mole (4.0 g) of lauric acid dissolved in 30 ml of ether was added 0.044 mole of methyllithium. The solution was boiled under reflux for 1 hour, when the precipitate first formed partly dissolved. Isolation gave 2.5 g of 2-tridecanone; m.p. $\sim 25°$. 2,4-dinitrophenylhydrazone from alcohol m.p. 68° corr.; yield 63 %.

Cinnamic acid. To a solution of 0.0088 mole (1.3 g) of cinnamic acid in 50 ml of ether was added 0.018 mole of methyllithium. The yield of benzalacetone was 1.0 g. 2,4-dinitrophenylhydrazone from alcohol m.p. 226—227° corr.; yield 77 %.

Methoxyacetic acid. Methoxyacetonitrile was prepared [16] and hydrolysed. On purification by distillation *in vacuo* a fraction of constant boiling point was collected; b.p. 96°/13 torr; $n_D^{20} = 1.4168$.

0.043 mole of methyllithium was added to 0.020 mole (1.8 g) of methoxyacetic acid in 50 ml of ether. A weakly yellow precipitate was formed, which partly dissolved on boiling under reflux for 30 minutes. Isolation of the ketone yielded 0.27 g of methoxyacetone; yield 16 %.

Hydrocinnamic acid. 0.050 mole (7.5 g) of hydrocinnamic acid was dissolved in 50 ml of ether and was allowed to react with 0.12 mole of methyllithium. The solution was boiled under reflux for 1 hour. 7.5 g benzylacetone was obtained; yield 100 %. (Test for tertiary alcohol was negative.)

Benzoic acid. A solution of 0.027 mole (3.3 g) of benzoic acid in 50 ml of ether was coupled with 0.055 mole of methyllithium. On isolating the ketone, the water layer was extracted twice with 50 ml of ether and the ethereal solutions were treated according to the general scheme. 2.7 g of acetophenone was isolated; yield 82 %.

Phenylacetic acid. 0.026 mole (3.5 g) of phenylacetic acid was allowed to react with 0.055 mole of methyllithium. A white precipitate separated, which partly dissolved. Isolation gave 2.65 g of phenylacetone; yield 76 %.

Preparation of methyl ketone using the lithium salt

Cinnamic acid was dissolved in the calculated amount of 1.98 *N* lithium hydroxide in water. The solution was evaporated to dryness and the solid residue finely divided in a mortar. The lithium salt was then washed with ether and dried at 120° for 10 hours.

To a suspension of 0.013 mole of finely divided lithium cinnamate in 25 ml of ether was added 0.015 mole of an ethereal methyllithium solution. On refluxing for 2 hours the main part of the salt had reacted. Isolation (as above) of the ketone gave 1.3 g of benzalacetone; yield 69 %.

In another run the time of reflux was increased to 6 hours. In this case the yield was 79 %.

SUMMARY

1. Methyllithium has been prepared in a nitrogen atmosphere in yields between 90—95 %.

2. Comparison has been made between the lithium salt of an acid and the free acid in the preparation of methyl ketone. No differences in yield have been obtained.

3. The preparation of methyl ketones by the action of methyllithium on carboxylic acids has been shown to give generally excellent yield.

I wish to express my sincere gratitude to Professor Karl Myrbäck and Docent Nils Löfgren for invaluable aid and discussions.

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The Chlorine Oxidation of Glycosides

II.* Oxidation of Methyl Glycosides of Galactose, Mannose and Xylose

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This paper describes the continuation of work aimed at elucidating the mechanism of the oxidative degradation of cellulose by chlorine, a problem which is of considerable interest to the wood pulp and cellulose industry. Part I dealt with an investigation of the action of chlorine water on a simple model substance for cellulose, methyl β-glucoside; it was shown that this leads to the formation of D-gluconic acid, which in turn is further oxidised to 5-keto-gluconic acid. It was also shown that methyl α-glucoside undergoes a similar reaction, but at a very much slower rate than the β-isomeride.

This work has now been extended to include glycosides of two other hexoses, namely galactose and mannose, and of a pentose, namely xylose. The latter is of interest owing to the presence of xylans in the hemicellulose constituents of wood. Both the α- and β-isomerides of each glycoside have been studied. The oxidations were carried out by treating an approximately 0.5 *M* aqueous solution of the glycoside with a slow stream of chlorine gas at room temperature in the absence of light. The reactions were followed by measurement of the optical rotation, estimation of the hydrochloric acid and other acids formed, and determination of the consumption of periodic acid by the solution. At suitable time intervals portions of the reaction mixture were withdrawn for qualitative examination. This involved first analysis of the solution on the paper chromatogram by the standard methods, using in general ammoniacal silver nitrate or alkaline potassium permanganate to detect aldonic acids and their

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* Part I, *Acta Chem. Scand.* **5** (1951) 253.

** Billeruds AB Research Fellow, 1950–1951.

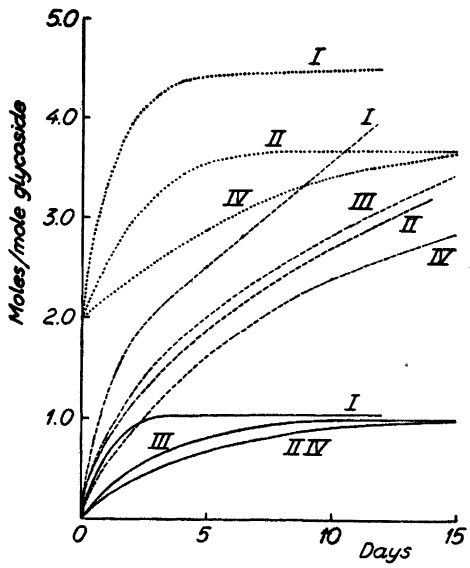


Fig. 1. Rate of chlorine oxidation of β-glycosides.

- - - - - - - - *Concentration of hydrochloric acid*
———— » » *other acids*
. . . . . *Consumption of periodic acid*
I. Galactoside *III. Mannoside*
II. Glucoside *IV. Xyloside*

lactones, and resorcinol in alcoholic hydrogen chloride for keto-acids. This analysis was followed whenever possible by isolation of the reaction products so indicated in the form of suitable derivatives.

Methyl β-galactoside was found to be oxidised by chlorine in a similar manner to the corresponding glucoside, but at a considerably greater rate than the latter (see Fig. 1). After the reaction had proceeded for only 3 days the amount of acids formed other than hydrochloric acid reached a value of 1 mole per mole of galactoside, and thereafter increased only very slightly. The amount of hydrochloric acid present in the solution at this stage was about 2 moles per mole, and the periodic acid consumption approximately 4 moles per mole of galactoside. These facts all indicated the formation of a monobasic organic acid, and by analogy with the results of the oxidation of methyl β-glucoside this was expected to be D-galactonic acid. Chromatographic analysis of the reaction mixture did in fact show the presence of galactonic acid and its lactone, and confirmation of this was obtained by isolation of the acid as the phenylhydrazide. After the chlorine oxidation had proceeded for 8 days, a white precipitate began to form in the solution and steadily increased in amount as the reaction was prolonged further. This substance was identified as the dicarboxylic acid mucic acid. The fact that titration still showed the solution to contain not appreciably more than 1 equivalent of other acids even at this stage can be ascribed to the low solubility of mucic acid, which was probably further diminished by the presence of hydrochloric

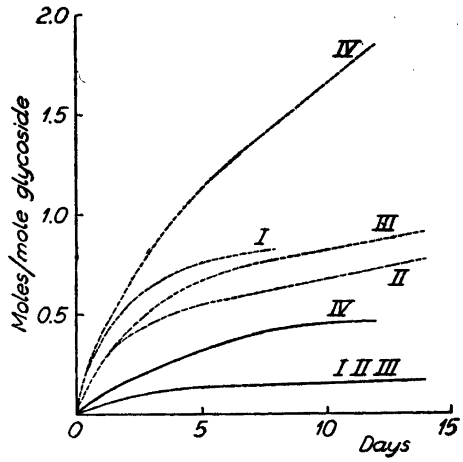


Fig. 2. Rate of chlorine oxidation of α-glycosides.

-------- Concentration of hydrochloric acid

———— » » other acids

I. Galactoside *III. Mannoside*
II. Glucoside *IV. Xyloside*

acid. It is of interest to note that the isomeric acid, saccharic acid, could not be detected as a product of the oxidation of methyl β-glucoside. No evidence of the formation of keto-acids on prolonged oxidation of methyl β-galactoside could be obtained.

An experiment was carried out to determine the stability of methyl β-galactoside to acid hydrolysis; after treatment with 2 *N* hydrochloric acid at room temperature for 21 days a 66 % recovery of the unchanged galactoside was obtained.

The action of chlorine water on methyl α-galactoside was found to be extremely slow, the reaction curves being closely similar to those for methyl α-glucoside (see Fig. 2). After treatment for 12 days, only 0.16 equivalent of other acids was formed and it was possible to recover the unchanged galactoside in 88 % yield.

Methyl β-mannoside showed a closely similar behaviour to the corresponding glucoside when treated with chlorine, the reaction rates for the two compounds differing only very slightly (Fig. 1). The results of the determination of periodic acid consumption in this case were extremely erratic, however, and of no interpretative value; no explanation of this fact is apparent. Chromatographic analysis of the reaction mixture after a period of 10 days showed the presence of mannonic acid and its lactone, which was confirmed by preparation of the phenylhydrazide. No evidence of keto-acids was obtained at this stage, but after the oxidation had continued for a total of 29 days the solution gave a faint positive reaction with the resorcinol reagent.

Methyl α-mannoside also behaved very similarly to the corresponding glucoside; after treatment with chlorine water for 14 days only 0.16 equivalent

of other acids was formed (Fig. 2) and it was possible to recover 72 % of the starting material unchanged.

Rather unexpectedly, the oxidation of methyl β-xyloside closely paralleled that of the β-glucoside (Fig. 1). After the reaction had proceeded for 10 days, chromatographic analysis of the solution indicated the presence of xylonic acid and its lactone. Isolation of the acid as the phenylhydrazide was not satisfactory owing to the difficulty of purifying the derivative, but good results were obtained by preparation of the benzimidazole according to the method of Moore and Link [1]. Only a small amount of keto-acids was detected in the reaction mixture, and this did not increase appreciably when the oxidation was prolonged for a total of 20 days.

With methyl α-xyloside, a profound difference was observed from the other α-glycosides investigated, since it reacted with chlorine water at an appreciable rate roughly intermediate between that for the other α-forms and that for the β-xyloside (Fig. 2). After a reaction time of 12 days, chromatography of the solution showed the presence of small amounts of xylonic acid and its lactone and also of keto-compounds. No reaction products were isolated in this case.

To summarise the results of these experiments and those of the preceding paper, it is clear that the oxidation of methyl glycosides by chlorine to form the corresponding aldonic acids may be regarded as a general reaction for the series. It may furthermore be said that in each pair of anomeric glycosides one form, in the examples studied the β-form, reacts comparatively rapidly while the other is only slowly oxidised. The corresponding anomeric forms of the free sugars show analogous differences in the rate at which they are oxidised by bromine [2]. The reason for these differences is at present obscure. It may, however, be noted that under conditions where a reversible transformation between α- and β-glycosides can take place, *e.g.* in solution in methanolic hydrogen chloride, the equilibrium mixture always contains a predominating proportion of one of the anomerides and a comparatively small proportion of the other. In the case of the sugars concerned in this investigation it is the α-glycoside which predominates. It is highly probable that the same configurational relationships which determine this difference in stability also cause the α-glycoside to be more stable than the β-isomeride towards chlorine oxidation.

EXPERIMENTAL

General procedure

The detailed procedure adopted in the oxidation experiments was the same as that described in Part I. The chromatographic analyses were also carried out in the same manner as in the earlier investigations.

Action of chlorine on the β-glycosides

Methyl β-galactoside. A 0.5 *M* solution of the galactoside was treated with chlorine for 3 days; a portion (10 ml) was then withdrawn, neutralised with silver carbonate, filtered, the filtrate saturated with H_2S, filtered again and the solution aerated until free of H_2S. A spot of this solution on filter paper reduced the alkaline $KMnO_4$ reagent but did not react with the resorcinol reagent. A paper chromatogram of the solution (suitably diluted) together with a reference solution of authentic galactonic acid, showed two pairs of spots with identical R_F values after development with alkaline $KMnO_4$, one pair corresponding to the free acid and the other (lower) pair to a lactone.

Galactonic acid was isolated as the phenylhydrazide as follows. The neutral reaction solution was heated with phenylhydrazine (1 ml) and glacial acetic acid (1.5 ml) on the boiling water-bath for 1 hour. After cooling, the crystalline phenylhydrazide was collected, washed with water and ether, and recrystallised from hot water. It had m.p. 194.5—195° * (decomp.), undepressed on admixture with authentic material. Yield, 0.14 g.

After the oxidation had proceeded for about 8 days a white precipitate began to separate from the solution, and when a further 4 days had elapsed this was collected and washed with alcohol. Yield, 0.11 g. After recrystallisation from hot water it had m.p. 212° (decomp.), undepressed on admixture with an authentic specimen of mucic acid.

Methyl β-mannoside. The starting material was prepared as a syrup by Zemplén deacetylation of the tetraacetate, and the exact concentration of the prepared aqueous solution determined by measurement of its optical rotation. After the chlorine oxidation had proceeded for 11 days, the solution contained approximately 3 equivalents of hydrochloric acid and 1 equivalent of other acids. A portion of the mixture was withdrawn at this stage, freed from hydrochloric acid in the usual way and analysed chromatographically. Mannonic acid was isolated from the neutral solution (10 ml) as the phenylhydrazide by the usual method; the crude derivative (0.45 g) was recrystallised from water, after which it had m.p. 212°, undepressed on admixture with authentic material.

Methyl β-xyloside. After oxidation of a 0.5 *M* solution of methyl β-xyloside for 9 days a small portion was removed, freed from hydrochloric acid and analysed on the chromatogram. The reaction was then allowed to proceed for a total of 20 days; the solution then contained 3.3 equivalents of hydrochloric acid, 1 equivalent of other acids, and the periodate consumption was 3.7 moles per mole of xyloside. From a portion (10 ml) of this solution xylonic acid was isolated as the benzimidazole derivative (0.06 g) by the method of Moore and Link [1]; the product had m.p. 225—226°, undepressed on admixture with an authentic specimen.

Action of chlorine on the α-glycosides

Methyl α-galactoside. After treatment with chlorine for 12 days, the observed optical rotation of a 0.5 *M* solution of methyl α-galactoside changed only from + 17.4° to + 16.5°. The solution (18.4 ml) was then aerated until free from chlorine and treated with Amberlite IR-4B ion exchange resin until neutral. Evaporation of the solution under reduced pressure gave a crystalline residue of unchanged starting material; yield, 1.57 g (88 % recovery). The substance contained a small amount of impurities which depressed the

* All melting-points uncorrected.

melting-point considerably and could not be removed by repeated recrystallisations. Acetylation of the product with acetic anhydride — pyridine, however, gave after one recrystallisation from alcohol pure tetraacetyl methyl α-galactoside, m.p. 85.5—87°.

Methyl α-mannoside. Treatment of a 0.5 *M* solution of methyl α-mannoside with chlorine for 14 days caused the optical rotation to change only from + 15.39° to + 14.91°. The solution (50 ml) was aerated and then freed from acids present by treatment with Amberlite IR-4B resin; evaporation under reduced pressure yielded a crystalline residue (4.4 g) which was recrystallised from alcohol. Unchanged methyl α-mannoside, m.p. 190—192°, was obtained; yield, 3.5 g (72 % recovery).

Methyl α-xyloside. The free glycoside was prepared by deacetylation of the triacetate, and the concentration of an aqueous solution of the product determined by measurement of the optical rotation. After oxidation for 12 days the solution was analysed chromatographically. No reaction products were isolated.

Action of hydrochloric acid on methyl β-galactoside

A 0.5 *M* solution of methyl β-galactoside in 2 *N* hydrochloric acid was kept at room temperature and the optical rotation periodically determined. After 21 days the α_D value, originally + 0.06°, had changed only to + 0.64°. The solution (7.5 ml) was neutralised with silver carbonate, filtered and evaporated to dryness under reduced pressure. The crystalline residue was recrystallised from alcohol, giving unchanged starting material, m.p. 174—176°; yield, 0.48 g (66 % recovery).

SUMMARY

The investigation of the action of chlorine water on the methyl glucosides has been extended to include the galactosides, mannosides and xylosides. A general reaction appears to exist whereby one of the anomeric forms of each glycoside, in the examples studied the β-form, is comparatively readily oxidised to the corresponding aldonic acid, while the other undergoes a similar oxidation at a very much slower rate.

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Short Communications

On the Linkage of the Amino Group in Heparin

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In a previous paper [1] it was shown that one of the sulfate groups in heparin must be linked to the amino group in a $-NHSO_2OH$ linkage giving rise to a substituted amidosulfuric or sulfamic acid. Since this linkage as shown by Berglund [2] who first described the properties of the amidosulfuric acids, is quite stable in alkaline solution whereas the acetyl component of the aminoacetyl group, $-NH-Ac$, is easily split off, heparin and chondroitin sulfuric acid should behave differently when submitted to treatment with alkali. This could also be shown to be the case.

Less than 10 per cent of the total nitrogen of heparin is liberated as ammonia during treatment with *N* NaOH for two hours at 100° C while about 55 per cent of the total nitrogen of the chondroitin sulfuric acid are liberated under similar conditions.

Experimental. 200 mg lots of heparin and of chondroitin sulfuric acid were dissolved in 100 ml *N* NaOH in a 250 ml three-necked round flask with a Liebig's cooler, a thermometer and a glass capillar and heated to boiling on a metal bath. Nitrogen was continuously bubbled through the flask with a speed of about 2 l per minute and the ammonia formed was taken up in Erlenmeyer flasks, supplied with 5 ml of a 2 per cent boric acid solution. The flasks were changed at different intervals. The amount of ammonia distilling over was estimated by titration with 0.01 *N* HCl using the ordinary Kjeldahl indicator.

Table 1. Analysis of the material used.

Preparation	Per cent of dry substance			
	N	S	Ash	Amino-N
I. Glucosamine HCl	6.31	—	0.49	—
II. Sodium salt of chondroitin sulfuric acid	2.19	6.69	22.94	0.02
III. Sodium salt of heparin	2.99	12.76	43.87	0.20
IV. Sodium salt of heparin partly hydrolyzed with 0.04 *N* HCl	2.93	10.29	18.20	1.27

The analyses of the preparations used are given in Table 1.

Preparation IV of the table was obtained in the following way.

1 g of sodium salt of heparin (preparation III) was dissolved in 40 ml 0.04 *N* HCl and hydrolyzed on a boiling water bath for 8 hours. After cooling the hydrolysate was dialyzed against running tap water overnight, concentrated *in vacuo* to a thick syrup, precipitated with 10 volumes of alcohol and treated with absolute alcohol and ether. Yield 600 mg.

The results are shown in Fig. 1. In the diagram the amount of ammonianitrogen liberated expressed in per cent of total nitrogen is plotted against time of hydrolysis in minutes.

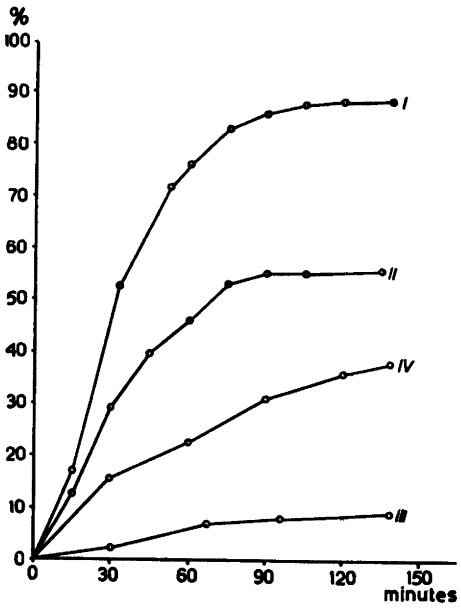


Fig. 1. Liberation of ammonia when heparin, chondroitin sulfuric acid and glucosamine are treated with N NaOH at 100° C. Distilled nitrogen in per cent of total nitrogen is plotted against time in minutes.
Curve I. Glucosamine hydrochloride.
Curve II. Sodium salt of chondroitin sulfuric acid.
Curve III. Sodium salt of heparin.
Curve IV. The same salt of heparin with about 60 per cent of the amino group free after 8 hours hydrolysis at 100° C with 0.04 N HCl.

In glucosamine (curve I) where the amino group is free, the formation of ammonia took place rapidly. After 135 minutes 90 per cent of the total nitrogen had distilled over as ammonia.

In chondroitin sulfuric acid (curve II) where the amino group is acetylated, the formation of ammonia was also remarkably rapid. After 135 minutes 55 per cent of the total nitrogen had distilled over.

In heparin (curve III) the formation of ammonia was very poor. After 135 minutes only about 10 per cent of the total nitrogen had distilled over as ammonia.

If the amino group in heparin, however, had been partly liberated by means of slightly acid hydrolysis with 0.04 *N* HCl the formation of ammonia was quite considerable (curve IV). After 135 minutes 38 per cent of the total nitrogen had distilled over as ammonia.

Consequently the linkage of the amino group in heparin is of another type than in the chondroitin sulfuric acid. The high resistance to alkaline hydrolysis may be considered as a further support for the assumption that the amino group in heparin is linked to sulfuric acid.

This investigation was aided by grants from the Wallenberg Foundation and the Vitrum Company, Stockholm.

1. Jorpes, E., Boström, H., and Mutt, V. *J. Biol. Chem.* **183** (1950) 607.
2. Berglund, E. *J. Chem. Soc.* **34** (1878) 643.

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Surface Balance Studies of the Multimolecular Layers of Isodextropimaric Acid

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The properties of isodextropimaric acid monolayers on water have been recently studied with a surface balance[1].* It was found that the surface pressure in the isodextropimaric acid monolayer began to increase on compression when the area per molecule was reduced to 54.1 Å^2, and the monolayer collapsed when the area was 42.6 Å^2 per molecule. After this an equilibrium surface pressure equal to 19.7 dynes per cm was established, the layer remained liquid.

* A paper read at the VII Nordiska kemistmötet in Helsingfors, August. 1950.

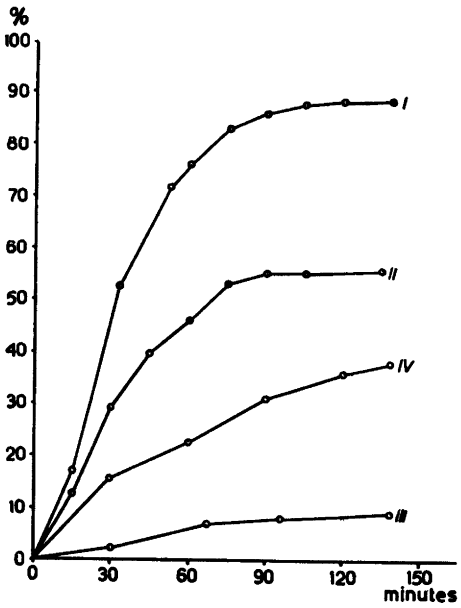


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When this liquid collapsed layer is further compressed until its area is approximately one half of the area at collapse, it is observed that the layer becomes solid within a narrow compression region. The surface balance curve for this phenomenon including also the monolayer compression range is of the form shown in Fig. 1. The monolayer collapses at the point marked a. In the range a—b the layer is liquid and the surface pressure remains practically constant. After the point b the surface pressure begins to increase and the layer becomes gradually more rigid in appearance. At c the layer has become solid. Further compression is accompanied by a pronounced pressure increase and it seems that the layer undergoes collapse at d, where the curve shows an inflexion. The surface pressure at d is about 37 dynes per cm.

The phenomenon is reversible between a and d. The curve shown in Fig. 1 was taken at a temperature of 20° C, but similar curves were obtained also at other temperatures between 5° and 40° C. The influence of the pH of the substrate was also investigated, whereupon it was found that the solidification of the isodextropimaric layer no longer takes place when the pH of the substrate exceeds 6. Between pH 4:2 and 5.5 the collapse pressure at d increases and attains its highest value, about 48 dynes per cm, at pH 5.5.

It is highly improbable that the molecules of isodextropimaric acid can be compressed in a monolayer to an area of only 21 Å^2 per molecule (at point b). Calculation of the size of the molecule indicates that the packing can not be much closer than that corresponding to the area, 42.6 Å^2 per molecule, observed at the collapse point a of the monolayer. If it is assumed that each molecule takes up in the region between a and b an area which is equal to the collapse area we come to the conclusion that at point b the layer corresponds to a complete di-

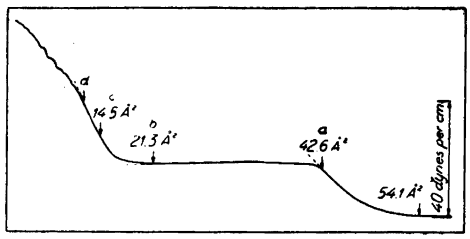


Fig. 1 The surface pressure-area curve for isodextropimaric acid on 0.001 M hydrochloric acid. Temperature 20° C.

layer. The area at point c, 14—15 Å^2, corresponds well with the value 14.2 Å^2 calculated for a complete trilayer. Since the transition of isodextropimaric layer from a liquid to a solid occurs gradually on compression between b and c, it appears that the formation of the third molecular layer brings about the solidification of the layer. The process taking place between b and d resembles to some extent that generally observed for monolayers on compression. There first occurs a closer packing of the molecules in the layer and then the layer collapses. It seems possible that a corresponding process takes place also in the isodextropimaric trilayer and that collapse of the trilayer occurs at d.

It was found that under the same conditions as used for isodextropimaric acid the monolayers of dextropimaric acid and the abietic acids (abietic, dehydroabietic, dihydroabietic and tetrahydroabietic acids) remain liquid on compression to very small areas per molecule and do not undergo a solidification process of the type described. Experiments conducted with isodextropimaric acid and dextropimaric acid mixtures and isodextropimaric and abietic acid mixtures showed that the solidification will not occur if the layer contains more than 20—30 per cent of the second component.

I am indebted to Professor Per Ekwall, the Head of the Institute for the opportunity

of using the facilities of the Institute and for discussions.

I am grateful to Dr. C. G. Harris of Hercules Powder Company, Wilmington, USA, for the sample of isodextropimaric acid received by the Institute.

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Identification of Galactose and Rhamnose in Birchwood and Aspenwood

K. J. BJÖRKQVIST and L. JÖRGENSEN

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In our last communication [1] we gave the carbohydrate composition of birch and spruce holocelluloses. In continuing our work on the hemicelluloses we have found the previous results to be incomplete, partly due to destruction of easily hydrolyzed sugars, when the standard quantitative saccharification method [2] is used. Better results have been obtained by employing a milder partial hydrolysis, followed by quantitative saccharification of the residue. The finely divided wood or holocellulose was heated for 15 hours at 95° C with dilute (0.5—1.0 *N*) sulfuric acid. The hydrolyzate was filtered through a sintered glass filter, and the residue was washed with water. The filtrate and washings were passed through a column of Amberlite IR 4 B anion-exchange resin, then concentrated *in vacuo* and examined by means of paper-partition chromatography. The residue from the partial hydrolysis was hydrolyzed by the standard method.

Applying this method we have found galactose to be present in birch — *Betula pubescens* and *Betula verrucosa* — and aspen — *Populus tremula* — to the extent of about one per cent. No variations in galactose content could be noticed in birch grown in widely different areas. Our results confirm the work of Gustafsson *et al.*[3], who also detected galactose in the same species, but found, however, that the amount varied. On the other hand, Jensen and Nuotio [4], using Schorger's method for determination of sugars, found galactose in *Betula pubescens* only.

Rhamnose was also detected in these partial hydrolyzates of the two birch species and the aspen. The two birches contained 0.3—0.4 per cent of rhamnose, and the aspen probably somewhat less. Recently Jones and Schoettler [5] identified L-rhamnose in aspenwood using a similar mild hydrolysis procedure. Up to now the presence of rhamnose as a constituent building unit in the wood hemicelluloses has not been proved [6]. Rhamnose has, however, long been known to be present in gums and mucilages of various origin [7,8]. Holocellulose contains the same amount of rhamnose as the original wood. It belongs to the easily hydrolyzable part of the hemicellulose.

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The Structure of Aqueous Solutions of Ammonium Nitrate

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An X-ray investigation of the structure of ammonium nitrate in aqueous solutions has been undertaken in connection with the structure determination of different aqueous solutions of nitric acid [1]. The experimental method is the same as in earlier works [2]. We have, however, reproduced the $\frac{\sigma(r)}{r}$-curves [3] instead of the $\sigma(r)$-curves.

Our two solutions contain 60 and 48 g ammonium nitrate per 100 g solution. This corresponds to about 3 and 5 water molecules per molecule ammonium nitrate, respectively. The intensity curves, together with theoretical backgrounds, are given in Fig. 1, and the corresponding $\frac{\sigma(r)}{r}$-curves in Fig. 2.

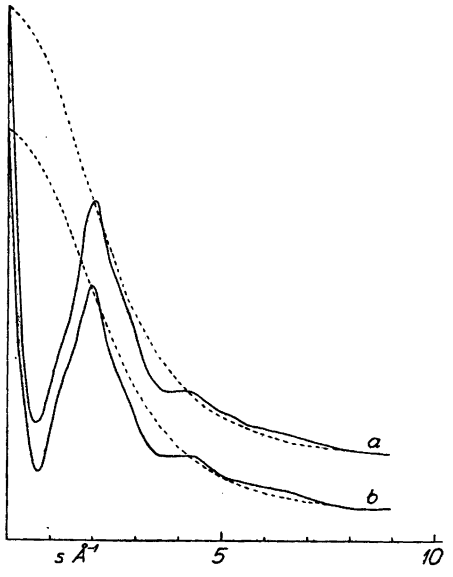


Fig. 1. Intensity curves (unbroken lines) and theorethical backgrounds (broken lines): a) 60 % solution, b) 48 % solution.

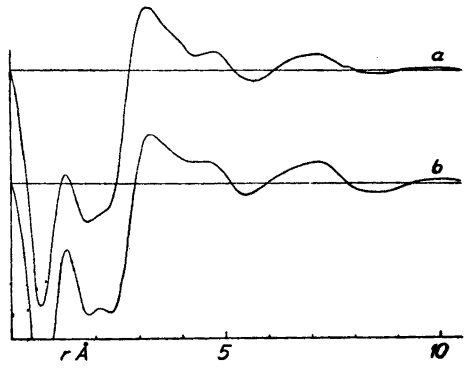


Fig. 2. $\frac{\sigma(r)}{r}$-functions corresponding to Fig. 1.

Both distribution functions have maxima at about 1.3 and 2.1 Å. In the nitrate ion, the average N—O distance is supposed to be 1.25 Å[4–7], leading to about 2.2 Å for the average O—O distance. Accounting for a displacement of the maxima due to mutual interaction, our results agree well with these values.

For greater values of r, our distribution curves are similar to the corresponding curve for liquid water [8]. We find maxima at about 3.2, 4.7 and 7.2 Å. In addition, we see an indication of a maximum at about 4 Å for the most concentrated of our solutions, and no distinct minimum at this point for the other one. The curve for water has maxima at about 3.0 and 4.5 Å, with a sharp minimum between them, and a third maximum at about 7.0 Å.

Because of the limited content of ammonium nitrate in aqueous solutions, we can only expect to find the structure relative to the structure of water. The shift in position of the maximum at 3.0 Å (water) to 3.2 Å (ammonium nitrate) is easily explained (see Fig. 3): An ammonium group, or a water molecule, (A) is connected with

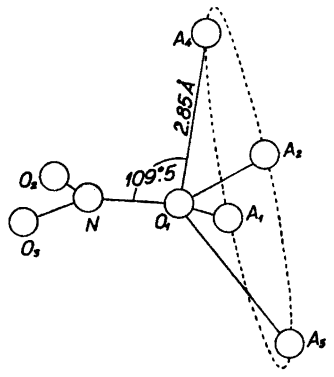


Fig. 3. Nitrate group ($NO_1O_2O_3$) with hydrogen bond to ammonium group or water molecule (A). The broken circle illustrates the rotation possibility of A.

an oxygen atom (O_1) of the nitrate ion by a hydrogen bond. With bond length 2.85 Å and bond angle 110°, the distance A—N in Fig. 3 is 3.47 Å. The distances A—O_2 and A—O_3 in Fig. 3 vary with the position of A on the broken circle in the figure. In positions A_1 or A_2 the hydrogen bond lies in the plane of the nitrate group and the distances are 3.2 and 4.7 Å. In positions A_3 or A_4 the hydrogen bond lies in a plane perpendicular to the nitrate group and both distances are about 4 Å. From the shape of our distribution curves in relation to that of water, we conclude that the latter structure is present, but evidently the presence of the first one cannot be eliminated. An equilibrium between the two and the intermediate positions, caused by a rotation around the bond N—O_1, is not unlikely.

The displacements of the maxima at 4.5 and 7.0 Å (water) to 4.7 and 7.2 Å in the present case are explained by analogeous reasoning.

However, we may mention that other possible arrangements may be partly responsible for the displacements of the maxima on the distribution curves: Water molecules packed around the nitrate group give distances from 3.2 Å upwards. The ammonium groups may have greater packing radii than the water molecules. And lastly, the hydrogen bonds may be longer in the present case than in the case of liquid water. Investigations of crystal structures indicate that this is possible for hydrogen bonds between nitrate and ammonium ions [7], but hardly between nitrate ions and water, where our value seems perhaps too great [5].

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X-ray Crystallographic Data on Tetrathionates

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This note completes a preliminary X-ray survey on the crystal structure of polythionic compounds. Earlier reports give unit cell and space group data on salts of pentathionic, selenopentathionic and telluropentathionic acids [1], divalent sulphur, selenium and tellurium methanethiosulphonates [2], selenium and diselenium disulphinates [3], and divalent selenium and tellurium benzene- and *p*-toluenethiosul-

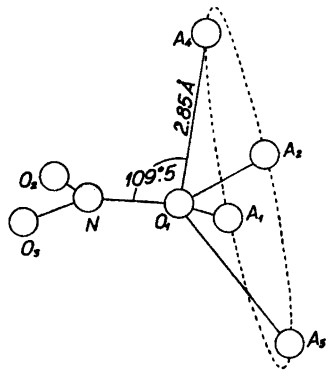


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X-ray Crystallographic Data on Tetrathionates

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This note completes a preliminary X-ray survey on the crystal structure of polythionic compounds. Earlier reports give unit cell and space group data on salts of pentathionic, selenopentathionic and telluropentathionic acids [1], divalent sulphur, selenium and tellurium methanethiosulphonates [2], selenium and diselenium disulphinates [3], and divalent selenium and tellurium benzene- and *p*-toluenethiosul-

phonates and the corresponding triselenium disulphinates [4]. The use of tellurium as a heavy atom has made possible a structure determination of the methanethiosulphonates [5].

Single-crystal oscillation and Weissenberg photographs were taken with CuK_α radiation, $\lambda = 1.54$ Å. The values recorded below for axial lengths are believed to be accurate to within 0.5 %. Density determinations were made by a flotation method.

Sodium tetrathionate dihydrate [6–8], $Na_2S_4O_6 \cdot 2H_2O$. Monoclinic crystals, submicroscopically twinned on (100) and having an orthorhombic appearance. $a = 14.46$ Å, $b = 6.37$ Å, $c = 5.44$ Å, $\beta = 105\frac{1}{2}°$. Two molecules per unit cell; density, calc. 2.11, found 2.10. Absent reflections, (hkl) when $h + k$ is odd. Of the three possible space groups, C_s^3-Cm and C_{2h}^3-C2/m would require that the tetrathionate ions lie in symmetry planes, while C_2^3-C2 requires that the tetrathionate ions possess a two fold axis of symmetry. The last requirement appears to be more probable. The salt, at first erroneously named 'disulphopersulphate' and listed as such by Groth [9], was described by Villiers [7] as orthorhombic.

Ammonium tetrathionate [8], $(NH_4)_2S_4O_6$. Monoclinic prismatic, $a = 5.56$ Å, $b = 11.86$ Å, $c = 14.10$ Å, $\beta = 106°$. Four molecules per unit cell; density, calc. 2.08, found 2.03. Absent reflections, ($h0l$) when l is odd, ($0k0$) when k is odd. Space group, $C_{2h}^5-P2_1/c$. The crystals occur as prisms, elongated along the a axis and bounded by {011}.

Rubidium tetrathionate [10], $Rb_2S_4O_6$. $a = 22.74$ Å, $b = 8.23$ Å, $c = 10.44$ Å, $\beta = 104°$. Eight molecules per unit cell; density, calc. 2.77, found 2.77. Absent reflections, (hkl) when $h + k$ is odd, ($h0l$) when h is odd or when l is odd. The habit of the crystals is monoclinic domatic, which indicates that the space group is C_s^4-Cc. The salt is isomorphous with potassium tetrathionate [11].

Barium tetrathionate dihydrate [6,12], $BaS_4O_6 \cdot 2H_2O$. Monoclinic prismatic, $a = 5.17$ Å, $b = 9.46$ Å, $c = 19.07$ Å, $\beta = 96°$. Four molecules per unit cell; density, calc. 2.84, found [12] 2.777. Absent reflections, ($h0l$) when l is odd, ($0k0$) when k is odd. Space group, $C_{2h}^5-P2_1/c$. The crystals were in most cases observed as prisms, elongated along the a axis.

Furthermore, data on a pentathionate are reported here.

Cesium pentathionate, $Cs_2S(S_2O_3)_2$, was prepared from the potassium salt by metathesis with sodium perchlorate and subsequently with cesium chloride, as described previously for rubidium pentathionate [1]. The cesium salt is apparently orthorhombic, with the unit cell dimensions, $a = 6.30$ Å, $b = 18.01$ Å, $c = 9.68$ Å. Four molecules per unit cell; density, calc. 3.16, found 3.12. The absent reflections are the same as for cesium selenopentathionate [1], and the two salts appear to be isomorphous.

Some of the salts will be the subject of further study.

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On the Determination of P^{31} and P^{32} in Biological Material

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Two years ago, an adaptation of the Martin and Doty method for the determination of phosphate [1], to be applied for metabolic studies with P^{32}, was published from this laboratory [2]. The method is based upon the fact that phosphomolybdic acid, formed in acid solution, can be quantitatively extracted by shaking the sample for 15 seconds with a mixture of equal parts of isobutanol and benzene. P^{31} and P^{32} can then be determined in aliquots of the organic phase.

Since that time, the method has been used for routine analyses in this laboratory in a very large number of experiments [3–5]. It has proved to be especially advantageous as it is not necessary to deproteinise samples with relatively low protein content, *e.g.* samples from enzymatic tests or incubation mixtures. Recently, in a series of experiments [5] on mitochondria suspensions, it was possible to follow the phosphate uptake during incubation, due to the fact that the phosphate determination can be performed very rapidly.

On the other hand, deproteinizing agents commonly used for tissue extractions, *i.e.* trichloroacetic and perchloric acids interfere with the determination. In the case of TCA, however, this difficulty may be easily overcome by removing the TCA from the extract with ether. Traces of ether can then be expelled from the extract by aeration.

In a recent publication [6], Ennor and Rosenberg raised the objection that organic phosphate compounds which are highly labile to acid may be hydrolyzed during the time of contact between the aqueous and organic phases. The accompanying table shows the influence on the resulting phosphate value of the time of contact between the two layers. The table clearly indicates that in spite of the fact that, as pointed out earlier [2], creatine phosphate undergoes rather rapid hydrolysis in the presence of molybdate and sulfuric acid, no significant part of the liberated orthophosphate migrates spontaneously into the organic layer in a standing sample. In general, there is no reason why the upper layer should not be removed immediately after separation, but even if a short time interval elapses between separation and removal no appreciable error ensues. In the case of creatine phosphate (*cf.* table), this error amounts to less than one per cent after one minute, and about four per cent after half an hour at room temperature. In the case of adenosine polyphosphates, it is evidently still lower.

Another objection raised against the present method by the authors mentioned above is the etching effect of the $SnCl_2$-sulfuric acid-ethanol mixture on glassware. During the past two years, several thousands of such samples have been handled in this laboratory in glass vessels of the most varying types, *i.e.* Pyrex (American, British and French), Jena glass, standard Klett tubings and Beckman Corex cells, and no signs of etching whatsoever have been noticed.

Table 1.

Time min.	Series A	Series B
	Ext. 730 mμ	
0	.158	.158
1	.167	.315
5	.178	.730
10	.187	1.050
15	.205	1.075
20	.215	1.150
30	.193	1.250

3 ml-samples of a solution of creatine phosphate (Ca-salt) were shaken, in the presence of 0.5 ml of 10 N H_2SO_4 and 0.5 ml of 10 % $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, with 5 ml of isobutanol-benzene mixture (1 : 1), for 15 sec. Temp. 22° C. — Series A: shaken immediately after the addition of the creatine phosphate, whereafter the samples were allowed to stand for various periods of time. — Series B: shaken after the addition of creatine phosphate, and again after various periods of time. — At the end of each period, a 1.0 ml sample of the organic layer was removed for the phosphate determination. Colour intensity was measured in a Beckman spectrophotometer at 730 mμ.

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Aminosäuren in Meeresalgen

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Die Aminosäuren in Meeresalgen sind früher schon Gegenstand von Untersuchungen von Mazur und Clarke [1,2] sowie von Lugg [3] gewesen. Haas [4] wie auch Dekker, Stone und Fruton [5] haben gewisse in Algen vorkommende Peptide isoliert. Roche und Lafon [6] haben das Vorkommen von Dijodtyrosin in *Laminaria flexicaulis* und *Laminaria saccharina* nachgewiesen. Die Aminosäurezusammensetzung in gewissen einzelligen Grünalgen ist von Fowden [7,8] und Stepka, Benson und Calwin [9] ermittelt worden.

In der vorliegenden Arbeit sind drei Braunalgen *(Phaeophyceae) — Sphacelaria arctica (Sphacelariales), Laminaria saccharina (Laminariales) Fucus vesiculosus (Fucales)* — hinsichtlich ihres Aminosäureinhaltes qualitativ untersucht worden. Die letztgenannte Alge ist in der Nähe von Fiskebäckskil im Skagerrak im Monat November 1951 geerntet worden, die zwei übrigen im Oktober desselben Jahres ausserhalb Simpnäs in der Ostsee. Zur Identifizierung der Aminosäuren haben wir ein- und zweidimensionale Papierchromatographie angewandt [10].

Zur Verminderung des Salzgehaltes wurden die Algenproben vor der Hydrolyse ca. 12 Stunden mit Wasser bei gewöhnlicher Temperatur behandelt; auch dieses Wasser wurde nach Eindampfen auf kleineres Volumen chromatographiert. Die entsalzten Algenproben — ungefähr 1 Gramm — wurden dann mit 6 *N* HCl oder mit 14 % $Ba(OH)_2$ 24 Stunden lang bei 100° C hydrolysiert. Die Salzsäure wurde vor dem Chromatographieren durch Eindampfen im Vakuum entfernt, das Bariumhydroxyd mit Schwefelsäure gefällt. Eine qualitative Veränderung der Aminosäurezusammensetzung konnte nicht festgestellt werden, wenn die Hydrolyse auf 48 Stunden ausgedehnt wurde. Das Entsalzen der Algenproben führte zu einem besser aufgeteilten Chromatogramm, bewirkte aber keine qualitative Veränderung. Das zur Entsalzung verwendete Wasser enthielt keine Aminosäuren, die nicht auch im Hydrolysat nachweisbar waren.

Bei zweidimensionaler Chromatographie wurde in der einen Richtung mit Wasser gesättigtes Phenol, in der anderen ein Gemisch von Pyridin (35), Isoamylalkohol (35) und Wasser (30) verwendet; für eindimensionale Chromatographie bedienten wir uns eines Gemisches von *n*-Butanol (50), Essigsäure (15) und Wasser (50). Die angewandte Papiersorte war Whatman Nr. 1, die Probemengen entsprachen 10 bis 50 γ Aminostickstoff. Die zweidimensio-

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nalen Chromatogramme wurden nach Trocknen in einem 40° C warmen Luftstrom mit einer 0,5 %-igen Lösung von Ninhydrin in *n*-Butanol besprüht und hierauf wieder im Trockenschrank bei einer Temperatur zwischen 80 und 100° C 5 Min. lang getrocknet. Die eindimensionalen Chromatogramme wurden teils mit Ninhydrin, teils mit für verschiedene Aminosäuren spezifischen Reagentien entwickelt. Um Tryptophan nachzuweisen, wurden Chromatogramme von Alkalihydrolysaten mit einer 1 %-igen Lösung von *p*-Dimethylaminobenzaldehyd in mit Wasser gesättigtem *n*-Butanol, das 5 % HCl enthielt, besprüht. Für Prolin und Oxyprolin wurde eine Lösung, bestehend aus 0,2 % Isatin in einem Gemisch vom 96 Vol. % *n*-Butanol und 4 Vol. % Essigsäure, angewandt. Histidin und Tyrosin wurden mit Hilfe von diazotiertem *p*-Bromanilin (1 %) in Sodalösung; die schwefelhaltigen Aminosäuren Cystin und Methionin durch Aufsprühen einer Mischung von KJ und H_2PtCl_6 [11] auf die eindimensionalen Chromatogramme, nachgewiesen. Das Vorkommen von Arginin wurde u.a. mit α-Naphtol in einer alkalischen Lösung vom Natriumhypochlorit nach Sakaguchi festgestellt. Um Leucin und Isoleucin zu trennen, führten wir die Chromatographie auf gepuffertem Whatman-Papier aus [12].

Kein qualitativer Unterschied in der Aminosäurezusammensetzung der drei Algen konnte beobachtet werden, obgleich diese innerhalb der Division der Braunalgen drei wohldifferenzierte Ordnungen repräsentieren. Die chromatographisch identifizierten Säuren waren folgende:

Glycin, α-Alanin, Valin, Leucin, Isoleucin, Phenylalanin, Tyrosin, Serin, Threonin, Cystin, Methionin, Arginin, Lysin, Prolin, Histidin, Asparaginsäure und Glutaminsäure.

Sämtliche Aminosäuren konnten sowohl mit Hilfe von Ninhydrin als auch mit den betreffenden Spezialreagentien nachgewiesen werden. Innerhalb des Dreiecks, welches auf einem zweidimensionalen, mit Ninhydrin entwickelten Chromatogramm von den Flecken von α-Alanin, Tyrosin und Valin gebildet wird, wurde ein schwacher, aber gut sichtbarer blauer Fleck beobachtet, welcher wahrscheinlich von Methioninsulfon herrührt.

Oxyprolin konnte in den genannten Algen weder mit Ninhydrin noch mit Isatin nachgewiesen werden. Diese Tatsache steht nicht in Übereinstimmung mit den Befunden von Mazur und Clarke. Nach den Angaben in der Literatur scheint Oxyprolin in Landpflanzen sehr selten vorzukommen. Mazur und Clarke sind der Meinung, dass in *Laminaria* (ohne nähere Bestimmung) [1] Lysin und Methionin fehlen, und dass *Fucus furcatus* [2] kein Arginin enthält. Nach unseren Ergebnissen enthalten Braunalgen aus verschiedenen Ordnungen sowohl Arginin, Methionin als auch Lysin, und es ist deshalb wenig wahrscheinlich, dass Algen von ein und derselben Ordnung sich in Hinsicht auf diese Aminosäuren unterscheiden. Tryptophan, welches nach Mazur und Clarke in *Laminaria* [1] und *Fucus furcatus* [2] sowie in der Grünalge *Ulva lactuca* (vgl. auch Lugg [3]) vorkommt, konnten wir in den Alkalihydrolysaten der von uns untersuchten Algen nicht finden. Dagegen beobachteten wir in *Laminaria saccharina* und *Fucus vesiculosus* andere Substanzen, welche eine stark blaue Farbe mit *p*-Dimetylaminobenzaldehyd gaben, deren R_F-Werte aber mit dem des Tryptophans nicht übereinstimmten und die keine Ninhydrinreaktion gaben. Diese Substanzen traten innerhalb desselben Bereichs auf, in welchen sich die braunen Algenfarbstoffe befinden und man könnte deshalb vielleicht vermuten, dass sie mit den Farbstoffen in Zusammenhang stehen, Dijodtyrosin, das eine schwache Ninhydrinreaktion gibt [13], konnte ebenfalls nicht nachgewiesen werden.

Es soll darauf hingewiesen werden, dass der Proteingehalt in Meeresalgen sehr stark mit der Jahreszeit schwankt, für *Laminaria*

saccharina von ca. 4 % im Spätsommer bis zu 14 % im Winter. Nach Haas [4] tritt das von ihm aus der Rotalge *Griffithsia flosculosa* isolierte Peptid nur im Winter und Frühling auf. Man kann deshalb annehmen, dass auch die Aminosäurezusammensetzung bei Meeresalgen mit der Jahreszeit variiert. Die Abweichungen unserer Resultate von denen anderer Verfasser können möglicherweise auf solchen jahreszeitlichen Schwankungen beruhen.

Die Aminosäuren der Braunalgen scheinen mit den gewöhnlich in Landpflanzen gefundenen übereinzustimmen. Von den essentiellen Aminosäuren konnte Tryptophan von uns nicht nachgewiesen werden.

Die Verfasser sind Herrn Dr. Mats Waern, Växtbiologiska Institutionen, Uppsala, herzlichen Dank schuldig für die Hilfe bei der Einsammlung der Algen und deren botanischer Klassifizierung, sowie Herrn Ing. Lars Ljungdahl, A.B. Stockholms Bryggerier, für die Kontrolle der Chromatogramme.

Professor Dr. Harry Lundin, Vorstand des Instituts, zeigte förderndes Interesse an vorliegender Arbeit und gab uns Gelegenheit zu fruchtbaren Diskussionen.

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Determination of Ethylenediamine in Cupriethylenediamine Solutions

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During the last ten years the cupriethylenediamine (CED) reagent has found extended use as a solvent for cellulose, especially in connection with measurements of its degree of polymerisation. It is therefore not surprising that many attempts have been made to work out methods for the analysis of this reagent

All the procedures described in the literature [1–7] are based on the same principle. Copper is first determined by an iodometric titration, and the CED solution is then titrated with acid to an end point at about pH 3.0 – 3.5. The ethylendiamine content is then related to the difference in the number of equivalents of the titrants used in the two titrations. However, it appears that no published method is free from more or less serious objections.

If the cupric ions are precipitated with hydrogen sulfide prior to the acid titration the equivalence point will be much more easily indicated, and as one equivalent of hydroxyl ions is used up for each equivalent of copper present in the original solution the acid consumption will be a direct measure of the amount of ethylenediamine present. When analytically pure cupric hydroxide is used in the preparation of the reagent this new method appears to be much superior to the previous ones. However, the commercial cupric hydroxide now sometimes employed often contains appreciable amounts of anions of strong

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saccharina von ca. 4 % im Spätsommer bis zu 14 % im Winter. Nach Haas [4] tritt das von ihm aus der Rotalge *Griffithsia flosculosa* isolierte Peptid nur im Winter und Frühling auf. Man kann deshalb annehmen, dass auch die Aminosäurezusammensetzung bei Meeresalgen mit der Jahreszeit variiert. Die Abweichungen unserer Resultate von denen anderer Verfasser können möglicherweise auf solchen jahreszeitlichen Schwankungen beruhen.

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During the last ten years the cupriethylenediamine (CED) reagent has found extended use as a solvent for cellulose, especially in connection with measurements of its degree of polymerisation. It is therefore not surprising that many attempts have been made to work out methods for the analysis of this reagent

All the procedures described in the literature [1–7] are based on the same principle. Copper is first determined by an iodometric titration, and the CED solution is then titrated with acid to an end point at about pH 3.0 – 3.5. The ethylendiamine content is then related to the difference in the number of equivalents of the titrants used in the two titrations. However, it appears that no published method is free from more or less serious objections.

If the cupric ions are precipitated with hydrogen sulfide prior to the acid titration the equivalence point will be much more easily indicated, and as one equivalent of hydroxyl ions is used up for each equivalent of copper present in the original solution the acid consumption will be a direct measure of the amount of ethylenediamine present. When analytically pure cupric hydroxide is used in the preparation of the reagent this new method appears to be much superior to the previous ones. However, the commercial cupric hydroxide now sometimes employed often contains appreciable amounts of anions of strong

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Table 1. Results of distillation experiments for the determination of ethylenediamine in CED-solution. The receiver was charged with 50 ml of 1 N HCl.

Treatment	Ml of 0.946 *N* NaOH consumed	Difference from blank
1. Blank (50 ml of acid)	50.50	
2. 25 ml of ethylenediamine solution added to 50 ml of acid	16.00	34.50
3a. 25 ml of ethylenediamine solution made alkaline with NaOH and distilled without xylene	33.00	34.55
3b. Residue from 3a. distilled with xylene	33.45	
4. 25 ml of ethylenediamine solution made alkaline with NaOH and distilled with xylene	16.02	34.48
5. 25 ml of ethylenediamine solution + equivalent amount of $CuCl_2$ solution precipitated with H_2S, filtered, and distilled with xylene	15.90	34.60
	16.02	34.48

acids which cause erroneous results, and due to the presence of alkali metal ions the method can not be much improved with the aid of anionexchangers (generally somewhat low results were obtained).

To overcome these difficulties the present authors have precipitated the cupric ions with hydrogen sulfide and recovered the ethylenediamine by distillation of the alkalized solution, xylene being added to facilitate the process. In this way the ethylenediamine passes over quantitatively in a reasonably short time — 30—60 minutes. Some data demonstrating the accuracy of the new process are shown in Table 1.

Recommended procedure. Twentyfive ml of CED reagent (1 *M* in $CuEn_2(OH)_2$) is pipetted into a 250 ml Erlenmeyer flask. Hydrochloric or sulfuric acid is added in slight excess as judged from the colour, and hydrogen sulfide is passed into the solution for 5—10 minutes. The precipitate is filtered off and washed with water, the filtrate and washings being collected in a 250 ml volumetric flask. The flask is filled to the mark with distilled water, and the contents are thoroughly mixed by shaking. One hundred ml of the mixture is pipetted into a Kjeldahl distillation apparatus, the receiver of which is charged with 50 ml of 1 *N* hydrochloric or sulfuric acid. An excess of strong soda lye is added to the test solution and most of it is distilled into the receiver. Then about 100 ml of pure, redistilled xylene is added to the residue in the distillation flask, and most of the xylene is distilled over. The contents of the receiver are then back-titrated either potentiometrically or using bromophenol blue as indicator.

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The Higher Normal Chain DL-β-Hydroxy Acids. Synthesis and Investigation of the Crystal Behaviour of Seventeen Homologous Acids with 8 to 24 Carbon Atoms

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The only practical synthetic route to normal chain β-hydroxy acids previously available has been the Reformatzky reaction, *i.e.* the condensation between aldehydes and bromoacetic ester in the presence of zinc [cf. 1]

$$RCHO \xrightarrow[Zn]{BrCH_2COOC_2H_5} RCHCH_2COOC_2H_5 \quad (OH) \xrightarrow{\text{hydrolysis}} RCHCH_2COOH \quad (OH)$$

Various side reactions occur, however, and the yields of pure β-hydroxy acids are not very satisfactory. Thaler and Geist [2], and Adickes and Andresen [3], state that the yields of pure products in their experiments amounted to 10–12 % of the theoretical.

The development of satisfactory methods for the preparation of long normal chain β-keto esters [4,5] opens up a new and convenient synthetic route, *viz.* hydrogenation of the β-keto esters to the corresponding β-hydroxy esters and hydrolysis of the latter.

Several methods of hydrogenation of the β-keto esters were tried in the course of the work to be described. The use of copper-chromium oxide catalyst has been reported by Adkins [6] to be satisfactory for the hydrogenation of ethyl acetoacetate. This method was tried, but did not prove satisfactory in the case of higher β-keto esters. Some side reactions evidently occurred, involving chain-splitting of the β-hydroxy ester produced. Mozingo and Folkers [7], by using relatively large amounts of this catalyst and temperatures of 150–180° C,

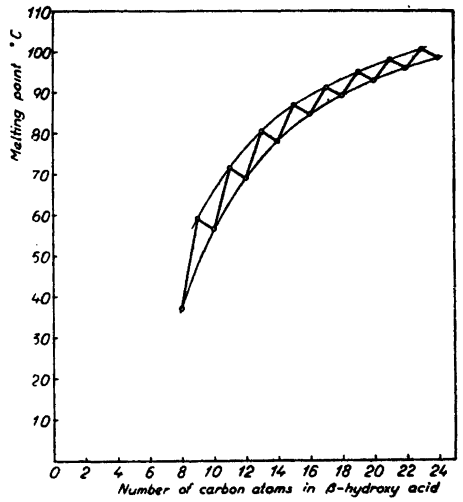


Fig. 1.

have converted ethyl acetoacetate into 1,3-butylene glycol in moderate (30 %) yield. For the preparation of β-hydroxy acids this represents an undesirable side reaction. Raney nickel was therefore tried, and good results were obtained when carrying out the reaction at a temperature of 85—210° C and a pressure of 70—140 kg per sq.cm.

After hydrolysis, the pure β-hydroxy acids were obtained in yields of 50—70 % of the theoretical, calculated on the β-keto esters used. The complete synthesis, starting from the carboxylic acid with two carbon atoms less than the final compound, gives an overall yield of 30—40 % of the theoretical. The following steps are involved:

$$\mathrm{RCOOH} \xrightarrow{\mathrm{SOCl_2}} \mathrm{RCOCl} + \mathrm{CH_3COCH_2COOCH_3} \xrightarrow[\mathrm{C_6H_6}]{\mathrm{Na}}$$

$$\mathrm{RCOCHCOOCH_3}\ (\mathrm{CO}\ \mathrm{CH_3}) \xrightarrow[\mathrm{CH_3OH}]{\mathrm{NaOCH_3}} \mathrm{RCOCH_2COOCH_3} \xrightarrow[\mathrm{H_2\,(70—140\,atm)}]{\text{Raney nickel}}$$

$$\mathrm{RCHCH_2COOCH_3}\ (\mathrm{OH}) \xrightarrow{\text{hydrolysis}} \mathrm{RCHCH_2COOH}\ (\mathrm{OH})$$

In this way the complete series of DL-β-hydroxy acids, from the C_8 (β-hydroxyoctanoic acid) up to and including the C_{24} member (β-hydroxytetracosanoic acid), was synthesized. As indicated in Table 1, several of these acids

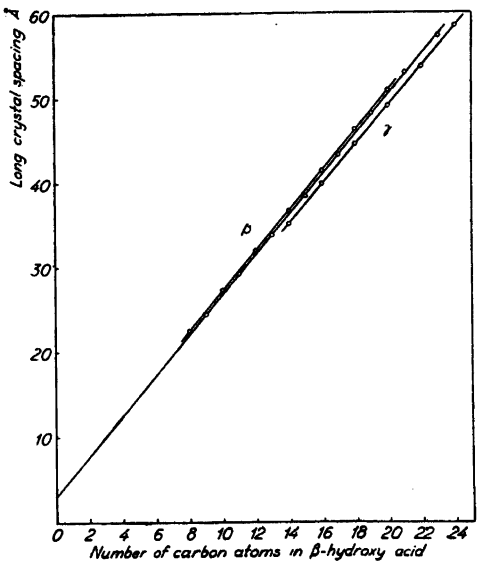


Fig. 2.

have been described previously on one or more occasions in the literature, but in several cases the melting point data given by different authors do not agree. The melting points of the acids now prepared, when plotted against the number of carbon atoms in the chain, appear to fall on two smooth curves (Fig. 1), that of the odd-numbered acids lying above that of the acids containing an even number of carbon atoms. As pointed out by Adickes and Andresen[3], this is the opposite to the manner in which the melting points of the normal chain acids alternate.

The crystal behaviour of the acids has also been studied, in order to detect the existence of polymorphism. The crystal spacings corresponding to the $00l$, and some of the stronger $hk0$ reflections, have been calculated from diffraction patterns of the powder type. The results are given in Fig. 2 and Table 3.

The long crystal spacings of the odd-numbered acids from C_9 to C_{23}, when plotted against the number of carbon atoms in the molecules, all fall on the same straight line (Fig. 2). The general appearance of the side spacing pattern from the β-hydroxy acids at room temperature remains the same throughout the series, but there are minor changes in the numerical values of the spacings. The same crystal structure is found for acids crystallized from light petroleum or benzene (low boiling light petroleum is the only solvent found from which it has been possible to crystallize the lowest members), as for specimens crystallized from the melt.

Table 1.

Systematic name	Yield %	m. p. °C	m.p. from literature °C
DL-3-hydroxyoctanoic acid	34	37	38—38.5 (3)
DL-3-hydroxynonanoic acid	37	58.8—59.0	60 (3)
DL-3-hydroxydecanoic acid	54	56.4—56.6	56—56.5 (3); 53—54 (2)
DL-3-hydroxyhendecanoic acid	59	71.2—71.4	73—73.5 (3); 73 (8)
DL-3-hydroxydodecanoic acid	48	68.8—69.1	70—70.5 (3); 78—79 (8)
DL-3-hydroxytridecanoic acid	66	80.4—80.6	81 (8)
DL-3-hydroxytetradecanoic acid	72	77.8—77.9	72—73 (2)
DL-3-hydroxypentadecanoic acid	63	86.7—87.0	
DL-3-hydroxyhexadecanoic acid	61	84.5—84.7	83—83.5 (9)
DL-3-hydroxyheptadecanoic acid	50	91.1—91.3	
DL-3-hydroxyoctadecanoic acid	55	89.4—89.6	89 (10); 90 (11)
DL-3-hydroxynonadecanoic acid	69	95.1—95.3	
DL-3-hydroxyeicosanoic acid	47	93.2—93.4 [a]	
DL-3-hydroxyheneicosanoic acid	81	97.9—98.1	
DL-3-hydroxydocosanoic acid	69	95.8—96.0	
DL-3 hydroxytricosanoic acid	68	100.6—100.9	
DL-3-hydroxytetracosanoic acid	67	98.7—98.9	

[a] After resolidification most of the sample in the tube melted between 91.9—92.5, but a small residue did not melt until 93.6°.

The long spacings of specimens of the even-numbered acids C_8—C_{20} obtained from solvents when plotted fall on a straight line (*cf.* Fig. 2), which lies slightly above the line for the odd-numbered acids. The side spacing patterns are very similar to those of the latter acids. However, melted specimens of the C_{14}, C_{16}, C_{18}, and C_{20} acids show, in addition to the form mentioned (marked β in Fig. 2), the presence of a second crystalline form with slightly shorter long spacings. This form (γ in Fig. 2) is the only one found for specimens of the C_{22} and C_{24} acids crystallized from light petroleum and benzene. Previously-melted specimens of the two acids just mentioned also show the spacings of the γ modification, but melted specimens of all C_{18} to C_{24} acids show a few very weak lines, which appear to correspond to a third modification of a still shorter long spacing. (ζ)

As indicated in Table 1, specimens obtained by crystallization from solvents, and on solidification of the molten acid, show the same m.p., with the exception of the C_{20} acid. It was mentioned above that previously-melted specimens of several of the higher even-numbered acids showed the presence of two crystalline forms at room temperature, and it was therefore of interest to follow the appearance of the diffraction pattern right up to the melting point.

Table 2.

Number of carbon atoms in β-keto ester	Hydrogenation: Keto-ester g	Solvent	ml	Raney nickel ml	Hydrogen pressure kg/sq. cm. (initially)	Reaction-temp °C	Reaction-time hrs	Hydrolysis: Potassium hydroxide g	Ethanol ml	Time of reflux hrs	Crystallization: Solvent	Number of carbon atoms in β-hydroxy acid	Yield g
9	0.9	methanol	12	1	140	210	5	5		5.5	light petroleum, b.p. 35—60°	8	0.26
10	1	»	10	1	115	145	2.5	5	22	12	petroleum	9	0.35
11	2	ethanol	6	1	120	120	4.5	10	50	6	»	10[a]	1.02
12	1	methanol	10	1	135	210	4	5	80	9	»	11	0.55
13	0.74	»	10	1	115	145	2.5	5	25	4.5	»	12	0.34
14	2	»	8	1	70	85	4	10	50	5	»	13	1.25
15	2	» + dioxan	10 + 5	1	100	100	2.5	10	50	8	»	14	1.37
16	2	methanol	20	1	100	100	5.5	10	50	6	»	15	1.20
17	2	» + dioxan	6 + 10	1	100	100	7	10	50	5	»	16	1.17
18	1	methanol	15	1	120	145	2.5	5	25	12	»	17	0.47
19	2	»	15	1	120	145	1	10	50	5	»	18	1.05
20	0.6	»	10	1	110	145	4	5	25	7	»	19	0.40
21	1	»	10	1	140	190	5	5	35	5	»	20	0.45
22	0.5	»	10	1	110	145	3.5	5	25	6	»	21	0.39
23	0.6	»	12	1	115	145	2.5	5	25	5	»	22	0.40
24	0.7	»	11	1	135	145	5.5	3	30[b]	4	benzene	23	0.50
25	0.4	»	9	1	140	210	3	5	25	5.5	light petroleum, b.p. 80—100°	24	0.26

(For compounds 15, 17, 21, 22: » + dioxan = methanol + dioxan; » = methanol. For compounds 15, 17, 21, 22: » = b.p. 60—80°; for compound 22: » = b.p. 80—100°.)

a) Prepared by S. Ställberg-Stenhagen, *cf.* ref. 13.
b) Methanol.

With an automatically recording X-ray camera [12], this was done for the whole series of acids crystallized from the melt, and for the C_9, C_{14}, C_{20}, C_{22} and C_{24} acids crystallized from solvents. In all cases where two crystalline forms co-existed at room temperature, the form with the shorter long spacing gradually disappeared within a certain temperature interval, transforming into the one with the longer spacing.

Except for the C_{20} acid obtained from the melt, the longer (β) form persisted up to the melting point, as could be expected from the melting point data. The C_{20} acid crystallized from light petroleum behaved in the same manner, but a previously-melted specimen, first transformed into the γ form, and then gradually back into a shorter form, evidently not identical with the ζ-form.

Table 3. X-ray diffraction data of β-hydroxy acids.

Number of carbon atoms in β-hydroxy acid	Long crystal spacing in Å S β	S γ	M γ	M ζ	Side spacings in Å S	Side spacings in Å M
8	22.4				*4.64*, 4.42, *3.88*, *3.53*	*4.64*, 4.40, 4.07, *3.87*, *3.53*
9	24.4				*4.68*, 4.31, *3.90*, *3.44*	*4.68*, 4.32, *3.90*, *3.46*
10	27.5				*4.64*, 4.45, *3.89*, *3.55*	*4.64*, 4.45, *3.89*, *3.55*
11	29.1				*4.68*, 4.38, *3.83*	*4.68*, 4.38, *3.83*
12	32.0				*4.64*, 4.47, *3.89*, *3.55*	4.64, 4.47, *4.08*, *3.93*, 3.58
13	33.8				*4.67*, 4.41, *3.90*	*4.66*, 4.41, *3.90*
14	36.8		35.2		*4.63*, 4.49, 4.05, *3.89*, *3.55*	*4.63*, 4.46, *4.08*, *3.90*
15	38.5				*4.66*, 4.44, *3.89*	*4.66*, 4.45, *3.89*
16	41.4		40.0		*4.66*, 4.49, *3.88*	4.40, 4.26, *4.09*, *3.89*
17	43.2				*4.64*, 4.46, *3.89*	*4.66*, 4.45, *3.88*
18	46.5	44.6		36.0	*4.11*, *3.89*	*4.11*, *3.87*
19	48.2			38.3	*4.61*, 4.47, *3.88*	*4.66*, 4.45, *3.86*
20	50.9	49.0		40.0	4.62, 4.49, *4.09*, *3.86*	*4.10*, *3.83*
21	53.0			42.0	*4.63*, *3.89*, 3.56	*4.66*, 4.46, *3.86*
22		53.6		43.9	*4.10*, *3.85*	*4.12*, *3.85*
23	57.3			45.6	*4.64*, 4.51, *3.88*	*4.66*, 4.47, *3.85*
24		58.4		47.5	*4.10*, *3.82*	*4.12*, *3.82*

S = Specimens crystallized from solvents.
M = Specimens crystallized from the melt.
Strong side spacings in italics.

Crystal spacings are given in Table 3. Fig. 3 shows the relative intensities of the low order 00*l* reflections, estimated visually from diffraction patterns of acids crystallized from solvents.

EXPERIMENTAL *

As all the β-hydroxy acids were produced by a series of more or less identical procedures, a detailed description is given only of the synthesis of the C_8 and the C_{23} acids. Other experimental data are collected in Table 2 and the analyses are given in Table 4.

DL-*3-Hydroxyoctanoic acid.* Methyl 3-ketooctanoate (0.9 g), dry methanol (12 ml), and a suspension of finely-dispersed Raney nickel in ethanol (1 ml) were placed in a high-pressure vessel, which was then filled with hydrogen from an ordinary commercial hydrogen container, giving a pressure of 140 kg/sq.cm. The temperature was raised up to

* For reference to melting point determination and X-ray technique employed, see Ställberg-Stenhagen, S., and Stenhagen, E. *J. Biol. Chem.* **173** (1948) 389.

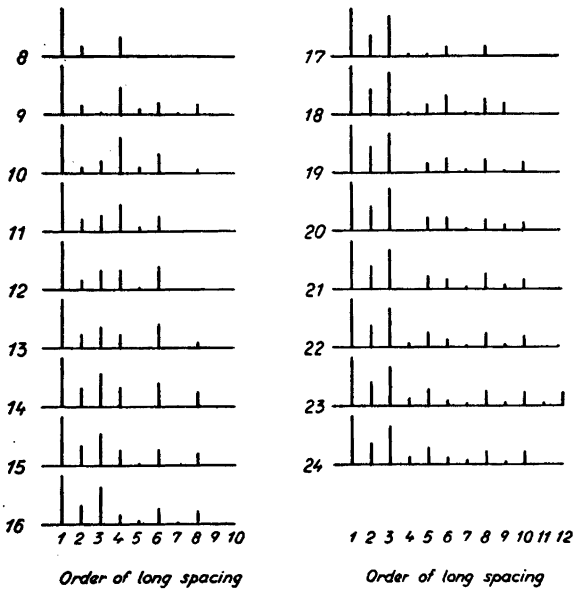


Fig. 3.

210° C, and the hydrogenation was allowed to continue at this temperature for about 5 hours under continuous shaking of the mixture.

When the contents had cooled down to room temperature, the catalyst was filtered off, and the solvents evaporated. Potassium hydroxide (5 g) in ethanol (25 ml) was added, and the material refluxed for 9 hours. After concentrated hydrochloric acid (8 ml) had been added, the solution was extracted with ether, and the ether washed with dilute hydrochloric acid, and several times with water. The ether layer was dried over sodium sulphate, and the solvent evaporated. The residue was dissolved in light petroleum (b.p. 35—60° C), from which 0.23 g of pure acid crystallized when placed in a refrigerator.

DL-*3-hydroxytricosanoic acid.* Methyl 3-ketotricosanoate ** (0.7 g) in dry methanol (11 ml) was hydrogenated with Raney nickel (1 ml) in the same manner as described above. The initial hydrogen pressure was 135 kg/sq.cm and the reaction temperature was kept constant at 145° C for 5.5 hours. The apparatus was allowed to cool down before being disconnected, whereafter the contents were extracted with ether and filtered. The ether was evaporated, and the remainder hydrolysed with potassium hydroxide (3 g) in methanol (30 ml) and water (3 ml) under reflux for 4 hours. Excess hydrochloric acid was added and the mixture heated and then extracted with warm benzene. The benzene layer was washed twice with water and partly distilled off. 0.5 g of pure hydroxy acid crystallized from the benzene.

All hydroxy acids, except C_8, C_{21} and C_{23}, were treated with active charcoal in ethanol solution before crystallization.

** This β-keto ester was prepared by Miss G. Ställberg, who also carried out the greater part of the synthesis of C_{23} hydroxy acid.

Table 4. Analysis of DL-*β-hydroxy acids. Per cent composition.*

Formula	C		H	
	Calcd.	Found	Calcd.	Found
$C_8H_{16}O_3$	59.97	59.90	10.07	10.01
$C_9H_{18}O_3$	62.04	61.95	10.41	10.39
$C_{10}H_{20}O_3$	63.80	63.90	10.71	10.81
$C_{11}H_{22}O_3$	65.31	65.52	10.96	10.92
$C_{12}H_{24}O_3$	66.63	66.53	11.18	11.21
$C_{13}H_{26}O_3$	67.78	67.80	11.38	11.27
$C_{14}H_{28}O_3$	68.81	68.74	11.55	11.50
$C_{15}H_{30}O_3$	69.72	69.95	11.70	11.69
$C_{16}H_{32}O_3$	70.54	70.43	11.84	11.87
$C_{17}H_{34}O_3$	71.28	71.09	11.97	11.95
$C_{18}H_{36}O_3$	71.95	71.90	12.08	12.12
$C_{19}H_{38}O_3$	72.56	72.53	12.18	12.22
$C_{20}H_{40}O_3$	73.12	73.11	12.27	12.22
$C_{21}H_{42}O_3$	73.63	73.62	12.36	12.41
$C_{22}H_{44}O_3$	74.10	73.80	12.44	12.39
$C_{23}H_{46}O_3$	74.54	74.55	12.51	12.51
$C_{24}H_{48}O_3$	74.94	75.14	12.58	12.59

Throughout the whole series the crystals appear as very thin rhombic plates.

The acids with an even number of carbon atoms are much more soluble in light petroleum than are the odd-numbered ones. The solubility decreases with increasing length of the carbon chain. The DL-3-hydroxytricosanoic acid was not soluble enough to allow crystallization even from high-boiling light petroleum.

SUMMARY

A series of normal chain DL-β-hydroxy acids, containing from 8 to 24 carbon atoms, has been synthesized by high pressure hydrogenation of the corresponding methyl β-keto esters, using Raney nickel as a catalyst. The crystal spacings have been determined by the X-ray powder technique, and the crystal polymorphism has been studied at various temperatures by using an automatically recording X-ray camera.

The yield of β-hydroxy acid obtained from the corresponding β-keto ester ranges from 50 to 70 % of the theoretical.

I am indebted to Dr. E. Stenhagen for advice, and to Mrs. S. Ställberg-Stenhagen for placing the β-keto esters used at my disposal.

The analyses were carried out by Mr. W. Kirsten in the Microanalytical Laboratory of this Institute.

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Studies on the Chemistry of Lichens

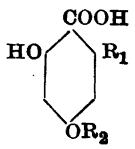
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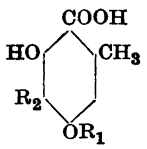
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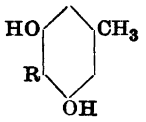
Organisk-kemiska Institutionen, Kungl. Tekniska Högskolan, Stockholm, Sweden

It has long been known that lichens contain a large variety of acidic constituents, generally termed "lichen acids", the structures of many of which have been elucidated. (For recent reviews see refs. 1 and 2.) The lichen acids may be divided into a number of groups, one of which comprises substances derived from orsellinic acid (I) and its homologues in various states of oxidation. The acids may occur (a) as simple depsides, (b) as depsidones which contain a diphenyl ether structure and are probably formed by a dehydrogenation process, or (c) as more condensed systems, obviously formed by a dehydrogenative coupling of monocyclic acids, of which the heterocyclic diphenyl derivative didymic acid [3] is an example.

Several attempts have been made to base a botanical classification of the lichens on the occurrence of different types of lichen acids, and Asahina has developed micro-chemical methods which have proved of value [4, 5] for this type of work. However these techniques which involve the use of a polarising microscope have not yet been widely used by botanists, and it appeared that an alternative method of identifying the lichen acids in small amounts of material would be desirable. This communication describes paper chromatographic methods which have been developed for the separation and characterisation of ten (I–X) of the 25 phenolic carboxylic acids known to be components of depsides.







I	$R_1 = CH_3$, $R_2 = H$	VI	$R_1 = H$, $R_2 = CH_3$	XI	$R = CH_3$
II	$R_1 = R_2 = CH_3$	VII	$R_1 = R_2 = CH_3$	XII	$R = CHO$
III	$R_1 = n\text{-}C_3H_7$, $R_2 = H$	VIII	$R_1 = H$, $R_2 = CHO$		
IV	$R_1 = n\text{-}C_3H_7$, $R_2 = CH_3$	IX	$R_1 = CH_3$, $R_2 = CHO$		
V	$R_1 = n\text{-}C_7H_{15}$, $R_2 = H$	X	$R_1 = CH_3$, $R_2 = COOH$		

In the solvent systems generally used for acids, *e. g.* benzene — acetic acid — water [6], the substances above often have R_F values which are too high to give satisfactory separations. However, the method of Goodall and Levi [7, 8], using buffer-impregnated paper, proved to be very useful, and by suitable choice of pH and solvents the degree of separation could be varied within wide limits. Trailing of the spots, often a disturbing factor in the case of acids, was greatly reduced.

The positions of the spots may be determined in several ways, the simplest being by examination of the chromatograms in ultra-violet light. The anions of all the phenolic carboxylic acids investigated, except those containing free aldehyde groupings, show a bluish-white fluorescence — the free acids on the other hand exhibit only a very faint fluorescence[Cf. 9]. Some of the acids related to atranol (XII) give a yellowish or green fluorescence on paper buffered at pH $>$ 8 and this behaviour appears to be characteristic for some *o*-hydroxy-aldehydes *e. g.* salicylaldehyde and *o*-vanillin although atranol itself does not fluoresce.

Many of the phenolic acids may be detected by spraying the chromatograms with a solution of bis-diazotised benzidine [10] and the colours produced with this reagent give some indication of the structure of the compounds involved. Ammoniacal silver nitrate solution [9] is reduced by 2,4-dihydroxy acids on heating at 80—100° for a short time; acids having only one free hydroxyl group do not react. *p*-Phenylene diamine [4] gives intense yellow spots with all the acids derived from atranol — a reaction which appears to be given by all *o*- and *p*-hydroxy aldehydes. Finally, the same acids have a faint yellow colour when run on paper buffered to pH $>$ 7 and this may be of diagnostic value, but the colour is often too faint to be observed.

The colour reactions given by the various acids are summarized in Table 1.

Some examples of the choice of conditions for separating phenolic carboxylic acids are given in Table 2. With the solvent system *n*-butanol — water, greatly improved separations may be achieved by increasing the pH of the phosphate buffer used for impregnating the strips. The most lipophilic acids move at nearly identical speed even at pH 11.7, but butanol — benzene — water (1 : 1 : 1), at the same pH, gives a god separation, although the hydrophilic acids then move very slowly. In this case a lower pH gives better results. On unbuffered paper benzene — water was found to give some separation of the lipophilic acids but extensive trailing was observed.

The use of papers buffered to high pH values causes partial oxidative destruction of the most sensitive acids (I, VI and VIII), yielding products with very low R_F values. In this respect β-orcinol-carboxylic acid (VI) is interesting since a spot of this compound on paper buffered to pH 11.7 after one day in a

Table 1. Colour reactions.

Phenolic carboxylic acids	Fluorescence in ultra-violet	Colour with		
		Benzidine reagent	*p*-Phenylene-diamine	$AgNO_3 - NH_3$
Orsellinic acid (I)	Bluish-violet	Wine-red	—	+
Everninic acid (II)	» »	Brown	—	—
Divaric acid (III)	» »	Wine red	—	+
Divaricatinic acid (IV)	» »	Reddish brown	—	—
Sphaerophorol-carboxylic acid (V)	» »	Wine red	—	+
β-Orcinol-carboxylic acid (VI)	» »	Buff-coloured	—	+
Rhizoninic acid (VII)	» » (intense)	» *	—	—
Haematommic acid (VIII)	Yellowish-green	Orange brown	+	+
Haematommic acid monomethyl ether ** (IX)	Green (intense)	Yellowish * brown	+	— ?
Orcinol-dicarboxylic acid monomethyl ether ** (X)	Bluish violet (intense)	» *	—	—

* Distinct colours are obtained only on paper buffered to pH > 8.

** Not isolated in the pure state but obtained as hydrolysis products of depsides of known composition.

Table 2. Approximative R_F values at 20° on buffered paper.

Substance	*n*-Butanol — water				*n*-Butanol — benzene water (1 : 1 : 1)		
	pH				pH		
	4.5	6.8	9.0	11.7	6.8	9.0	11.7
Orsellinic acid (I)	0.76	0.58	0.55	0.19	0.28	0.08	0.02
Everninic acid (II)	0.90	0.78	0.74	0.73	0.57	0.30	0.27
Divaric acid (III)	0.94	0.87	0.84	0.63	0.74	0.44	0.15
Divaricatinic acid (IV)	0.95	0.94	0.93	0.92	0.87	0.75	0.74
Sphaerophorol-carboxylic acid (V)	0.96	—	0.97	0.97	0.95	0.90	0.87
β-Orcinol-carboxylic acid (VI)	0.91	0.68	0.60	0.39	0.46	0.16	0.05
Rhizoninic acid (VII)	0.90	0.85	0.83	0.82	0.75	0.51	0.50
Haematommic acid (VIII)	0.96	0.86	0.83	0.32	0.58	0.38	0.05
Haematommic acid monomethyl ether (IX)	—	0.93	—	0.62	—	—	—
Orcinol-dicarboxylic acid monomethyl ether (X)	0.66	0.17	0.04	0.0	0.0	0.0	0.0

moist atmosphere turns dark violet. With butanol — water as solvent the violet coloured substance divides into two spots with $R_F \sim 0$ and 0.04 respectively, and spraying with the benzidine reagent or examination in ultraviolet light reveals a third spot, deriving from the unchanged acid ($R_F \sim 0.39$).

Table 2 shows some relationships between structure and R_F values. As would be expected, the 2,4-dihydroxy acids and their 4-methyl ethers show increasing R_F values with increasing number of methyl and methylene groups. Increasing pH decreases the R_F values of the dihydroxy acids to a far greater extent than those of the methoxylated ones. Thus divaric acid (III) runs faster than everninic acid (II) at pH 9.0, whereas at pH 11.7 the sequence is reversed.

At a low pH (< 10) haematommic acid (VIII) runs much faster than the related β-orcinol-carboxylic acid (VI), and the strongly lipophilic character of the former must be due to intramolecular hydrogen bonding between the hydroxyl groups and the aldehyde group. When the pH is increased to 11.7, however, the R_F values of the two acids lie close together. At this high pH the ionization of the hydroxyl groups is a factor of importance. The fact that the methyl ester of haematommic acid can be titrated with phenolphtalein as indicator shows that one phenolic hydroxyl group in the free acid is markedly acidic.

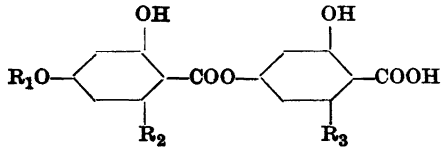
For comparison, the chromatographic behaviour of the analogous compounds atranol (XII) and β-orcinol (XI) was studied. As shown in Table 3, influence of hydrogen bonding is again evident.

Before the components of a depside can be investigated chromatographically, the depside must first be hydrolyzed and this step may present difficulties because of the ease with which some of the acids so formed, especially 2,4-dihydroxy acids, undergo decarboxylation even under the mildest conditions. However the resultant phenols can be readily distinguished from the phenolic acids, by virtue of their lack of fluorescence in ultra-violet light and their very high R_F values in the solvent systems described. In 0.1 *M* trisodium phosphate solutions in the absence of air (to prevent oxidative degradation) lecanoric acid (XIII), evernic acid (XIV), divaricatic acid (XV) and sphaerophorin (XVI) are completely hydrolyzed in 1—2 days at room temperature and give the expected products, but the more resistant compounds squamatic acid (XVIII), baeomycessic acid (XVII) and atranorin (XIX) which belong to the β-orcinol group of depsides require 6—7 days at room temperature or 1—2 days at 40°. In the case of atranorin, subsequent chromatography revealed only one distinct fluorescent spot due to β-orcinol carboxylic acid (VI), and haematommic acid (VIII) was destroyed.

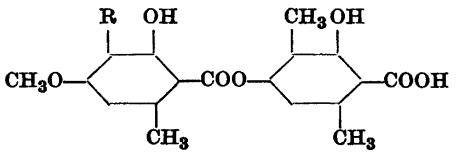
Cold concentrated sulphuric acid has been used to hydrolyse certain sterically hindered esters [11, 12] but does not appear to have been employed in the

Table 3. R_F values on buffered paper. Solvent system: benzene-water.

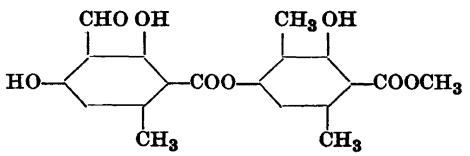
	pH		
	6.8	9.0	11.7
β-Orcinol (XI)	0.25	0.25	0.25
Atranol (XII)	0.65	0.40	0.02



XIII $R_1 = H$, $R_2 = R_3 = CH_3$
XIV $R_1 = R_2 = R_3 = CH_3$
XV $R_1 = CH_3$, $R_2 = R_3 = n\text{-}C_3H_7$
XVI $R_1 = R_2 = CH_3$, $R_3 = n\text{-}C_7H_{15}$



XVII R = CHO
XVIII R = COOH



XIX

case of depsides. Atranorin and squamatic acid have been succesfully hydrolyzed with this reagent, and although the hydrolysis is generally not complete the unchanged water-insoluble depside is easily removed and the expected products of hydrolysis have been identified.

Satisfactory hydrolysis may be obtained if the depside in acetone solution is applied to a paper strip buffered to a high pH and the paper is allowed to stand for at least 6—7 hours before irrigation with the solvent. Hydrolysis is seldom complete, but spots corresponding to the original depside and its fission products may be obtained in this way.

For the complete analysis of an unknown mixture of depside components it is usually necessary to run chromatograms with several different solvent systems and with papers buffered to different pH values. Final identification, of course, must always involve comparison with authentic reference substances. Even if unknown acids are encountered, considerations of the colours they produce with the various reagents together with their R_F values often enable valuable deductions regarding their structures to be made.

The methods described here may prove of value for the identification of the natural depsides as well as for the elucidation of their structures, and as is shown by the chromatograms obtained from β-orcinol (XI) and atranol (XII) (Table 3), the use of buffered paper is advantageous for the separation and identification of the phenols and hydroxyaldehydes which are often obtained as degradation products of lichen acids.

EXPERIMENTAL

Isolation of lichen acids

The following depsides have been isolated: Lecanoric acid (XIII) from *Parmelia fuliginosa* (Fr.) Nyl., Evernic acid (XIV) from *Evernia prunastri* (L.) Ach., Divaricatic acid (XV) from *Haematomma ventosum* (L.) Mass., Sphaerophorin (XVI) from *Sphaerophorus fragilis* (L.) Pers., Atranorin (XIX) from *Stereocaulon*-sp., Squamatic acid (XVIII) and Squamatic acid + Baeomycessic acid (XVII)-mixture from *Thamnolia vermicularis f. taurica* Shaer.

The isolation of the depsides was carried out mainly according to the methods referred to by Asahina[1]. Purification was often facilitated by filtering the crude ether or acetone extracts through a short column of alumina. The purity of the depsides was established by melting point and eqvivalent weight determinations.

Preparation of depside components

The acids I—V were obtained from the depsides by methanolysis and subsequent hydrolysis as described by Asahina[13].

The methyl esters of β-orcinol-carboxylic acid (VI) and haematommic acid (VIII) were prepared from atranorin by the method of Robertson *et al.*[14]. Hydrolysis of the esters with concentrated sulphuric acid by Sonn's method[12] yielded the free acids.

Rhizoninic acid (VII) was prepared from β-orcinol-carboxylic acid methyl ester by methylation with diazomethane and hydrolysis with sulphuric acid[12].

The acids I—VIII were all purified by treatment with alumina and recrystallisation. As these acids often melt with decomposition their purity was established by paper chromatography.

β-Orcinol (XI) and atranol (XII) were prepared from atranorin by the method of Pfau[15] and purified by vacuum sublimation.

Hydrolysis of depsides on a microscale

The depside or depside mixture (2 mg) was dissolved in 0.1 M trisodium phosphate (1 ml) and the mixture was set aside in the absence of air. All the depsides which have been investigated were completely hydrolyzed after 6—8 days at 20° or 1—2 days at 40°. The solutions were then acidified with 2 N sulphuric acid and extracted with ether (0.3 ml).

Atranorin (XIX) (20 mg) was dissolved in 100 % sulphuric acid (0.4 ml). After 10 minutes at room temperature the yellow solution was poured with stirring into ice-water

(30 ml). The resulting solution was filtered to remove a white, amorphous precipitate, the filtrate was extracted with ether (2 × 5 ml) and the ether solution was evaporated to a volume of 1 ml.

Squamatic acid (XVIII) was hydrolyzed in the same manner.

Paper chromatography

Filter paper (Whatman No. 1), cut into strips 54 × 9 cm, was soaked in buffer solution, pressed between sheets of thick filter paper to remove excess liquid and dried in the air. The buffers used were as follows: 0.1 *M* NaH_2PO_4 (pH 4.5), 0.1 *M* NaH_2PO_4 (1 volume), + 0.1 *M* Na_2HPO_4 (1 volume) (pH 6.8), 0.1 *M* Na_2HPO_4 (pH 9.0), 0.1 *M* Na_3PO_4 (pH 11.7).

Freshly prepared acetone solutions of the pure acids, 1 % with respect to each component, were used. The crude fission products of the depsides, obtained as described above, were used without further purification.

A thick sheet of filter paper, soaked with the solvent system used, was suspended in the tank to facilitate the vaporization of the solvents and the strips were generally allowed to remain in the tank for 6—7 hours before irrigation with the solvent. This time was found to be sufficient to prevent trailing and to produce comparatively sharp spots, while oxidative destruction of the alkali-sensitive acids even on papers buffered to high pH was negligible. When the time was reduced to 2 hours, some trailing was noticed. The chromatograms were run for 7—8 hours in the case of butanol — water and about 3 hours in the case of butanol — benzene — water (1 : 1 : 1), the solvent advancing 27—30 cm.

The air-dried chromatograms were examined in ultra-violet light, the positions of the spots being marked and the intensities of the fluorescence being noted. A small fluorescent band at the solvent front interferred with the detection of compounds having very high R_F values, but in other cases spots containing 1 μg of substance were easily detectable. The strips were then dipped in bisdiazotised benzidine solution [10], set aside if necessary for 1—2 minutes to allow the coloured spots to appear, then washed with water and allowed to dry in the air. With papers buffered to high pH values the spots appeared immediately and the reaction is extremely sensitive, a distinct spot being obtained with, for example, 0.01 ml of 0.01 % orsellinic acid solution; at a pH of about 7 the spots may take some minutes to develop and the sensitivity is somewhat less. Some acids do not give any appreciable colouration under neutral or acidic conditions so that the paper may be sprayed with an alkaline buffer prior to treatment with the benzidine reagent.

Freshly prepared 0.5 % *p*-phenylene diamine in acetone solution is about as sensitive as the benzidine reagent, but the yellow spots formed are not stable, and after some days the paper becomes dark in colour. Ammoniacal silver nitrate solution was also used as a spraying reagent but appeared to be less sensitive than the other reagents.

Generally, the R_F value of an acid was reproducible to within 5—10 %, when different strips were compared. On one single strip, however, the variation of the R_F values was considerably less, not exceeding 2 %.

SUMMARY

Methods are described for the separation and identification by paper chromatography of a number of phenolic acids, known to be components of depsides. The development of a method of hydrolysing certain depsides by means of concentrated sulphuric acid is recorded.

The author is indepted to fil. lic. T. E. Hasselrot, Naturhistoriska Riksmuseet, Stockholm, for the identification of some of the lichens and to AB Ferrosan, Malmö, for financial support.

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Gradient Elution Analysis

I. A General Treatment

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In a previous paper [1] the limitations of the two most common procedures for chromatographic analysis, displacement analysis and elution analysis, have been discussed. It is the purpose of this paper to introduce a technique, gradient elution analysis, which has only received scant mention in the literature [2,3] and has not been treated in any way theoretically.

It is usual to point out that elution analysis can not be successfully applied to those substances which have curved isotherms because of the spreading of the zones and the high losses of material on the column ***. To some extent this has been overcome by applying a succession of different eluting solutions [4,5] but in such a case there are a number of grave complications. Firstly many substances are eluted partially by one eluting agent and then appear again in the front of the next eluting solution. It appears that the zones are spread widely and all the material is not elutable from the column in the first eluting agent but is picked up again by the second (Fig. 1). Clearly this can result in poor separation (see later). Again the position in which the substance travels in the eluting agent is dependent upon (a) the column length (b) the amount of the material, (c) the step length. Thus the recognition of an individual compound is not predictable from the position in which it travels in the eluting

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*** For very small concentrations of the substances to be applied to the column, the isotherms are linear, of course. Working in the extremely low concentration regions it is possible to apply elution analysis as in the well-known example of Moore and Stein's analysis of amino-acids. Even in such low concentrations elution analysis may not be always possible *e.g.* with carbon, because of irreversible adsorption.

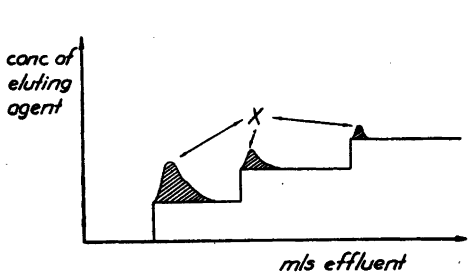


Fig. 1. A schematic representation of a stepwise elution experiment in which one substance occurs three times.

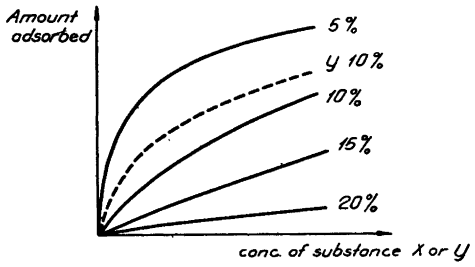


Fig. 2. Adsorption isotherms of two substances, X and Y, in different solutions of ethanol in water. The full lines represent isotherms for X and the percentages are the % ethanol in water in the solvent.

solutions. It was of interest to enquire therefore how substances would travel in a column in which the eluting power was being continuously increased.

The first approach to this problem has been made through the observation of the behaviour of the oligo-saccharides in a gradient of increasing ethanol concentration in water [6].

However, the general effect of eluting agents on the isotherms of a number of different substances [7] is similar and therefore the approach through the isothermal data instead of through column procedure will be outlined as it presents a more widely applicable picture of the method. The data will be treated in a purely qualitative fashion.

THE ISOTHERMS

As was shown by Hagdahl, Williams and Tiselius [1] a saturator depresses the maximum possible adsorption (as shown by the isotherms) of many substances such that the isotherms become linear only at very low adsorption. An eluting agent has a somewhat different effect in that the maximum adsorption remains the same but an adjustment of the isotherm to become more linear occurs already at much higher concentrations of the substance being examined than with a saturator. Fig. 2 shows the way in which the isotherms change for substances such as the sugars and amino-acids adsorbed on carbon from different concentrations of ethanol, or many other eluting agents, in water. Changes of the isotherms of other groups of substances are somewhat different but not sufficiently so to warrant a separate general discussion [6].

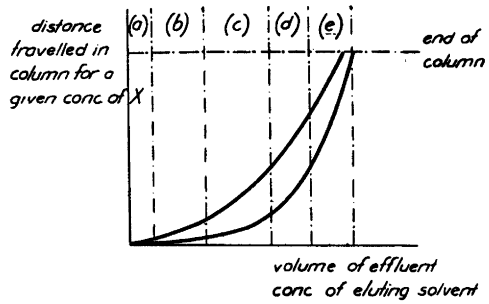


Fig. 3. Diagrammatic representation of the way in which two different concentrations of the same substance would travel in the same gradient. The lower curve is for the lower concentration. The curves can be deduced from a set of isotherms as in Fig. 2. The letters refer to Fig. 4.

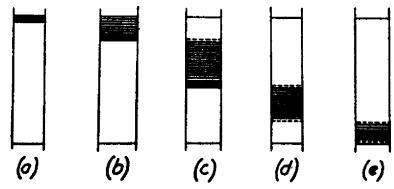


Fig. 4. The development of a zone (see Fig. 3).

This suggests that for a substance which has a curved isotherm in the initial pure solvent, *e.g.* water, the effect of the second eluting agent is generally to decrease the adsorption until the isotherm is linear to the origin — a continuous change of the isotherm being seen [7]. For different substances the isotherm will become linear at different concentrations of the second eluting agent. Now when a step-wise change in the eluting agent, *e.g.* alcohol, concentration is made in eluting a substance X (Fig. 2) from a column of adsorbent taking for example 5 %, 10 % and 20 % ethanol, it may well be that at the first concentration a little of X is eluted, and then at 10 % ethanol most of X will be washed from the column and finally at 20 % all of it will be eluted, see Fig. 1. Only if the steps are made very large can the substance be completely eluted in a concentration of eluting agent less than that of the condition where the isotherm is linear (*i.e.* in this case, in 15 % alcohol). Unfortunately if such a procedure is adopted other compounds may occur in the step. This is illustrated with reference to Fig. 2 where it is clear that Y will be eluted to some extent in 10 % ethanol *i.e.* before all of X is certainly eluted. This explains the painstaking methods which have been found to be necessary in step-wise elution methods. An example is provided by steroid analysis [5].

In order to appreciate the advantages of the gradient procedure consider the movement of the zone of a substance in an increasing concentration of an eluting agent. In the first instance let it be assumed that the eluting agents move without retention volume. This is the case for most eluting agents at the relatively high concentrations used. Then the concentration of the eluting

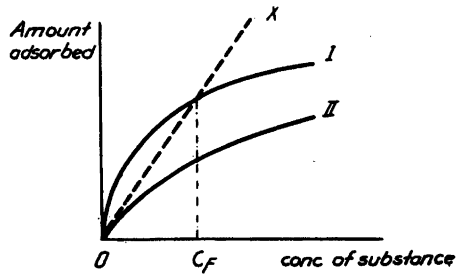


Fig. 5. Two adsorption isotherms taken from Fig. 2 and used to discuss the speed of movement of the front and rear of a zone.

agent, which is assumed for a moment, to increase in a linear manner at the top of the column will also increase linearly with the effluent volume. The movement of the substance X can now be considered in this gradient. There are two aspects of major importance, firstly the zone spreading and secondly the separating power of the gradient.

ZONE SPREADING

On applying the substance X to the adsorbent its spreading during movement down the column in a constant eluting solution depends upon the shape of its isotherm. In the first place if the isotherm is linear there will be the theoretical spreading (discussed in ref. 8 and 9) but if the isotherm is curved the substance will travel as a "tailing" elution peak. In normal elution methods there are no features opposing this zone spreading and tailing. In the gradient two factors adjust these effects. Firstly the gradient itself, which will exist in the column, is such that for all isotherms the "tail" moves in a region of

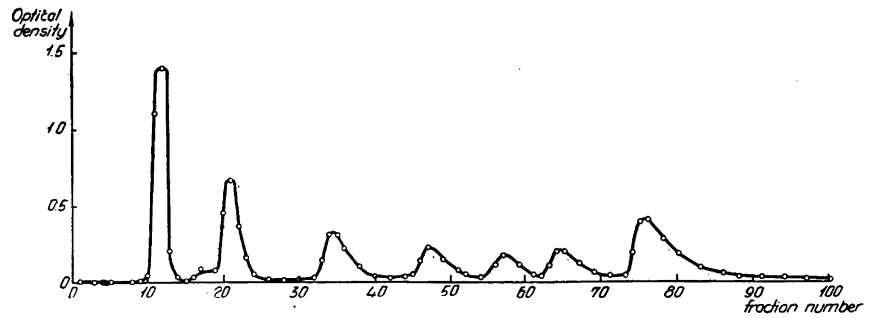


Fig. 6. A plot, optical density (which is proportional to the amount of each sugar) against fraction number. It shows the resolution of an oligosaccharide mixture by a gradient elution analysis. The oligosaccharide mixture, an α-Schardingerdextrine hydrolysate was applied to a pretreated column (carbon Darco G 60) and the elution carried out with 0—20 % ethanol. The experiment will be described in detail in a later publication.

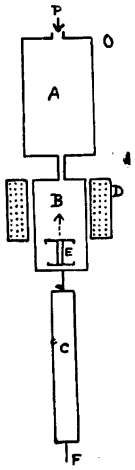
higher eluting power than the front of the elution peak, *i.e.* the peak will be constrained to move in a smaller volume than in a normal elution experiment. The peaks will be somewhat disturbed, of course, from their usual shape. This effect has been mentioned in a theoretical fashion by Strain [2]. Secondly the gradient moves as a whole with no, or little, retention volume so that the zone of the substance is finally moving in an eluting concentration in which it moves rapidly, *i.e.* it does not remain in the column for very large volumes of liquid. The discussion can be clarified by the use of Fig. 3 which illustrates the movement of two concentrations of substance, X, in a column. The isotherms of X in different eluting agent concentrations are assumed to be as in Fig. 2.

When development of such a compound, X, on a column occurs under the influence of a gradient then at first, *i.e.* at low concentrations of the eluting agent, the movement in the column is slight, Fig. 3a, and the zone spreading which takes place is not very different from that to be expected in a normal elution experiment, Fig. 4b. The tail of the peak hardly moves at this stage as the adsorption is very strong at low concentration of the eluting agent. When higher concentrations are reached the zone begins to move as a whole but the tail still moves more slowly than the front. The zone continues to spread. At this time the zone may occupy a considerable region of the column and the concentration in which the tail travels is noticeably higher than that in which the front moves, Fig. 3c and 4c.

A stage will be reached when this difference causes the tail and the front to move at equal rates although the isotherm is still curved. In Fig. 5 this is illustrated with reference to two isotherms from Fig. 2. Isotherm I is that from a solvent of the same composition as that in which the front of the zone is moving. Isotherm II is that from a solvent of the same composition as that in which the tail moves at this stage. Now let the concentration at the front of the zone be C_F, the speed line OX [1], through the point on the isotherm, I, corresponding to this concentration, will not cut isotherm II and is a tangent to it at the origin. The point C_F on isotherm I travels at the same speed as a very low concentration on isotherm II, *i.e.* the tail and the front move at equal speeds. At this stage the spreading of the zone ceases.

The eluting agent concentration is still being linearly increased and as the isotherms are further depressed the tail begins to move faster than the front, Fig. 3(d) and 4(d). Finally the zone travels as an almost symmetrical elution peak Fig. 4(e) as the isotherm becomes linear. The zone spreading is still controlled, however, by the gradient in which the peak travels.

The factor which is clearly important in the above reasoning is the relationship between the column length and the steepness of the gradient. With a



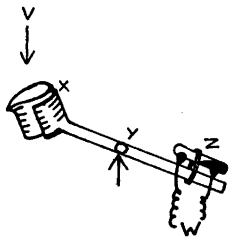


Fig. 8. Simple arrangement for an intermittent current supply. Water runs slowly into the container X until its weight is sufficient to overbalance the mercury switch Z. The swing pivots about Y and empties X. The swing returns to its previous position. The leads W, go to the coil D.

Fig. 7. The arrangement of the column. The effluent from F is collected in fractions.

steep gradient the zone development will occur rapidly and the column length need not be excessive. The less steep the gradient the longer the column must be for a full development of the zone as described above. Again for a slight gradient there is the danger that the compound will leave the column before stage (e), Fig. 4, is reached. Apart from the poor definition of the zone before this stage, the "break through" volume and the "break through" concentration will be ill-defined. A detailed analysis of such points is being undertaken and will be described elsewhere. It is already clear, however, that in many cases the "break through" volume is well defined and that the concentration of the eluting agent at which break through occurs is characteristic of a given compound and independent of the mutual influence of the column length and the gradient steepness. A component eluted from a chromatographic column must be recognisable by the position in which it travels if the technique is to be used analytically. In normal elution experiments this is clearly the case. In gradient elution the "break through" volume of a component will be controlled by factors discussed in later papers and it appears that the normal elution technique is somewhat more convenient as it results in well defined positions of a component relative to such conditions as the eluting medium and the column length. Examples of this are given in ref. 6.

Naturally the zone spreading depends upon the amounts of the substance applied to the column. It has been found that the gradient elution technique is particularly suitable for the analysis of small quantities of material.

Before discussing factors controlling separation a further point must be made. If the eluting agent employed has a curved isotherm itself then instead of a gradient of eluting concentration emerging from the column a step will occur covering the low concentration range. After this initial step a gradient will still be obtained. It is important that the substances to be examined are not eluted in concentrations of the eluting agent lower than that of the initial step. Thus if the eluting agent has not a linear isotherm it must be chosen so that its adsorption is considerably less than that of the examined substances.

SEPARATION OF SUBSTANCES

The only effect which has been mentioned so far as effecting a separation of two substances is that of the different rate of travelling in the gradient of eluting concentration. However, as the substances are applied to the column a displacement of the more weakly adsorbed by the more strongly adsorbed compounds will occur especially for those cases in which the substances to be separated belong to the same homologous series or to the same group of compounds [1]. It must be clear that displacement occurs first and then the zones of the different compounds are separated by the eluting agent. The displacement will be very strong as the isotherms are initially very curved.

The main limitation to separation of two substances which have a given difference in adsorption is the zone spreading.

An example of the experimental recordings is given in Fig. 6 which shows a typical analysis of a mixture of oligo-saccharides. It should be observed that the compounds emerge as almost symmetrical elution peaks. This probably means that the length of the column has been correctly adjusted to the given gradient so that complete development of the zones has occurred.

The application of a gradient elution can not increase the separation over that of the simpler elution techniques already devised in those cases where the substances have linear isotherms.

Apart from the adjustment of the concentration of the eluting agent the choice of this agent is important. It has been found for adsorption on silica gel and alumina that a series of eluting agents of increasing power can be utilised. This series, the Trappe series, can be used here too. Experiments on carbon have been made which show the effect of different eluting agents. A discussion of this problem will be given in a later paper [6]. However, other data [10] already exists in the literature which shows that the choice of the eluting agent can have a great influence on the rate of movement, the separation and the zone spreading in a chromatogram of the usual type. Similar somewhat specific factors are to be expected here.

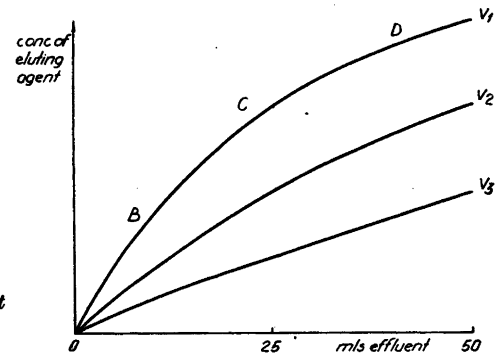


Fig. 9. Three gradient curves for different volumes of the mixing chamber.

The mechanism of the adjustment of the adsorption of compounds by eluting agents can be described under two headings. In the first instance the eluting agent may alter the solubility of the compounds to be examined in the moving phase. Secondly the adsorption of the eluting agent may well produce a change in the adsorbent surface. At present an investigation is being carried out into the relative power of eluting agents on carbon in order to elucidate this aspect of the problem.

PRETREATMENT OF THE ADSORBENT

The effect of pretreatment of the adsorbent has been discussed in several previous papers [1,11,12]. Its first successful application by the gradient elution procedure will be described in connection with the separation of sugars [5]. It has been found in this work and more recently in the separation of peptides [7] that saturation or pretreatment improves the shape of the zones, increases the recovery to a value very close to 100 % and lastly permits the extension of the technique to very strongly adsorbed compounds such as the higher oligosaccharides [6] and the peptides [7]. The combination of a suitable saturation and a gradient elution seems at present to be of extraordinary power in its range of application.

APPLICATION OF THE GRADIENT TO A COLUMN

The above has been concerned in demonstrating the method of gradient elution from a theoretical stand-point. The application of the gradient to a column is not simple as the column must be operated under pressure. The diagram Fig. 7 shows the special construction which has been utilised.

There are two distinct chambers: the first of which A, contains the solution of the eluting agent which is to be used for development, and the second, B,

of volume V, the mixing chamber, contains the solvent in which the column C was packed. The mixing chamber is equipped with a magnetic stirrer which consists of an iron rod enclosed in plastic material which is not attacked by the eluting solvent. The chamber itself can be made of brass or plastic. An intermittent magnetic field produced in a coil, D, surrounding the mixer, is sufficient to lift the iron rod, D, in the mixer. The up and down movements of the rod mixes the liquid in the chamber. The intermittent current is produced by the simple arrangement shown in Fig. 8.

The operational procedure is simple. The column is set up and the substances to be examined are applied to the top of the column in the first solvent which has little if any eluting action on the compounds. The column is then connected to the mixing chamber, which should be made in pieces so that its volume can be adjusted, and then to the chamber A. Naturally the chamber B must be completely filled. When pressure is applied to A the first solvent starts to move down the column and the second solvent moves into the mixing chamber. The gradient of the second solvent applied to the column in this way is expressed by the formula:

$$2.303 \log_{10} \frac{X}{X-x} = \frac{v}{V}$$

x is the concentration of the second solvent in the mixing chamber when a volume, v, has flowed into this chamber, and X is the concentration of the second solvent in the chamber A. V is the volume of the mixing chamber. A plot can be made of $\frac{x}{X}$ in the mixing chamber against v. The shape of this plot is independent of X but is dependent upon V. A set of such curves can be obtained for different V values. Thus the steepness of the gradient is adjustable by an alteration of the value of V. This is shown in Fig. 9 where $V_3 > V_2 > V_1$.

The second adjustment of the gradient that is possible lies in the choice of the initial solutions in A and B. Thus it is possible to choose a gradient of any desired slope in any required concentration range.

In Fig. 9, and the above equation, v can be replaced by $v_1 - v_2$ where v_1 is the volume of the effluent and v_2 the dead volume of the column, providing the eluting agent travels without retention volume.

Now in Fig. 9 if the curve for the volume of the mixing chamber is V_2 and the curve for this volume is divided into three parts OB, BC and CD then it is seen that the gradient is steep over the region OB, moderate from B to C and slight from C to D. Substances eluted in the region OB will be somewhat crowded together and substances eluted in the region CD will be well separated but the peaks will be more spread than those eluted in the earlier parts of the

gradient. Clearly a small gradient should be applied to similarly adsorbed substances and a steep gradient to compounds adsorbed in very different strengths. However, as a saturator can be used to adjust the strength of the adsorption the further question arises as to whether a small gradient should be employed together with high saturation or a steep gradient together with low saturation. These features will be discussed in detail for specific cases in later papers.

It is obvious too that an improvement in the existing technique can be immediately made by the production of a linear gradient in the mixer. Methods for this are under consideration. It is of interest to note devices suggested by Desreux [15] in a somewhat parallel apparatus for the production of gradient changes in solvent composition.

The gradient elution technique has a value apart from its immediate application to separation problems. It can be utilised in initial experiments in order to obtain a survey of the chromatographic behaviour of an unknown mixture of compounds.

The introduction of gradient elution procedures has already met with considerable success which will be described elsewhere. However, the application of the method is very wide and its full value has not been appreciated so far. Thus a gradient can be applied in many ways, a continuous change of pH, of salt concentration [13], of displacing strength [1,7], are all feasible and a similar variation is possible in partition extraction procedures [14]. All these methods must be thoroughly examined before their usefulness can be accessed. It is known however, that very good separations can readily be achieved in the oligosaccharides, fatty acids, sterols, peptides, and amino acids. The method is being tried also for proteins and alkaloids. Full reports of these experiments will appear shortly.

SUMMARY

A new general procedure for the chromatographic analysis of mixtures of compounds is outlined. The method consists of the use of a continuously changing eluting medium which is produced external to the column in a mixing chamber. The mode of operation of this "gradient elution method" (as we propose it should be named) is outlined and factors such as the spreading of zones, the separation of components and the pretreatment of the adsorbent are discussed. From the experiments which will be published elsewhere it is clear that the method has widespread application.

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Potentiometric and Spectrophotometric Studies on 8-Quinolinol and Its Derivatives. IV. 8-Quinolinol Chelates of Cadmium, Zinc and Copper in Aqueous Solutions

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In the two preceding papers [2,3] the 8-quinolinol chelates of the alkaline earth metals have been dealt with. These chelates cannot be precipitated until the pH is so high that the 8-quinolinol in the solution exists mainly in the neutral form, HOx[1]. The chelates of the other divalent metals, however, can be precipitated at a pH so low that the main constituent is the positive ion H_2Ox^+. This fact leads to a somewhat different treatment of the solubility equilibria in the two cases.

The essence of the method used in both cases is to regulate the pH to such a value that, whilst a sufficient amount of the precipitate is formed, the solution contains amounts of 8-quinolinol and metal ions such that the concentrations of all the constituents can be calculated accurately from the stoichiometric concentrations, from the pH measured and from dissociation constants of 8-quinolinol [1]. The pH may be regulated by adding sodium hydroxide or perchloric acid.

The solubility equilibria can be represented at higher pH's by

$$MOx_2(s) + 2\ H^+ = M^{++} + 2HOx \qquad (1)$$

and at lower pH's by

$$MOx_2(s) + 4H^+ = M^{++} + 2H_2Ox^+ \qquad (2)$$

In the latter case, dealt with in the present paper, the law of mass action gives

$$\frac{[M^{++}][H_2Ox^+]^2}{(H^+)^4} = K_s' \qquad (3)$$

One can easily see that[2]

$$K_s' = S/K_1'^2\ K_2'^2 \tag{4}$$

where S is the solubility product,

$$S = [M^{++}][Ox^-]^2 \tag{5}$$

and K_1' and K_2' are the dissociation constants of 8-quinolinol[1].

The complexes between metal ions and the ligand Ox^- display a great stability. Their role in the solubility equilibria is, however, slight beacause of the very low concentration of the ligand. Only the first complex MOx^+ is of any significance. The equilibrium in question can be represented at higher pH's by

$$MOx^+ + H^+ = M^{++} + HOx \tag{6}$$

and at lower pH's by

$$MOx^+ + 2H^+ = M^{++} + H_2Ox^+ \tag{7}$$

In the latter case the law of mass action gives

$$\frac{[MOx^+]\ (H^+)^2}{[M^{++}][H_2Ox^+]} = k_1'' \tag{8}$$

One can easily see that [2]

$$k_1'' = k_1 K_1' K_2' \tag{9}$$

where

$$k_1 = [MOx^+]/[M^{++}][Ox^-] \tag{10}$$

For the calculation of the solubility equilibrium the following equations were obtained:

$$[H_2Ox^+] = \frac{c_{Ox} + c_A - c_B + [OH^-] - [H^+]}{2 + K_1'/(H^+)} \tag{11}$$

$$c_{Ox}' = c_{Ox} - [H_2Ox^+]\{1 + K_1/(H^+) + K_1'K_2'/(H^+)^2\} \tag{12}$$

$$\frac{1}{K_s'} = \frac{(H^+)^4}{(c_M - 0.5\ c_{Ox}')[H_2Ox^+]^2} + \frac{0.5\ (H^+)^2\ k_1''}{(c_M - 0.5\ c_{Ox}')[H_2Ox^+]} \tag{13}$$

(For an explanation of the notation see refs. 1, 2, 3.) The second term on the right hand side of equation (13) was generally negligible.

The pH was generally measured but in the case of copper chelate, it was so low, (about 2) that the spectrophotometric determination of $[H_2Ox^+]$ gave better results. This analysis was carried out as follows: A sample of clear equilibrium solution was diluted to a suitable concentration with a 0.1 molar solution of perchloric acid and the extinction was measured at 365 mμ.

From the total concentration obtained, the concentration of H_2Ox^+ was calculated and thence, using equation (11), the concentration of hydrogen ion.

The constants k_1'' were determined spectrophotometrically, using the equation

$$\varepsilon k_1'' - \varepsilon_{MOx^+} k_1'' + \left\{(\varepsilon - \varepsilon_{H_2Ox^+})(H^+)^2 + (\varepsilon - \varepsilon_{HOx})K_1'(H^+)\right\}/[M^{++}] = 0 \qquad (14)$$

In this equation $\varepsilon = e/c_{Ox}$ (e the extinction when 10 mm cells were used), ε_{MOx^+}, $\varepsilon_{H_2Ox^+}$, ε_{HOx} are molar extinction coefficients as indicated. The values of K_1', $\varepsilon_{H_2O^+}$ and ε_{HOx} have been determined previously [1]. The wavelength range 340 mμ—380 mμ was used. The conditions were not very favourable because the light absorption of H_2Ox^+ at this wavelength range is relatively large but the other methods seemed to be still worse. From a definite pH value upwards practically all the 8-quinolinol exists as MOx^+ if the metal salt is in excess. On the other hand from a definite pH value downwards the complex ions practically vanish from the solution. The measurements must therefore be made over a relatively narrow pH range characteristic of each chelate. Generally four or five pH values were used. For each pH an equation (14) was obtained. These equations were then solved by the method of least squares. The desired pH's were obtained by adding perchloric acid or sodium hydroxide.

The results relating to the complex equilibria are recorded in Table 1.

Table 1. Spectrophotometric determination of equilibrium constants k_1'' for $CdOx^+$, $ZnOx^+$ and $CuOx^+$ at 20° C.

	ε				
pH	350 mμ	360 mμ	370 mμ	380 mμ	
4.739	1530	1540	1460	1195	$c_{Cd} = 4.79 \cdot 10^{-3}$
4.983	1595	1650	1560	1310	$c_{Ox} = 2.00 \cdot 10^{-4}$
5.462	1910	2050	1990	1690	$\sqrt{I} = 0.127$
6.978	2175	2358	2265	1935	$pk_1'' = 7.35$

	ε				
pH	350 mμ	355 mμ	360 mμ	365 mμ	
4.417	1715	1760	1750	1700	$c_{Zn} = 4.20 \cdot 10^{-3}$
4.744	1890	1940	1940	1910	$c_{Ox} = 2.00 \cdot 10^{-3}$
6.265	2140	2235	2260	2235	$\sqrt{I} = 0.115$
7.444	2180	2270	2325	2275	$pk_1'' = 6.56$

	ε					
pH	530 mμ	355 mμ	360 mμ	365 mμ	370 mμ	
2.31	1735	1790	1815	1770	1700	$c_{Cu} = 5.00 \cdot 10^{-3}$
2.39	1715	1790	1810	1790	1710	$c_{Ox} = 2.00 \cdot 10^{-4}$
2.68	1790	1885	1940	1960	1900	$\sqrt{I} = 0.123$
3.59	1940	2080	2160	2200	2100	$pk_1'' = 2.550$
5.88	1975	—	2190	2215	—	

For extrapolation to zero ionic strength the equation

$$pk''_{1.0} = pk''_1 - \frac{2.02\sqrt{I}}{1 + a\sqrt{I}} \qquad (15)$$

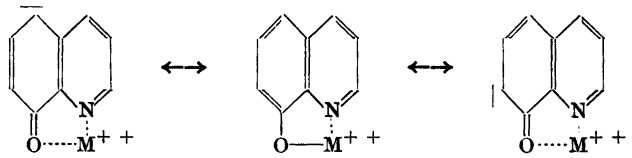
was used. The value $a = 1.54$, obtained previously for $CaOx^+$, was used [2]. The extrapolation gave

	$CdOx^+$	$ZnOx^+$	$CuOx^+$
$pk''_{1.0}$	7.135	6.363	2.361

Using the values $pK_{1.0} = 5.02$ and $pK_{2.0} = 9.90$, obtained previously [1], we obtained from equation (9) the complexity consants of MOx^+. With the complexity constants of the alkaline earth metals, previously determined [2, 3], we obtain the following summary:

	$BaOx^+$	$SrOx^+$	$CaOx^+$	$MgOx^+$	$CdOx^+$	$ZnOx^+$	$CuOx^+$
log $k_{1.0}$	2.07	2.56	3.27	4.74	7.78	8.56	12.56

For the complexes of the alkaline earth metals the absorption spectra (280—400 mμ) were obtained indirectly. The spectra of the cadmium, zinc and copper complexes, on the contrary, have been obtained directly because of the very much greater complex formation. The absorption spectra of the latter (300—400 mμ) are shown in Fig. 1. The maxima at about 360 mμ obviously correspond to the maximum of Ox^- at 355 mμ [1]. A shoulder at about 330 mμ in the spectra of $ZnOx^+$ and $CdOx^+$ obviously corresponds to the maximum at 335 mμ in the spectrum of Ox^-. Another shoulder occurs in the spectra of the cadmium and zinc complexes near 315 mμ, at which H_2Ox^+ has a maximum. The same maximum and the two shoulders also occur in the spectra of the complexes of the alkaline earth metals but the shoulders do not occur in the spectrum of the copper complex. In terms of the structures of 8-quinolinol HOx, its positive ion H_2Ox^+ and its negative ion Ox^-, as reported in the literature [4], the following structures may be suggested for MOx^+



I II III

The polar structure I probably corresponds to the maximum at about 360 mμ. Thus this structure seems to be predominant. The structures II and III

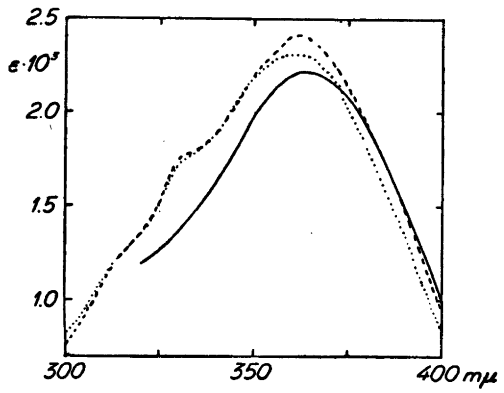


Fig. 1. Molar extinction coefficients of MOx^+. $CdOx^+$ – – –, $ZnOx^+$ . . . ., $CuOx^+$ ——.

probably corresponding to the shoulders at 315 mμ and 335 mμ, are of little significance.

We have assumed above that the higher complexes, particularly MOx_2, do not make any contribution to the equilibrium. In actual fact the spectrophotometric data show that this is true, as can easily be understood in view of the very low concentration of the ligand Ox^- at the pH's in question.

The results relating to the solubility equilibria of cadmium, zinc and copper chelates in sodium perchlorate solutions are recorded in Table 2. The measurements are performed at 2—4 ionic strengths. The values quoted for log K_s' are mean values.

Table 2. Solubility equilibria of cadmium, zinc and copper chelates in sodium perchlorate solutions.

		$CdOx_2$ (25° C)			
$\sqrt{I}$	$(c_A \cdot 10^3)$ $c_{Ox} \cdot 10^3$	$c_{Cd} \cdot 10^3$	$c_B \cdot 10^3$	pH	log K_s'
0.145	3.62—3.70	6.92—7.09	2.04—6.12	3.835—4.173	7.88
0.506	3.43—3.49	6.57—6.68	1.92—4.71	4.004—4.190	8.54
1.010	3.68—3.71	7.05—7.10	2.04—3.04	4.085—4.186	9.07
		$ZnOx_2$ (20° C)			
$\sqrt{I}$	$(c_A \cdot 10^3)$ $c_{Ox} \cdot 10^3$	$c_{Zn} \cdot 10^3$	$c_B \cdot 10^3$	pH	log K_s'
0.104	4.87—4.96	2.42—2.47	1.58—4.28	3.306—3.434	5.65
0.142	9.01—9.62	4.50—4.81	1.58—4.27	3.040—3.812	5.73
0.266	4.86—9.60	2.42—4.80	1.58—15.81	3.363—3.854	6.00
0.413	4.84—9.57	2.41—4.78	1.57—15.76	3.411—3.886	6.25
		$CuOx_2$ (25° C)			
$\sqrt{I}$	$c_{Ox} \cdot 10^3$	$c_{Cu} \cdot 10^3$	$c_A \cdot 10^3$	pH	log K_s'
0.176	3.75—4.00	10.59—16.25	6.25—6.67	1.916—2.050	0.342
0.460	3.75	10.00	6.25	2.110	0.870

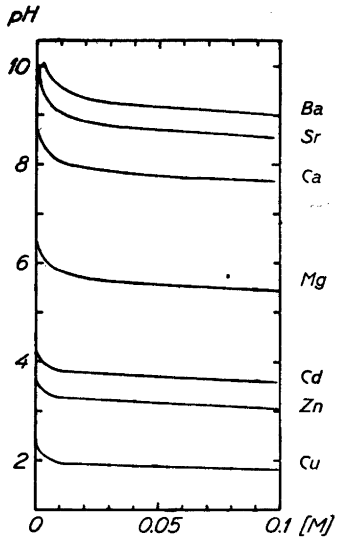


Fig. 2. Precipitation of some divalent metals with 8-quinolinol. $[Ox] = 3.78 \cdot 10^{-3}$. $\sqrt{I} = 0.4$.

from 3—6 experiments. The pH's in the measurements of copper chelate were calculated from $[H_2Ox^+]$ measured spectrophotometrically, using equation (11) and the values for the activity coefficient of the hydrogen ion determined previously [1]. The results can be represented by

$$\log K'_s = \log K'_{s,o} + \frac{3.06\sqrt{I}}{1 + a\sqrt{I}} \qquad (16)$$

Calculation by the method of least squares gave

	$CdOx_2$	$ZnOx_2$	$CuOx_2$
$\log K'_{s,o}$	7.49	5.34	−0.125
a	0.95	0.95	0.90

From equation (4) we obtain for the thermodynamic solubility products:

	$CdOx_2(25°)$	$ZnOx_2(20°)$	$CuOx_2(25°)$
pS_o	21.956	24.500	29.571

Recently Phillips and Price [5] have reported values for the solubility products of some 8-quinolinol chelates. These values have been computed from the solubility data available in the literature [6]. Like some other authors [6] they have assumed that the equilibrium in question is represented by (1). Since the data concern solubility in 1 *N* acetic acid, the pH is generally so low that the equilibrium is represented by (2). Therefore their values are incorrect.

A correct calculation gives solubility product values which are at least of the same order of magnitude as our values.

In Fig. 2 the total concentration of metal in the solution is represented as a function of pH when the excess of reagent is $[Ox] = 3.78 \cdot 10^{-3}$. In the case of cadmium, zinc and copper chelates the following equations were used:

$$[H_2Ox^+] = [Ox]/\left\{1 + K_1'/(H^+) + k_1'\ K_2'/(H^+)^2 + k_1'[M^{++}]/(H^+)^2\right\} \quad (17)$$

$$[M^{++}] = K_s'\ (H^+)^4/[H_2Ox^+]^2 \quad (18)$$

$$[M] = [M^{++}](1 + k_1'[H_2Ox^+]/(H^+)^2 \quad (19)$$

SUMMARY

The equilibria of the 8-quinolinol chelates of cadmium, zinc and copper in aqueous solution have been investigated. The complex formation involved has been studied spectrophotometrically and the complexity constants of the first complexes have been determined. The solubility products of these chelates have been determined potentiometrically. The ultra-violet spectra of the complex ions are discussed.

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Hydrolysis and Alcoholysis of Sulphonic Esters

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In a previous investigation [1] the hydrolysis and alcoholysis of sulphonic esters were studied. The experiments, however, were limited then to the esters of primary alcohols. Sulphonic esters in several respects behave like alkyl halides [Cf. 2]. It is well known [3] that the alkaline hydrolysis of methyl and ethyl halides in aqueous acetone or alcohol is predominantly bimolecular or a S_N2 reaction, while *iso*propyl and *tert.*-butyl halides undergo hydrolysis by a two-stage or S_N1 mechanism, an addition of alkali having no influence on the reaction velocity. Thus it was to be expected that the hydrolysis of the sulphonic esters of secondary and tertiary alcohols also takes place by the unimolecular mechanism, the first stage being a dissociation of the ester molecule. The main object of the investigation reported here was to elucidate this problem. In addition, the hydrolysis and alcoholysis of some other alkyl sulphonates and the solvent effect were studied.

EXPERIMENTAL

Chemicals. The esters were prepared from the respective sodium alcoholates and benzenesulphonyl chloride [Cf. 4]. To obtain products free of sulphonyl chloride, the sodium alcoholate was used in a slight excess. With *iso*propanol and *sec.*-butanol sodium reacts slowly, so that a great excess of the alcohol was needed. Finally, the mixture was heated on a water bath. On cooling the main part of these alcoholates separated as a crystal mass. The sulphonyl chloride was added to this mixture in small portions. During the process the reaction mixture was agitated and its temperature was kept between 30° and 50° C. When all sulphonyl chloride was added, the mixture was left to stay for about an hour and then heated to about 70° C. and kept at this temperature about 10 minutes. The mixture was left to stay at room temperature for an hour and then, to remove the last traces of unchanged sulphonyl chloride, vigorously shaken with a few milliliters of an approximately 10 *N* sodium hydroxide solution. The mixture was then poured into a large amount of cold water and the separated ester taken into a separating funnel with a small amount of ether and shaken repeatedly with pure cold water. Finally,

the ethereal solution was dried with anhydrous sodium sulphate and the ether evaporated at reduced pressure at room temperature. By this method, using benzenesulphonyl chloride and alcohols of the highest purity, esters could be obtained in a degree of purity satisfactory for this work. They were colourless and did not give any halogen reaction with silver nitrate; the total hydrolysis gave, within the experimental error, in all cases a purity of 100 per cent. Of the esters used in this work only ethyl and *n*-propyl benzenesulphonates distil at reduced pressure without decomposition. The boiling point of *n*-propyl benzenesulphonate is 153—154°/9 mm.

All efforts to obtain the *tert.*-butyl ester of benzenesulphonic acid failed. The following methods were tried. (*a*) Benzenesulphonyl chloride was dissolved into a large excess of *tert.*-butanol, and an equivalent amount of pyridine was added to the solution. At room temperature a slow evolution of a gas with the smell of a hydrocarbon (i-butylene) was observed, and as on the following day when the smell of the sulphonyl chloride had disappeared, the reaction mixture was poured into cold water, only a few drops of a colourless oil were separated. These drops which had an aromatic odour disappeared rather quickly. (*b*) The method of shaking the sulphonyl chloride and *tert.*-butanol with a concentrated sodium hydroxide solution did not give any positive result. (*c*) Silver salt of benzenesulphonic acid was shaken with *tert.*-butylbromide dissolved in dry acetone (6.5 g in 50 ml of acetone) for about an hour. An evolution of heat indicated that reaction was taking place. The liquid was separated by filtration. Analysis showed that the solid phase was AgBr. From the yellow solution, acetone and unchanged butylbromide were evaporated at reduced pressure at room temperature. The residue was an oil of a reddish brown colour. In titration, 131.3 mg of the substance required 29.3 ml of 0.02 *N* $Ba(OH)_2$. Assuming that the oil was the *tert.*-butyl ester, the purity of the product was thus 95.5 %. The alkali was neutralized immediately, and it was impossible to decide whether the ester was completely decomposed already in the brown liquid, or whether it was instantaneously hydrolysed when it came in contact with water. A conductivity measurement showed that when a few drops of the acetone solution, directly after the filtration of the reaction mixture, were added to water, its conductivity reached ist final value in less than five seconds.

The water used as solvent in the kinetic experiments was freshly distilled. Acetone and methanol were purified as described in the paper of Tommila and Lindholm. Dioxan was purified by the method of Hess and Frahm [5].

Method. The kinetic experiments were carried out in the same reaction vessels as were used in the work of Tommila and Lindholm, and also the method was essentially the same. At higher temperatures the reactions were conducted in sealed ampoules of Pyrex-glass. Since the velocity of the hydrolysis increases very rapidly with the water content of the solvent, the titrations in the experiments were made in the following manner. Into a 100 ml erlenmeyer flask from a burette an estimated amount of the titrating solution (0.02 *N* $Ba(OH)_2$ or 0.02 *N* HCl) was measured. The solution was cooled in a mixture of ice and water. A reaction vessel was taken from the thermostat, cooled quickly by immersing it into a mixture of ice and water, and the cold solution from the erlenmeyer flask was poured into the reaction mixture. After shaking, the solution was poured back into the erlenmeyer flask and titrated with cresol red as indicator. In this titration generally only a few tenths of a ml were required. Finally the reaction vessel was washed with the titrated solution and, if needed, titrated again to the neutral point. The whole titration took only a few seconds, during which time, at a low temperature, the progress of the reaction was negligible.

RESULTS AND DISCUSSION

The hydrolysis of isopropyl benzenesulphonate. The course of the hydrolysis of *iso*propyl benzenesulphonate in various acetone-water mixtures is expressed by the usual first order formula, as shown in Tables 1 and 2, which record the results of two typical runs. The rate constant k_1 is calculated by the formula $k_1 = \frac{2.303}{t} \log \frac{100}{100\text{-}p}$, where p is the percentage change at the time t. The amount of the ester weighed into the leg of the reaction vessel varied between 90 and 110 mg and the volume of the solvent was so calculated that, after mixing, the initial concentration of the ester was 0.05 moles/liter. In these two and in all the following tables the expressions '56 per cent acetone' *etc.* mean acetone-water mixtures containing 56 weight per cent acetone, *etc.*

Table 1. The hydrolysis of isopropyl benzenesulphonate in 56 per cent acetone. Temperature 40.00° C.

Mins.	p	$k_1 \cdot 10^4$ sec.$^{-1}$
90	14.5	0.290
120	19.2	0.296
180	25.9	0.278
240	33.5	0.282
300	39.4	0.279
420	50.2	0.276
540	60.3	0.285
720	70.8	0.285

Mean = 0.2837

Table 2. The hydrolysis of isopropyl benzenesulphonate in 36.5 per cent acetone. Temperature 25.00° C.

Mins.	p	$k_1 \cdot 10^4$ sec.$^{-1}$
120	16.1	0.243
180	22.4	0.235
300	34.5	0.235
360	39.9	0.236
480	49.0	0.234
600	57.3	0.237
620	64.4	0.239
900	72.2	0.237

Mean = 0.2370

The results of experiments carried out in the same solvents and at the same temperatures but in the presence of sodium hydroxide are given in Tables 3 and 4. A comparison of the percentage changes p shows that an addition of alkali has hardly any influence on the rate of the hydrolysis. The reaction is of the first order even in the presence of alkali. The small differences in the values of p and k, between Tables 1 and 3, and 2 and 4, are fully accounted for by experimental errors in the kinetic measurements and by eventual variations of the composition of the solvent. As will be seen later on, the rate of the reaction is extremely sensitive to the water content of the medium. The independence of the rate of the hydrolysis on the alkali was further confirmed

by the experiments made with different initial concentrations c of the ester and alkali (Table 5).

Table 3. The hydrolysis of isopropyl benzenesulphonate in 56 per cent acetone in the presence of alkali. Ester: $c = 0.05$; NaOH: $c = 0.05$. Temperature 40.00° C.

Mins.	p	$k_1 \cdot 10^4$ sec.$^{-1}$
90	15.0	0.302
120	19.1	0.294
180	27.5	0.298
240	33.9	0.287
270	38.3	0.298
420	53.2	0.301
540	62.4	0.302
720	73.5	0.307

Mean 0.2986

Table 4. The hydrolysis of isopropyl benzenesulphonate in 36.5 per cent acetone in the presence of alkali. Ester: $c = 0.05$; NaOH: $c = 0.05$. Temperature 25.00° C.

Mins.	p	$k_1 \cdot 10^4$ sec.$^{-1}$
120	15.7	0.236
180	21.9	0.229
300	35.7	0.245
480	51.2	0.249
720	65.1	0.244
900	71.5	0.232

Mean 0.2391

Table 5. The hydrolysis of isopropyl benzenesulphonate in 56 per cent acetone at 50.00° C.

c_{Ester}	0.0712	0.0712	0.0674	0.0673	0.0648	0.0633	0.0607
c_{NaOH}	0.100	0.070	0.050	0.030	0.020	0.010	0.000
Mins.	90	90	90	90	90	90	90
p	39.0	37.9	37.7	37.2	36.4	37.6	36.6
$k_1 \cdot 10^4$sec.$^{-1}$	0.916	0.885	0.878	0.861	0.841	0.874	0.845

For a unimolecular reaction the time of half-completion must be independent of the initial concentration, according to the formula $t_{1/_2} = (\ln 2)/k_1$. That this condition is fulfilled is seen from Table 6. The time of half-completion

Table 6. The hydrolysis of isopropyl benzenesulphonate. Time of half-completion at 25° and 50° C. Solvent 56 per cent acetone.

c_{Ester}	c_{NaOH}	25.00°		50.00°	
		Mins.	p	Mins.	p
0.100	0.100	2 220	47.9	130	50.2
0.050	0.050	2 220	49.0	130	51.3
0.025	0.025	2 220	49.7	130	50.0
0.010	0.010	2 220	50.0	130	49.8

is quite independent of the initial concentration of the ester, and the presence of alkali has no effect.

All these results show that the hydrolysis of the *iso*propyl ester of benzenesulphonic acid is a two-stage reaction with the S_N1 mechanism, the rate-determining stage being ionization of the ester molecule:

$$RSO_2OCH(CH_3)_2 \rightarrow RSO_2O^- + {}^+CH(CH_3)_2 \qquad \text{slow}$$
$$(CH_3)_2HC^+ + HOH \rightarrow (CH_3)_2HCOH + H^+ \qquad \text{fast}$$

The hydrolysis of *n*-propyl ester of benzenesulphonic acid is much slower than that of the *iso*propyl ester. *E.g.* in 56 per cent acetone at 40° C, for the latter $k_1 = 0.284 \cdot 10^{-4}$ sec.$^{-1}$, for the former only $0.0163 \cdot 10^{-4}$ sec.$^{-1}$ (Table 7). Besides, the former reaction is powerfully catalysed by alkali *, the latter not at all. These facts, too, indicate that the mechanisms cannot be the same in the two cases. The *n*-propyl ester like the other *n*-alkyl esters undergoes hydrolysis by the one-stage or S_N2 reaction.

The hydrolysis of *sec.*-butyl ester of benzenesulphonic acid occurs faster than that of *sec.*-propylester. In 56 per cent acetone at 40° C the value of the rate constant is $k_1 = 0.390 \cdot 10^{-4}$ sec.$^{-1}$ (Table 7). An addition of sodium hydroxide had no influence on the speed of the reaction. Obviously all the sulphonates of secondary alcohols undergo hydrolysis by a two-stage mechanism.

The Arrhenius equation. — The mean values of k for various temperatures are given in Table 7. Activation energies and frequency factors were obtained from the variation of log k with $1/T$, the simple Arrhenius equation $k = Ae^{-E/RT}$ being obeyed within the limits of the experimental error in all cases. In the calculation the method of the least squares was used. Table 7 contains also the values of E and log A. Because of the poor solubility of *iso*propyl benzenesulphonate in water and the great rapidity of the reaction in this solvent, a direct measuring of the rate of its hydrolysis in water was not possible. The values of k_1 for pure water given in the Table are obtained by extrapolating to the acetone content zero on the ground of Fig. 1 which shows that log k_1 is practically a linear function of the acetone percentage of the solvent. From these extrapolated values, which give a good Arrhenius plot, E and log A were calculated.

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* In the presence of alkali the hydrolysis of the *n*-propyl ester is a reaction of second order; in 56 per cent acetone $k_2 = 0.000125$ l mol^{-1}sec.$^{-1}$ at 40.00 °C.

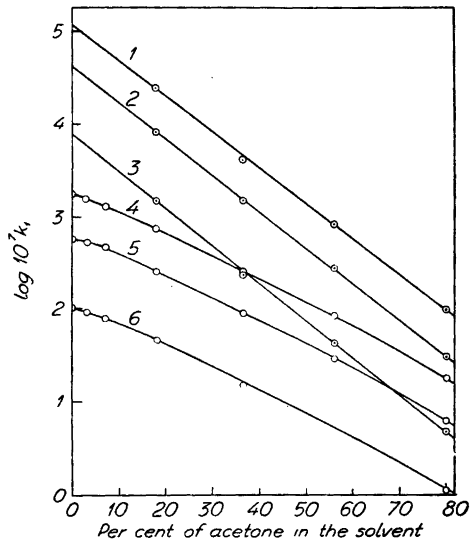


Fig. 1. Influence of solvent. 1, 2 and 3 the hydrolysis of isopropyl benzenesulphonate, 4, 5 and 6 the hydrolysis of ethyl benzenesulphonate at 50°, 40°, and 25° C.

The data of Table 7 give further justification for the following mechanism of the solvolysis of sulphonates of primary alcohols proposed in the paper of Tommila and Lindholm:

$$\mathrm{R \cdot SO_2 \cdot O - CH_2R' \rightarrow \left[\begin{matrix} R \cdot SO_2 \cdot O \ldots CH_2R' \\ \vdots \quad\quad\quad \uparrow \\ R''O \ldots H \quad\quad R''O-H \end{matrix} \right] \rightarrow RSO_2O^- + R''OCH_2R' + H^+} \qquad \text{(A)}$$

Transition state

($R'' = H$ or an alkyl radical), or in the presence of alkali:

$$\mathrm{R \cdot SO_2 \cdot O - CH_2R' \rightarrow \left[\begin{matrix} R \cdot SO_2 \cdot O \ldots CH_2R' \\ \uparrow \quad\quad\quad \vdots \\ OH^- \quad\quad\quad OH \end{matrix} \right]^- \rightarrow RSO_2 \cdot O^- + \begin{matrix} CH_2R' \\ | \\ OH \end{matrix}} \qquad \text{(B)}$$

In the same solvent, e.g. in 56 per cent aqueous acetone, the specific reaction rate follows the order

$$CH_3 > C_2H_5 > n\text{-}C_3H_7$$

i.e. the order of increasing electron-repulsive character of the alkyl group in the alcohol side. We have also seen, above, that the *sec.*-butylester is hydrolysed more rapidly than the *sec.*-propylester. This is in harmony with the predictions of the theory. Groups which facilitate the electron release from the C atom of the -O-C-group facilitate the ionization and have an accelerating effect on the S_N1 mechanism. In the one-stage bimolecular mechanism, such a group, on the one hand, weakens the bond which must be broken, on the

Table 7. The values of k, E and A.

Ester and solvent	$10^7 \cdot k_1$ sec.$^{-1}$					E cal.	log A
	25.00°	40.00°	50.00°	60.00°	75.00°		
$C_6H_5SO_2OMe$:							
56.0 % acetone		71.0	201	529		20 600	9.25
$C_6H_5SO_2OEt$:							
Pure water	103	577	1 740			22 030	11.13
3.0 % acetone	93.0	530	1 610			21 800	10.95
7.0 » »	79.3	465	1 290			21 410	10.60
17.8 » »	45.8	259	745			21 370	10.32
36.5 » »	15.1	90.4	257			21 730	10.11
56.0 » »		29.1	86.8		974	21 730	9.64
80.0 » »	1.10	6.40	18.2		201	21 460	8.78
$C_6H_5SO_2OPr(n)$:							
56.0 % acetone		16.3	48.2	21.7 (65.0°)		21 800	9.42
$C_6H_5SO_2OPr(iso)$:							
Pure water	7 750	42 200	120 000			21 000	12.27
17.8 % acetone	1 490	8 430	24 600			21 470	11.91
36.5 » »	237	1 400	4 180			22 000	11.50
56.0 » »	43.1	284	839			22 780	11.34
80.0 » »	4.75	30.3	100			23 310	10.76
65.9 » dioxan			646				
$C_6H_5SO_2OBu(sec.)$							
56.0 % acetone	66.7	390	1 230			22 280	11.15

other hand it augments the repulsion between the attacking reagent and the said C atom. The latter effect is more powerful and the net result is opposite to that in S_N1. Steric effects also may hinder S_N2 reaction.

When the repulsion between the attacking reagent and the seat of reaction is relatively small, the energy of activation is determined by this repulsion energy. Such is the case in the hydrolysis of the sulphonates of primary alcohols. If the group $-CH_2R'$ be sufficiently varied in the direction of increasing negative charge on C, the energy of activation will no longer be determined primarily by the repulsion between this C atom and attacking reagent, for it may now require less energy to extend the bond O—C than force a hydroxyl ion or the negative portion of a water dipole right up to C. This means a change of mechanism ($S_N2 \rightarrow S_N1$). The data of Table 7 actually show that in the

same solvent, e.g. in 56 per cent acetone, the energy of activation for the hydrolysis of esters of primary alcohols follows the order

$$CH_3 < C_2H_5 < C_3H_7$$

i.e. the order of increasing negative charge on the C atom, but is considerably lower than E for the hydrolysis of *iso*propyl ester. On the other hand, the energy of activation is, as required by the theory, less for the hydrolysis of *sec.*-butylester than for *sec.*-propylester.

The influence of the solvent. Table 7 shows that in acetone-water mixtures the specific reaction rate k_1 of the water hydrolysis decreases enormously with decreasing water content of the solvent. From Fig. 1 we see that in the range between 0 and 80 per cent acetone log k_1 for the *iso*propyl ester is practically a linear function of the acetone percentage. The effect of the solvent is appreciably greater in the hydrolysis of *iso*propyl benzenesulphonate than in that of ethyl benzenesulphonate. Although for both reactions the curves in Fig. 1 are greatly similar, the parameters of the Arrhenius equation behave differently in the two series. For the hydrolysis of *iso*propyl benzenesulphonate E increases continuously with the percentage of the acetone in the solvent, whereas for the hydrolysis of ethyl benzenesulphonate E in low acetone contents has a trough and in high acetone contents falls again. In the latter case A changes in the same direction as E, whereas in the hydrolysis of the *iso*propyl ester the changes of A and E take place in opposite directions. This entirely different behaviour of E and A is clearly an indication of different reaction mechanisms in the two cases. The trough in E is also found for the alkaline hydrolysis of carboxylic esters, which, too, is a bimolecular reaction.

The influence of the solvent may be assessed by considering the total solvation of the transition state in relation to those of the reactants, which solvation depends on the magnitude and distribution of the charges in the two states. If the transition state is solvated to a greater extent than the reactants, the activation energy is lowered. There is no doubt that solvation plays a part in the bond fission of the mechanism S_N1, and that the ionizing effect of the water-acetone mixtures increases with the water content. Thus the reaction is the faster the greater the water content of the solvent. In the case of the bimolecular (S_N2) mechanism the observed changes of E and A have an opposite effect on the reaction velocity, and thus the solvent effects are more complicated.

The inhibiting effect of dioxan is somewhat greater than that of acetone. Table 7 shows the value of k at 50° C in a 65.9 per cent dioxan-water, in which mixture the mole fraction of water is the same ($x_{water} = 0.717$) as in 56 per

cent aqueous acetone. This different effect is probably, partly at least, due to the formation of hydrogen bonds between water and the organic solvent component from which it comes, so that only a part of the water molecules are quite free to participate in the reaction or in the solvation phenomena. This part becomes smaller with the decrease of the water content of the solvent, and is, at the same water content, smaller in dioxan-water than in acetone-water, since a dioxan molecule with its two oxygen atoms can join two water molecules with hydrogen bonds, while an acetone molecule can join only one.

The alcoholysis. In pure absolute methanol the alcoholysis of *iso*propyl benzenesulphonate

$$C_6H_5SO_2OPr(i) + MeOH \rightarrow C_6H_5SO_2OH + MeOPr(i)$$

is a first order reaction (Table 8). The most probable values obtained are:

	25.00°	40.00°	50.00°	E cal	log A
10^6k_1 sec.$^{-1}$	1.95	12.5	39.2	22 980	11.13

This reaction is also much (at 50° about five times) faster than the corresponding reaction of ethyl benzenesulphonate. As in hydrolysis, the activation energy and the frequency factor are higher than for the reaction of ethyl benzenesulphonate, for which alcoholysis in methanol[1] $k_{50°} = 8.00 \cdot 10^{-6}$, $E =$ 22 090 cal, log $A = 9.84$. Alkali affects alcoholysis, as will be seen from Tables 8 and 9, but in the presence of an equivalet concentration of sodium hydroxide the second order formula gives for k values which continuously increase *,

Table 8. The alcoholysis of isopropyl benzenesulphonate in abs. methanol at 50.00° C.

t mins.	90	120	180	240	360	420	480
p	19.2	24.5	34.6	43.2	57.0	62.7	67.1
10^5k_1 sec.$^{-1}$	3.97	3.91	3.94	3.93	3.91	3.92	3.86

Table 9. The alcoholysis of isopropyl benzenesulphonate in abs. methanol in presence of alkali at 50.00°. $c_{Ester} = c_{NaOH} = 0.05$ moles/liter.

t mins.	45	60	90	120	150	180	210	300	360
p	15.3	20.5	26.9	34.2	41.1	46.3	49.7	61.9	66.4
10^5k_1 sec.$^{-1}$	6.14	6.39	5.81	5.82	5.88	5.76	5.45	5.36	5.05
10^5k_2 l mol^{-1} sec.$^{-1}$	133	134	137	145	155	160	157	181	183

* Owing to the equilibrium $CH_3OH + NaOH \rightleftharpoons CH_3ONa + H_2O$ the alkaline solution contains a little of water. However, experiments in absence of alkali, in methanol containing 60 mg of water in 50 ml, showed that in this solution the reaction velocity is approximately the same as in water free methanol. Thus the small water content of the solution cannot cause the shift in k

the first order formula values which continuously decrease. This indicates that in the presence of alkali, alcoholysis takes place partly by S_N1, partly by S_N2 mechanism. A transesterification, catalyzed by the alkali, may in a measure occur, but it cannot seriously disturb the picture. In the absence of alkali, the alcoholysis of *iso*propyl benzenesulphonate is very likely to occur predominantly by S_N1 mechanism, inasmuch as it is so much faster than the corresponding reaction of the ethyl ester.

In *iso*propyl alcohol the velocity of the alcoholysis of *iso*propylbenzene sulphonate is only about one tenth of that in methanol (observed: $k_1 = 0.388$ 10^{-5} sec.$^{-1}$ at 50°). The acceleration by alkali is greater (at 50° from the first order formula $k_1 < 20 \cdot 10^{-5}$, from the second order formula $k_2 \approx 45 \cdot 10^{-5}$, the latter early constant) than in methanol, in accordance with the fact that *iso*propanol is a less ionizing solvent than methanol.

With sodium *iso*propylate in isopropanol *iso*propyl benzenesulphonate reacts with about the same velocity as with sodium hydroxide and the reaction is mainly bimolecular (at 50° $k_2 = 46 \cdot 10^{-5}$ l mol^{-1} sec.$^{-1}$).

SUMMARY

The hydrolysis of *iso*propyl benzenesulphonate and *sec.*-butyl benzenesulphonate in aqueous acetone is a two-stage reaction. The alcoholysis of these esters takes place partly by a unimolecular, partly by a bimolecular mechanism. The solvolyses of *n*-alkyl sulphonates are presumably one-stage reactions. The solvent effects in the hydrolytic reactions of sulphonic esters were studied.

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The Chemistry of the Natural Order *Cupressales*

IX*. The Synthesis of Hexahydrothujic Acid

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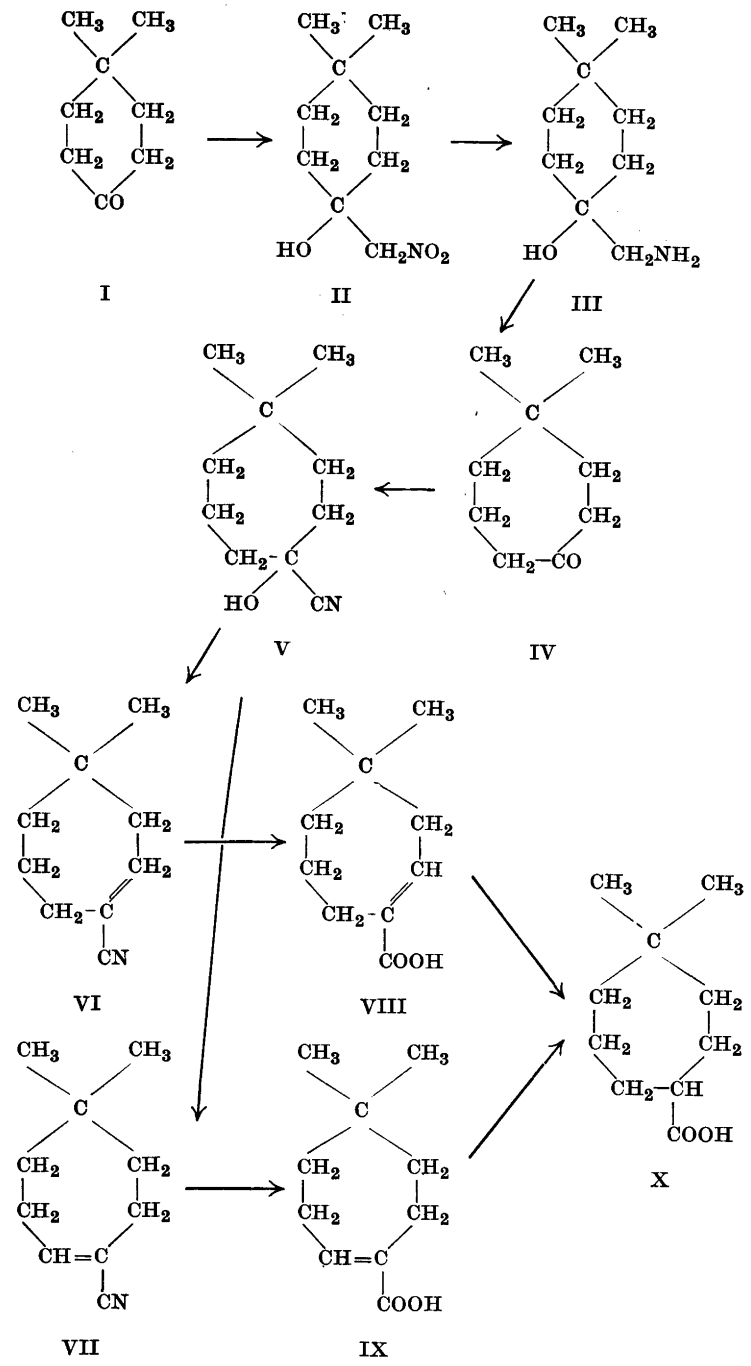
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In a previous communication [1] the partial synthesis of thujic acid from hexahydrothujic acid was reported. In order to complete the total synthesis of thujic acid the synthesis of hexahydrothujic acid remained to be carried out. This has now been done and forms the subject of the present paper.

A convenient way of obtaining the starting material for this synthesis *viz.* 4,4-dimethylcyclohexanone, has been described by Miller and Adams [2] who obtained it by catalytic hydrogenation of 4-methyl-4-dichloromethylcyclohexa-2,5-dienone and oxidation of the 4,4-dimethylcyclohexanol obtained. The drawback of this method is the low yield obtained in the preparation of 4-methyl-4-dichloromethylcyclohexa-2,5-dienone as described by Auwers and Keil. [3] We therefore investigated the hydrogenation of the more readily obtainable 4-methyl-4-trichloromethylcyclohexa-2,5-dienone [4]. However, in spite of several experiments with different catalysts under different conditions the desired material could not be obtained, and the reduction products always contained chlorine. These were not studied further, and this approach was abandoned, in favour of the original method of Miller and Adams [2].

The 4,4-dimethylcyclohexanone (I) was next converted into 4,4-dimethylcycloheptanone (IV). This was first carried out by diazomethane ring enlargement [5], but this method gave only a poor yield of the desired ketone and the method described by Dauben Jr., Ringold, Wade and Anderson Jr.[6] proved much more satisfactory. (The author wishes to express his thanks to Dr. Anderson for placing the information contained in this paper to his disposal prior to publication). This method, with slight modifications, gave a reasonable yield of 4,4-dimethylcycloheptanone (IV). This compound has been

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I
II
III
IV
V
VI
VII
VIII
IX
X

obtained as a degradation product of thujic acid [7] and comparison of the semicarbazones and bis-*p*-nitrobenzylidene derivatives established the identity of the two ketones.

The 4,4-dimethylcycloheptanone (IV) was converted via the cyanohydrin (V) and the unsaturated nitrile (presumably a mixture of VI and VII) into the unsaturated acid, which evidently was a mixture of (IX) and (X). These acids are the same as those obtained from hexahydrothujic acid and in view of the difficulty of separating them [1], the mixture was hydrogenated directly to 4,4-dimethylcycloheptanecarboxylic acid (X), a liquid which was characterised as its p-bromophenacyl ester and amide. These derivatives were identical (mixed m.p.) with the corresponding derivatives of hexahydrothujic acid obtained by the hydrogenation of thujic acid. The reactions used in this synthesis are outlined in the chart on p. 855.

These reactions thus complete the total synthesis of thujic acid.

EXPERIMENTAL

4,4-Dimethyl-1-nitromethylcyclohexanol (II). A mixture of dimethylcyclohexanone (8.4 g) and nitromethane (6.5 g) was added dropwise to a stirred solution of sodium (1.55 g) in absolute alcohol (35 ml). The temperature rose gradually to 40° and a thick white precipitate formed. Stirring was continued for an additional 4 hours. The mixture was cooled and the precipitate, collected by filtration, was dried on a porous plate and treated, with cooling, with acetic acid (5 g) in water (35 ml). The oil which separated was taken up in ether, and the ether solution was washed with a saturated salt solution, dried, and evaporated. The residue distilled as a thick colourless oil. (Found: C, 57.7; H, 8.9; $C_9H_{17}O_3N$ requires C, 57.7; H, 9.2 %.)

4,4-Dimethylcycloheptanone (IV). 4,4-Dimethyl-1-nitrometnylcyclohexanol (9 g) was dissolved in acetic acid (50 ml) and hydrogenated at 2 atm. pressure in the presence of Raney-nickel catalyst. The mixture was warmed to about 30°, to start the reaction and this temperature was maintained until 3.2 l of hydrogen had been absorbed (theoretical 3.3 l). Most of the acetic acid was evaporated under reduced pressure and water (60 ml) was added. The clear stirred solution of the acetate of 4,4-dimethyl-1-aminomethylcyclohexanol (III) was treated at −5° with sodium nitrite (6.5 g) dissolved in water (20 ml). Stirring was continued until evolution of nitrogen had ceased, and the mixture was set aside over night and then steam distilled. The product which distilled was taken up in ether and treated with a saturated solution of sodium bisulphite. The bisulphite compound was decomposed with sodium hydrogen carbonate and the oil thus obtained distilled, giving 4,4-dimethylcycloheptanone as a colourless oil (2.3 g; 35 %). b.p. 69−72°/7 mm (Found: C, 76.9; H, 11.7; $C_9H_{16}O$ requires C, 77.1; H, 11.5 %).

4,4-Dimethylcycloheptanone was characterised as its semicarbazone, m.p. 173−174° and bis-p-nitrobenzylidene derivative, m.p. 158−160°, both of which were identical (mixed m.p.) with the corresponding derivatives obtained by degradation of thujic acid [7].

4,4- and 5,5-Dimethylcycloheptenyl cyanides (VI and VII). A mixture of 4,4-dimethylcycloheptanone (2.0 g), sodium bisulphite (3.0 g) and water (5 ml) was stirred until it formed a thick mass. Thereafter a solution of potassium cyanide (1.5 g) in water (3 ml) was

added dropwise and the stirring was continued for a further 4 hours. The 4,4-dimethylcycloheptanone cyanohydrin thus formed was extracted with ether, and the ether solution was washed with dilute hydrochloric acid and evaporated. The residual oil, without further purification, was dissolved in pyridine (2 ml) and thionyl chloride (1.5 ml) was added. The mixture turned brown and heat was evolved. The following morning the excess of thionyl chloride was evaporated under reduced pressure and the residue was poured into water. The aqueous solution was extracted with ether and the ether solution was washed with hydrochloric acid and sodium bicarbonate and evaporated, yielding an oil which distilled at 92—104° /8 mm (yield 1.1 g). In view of the large boiling interval and the analysis (Found: C, 78.4; H, 9.9; $C_{10}H_{15}N$ requires C, 80.5; H, 10.2 %), this material was apparently not quite pure and apparently contained some unreacted 4,4-dimethylcycloheptanone cyanohydrin.

4,4- and 5,5-Dimethylcycloheptene carboxylic acids (VIII and IX). The crude unsaturated cyanide obtained above (1.0 g) was boiled with sodium hydroxide (2.0 g) in water (4 ml) and alcohol (10 ml) until no more ammonia was evolved (48 hours). The alcohol was distilled off and water was added to the residue. The oily acid which precipitated on acidification was taken up in ether, and the ether solution was extracted with sodium carbonate solution. The acid recovered from the sodium carbonate solution was still an oil and was distilled in vacuum giving 0.4 g of an oil that rapidly solidified, m.p. 45—55°. No attempt was made to separate the isomerides. (Found: C, 71.0; H, 9.6; $C_{10}H_{16}O_2$ requires C, 71.4; H, 9.6 %.)

4,4-Dimethylcycloheptanecarboxylic acid (X). The mixture of 4,4- and 5,5-dimethylcycloheptenecarboxylic acids (0.4 g) dissolved in alcohol was hydrogenated in the presence of a PtO_2-catalyst (30 mg). The uptake of hydrogen amounted to 51 ml (calc. 53 ml). The catalyst was removed and the alcohol was evaporated, giving 4,4-dimethylcycloheptanecarboxylic acid as a colourless oil. The substance was characterised as its p-bromophenacyl ester and amide. The former was first obtained as long needles, m.p. 69—70°, but after recrystallisation from dilute methanol formed leaflets, m.p. 66—67°. (Found: C, 58.8; H, 6.3; $C_{18}H_{23}O_3Br$ requires C, 58.5; H, 6.3 %.) The same dimorphism is observed with the p-bromophenacyl ester of hexahydrothujic acid [7] and the mixed m.p. was not depressed. The amide was obtained as leaflets, m.p. 146—147°. (Found: C, 71.2; H, 11.2; $C_{10}H_{19}ON$ requires C, 70.9; H, 11.3 %) undepressed in admixture with the amide of hexahydrothujic acid, m.p. 146.5—147.5 [7].

SUMMARY

The synthesis of 4,4-dimethylcycloheptanecarboxylic acid (hexahydrothujic acid) is described. This synthesis completes the total synthesis of thujic acid.

The analyses were carried out by Mr. J. Hukki of this Department.

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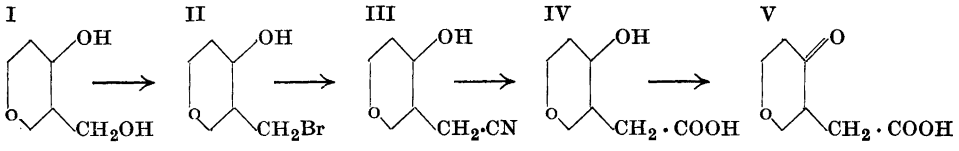
Über die Umsetzung von Formaldehyd mit Butadien *

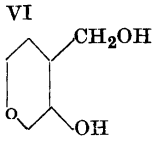
II. Zur Frage der Konstitution der Reaktionsprodukte

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Anlässlich einer vor kurzem durchgeführten Untersuchung über die Umsetzung von Formaldehyd mit Butadien bzw. Δ^3-Dihydropyran schrieben wir dem bei der Reaktion in beiden Fällen entstehenden C_6-Glykol die Formel eines 3-Oxymethyl-4-oxy-tetrahydropyrans (I) zu [1]. Als wesentlichstes Argument für die Richtigkeit dieser Formel führten wir an, dass sich das Glykol über das Bromid (II), das Nitril (III) und die Oxysäure (IV) in die Dihydro-desoxy-patulinsäure (V) überführen liess — eine Reaktionsfolge, die u.E. jede andere Formulierung ausschliesst.



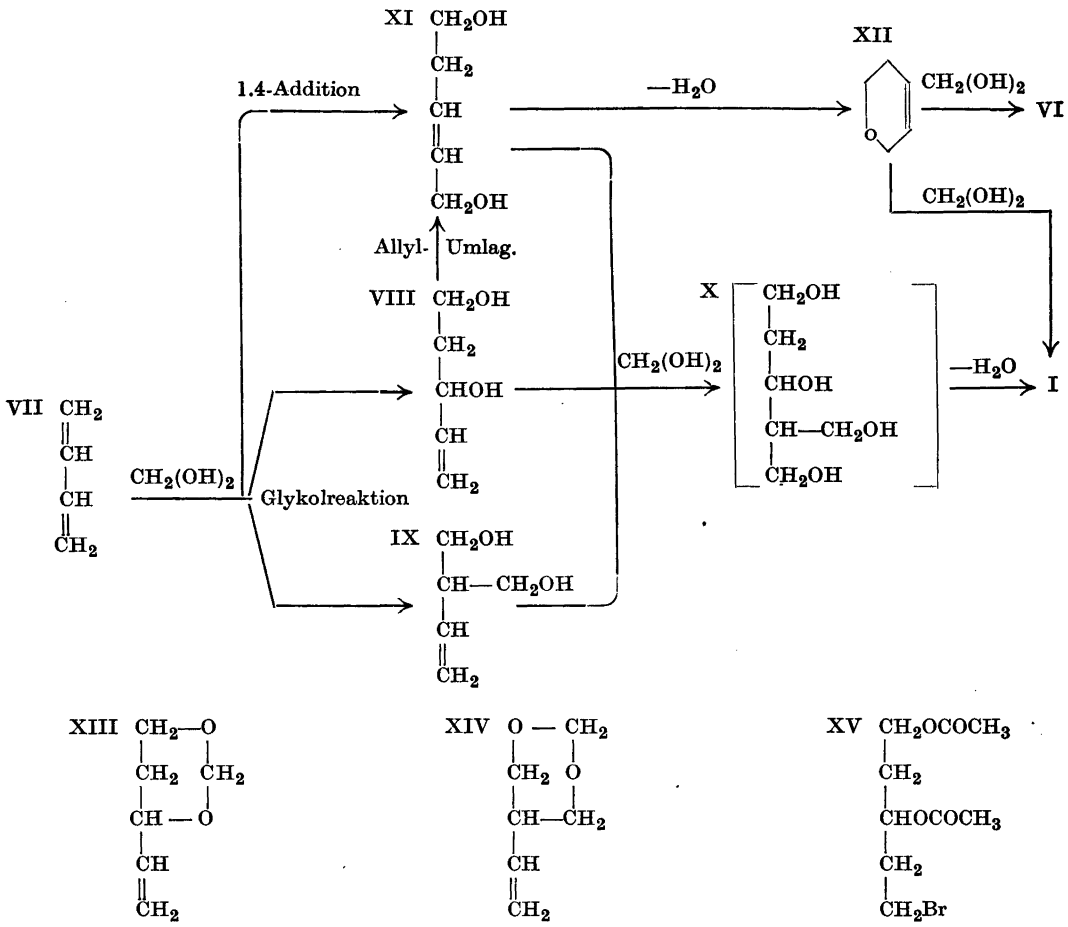


Wie wir erst jetzt erfahren, haben einerseits Gresham und Grigsby [2], andererseits Friedrichsen [3] die Umsetzung von Butadien mit Formaldehyd in wässriger Schwefelsäure zum Gegenstand von Patentanmeldungen gemacht, in denen für das Glykol neben der Formel I die strukturisomere Formel VI in

* 13. Mitteilung über Formaldehyd-Olefin-Reaktionen.

Betracht gezogen wird. Dermer, Kohn und Nelson [4], die die Reaktion näher studierten, neigen darüber hinaus auf Grund eines an einem unreinen Präparat beobachteten positiven Perjodattestes zu der Auffassung, dass dem Glykol die Formel VI zukommt.

Diese Unstimmigkeit veranlasste uns zu einer neuen Untersuchung. Bevor wir über deren Ergebnisse berichten, sei dem vorliegenden Gegenstand eine kurze theoretische Behandlung gegeben: Die Frage nach der Konstitution der Reaktions-*End*produkte (C_6-Glykol bzw. dessen Derivate) ist eng verknüpft mit der Natur der Reaktions-*Zwischen*produkte (C_5-Glykole bzw. deren Derivate), wobei man bedenken muss, dass in wässrigem Medium die freien Glykole bzw. deren Methylenäther, in Eisessig dagegen hauptsächlich die den Glykolen entsprechenden Acetate entstehen. Wenn äquimolekulare Mengen Formaldehyd (Methylenglykol!) und Butadien (VII) in wässriger Schwefelsäure miteinander in *normaler* Weise unter 1,3-Glykolbildung (»Glykolreaktion») reagieren, sind nur *z w e i* Reaktionsprodukte möglich: das Penten-(1)-diol-(3,5) (VIII) und das 2-Vinyl-trimethylenglykol (IX). Beide diese Glykole können durch erneute Reaktion mit Formaldehyd über das hypothetische Tetrol X nur *e i n*(!) Pyranderivat liefern, nämlich das 3-Oxymethyl-4-oxy-tetrahydropyran (I). Diese Tatsache allein ist jedoch für die Konstitution des C_6-Glykols nicht beweisend, nachdem von verschiedenen Autoren [2–4] unter den Reaktionsprodukten mit Sicherheit das Δ^3-Dihydropyran (XII) nachgewiesen wurde, das nicht ohne weiteres aus den Glykolen VIII bzw. IX hervorgegangen sein kann. Man muss daher neben dem »*normalen*» einen »*anomalen*» Reaktionsverlauf postulieren. Für einen solchen wären folgende Möglichkeiten diskutierbar: Man könnte annehmen, dass sich Butadien und Formaldehyd im Sinne einer Diensynthese unter Bildung von Δ^3-Dihydropyran vereinigen, jedoch reagierten die reinen Komponenten nicht miteinander [5]. Wahrscheinlicher wäre dann unter den vorliegenden Bedingungen schon eine 1,4-Addition des Methylenglykols an das Butadien unter Bildung des Penten-(2)-diol-(1,5) (XI), das durch Ringschluss in das Δ^3-Dihydropyran übergehen könnte. Eine dritte und vielleicht am nächsten liegende Möglichkeit wäre jedoch die, dass das Penten-(2)-diol-(1,5) durch Allylumlagerung aus dem Penten-(1)-diol-(3,5) hervorgeht (VIII → XI). Nur über das Penten-(2)-diol-(1,5) bzw. das durch Ringschluss daraus entstehende Δ^3-Dihydropyran, also die »anomalen» Zwischenprodukte, wäre die Entstehung des Glykols VI denkbar, während sich das Glykol I sowohl über die »normalen» als auch über die »anomalen» Zwischenprodukte bilden kann. Aus statistischen Gründen ist dessen Bildung daher von vorn herein wahrscheinlicher.



XIII

XIV

XV

Um die Natur der Reaktions-Zwischenprodukte kennen zu lernen, haben wir im Gegensatz zu den genannten Autoren und zu unseren früheren Versuchen Butadien und Formaldehyd im Molverhältnis ca. 1 : 1 in Eisessig-Schwefelsäure umgesetzt. Wir erwarteten, dass unter diesen Bedingungen nur eine Doppelbindung des Butadiens unter Entstehung von Penten-(1)-diol-(3,5)-diacetat (Diacetat des Glykols VIII) bzw. 2-Vinyl-trimethylenglykol-diacetat (Diacetat des Glykols IX) reagieren würde. Die Bildung wenigstens einer dieser Verbindungen war auch deshalb zu vermuten, weil alle die genannten Autoren aus dem Reaktionsgemisch ein 1,3-Dioxanderivat isoliert haben, dem Gresham und Grigsby sowie Dermer, Kohn und Nelson die Formel eines 4-Vinyl-1,3-dioxan (XIII) (Methylenäther des Glykols VIII!), Friedrichsen die Formel eines 5-Vinyl-1,3-dioxan (XIV) (Methylenäther des Glykols IX!) zuschreiben,

ohne jedoch einen bündigen Beweis für die Richtigkeit ihrer Annahme zu führen. — Unser Versuch brachte in zweierlei Hinsicht eine Überraschung: Wir erhielten nämlich einerseits als Hauptfraktion nicht die erwarteten Diacetate der Glykole VIII bzw. IX, sondern das Penten-(2)-diol-(1,5)-diacetat vom Sdp_{13} 124—128° (Diacetat des Glykols XI) und andererseits unter den höher siedenden Anteilen 3-Acetoxymethyl-4-acetoxy-tetrahydropyran vom Sdp_{13} 139—143° (Diacetat des Glykols I) — also das Reaktionsprodukt des Butadiens mit zwei (!) Molekülen Formaldehyd! — Von den in geringer Menge isolierten niedriger siedenden Anteilen verdient eine Substanz vom Sdp_{13} 105—110° Erwähnung, die nach ihren Kennzahlen, ihrer Molrefraktion, dem Ergebnis der Elementaranalyse und ihrem sonstigen chemischen Verhalten (Überführbarkeit in ein Glykol) eines der beiden isomeren Diacetate des Penten-(1)-diol-(3,5) (VIII) oder des 2-Vinyl-trimethylenglykol (IX) enthalten könnte. Diese Verbindung stimmt aber nun in allen Eigenschaften überein mit einem aus 1-Brom-pentandiol-(3,5)-diacetat (XV) durch Kochen mit Natriumacetat in Essigsäureanhydrid erhaltenen Pentendiol-diacetat, das auf Grund seiner Entstehung keine verzweigte Kohlenstoffkette enthalten und daher nicht mit dem 2-Vinyl-trimethylenglykol-diacetat identisch sein kann. Hieraus ergibt sich sowohl für das aus Butadien wie für das aus 1-Brom-pentandiol-(3,5)-diacetat erhaltene Diacetat eindeutig die Formel eines Penten-(1)-diol-(3,5)-diacetates. Bemerkenswert ist, dass sich dieses auch aus 1-Brom-pentandiol-(3,5)-diacetat in wesentlich geringerer Menge bildete als das Penten-(2)-diol-(1,5)-diacetat. Die Bildung von Penten-(2)-diol-(1,5)-diacetat beruht bei beiden Versuchen wahrscheinlich auf der gleichen Ursache, nämlich auf einer Verschiebung der Allylgruppierung in dem primär entstehenden Penten-(1)-diol-(3,5)-diacetat. — Das Penten-(2)-diol-(1,5)-diacetat liess sich zu dem von Paul und Tchelitcheff[6] beschriebenen Penten-(2)-diol-(1,5) (XI) umestern, durch Hydrierung mit Platinoxyd in Eisessig in das Pentandiol-(1,5)-diacetat und durch Umsetzung mit Formaldehyd in Eisessig-Schwefelsäure in das erwähnte 3-Acetoxymethyl-4-acetoxy-tetrahydropyran überführen. Es erscheint somit als ein Reaktions-Zwischenprodukt, das gleichzeitig eine Brückenstellung einnimmt bei der Bildung des Δ^3-Dihydropyrans. — Aus der unter den vorliegenden Bedingungen überraschend erfolgenden Bildung des 3-Acetoxymethyl-4-acetoxy-tetrahydropyrans schliessen wir, dass die zunächst entstehenden ungesättigten C_5-Glykole bzw. deren Acetate mit Formaldehyd sehr viel rascher reagieren als das Butadien selbst. — Die Frage, ob bei der Reaktion *neben* dem 3-Acetoxymethyl-4-acetoxytetrahydropyran auch das strukturisomere 3-Acetoxy-4-acetoxymethyl-tetrahydropyran entsteht, wäre dahingehend zu beantworten, dass wir hierfür keinerlei Anthaltspunkte gefunden haben. Sowohl bei der Umsetzung von Butadien als auch Δ^3-Dihydropyran

mit Formaldehyd in Eisessig-Schwefelsäure erhielten wir stets sehr einheitlich siedende Diacetatfraktionen. Aus der Butadien-Formaldehyd-Umsetzung im Molverhältnis 1 : 1 haben wir zum ersten Male das 3-Acetoxymethyl-4-acetoxy-tetrahydropyran in krystallinischer Form (Schmp. 43—46°) gewonnen. Das krystallisierte Diacetat liess sich zum freien Glykol vom Sdp_8 156,5° umestern, das im Brechungsindex und in der Dichte ausgezeichnet mit den von Gresham und Grigsby und Hatch und Evans [7] angegebenen Werten übereinstimmt und in das früher [1] beschriebene Bisphenylurethan vom Schmp. 144—145° überführbar ist. — Zwecks einer eindeutigen, von unserer früherer Beweisführung unabhängigen Sicherstellung der Formel I haben wir das Glykol nach der Vorschrift von Hatch und Evans aus Acrolein bereitet und nach Überführung in das Bisphenylurethan mit unserem eigenen Präparat identifiziert.

EXPERIMENTELLER TEIL

Umsetzung des Butadiens mit Formaldehyd. Eine Mischung aus 20 g Paraformaldehyd, 500 ml Eisessig und 5 ml konz. Schwefelsäure wurde unter Kühlung mit Kohlensäureschnee im Autoklaven mit 40 g kondensiertem Butadien versetzt und 2 ½ Stunden auf 150—180° erhitzt. Das erkaltete Reaktionsgemisch wurde nach Zusatz von 10 g wasserfreier Soda zunächst bei gewöhnlichem Druck destilliert, wobei eine geringe Menge nicht umgesetztes Butadien, dann zwischen 112—120° im wesentlichen die überschüssige Essigsäure (Destillat A) überging. Der Rückstand wurde zuerst an der Brücke, dann zweimal an der Widmerspirale unter vermindertem Druck destilliert. Man erhielt folgende Fraktionen:

				V.Z.	*J.Z.*	
1)	$Sdp._{13}$	67—105°	2,6 g	463,9	98,8	
2)	»	105—110°	3,2 »	552,0	129,6	$n_D^{20} = 1{,}4363$; $D_4^{20} = 1{,}0555$
3)	»	110—124°	2,1 »	575,2	141,3	
4)	»	124—128°	11,2 »	585,7	134,7	$n_D^{21} = 1{,}4444$; $D_4^{21} = 1{,}0657$
5)	»	128—139°	1,2 »	575,2	114,2	
6)	»	139—143°	2,4 »	—	—	

Die Fraktion 2) besteht aus *Penten-(1)-diol-(3,5)-diacetat* (Theorie V.Z. 602,6 J.Z. 136,3) MR = 46,18 (ber. 46.61)

$C_9H_{14}O_4$(168.2)	Ber.	C	58,05	H	7,58	
	Gef.	»	58,51	»	7,89	

Durch Umesterung des Diacetates erhielt man *Penten-(1)-diol-(3,5)* (VIII) Sdp_{14} 103—106°.

Bisphenylurethan aus Alkohol Schmp. 103—103.7°. Mischschmp. mit dem später beschriebenen Präparat unverändert!

$C_{19}H_{20}O_4N_2$(340.4)	Ber.	C	67,04	H	5,92	N	8,23
	Gef.	»	66,78	»	5,87	»	7,96

Die Fraktion 4) besteht aus *Penten-(2)-diol-(1,5)-diacetat* (Theorie V.Z. 602,6, J.Z. 136,3) MR = 46,39 (ber. 46,61)

$C_9H_{14}O_4$(186.2)	Ber.	C	58,05	H	7,58	
	Gef.	»	57,88	»	7,46	

Durch Umesterung des Diacetates erhielt man *Penten-(2)-diol-(1,5)* (XI) Sdp_{15} 136—138° (Paul u. Tchelitcheff geben $Sdp._5$ 85° an) *Bisphenylurethan* aus Alkohol Schmp. 159—160,5°

$C_{19}H_{20}O_4N_2$(340,4)	Ber.	C	67,04	H	5,92	N	8,23
	Gef.	»	66,94	»	6,05	»	8,28

Mischschmp. mit einem aus dem früher beschriebenen Crackversuch erhaltenen Präparat [8] zeigt keine Depression.

Durch *Hydrierung der Fraktion 4)* in Eisessig mit Platinoxyd erhielt man *Pentandiol-(1,5)*-diacetat, das durch Umesterung in das *Pentandiol-(1,5)* überführt wurde.

Bisphenylurethan aus Benzol Schmp. und Mischschmp. mit authentischem Präparat 172—174°.

Die Fraktion 6) besteht aus *3-Acetoxymethyl-4-acetoxy-tetrahydropyran.* Durch Umesterung erhielt man daraus das freie *3-Oxymethyl-4-oxy-tetrahydropyran* (I).

Bisphenylurethan aus Äthanol Schmp. und Mischschmp. 144—145°.

Aus dem Essigsäure enthaltenden Vorlauf (Destillat A) wurden 2 g einer farblosen, stark ungesättigten Flüssigkeit vom Sdp. 81—90°/760 mm herauspräpariert, die wahrscheinlich im wesentlichen aus Δ^3-*Dihydropyran* besteht.

Aus einer anderen Butadien-Formaldehyd-Umsetzung (Molverhältnis 1 : 1) erhielt man 17 g einer farblosen, beim Sdp_8 134—138° destillierenden Flüssigkeit, die bald krystallisierte. Die Verbindung wurde zweimal aus Alkohol umkrystallisiert, schmolz dann bei 43—46° und erwies sich als reines *3-Acetoxymethyl-4-acetoxy-tetrahydropyran* V.Z. 523 (ber. 518); $n_D^{21} = 1.4530$ (im unterkühlten Zustand!).

$C_{10}H_{16}O_7$(216.2)	Ber.	C	55,54	H	7,46	
	Gef.	»	55,66	»	7,49	

Daraus gewann man durch Umesterung das reine *3-Oxymethyl-4-oxy-tetrahydropyran* (I) als dickflüssiges, farbloses Öl vom Sdp_8 156,5°, $n_D^{22} = 1{,}4860$, $D_4^{22} = 1{,}1837$

Bisphenylurethan wie oben Schmp. und Mischschmp. 144—145°.

Beim Impfen der früher aus Δ^3-Dihydropyran erhaltenen flüssigen Diacetatfraktionen mit dem vorstehend beschriebenen krystallinen Diacetat erfolgte Krystallisation. Man gewann so das krystalline Diacetat in einer Ausbeute von ca. 45 %.

Umsetzung des Penten-(2)-diol-(1,5)-diacetates mit Formaldehyd. Durch Umsetzen von 14 g Penten-(2)-diol-(1,5)-diacetat mit Paraformaldehyd in Eisessig-Schwefelsäure erhielt man nach exothermer Reaktion und kurzem Kochen 10,7 g *3-Acetoxymethyl-4-acetoxy-tetrahydropyran.* Das daraus gewonnene *Bisphenylurethan* schmilzt bei der Mischprobe mit einem authentischen Präparat unverändert bei 144—145°.

3-Oxymethyl-4-oxy-tetrahydropyran (I). Eine Mischung aus ca. 60 ml Acrolein, 280 ml Wasser, 1 g Hydrochinon und 4 ml konz. Schwefelsäure wurde 20 Stunden auf 30°,

dann 6 Stunden auf ca. 50° erwärmt. Die Schwefelsäure wurde sodann durch Zusatz von 25 g Bariumcarbonat gebunden. Nach dem Abfiltrieren der Bariumsalze wurde das Filtrat im Vakuum bis zur Ölkonsistenz eingedampft. 47 g des dickflüssigen Rückstandes hydrierte man nach dem Auflösen in 100 ml Alkohol im Autoklaven mit 18 g Raney-Nickel bei einem Wasserstoff-Anfangsdruck von 145 atü und langsam bis 111° steigender Temperatur. Nachdem der Druck auf 70 atü gefallen war, wurde der ursprüngliche Wasserstoffdruck durch Nachpressen wiederhergestellt und solange hydriert, bis der Druck bei 120 atü konstant blieb. Die Hydrierung dauerte etwa 3 Stunden. Nach dem Abfiltrieren des Katalysators gewann man neben einer grösseren Menge niedriger siedender Produkte 10,5 g des *Glykols* vom Sdp_{10} 158—160° als dickflüssiges, farbloses Öl, $n_D^{20} = 1{,}4825$, $D_4^{20} = 1{,}1736$, MR = 32,14 (ber. 32,42). *Bisphenylurethan* aus Akolhol Schmp. und Mischschmp. mit dem aus Butadien gewonnenen Präparat unverändert 144—145°.

Umsetzung des 1-Brom-pentandiol-(3,5)-diacetates mit Natriumacetat

(Experimentell bearbeitet von Jacob Gundersen)

Eine Mischung aus 957 g 1-Brom-pentandiol-(3,5)-diacetat [9], 600 g Essigsäureanhydrid und 590 g wasserfreiem Natriumacetat kochte man 14 Stunden unter Rückflusskühlung. Das Salz wurde abgesaugt und das Filtrat erneut mit 400 g wasserfreiem Natriumacetat 17 Stunden gekocht. Nun wurde unter vermindertem Druck vom Salz abdestilliert und das Destillat mehrmals fraktioniert. Als Hauptfraktionen erhielt man:

				V.Z.	*J.Z.*	
1)	$Sdp._8$	96 — 97,5°	16,6 g	—	—	$D_4^{21} = 1.0475$
2)	»	110,5—112,5°	129,8 »	602,4	126,7	$D_4^{20,3} = 1{,}0585$, $n_D^{20,5} = 1{,}4421$, MR = 46,55
3)	»	150 —151°	481,5 »	683,6	0,0	$D_4^{21,5} = 1{,}1209$, $n_D^{21,5} = 1{,}4360$, MR = 57,44

Die Fraktion 1) besteht aus *Penten-(1)-diol-(3,5)-diacetat.* Durch Verseifung mit methylalkoholischer Kalilauge und anschliessende Destillation gewann man das *Penten-(1)-diol-(3,5)* als farbloses Öl, das im wesentlichen beim $Sdp._{10}$ 105—106,5° ($n_D^{21} = 1{,}4500$) überging. *Bisphenylurethan* aus wasserfreiem Benzol Schmp. und Mischschmp. mit dem aus Butadien erhaltenen Präparat 103—103,7°. *Die Fraktion 2)* besteht aus *Penten-(2)-diol-(1,5)-diacetat* (Theorie: V.Z. 602,6, MR = 46,61).

Die Fraktion 3) besteht aus *Pentantriol-(1,3,5)-triacetat,* das nach dem Ergebnis der Cariusbestimmung ca. 1 % gebundenes Brom enthält (Theorie: V.Z. 683,6, MR = 57,97).

ZUSAMMENFASSUNG

Butadien und Formaldehyd in Eisessig-Schwefelsäure reagieren in normaler Weise unter Bildung von Penten-(1)-diol-(3,5)-diacetat. Wahrscheinlich aus diesem durch Allylverschiebung entsteht als sekundäres Zwischenprodukt

Penten-(2)-diol-(1,5)-diacetat und daraus durch Ringschluss Δ^3-Dihydropyran. — Alle diese Intermediärprodukte vermögen mit Formaldehyd in Eisessig-Schwefelsäure unter Bildung von 3-Acetoxymethyl-4-acetoxy-tetrahydropyran zu reagieren. Für die gleichzeitige Entstehung des isomeren 3-Acetoxy-4-acetoxymethyl-tetrahydropyrans liegen keine Anzeichen vor.

Der Eine von uns dankt *Norges Almenvitenskapelige Forskningsråd* für finanzielle Unterstützung.

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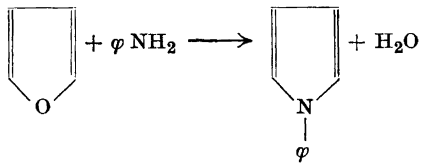
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The Preparation of Pyrroles from Furans

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Yur'ev et al. have described a method for transforming a furan into the corresponding pyrrole or N-substituted pyrrole [1–7]. The furan and a large excess of ammonia or an amine were passed, in the vapor phase, over aluminum oxide at about 400°, whereby the pyrrole and water were formed. In this way Yur'ev prepared 14 pyrroles in moderate yields (3—41 per cent). Thus furan and aniline gave a 24 per cent yield of 1-phenylpyrrole.



Another route from furans to pyrroles has recently been reported [8]. 2-(Acetamidomethyl)-furan (I) was methoxylated to 2,5-dimethoxy-2-(acetamidomethyl)-2,5-dihydrofuran II, this compound was hydrogenated catalytically to 2,5-dimethoxy-2-(acetamidomethyl)-tetrahydrofuran III, which with aniline in acetic acid gave 1-phenyl-2-(acetamidomethyl)-pyrrole IV. The overall yield of this 3-step reaction was 81 per cent.

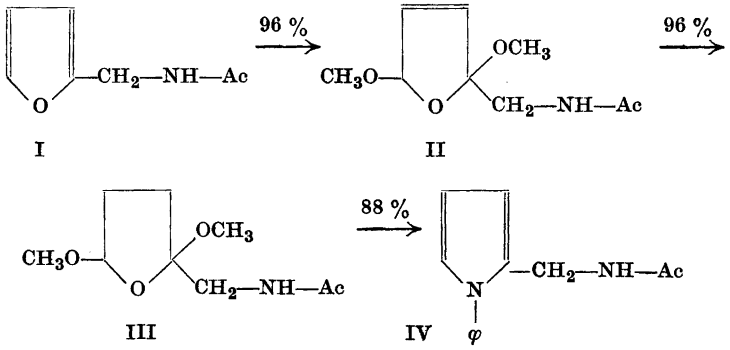


Table 1. Pyrrole compounds from furans. New compounds are marked with an asterisk.

Furan compound	RNH_2 compound	Pyrrole compound	Yields (%) based upon the furan compound	
			Yur'ev's method (one step)	Our method (three steps)
Furan	Ammonia	Pyrrole	30 [1]	40
—	Methylamine	1-Methylpyrrole	25 [6]	
—	Ethylamine	1-Ethylpyrrole	27 [6]	
—	*n*-Butylamine	1-*n*-Butylpyrrole		21
—	*n*-Amylamine	1-*n*-Amylpyrrole	14 [2]	28
—	Aniline	1-Phenylpyrrole	24 [3]	59
—	*o*-Toluidine	1-*o*-Tolylpyrrole	41 [3]	54
—	*p*-Toluidine	1-*p*-Tolylpyrrole	21 [3]	
—	Benzylamine	1-Benzylpyrrole	3 [7]	
—	*p*-Phenylene-diamine	1,1′-*p*-Phenylene-dipyrryl *		31
2-Methylfuran	Ammonia	2-Methylpyrrole	24 [4]	25
—	Aniline	1-Phenyl-2-methyl-pyrrole	12 [5]	51
—	*o*-Toluidine	1-*o*-Tolyl-2-methyl-pyrrole	10 [5]	
—	*p*-Toluidine	1-*p*-Tolyl-2-methyl-pyrrole	10 [5]	
2-(Acetamidomethyl)-furan	*n*-Amylamine	1-*n*-Amyl-2-(acetam-idomethyl)-pyrrole*		69
Furfuryl methyl ether	Aniline	1-Phenyl-2-(methoxy-methyl)-pyrrole *		17
Furfural dimethyl acetal	Ammonia	2-Pyrrolecarboxal-dehyde		24
—	Aniline	1-Phenyl-2-pyrrole-carboxaldehyde *		35
Methyl 2-furoate	Ammonia	Methyl 2-pyrrole-carboxylate		11
—	Aniline	Methyl 1-phenyl-2-pyrrolecarboxylate		36
2,5-Dimethylfuran	Ammonia	2,5-Dimethylpyrrole	16 [7]	
2-Ethylfuran	Ammonia	2-Ethylpyrrole	5 [2]	
3-Isopropylfuran	Aniline	1-Phenyl-3-isopropyl-pyrrole *		35

The transformation of furans into pyrroles by way of the 2,5-dimethoxytetrahydrofurans has now been used for the preparation of 15 pyrroles. The final step, the reaction between the tetrahydrofuran and ammonia or an amine, was usually carried out in acetic acid as mentioned above, but some pyrroles were also prepared in the liquid phase without a solvent, or in the vapor phase over aluminum oxide. Our results, together with those of Yur'ev, are summarized in Table 1. Five of the pyrroles prepared by us are new compounds.

The methoxylation of furfural dimethyl acetal has been described previously [9], but the structure of the resulting dimethoxy compound was not determined. Since dimethoxytetrahydrofurfural dimethyl acetal now has been transformed into 2-pyrrolecarboxaldehyde and 1-phenyl-2-pyrrolecarboxaldehyde, the methoxy groups must be in the 2,5-positions.

It will be seen from the above that the new method, apart from the preparation of pyrroles, may also be used to prove the structure of new dimethoxydihydro- and dimethoxytetrahydrofurans.

EXPERIMENTAL

Microanalyses by E. Boss and K. Glens

Materials. a) *2,5-Dimethoxytetrahydrosilvan.* 30.0 g of dimethoxydihydrosilvan [9] and 100 ml of methanol (technical product) were shaken with 1.0 g of Raney nickel under 100 atmospheres of hydrogen for 1 hour. The hydrogenation product was isolated by distillation through a short packed column. Yield 24.5 g of dimethoxytetrahydrosilvan = 81 %; colourless liquid, b.p.$_{759}$ = 147—155°; n_D^{25} = 1.4166.

$C_5H_8O(OCH_3)_2$ (146.2)	Calc.	C 57.5	H 9.7	OCH_3 42.5
	Found	» 57.5	» 9.5	» 42.8

b) *2,5-Dimethoxy-3-isopropyltetrahydrofuran.* 3.00 g of 2,5-dimethoxy-3-isopropyl-2,5-dihydrofuran [10] and 15 ml of anhydrous methanol were shaken with 0.2 g of Raney nickel under 100—110 atmospheres at 70—90° for 3.5 hours. Yield 1.68 g of dimethoxyisopropyltetrahydrofuran = 56 %; colourless liquid, b.p.$_{14}$ = 74—76°; n_D^{25} = 1.4240.

$C_7H_{12}O(OCH_3)_2$ (174.2)	Calc.	C 62.0	H 10.4	OCH_3 35.6
	Found	» 62.0	» 10.4	» 34.5

c) *2,5-Dimethoxytetrahydrofurfural, dimethyl acetal.* 17.4 g of dimethoxydihydrofurfural dimethyl acetal [9] and 35 ml of anhydrous methanol were shaken with 2.0 g of Raney nickel under 100 atmospheres for 4 hours. The product was isolated by distillation.

Fraction (g)		B.p.$_{10}$	n_D^{25}
1	(6.1)	95°	1.4327
2	(6.8)	95—96°	1.4328
3	(3.2)	96—102°	1.4331

Yield (all fractions) 16.1 g of dimethoxytetrahydrofurfural dimethyl acetal = 92 % (colourless liquid). Fraction 2 was analyzed.

$C_5H_6O(OCH_3)_4$ (206.2)	Calc.	C 52.4	H 8.8	OCH_3 60.2
	Found	» 52.5	» 8.9	» 59.8

The preparation of the 2,5-dimethoxytetrahydrofurans from furan, 2-(acetamidomethyl)-furan, furfuryl methyl ether and methyl 2-furoate has been described previously [11, 8, 12, 13].

Pyrrole. 6.60 g of dimethoxytetrahydrofuran was, during 50 minutes, distilled in a stream of ammonia through a pyrex tube (40 × 1.4 cm) filled with activated aluminum oxide (8—14 mesh, Fisher Scientific Co.). The tube was kept at 250° and connected to a receiver kept at —80°. After the reaction the receiver contained about 10 ml of a liquid, which was heated to 50°, taken up in ether, dried with magnesium sulfate and distilled. Yield 1.78 g of pyrrole = 53 % (yield based upon furan 40 % *Cf.* [14,11]); $b.p._{760}$ = 131—133°; n_D^{20} = 1.5085, n_D^{25} = 1.5068.

C_4H_5N (67.1)	Calc.	N 20.9
	Found	» 20.9

1-n-Butylpyrrole. 13.2 g of dimethoxytetrahydrofuran (0.10 mole) and 7.30 g of *n*-butylamine (0.10 mole) were dissolved in 20 ml of acetic acid and the solution refluxed for 1 hour. The pyrrole was isolated by addition of 150 ml of water, extraction with 75 ml of ether, washing with water, 2 *M* aqueous potassium hydrogen carbonate and again with water, drying with sodium sulfate and distillation. Yield 3.44 g of 1-*n*-butylpyrrole = 28 % (yield based upon furan = 21 %); colourless liquid with a characteristic odour; $b.p._9$ = 52—53°; n_D^{25} = 1.4700. Previously found $b.p._{11}$ = 53—54° [15].

$C_8H_{13}N$ (123.2)	Calc.	C 78.0	H 10.6	N 11.4
	Found	» 77.6	» 10.2	» 11.3

1-n-Amylpyrrole. Prepared as 1-*n*-butylpyrrole from 3.30 g of dimethoxytetrahydrofuran (0.025 mole), 2.18 g of *n*-amylamine (0.025 mole) and 5 ml of acetic acid (refluxing time 2 hours). Yield 1.26 g of 1-*n*-amylpyrrole = 37 % (yield based upon furan = 28 %); colourless liquid with a characteristic odour; $b.p._9$ = 68—69°; n_D^{20} = 1.4728; n_D^{25} = 1.4701. Previously found [2] $b.p._{28}$ = 95—96° and n_D^{20} = 1.4731.

$C_9H_{15}N$ (137.2)	Calc.	C 78.8	H 11.0	N 10.2
	Found	» 78.6	» 10.8	» 9.9

1-Phenylpyrrole. 3.30 g of dimethoxytetrahydrofuran (0.025 mole) and 2.33 g of aniline (0.025 mole) were dissolved in 5 ml of acetic acid and the solution refluxed for 1.5 hours and then distilled. Yield 2.68 g of 1-phenylpyrrole = 75 %; $b.p._{30}$ = 131—133°; m.p. 57—59° (Hershberg apparatus, corr.). Previously found $b.p._{38}$ = 140° [16] and m.p. 62° [17].

$C_{10}H_9N$ (143.2)	Calc.	N 9.8
	Found	» 9.9

In another experiment the reactants were heated together without a solvent in a sealed glass tube to 265° for 2 hours. The dark crystalline residue was distilled. Yield 2.80 g of 1-phenylpyrrole = 78 % (yield based upon furan = 59 %); m.p. 55—58°.

1-o-Tolylpyrrole. 6.60 g of dimethoxytetrahydrofuran (0.050 mole) and 5.35 g of *o*-toluidine (0.050 mole) (n_D^{25} = 1.5697) were dissolved in 10 ml of acetic acid and the

solution refluxed for 1.5 hours and then distilled. Yield 5.55 g of 1-*o*-tolylpyrrole = 71 % (yield based upon furan = 54 %); b.p.$_9$ = 97—100°; n_D^{20} = 1.5711; n_D^{25} = 1.5689. Previously found[3] b.p.$_{12}$ = 114° and n_D^{20} = 1.5749.

$C_{11}H_{11}N$ (157.2)	Calc.	C 84.0	H 7.1	N 8.9
	Found	» 84.0	» 7.1	» 8.8

1,1'-p-Phenylenedipyrryl. 6.60 g of dimethoxytetrahydrofuran (0.050 mole) and 2.70 g of *p*-phenylenediamine (0.025 mole) were dissolved in 100 ml of acetic acid and the solution refluxed. A precipitate was formed immediately. After 5 minutes another 50 ml of acetic acid was added, 10 minutes later some carbon black was added and again 5 minutes later heating was discontinued and the hot liquid filtered through paper without suction. The filtrate was left standing for 2 hours whereby a crop of crystals separated. The light brown crystals were filtered off, washed with acetic acid and ether and dried. Yield 2.13 g of 1,1'-*p*-phenylenedipyrryl = 41 % (yield based upon furan = 31 %); m.p. 227—230° after sintering at 210°.

$C_{14}H_{12}N_2$ (208.3)	Calc.	C 80.7	H 5.8	N 13.5
	Found	» 80.3	» 5.7	» 13.2

Three crystallizations from acetic acid gave perfectly white crystals, m.p. 229—231°. Sublimation of this product at 0.05 mm did not change the m.p.

	Found	C 80.9	H 6.2	N 13.0

2-Methylpyrrole. 7.30 g of dimethoxytetrahydrosilvan was treated with ammonia and worked up as described for the preparation of pyrrole. Yield 2.14 g of a pale yellow liquid, b.p.$_{10}$ = 43—48°. Redistillation gave 1.51 g of 2-methylpyrrole = 37 % (yield based upon silvan = 25 %[Cf.9]); colourless liquid, b.p.$_{10}$ = 45—47°; n_D^{20} = 1.5012; n_D^{25} = 1.4992. Previously found b.p.$_{760}$ = 149—150° and n_D^{20} = 1.5012[4].

C_5H_7N (81.1)	Calc.	C 74.0	H 8.7	N 17.3
	Found	» 74.0	» 8.7	» 17.3

1-Phenyl-2-methylpyrrole. 3.65 g of dimethoxytetrahydrosilvan (0.025 mole) and 2.33 g of aniline (0.025 mole) were dissolved in 5 ml of acetic acid and the solution refluxed for 1.5 hours and then distilled. Yield 3.03 g of 1-phenyl-2-methylpyrrole = 77 % (yield based upon silvan = 51 %); colourless liquid, b.p.$_{11}$ = 109—111°; n_D^{20} = 1.5810; n_D^{25} = 1.5786. Previously found b.p.$_{14}$ = 118—119° and n_D^{20} = 1.582[5].

$C_{11}H_{11}N$ (157.2)	Calc.	C 84.0	H 7.1	N 8.9
	Found	» 84.2	» 7.1	» 9.0

1-n-Amyl-2-(acetamidomethyl)-pyrrole. 5.35 g of dimethoxy-2-(acetamidomethyl)-tetrahydrofuran (0.026 mole) and 2.28 g of *n*-amylamine (0.026 mole) were dissolved in 6 ml of acetic acid and the solution refluxed for 30 minutes and then distilled. Yield 4.01 g of 1-*n*-amyl-2-(acetamidomethyl)-pyrrole = 75 % (yield based upon 2-(acetamidomethyl)-furan = 69 % [Cf.8]); b.p.$_{0.1}$ = 131—134°; m.p. 52—54° (greenish crystals). Redistillation gave 3.44 g of almost colourless crystals with the same m.p. Crystallization of this product from ether gave 2.54 g of white crystals, m.p. 53—55°.

$C_{10}H_{17}N_2(COCH_3)$ (208.3)	Calc.	C 69.2	H 9.7	N 13.5	$COCH_3$ 20.7
	Found	» 68.8	» 9.5	» 13.2	» 20.1

Another crystallization from ether gave m.p. 54—56°.

1-Phenyl-2-(methoxymethyl)-pyrrole. 4.40 g of dimethoxytetrahydrofurfuryl methyl ether (0.025 mole) and 2.33 g of aniline (0.025 mole) were mixed and heated to 250° in a sealed glass tube for 3 hours. Distillation gave 1.02 g of crude 1-phenyl-2-(methoxy-

methyl)-pyrrole = 22 % (yield based upon furfuryl methyl ether = 17 % *Cf.*[12]); colourless liquid, b.p.$_{0.1}$ = 71—73°; n_D^{25} = 1.5668. Redistillation gave 0.61 g; b.p.$_{0.1}$ = 72—73°; n_D^{25} = 1.5682. Another distillation did not change the refractive index.

$C_{11}H_{10}N(OCH_3)$ (187.2)	Calc.	C 77.0	H 7.0	N 7.5	OCH_3	16.6
	Found	» 77.3	» 7.1	» 7.6	»	16.6

2-Pyrrolecarboxaldehyde. 4.12 g of dimethoxytetrahydrofurfural dimethyl acetal (0.020 mole) and 1.60 g of ammonium acetate (0.021 mole) were dissolved in 10 ml of acetic acid and the solution refluxed for 15 minutes. The pyrrole was isolated by addition of 20 ml of water, extraction with three 50 ml-portions of ether, drying with sodium sulfate and distillation. Yield 0.61 g of crude 2-pyrrolecarboxaldehyde = 32 % (yield based upon furfural dimethyl acetal = 24 % *Cf.*[9]); pale-yellow liquid, which crystallizes on cooling; b.p.$_{0.1}$ = 55—59°. Crystallization from petroleum ether gave 0.19 g of white crystals, m.p. 43—45°. Previously found 50—51° [17].

C_5H_5ON (95.1)	Calc.	C 63.1	H 5.3	N 14.7
	Found	» 62.7	» 5.4	» 15.0

A phenylhydrazone was prepared in the usual way. M.p. after two crystallizations from petroleum ether = 139—140°. Previously found 139—140° [18].

$C_{11}H_{11}N_3$ (185.2)	Calc.	C 71.3	H 6.0	N 22.7
	Found	» 71.7	» 5.9	» 22.4

1-Phenyl-2-pyrrolecarboxaldehyde. 2.06 g of dimethoxytetrahydrofurfural dimethyl acetal (0.010 mole) and 0.93 g of aniline (0.010 mole) were dissolved in 5 ml of acetic acid and the solution refluxed for 30 minutes and then distilled. The resulting pale-yellow liquid (1.14 g) contained 8.4 % of OCH_3 and was apparently a mixture of 1-phenyl-2-pyrrolecarboxaldehyde and the corresponding dimethyl acetal. 0.79 g of the product was refluxed with 3 ml of dioxane and 1 ml of 2 % hydrochloric acid for 3 minutes. 10 ml of water was added and the solution extracted with 10 ml of ether. The etheral extract was washed quickly with a few ml of 2 *M* aqueous potassium hydrogen carbonate and with water, dried over sodium sulfate and distilled. Yield 0.55 g of 1-phenyl-2-pyrrolecarboxaldehyde = 46 % (yield based upon furfural dimethyl acetal = 35 %); almost colourless liquid, which crystallizes on cooling; b.p.$_{0.1}$ = 90—93°; m.p. 27—30°.

$C_{11}H_9ON$ (171.2)	Calc.	C 77.2	H 5.3	N 8.2
	Found	» 76.9	» 5.5	» 7.9

A phenylhydrazone was prepared in the usual way and crystallized twice from ethanol; white crystals, m.p. 130—131°.

$C_{17}H_{15}N_3$ (261.3)	Calc.	C 78.1	H 5.8	N 16.1
	Found	» 78.0	» 5.7	» 16.3

Methyl 2-pyrrolecarboxylate. 4.75 g of methyl dimethoxytetrahydrofuroate (0.025 mole) and 2.00 g of ammonium acetate (0.026 mole) were dissolved in 6 ml of acetic acid and the solution refluxed for 30 minutes. The pyrrole was isolated by addition of 75 ml of water, extraction with 50 ml of ether, washing with water, 2 *M* potassium hydrogen carbonate and again with water, drying with sodium sulfate and distillation. Yield 1.26 g of a colourless liquid, which crystallized on cooling; b.p.$_9$ = 104—105°. Crystallization from *n*-hexane gave 530 mg of methyl 2-pyrrolecarboxylate = 17 % (yield based upon methyl 2-furoate = 11 % *Cf.*[13]); m.p. 75—76°. Previously found 73° [19].

$C_5H_4ON(OCH_3)$ (125.1)	Calc.	C 57.6	H 5.6	N 11.2	OCH_3	24.8
	Found	» 58.0	» 5.4	» 11.2	»	25.0

Methyl 1-phenyl-2-pyrrolecarboxylate. 4.75 g of methyl dimethoxytetrahydrofuroate (0.025 mole) and 2.33 g of aniline (0.025 mole) were dissolved in 5 ml of acetic acid and the solution refluxed for 10 hours. The pyrrole was isolated as in the preceding experiment. Yield 2.87 g of crude methyl 1-phenyl-2-carboxylate = 57 % (yield based upon methyl 2-furoate = 36 %); colourless liquid, which crystallizes; $b.p._{0.4}$ = 105—112°; m.p. 72—78°. Crystallization from methanol gave 1.78 g, m.p. 88—90°.

$C_{11}H_8ON(OCH_3)$ (201.2)	Calc.	C	71.6	H	5.5	N	7.0	OCH_3 15.4
	Found	»	71.9	»	5.8	»	7.1	» 15.6

Another crystallization from methanol gave m.p. 90—91°. Previously found 88°[20].

1-Phenyl-3-isopropylpyrrole. 0.90 g of dimethoxy-3-isopropyltetrahydrofuran (0.0052 mole) and 0.48 g of aniline (0.0052 mole) were dissolved in 1.2 ml of acetic acid. The solution was refluxed for 1.5 hours and then distilled. Yield 0.80 g of crude 1-phenyl-3-isopropylpyrrole = 83 % (yield based upon 3-isopropylfuran = 35 %[Cf.10]); pale-yellow liquid, which crystallizes on cooling; $b.p._{0.1}$ = 89—90°.

$C_{13}H_{15}N$ (185.3)	Calc.	N	7.6
	Found	»	8.0

In order to get the correct analytical values, the compound should be analyzed immediately after distillation. Our purest sample (almost colourless liquid) showed the following data: $b.p._{0.1}$ = 90°; n_D^{25} = 1.5656; m.p. 0—2°.

$C_{13}H_{15}N$ (185.3)	Calc.	C	84.3	H	8.2	N	7.6
	Found	»	84.2	»	8.4	»	7.6

Colour reactions. All the above pyrroles gave positive Ehrlich and pine shaving reactions.

SUMMARY

A new method for the transformation of furans into the corresponding pyrroles or N-substituted pyrroles is described.

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Electron Diffraction Investigation of $\alpha, \beta, \gamma, \delta$ and ε Benzene Tetrachloride

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The structure of the five isomers of benzene tetrachloride (BTC) isolated by Calingaert, Kolka, Orloff *et al.* at the Research Laboratories of Ethyl Corporation, Detroit, have been studied by pure chemical means by the Ethyl Corporation team [1,2] and by dipole moment measurements at this institute [3]. To obtain a direct and conclusive determination of the structure of these five isomers the present electron diffraction work based on the rotating sector method was started.

The electron diffraction diagrams of the five isomers were all very similar. By visual inspection we were not able to distinguish between the diagrams of the α, β, γ and ε isomers; however, the diagrams of the δ isomer could easily be separated from the other ones. In Fig. 1 the intensity curves for the five isomers are given. The curves are multiplied by the usual modification factors [4]. The $\frac{\sigma(r)}{r}$ curves calculated by Fourier transformation are given in Fig. 2. The curves have been normalized so that the peaks having their maximum at 2.73 Å are of the same height for all curves. The inner parts of the curves are practically identical as might be expected. The outer parts of the curves, however, are markedly different. The inner parts of the curves give the average carbon carbon bond distance, the carbon chlorine bond distance and the average values for the distances of the type C_2-Cl_3. The corresponding peaks have their maximum values at 1.50, 1.80 and 2.73 Å respectively, suggesting the following values for the structure parameters: C = C distance 1.35 Å, C-C distance 1.54 Å, C-Cl distance 1.80 Å and the average C-C-Cl angle 109.5°. The distances corresponding to the peak at 2.73 Å might to some extent be split up particularly for less symmetric molecules. For the δ isomer this peak is somewhat higher compared to the peak at 1.80 Å than for the other isomers. This might be related to the assumption of a high symmetry for the δ isomer.

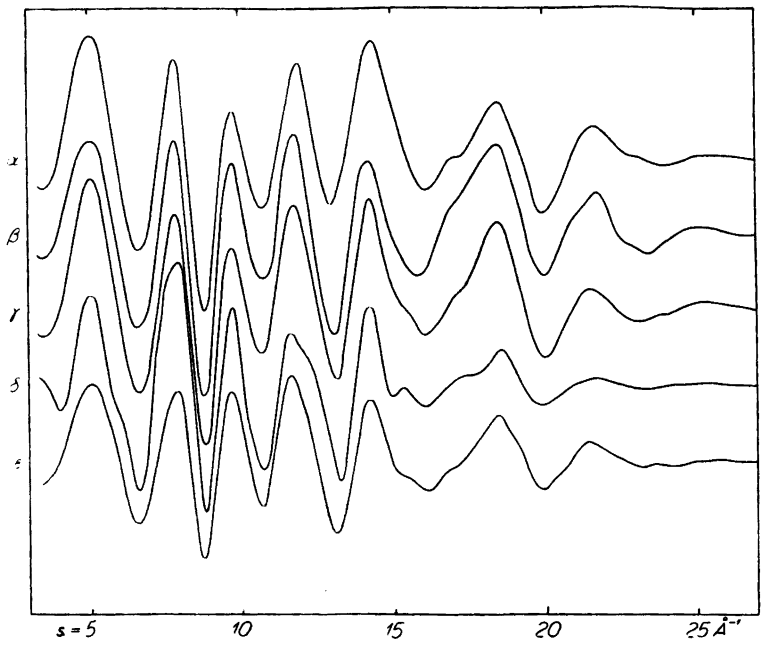


Fig 1. Intensity curves of α, β, γ, δ and ε BTC.

Adopting the ideas and designations used for cyclohexane derivatives [5] the possible configurations of the BTCs can be characterized by the scheme given in Table 1.

Table 1. Possible structure forms for the BTCs.

I	$\varkappa\varkappa\varkappa\varkappa \rightleftharpoons \varepsilon\varepsilon\varepsilon\varepsilon$
II	$\varepsilon\varkappa\varkappa\varkappa \rightleftharpoons \varkappa\varepsilon\varepsilon\varepsilon$
III	$\varkappa\varepsilon\varkappa\varkappa \rightleftharpoons \varepsilon\varkappa\varepsilon\varepsilon$
IV	$\varepsilon\varepsilon\varkappa\varkappa \rightleftharpoons \varkappa\varkappa\varepsilon\varepsilon$
V	$\varepsilon\varkappa\varkappa\varepsilon \rightleftharpoons \varkappa\varepsilon\varepsilon\varkappa$
VI	$\varepsilon\varkappa\varepsilon\varkappa \rightleftharpoons \varkappa\varepsilon\varkappa\varepsilon$

The $\frac{\sigma(r)}{r}$ curves of the five isomers exclude $\varepsilon\varepsilon\varepsilon\varepsilon$ and $\varkappa\varepsilon\varepsilon\varepsilon$ as possible forms for any of the known isomers. Even if rather marked deformations from ideal structures are assumed the $\frac{\sigma(r)}{r}$ curves of these two forms should have a pronounced peak at an r-value of approximately 4.3 Å. None of the experi-

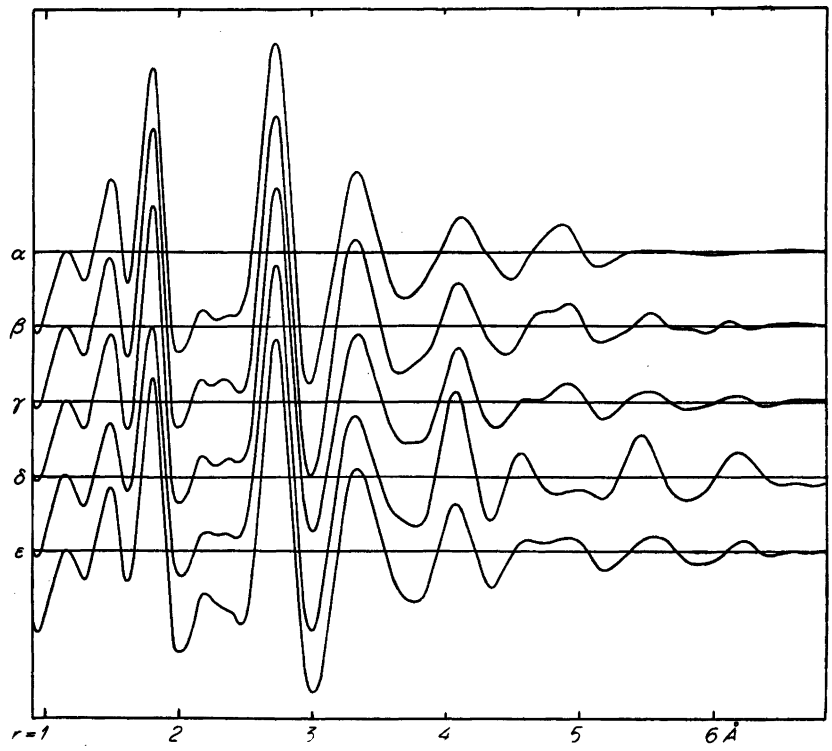


Fig. 2. $\frac{\sigma(r)}{r}$ *curves of* α, β, γ, δ *and* ε *BTC.*

mental $\frac{\sigma(r)}{r}$ curves exhibits such a peak. (Minor contribution of these forms to an equilibrium of the kind indicated in Table 1 can of course not be excluded by this argument.)

By inspection of the $\frac{\sigma(r)}{r}$ curves the δ isomer can easily be identified with the κκκκ form. The peaks at 4.12, 4.57 and 5.45 Å of the $\frac{\sigma(r)}{r}$ curve of the δ isomer are higher than the corresponding peaks of any of the other isomers. This should indeed be the case for the κκκκ form. The lack of the peak at about 4.9 Å is also a characteristic feature of the $\frac{\sigma(r)}{r}$ curve of the δ isomer. Beside the κκκκ form the εκεκ form is the only one not excluded for other reasons that should be expected to lack the peak at about 4.9 Å. This fact should therefore be an argument for excluding the εκεκ form. Experiences

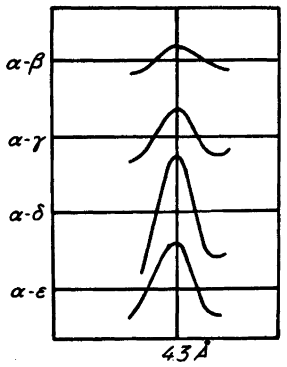


Fig. 3. $\frac{\sigma(r)}{r}$ *difference curves in the region of r equal to 4.3 Å.*

from chlorinated cyclohexanes are in accordance with this result as the presence of $1\varepsilon-3\varepsilon$ chlorine atoms seems to be less favourable [6].

The next isomer to be studied is the α isomer. The lack of peaks on the $\frac{\sigma(r)}{r}$ curve beyond r equal to 5 Å makes us believe that this isomer has the form $\varepsilon\varepsilon\varkappa\varkappa$. All the other forms of Table 1 not yet excluded require maxima beyond this point. To obtain further evidence we have calculated the $\frac{\sigma(r)}{r}$ difference curves in the neighbourhood of $r = 4.3$ Å. This r-value corresponds to the $1\varepsilon-2\varepsilon$ distance which should occur for the following three forms not yet excluded: $\varepsilon\varepsilon\varkappa\varkappa$, $\varkappa\varepsilon\varepsilon\varkappa$ and $\varepsilon\varkappa\varepsilon\varepsilon$. (The last one of these forms will be excluded as we shall see later on). The $1\varepsilon-2\varepsilon$ distance interferes with other distances of somewhat lower r-value so that no isolated peak will occur exactly at $r = 4.3$ Å. In the difference curves, however, the influence of the neighbouring distances should to a certain extent be subtracted and the peak at 4.3 Å should show up. In Fig. 3 the difference curves are given for the region mentioned. The $\frac{\sigma(r)}{r}$ curve for each of the four isomers β, γ, δ and ε has been subtracted from that of the α isomer. All these difference curves have maxima at 4.3 Å corresponding to the assumption that the α isomer has the form $\varepsilon\varepsilon\varkappa\varkappa$. The relatively small value of the height of the peak corresponding to the difference $\alpha - \beta$ is explained by assuming the β isomer to be of the form V (Table 1) characterized by the equilibrium $\varepsilon\varkappa\varkappa\varepsilon \rightleftharpoons \varkappa\varepsilon\varepsilon\varkappa$. The relatively large height of the peak for the difference $\alpha - \delta$ is easily explained by the fact that the $\frac{\sigma(r)}{r}$ curve of the δ isomer itself has a pronounced minimum at 4.3 Å introduced by neighbouring distances.

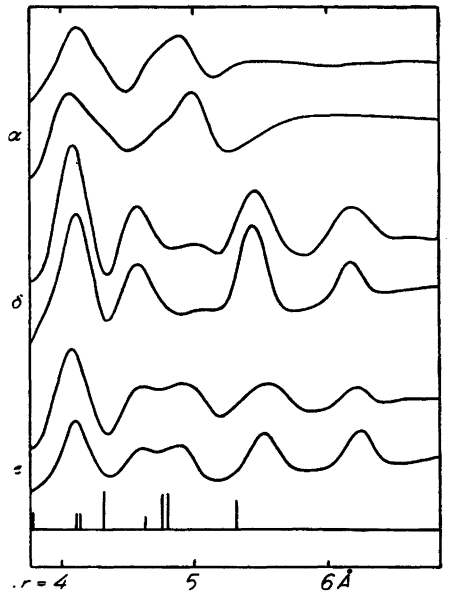


Fig. 4. Experimental and theoretical $\frac{\sigma(r)}{r}$ *curves for the outer parts of* α, δ *and* ε *BTC.*

To provide further evidence concerning the structure of the β isomer and to determine the structure of the γ and ε isomers the relative values for the areas and heights of pronounced peaks of the $\frac{\sigma(r)}{r}$ curves of the different isomers were studied. First of all the areas below the peak at about 4.12 Å were compared. The results are listed in Table 2. In the second column of the table the observed areas for the five isomers are given in an arbitrary unit. In the third column the calculated values for the various possibilities are listed in a sequence so that the correspondence with the values in the second column is as good

Table 2. Experimental and theoretical relative values for the areas under the peak at $r = 4.12$ Å for the various isomers.

Isomers	Experimental values	Theoretical values	Structure forms
α	5.0	5	$\varepsilon\,\varepsilon\,\varkappa\,\varkappa$
β	5.7	$6 \rightleftharpoons 4$	$\varepsilon\,\varkappa\,\varkappa\,\varepsilon \rightleftharpoons \varkappa\,\varepsilon\,\varepsilon\,\varkappa$
γ	6.7	7	$\varepsilon\,\varkappa\,\varkappa\,\varkappa$
δ	11.8	8	$\varkappa\,\varkappa\,\varkappa\,\varkappa$
ε	6.3	6	$\varkappa\,\varepsilon\,\varkappa\,\varkappa$

as possible. In the fourth column the corresponding structure forms are given. The present consideration seems to indicate that the structures of the two somers γ and ε are characterized by the forms $\varepsilon\,\varkappa\,\varkappa\,\varkappa$ and $\varkappa\,\varepsilon\,\varkappa\,\varkappa$ respec-

tively. It further indicates that the β isomer probably has the structure $\varepsilon\varkappa\varkappa\varepsilon \rightleftharpoons \varkappa\varepsilon\varepsilon\varkappa$ the former being the predominating form.

Similar studies were carried through for the peak at r equal to 4.90 Å, and the results are listed in Table 3. The areas below this maximum are much influenced by neighbouring maxima as can be easily seen in Fig. 2. We have therefore found it more correct in this case to measure the heights of the peaks instead of the areas. The value 2 + for the $\varepsilon\varepsilon\varkappa\varkappa$ form and 1 + for

Table 3. Experimental and theoretical relative values of the heights of the peak at $r = 4.90$ Å for the various isomers.

Isomers	Experimental values	Theoretical values	Structure forms
α	2.4	2 +	$\varepsilon\varepsilon\varkappa\varkappa$
β	2.0	$2 \rightleftharpoons 2$	$\varepsilon\varkappa\varkappa\varepsilon \rightleftharpoons \varkappa\varepsilon\varepsilon\varkappa$
γ	1.6	1 +	$\varepsilon\varkappa\varkappa\varkappa$
δ	—	0	$\varkappa\varkappa\varkappa\varkappa$
ε	1.3	1	$\varkappa\varepsilon\varkappa\varkappa$

the $\varepsilon\varkappa\varkappa\varkappa$ form mean that the calculated heights are somewhat greater than 2 and 1 respectively. An exact value can not be given unless information concerning the deviation from the ideal structures is obtained. The conclusions drawn from Table 3 support the ideas already given concerning the structure of the five isomers.

In the preceding discussion ideal structures as described in an earlier work [3] were tacitly applied. The fine structures of the molecules were of less importance for these discussion though some of the discrepancies between observed and calculated values in Tables 2 and 3 must be ascribed to the deviations from the ideal structures. To make a comparison of experimental and calculated $\frac{\sigma(r)}{r}$ curves possible, deformations from the ideal structures must be taken into account. In Fig. 4 experimental and theoretical pairs of curves are given for the isomers α, δ and ε. Only the outer parts of the curves are reproduced. The theoretical curves are calculated by assuming the forms already found and by introducing deformations of the same kind and magnitude as those known for corresponding cyclohexane derivatives [7,8]. The correspondence between experimental and calculated curves adds further evidence to our ideas of the structure of the three isomers α, δ and ε. Below the $\frac{\sigma(r)}{r}$ curve of the ε isomer the line diagram for the "conversion form" $\varepsilon\varkappa\varepsilon\varepsilon$ not yet excluded is given. The line diagram can not be brought to agreement with the

$\frac{\sigma(r)}{r}$ curve of the ε isomer or that of any of the other isomers. This form may therefore be excluded.

The similarities of the $\frac{\sigma(r)}{r}$ curves for the β and γ isomers are so great that calculation of theoretical curves should not be expected to elucidate much further the structures of these two isomers. The only obvious difference in the general appearance is related to the shape of the shoulder at approximately 4.8 Å. This point is indeed in accordance with what should be expected from the structures already ascribed to these two isomers.

The above arguments concerning the structure of the α, δ and ε BTC can be regarded as conclusive. Concerning the β and γ isomers the electron diffraction work alone might leave some doubt, though strong arguments are in favour of the structures given. Fortunately the dipole moment measurements, on the other hand, must be regarded as quite conclusive for these two isomers [3].

The structure forms containing more than two ε bonds were all excluded. Of course it is not possible from the electron diffraction studies alone to exclude the possibility of minor contribution of these forms to the equilibria indicated in Table 1. It should, however, be obvious from the above arguments that these less stable forms are not contributing to any great extent.

Finally it can be mentioned that the result of this work as well as the result of the dipole moment measurements [3] is in agreement with the view expressed by the Ethyl Corporation team [2].

SUMMARY

The configuration of the five isomers of benzene tetrachloride α, β, γ, δ and ε have been studied by the aid of the electron diffraction sector method. The five isomers were attributed to the following forms: α ($\varepsilon\,\varepsilon\,\varkappa\,\varkappa$), β ($\varepsilon\,\varkappa\,\varkappa\,\varepsilon \rightleftharpoons \varkappa\,\varepsilon\,\varepsilon\,\varkappa$), γ ($\varepsilon\,\varkappa\,\varkappa\,\varkappa$), δ ($\varkappa\,\varkappa\,\varkappa\,\varkappa$) and ε ($\varkappa\,\varepsilon\,\varkappa\,\varkappa$).

The author wishes to express his gratitude to the Research Laboratories of Ethyl Corporation, Detroit, for having supplied him with samples of the five isomers of BTC, to Mr. E. Risberg for taking the sector photographs, and to the I.B.M. office in Oslo for their valuable help by placing their calculation machines at the disposal of our institute.

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Studies Related to Naturally Occurring Acetylene Compounds. IX. The Occurrence of Methyl dec-8-*cis*-en4:6-diynoate (= *a*, *β*-Dihydro-Matricaria Ester) and 2-*cis*; 8-*trans*-Matricaria Ester in Nature

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The methyl-deca-2-*cis*: 8-*cis*-diene-4 : 6-diynoate or matricaria ester discovered in 1941 in the essential oil of scentless mayweed (*Matricaria inodora* L.)[1] has so far turned out to be the most widely distributed acetylenic component of essential oils. The preliminary investigation of some 94 essential oils from plants belonging to the plant family of the *Compositae*, the results of which will be treated in a separate paper in this series, has established the occurrence of the matricaria ester in some 34 different plants. In all these cases the dienediyne ester was crystallized and the pure compound characterized through mixed m.p. and U.V.-absorption.

Some of the essential oils investigated, especially those amongst the fleabane oils, consisted entirely of matricaria ester. The scentless mayweed oil, however, contains an appreciable liquid fraction in the mother liquors after low temperature crystallization of matricaria ester. The essential oil from the flowers contains only small amounts of the hexahydro matricaria ester characteristic of the leaves. When all the *cis* : *cis*-matricaria ester which can be crystallized out from pentane solution at — 20° has been removed, the mother liquor still exhibits a U.V.-spectrum above 2900 ÅU similar to matricaria ester with the intensity of absorption depressed only some 20 to 30 %. Below 2900 ÅU there occur new, sharp peaks at 2815, 2650 and 2510 ÅU.

HYDROCARBONS

We have tried to separate the components in these mother liquors through chromatography on slightly activated alumina. Small amounts of a hydrocarbon passed with the solvent, petroleum ether, through the column. This hydrocarbon showed the composition of a sesquiterpene $C_{15}H_{24}$. Density and refraction $d_4^{20} = 0.8505$ and $n_D^{20} = 1.4912$, which gives a molecular refraction of $M_{D,\,obs.} = 69.56$. This value is very close to the calculated value for an aliphatic sesquiterpene $M_{D.calc.}$ $C_{15}H_{24}$, $4/^{=} = 69.54$. However, an aliphatic sesquiterpene should have at least 2 double bonds in conjugation and an exaltation of about 1.5—1.6 to be added. So the molecular refraction is intermediate between an aliphatic and a monocyclic sesquiterpene ($M_{D.calc.} = 67.81$). Catalytic hydrogenation seemed to confirm this view, since a saturated hydrocarbon was obtained after consumption of 3.1 moles of hydrogen.

As all the known monocyclic sesquiterpenes have density and refraction considerably higher than this hydrocarbon from scentless mayweed and the quantity of material was insufficient for further fractionation, the hydrocarbon and its perhydrocompound was sent to Dr. Josef Plíva, Praha, for examination of their infrared spectra. As will be seen from Fig. 1 the infrared spectra of the sesquiterpene from scentless mayweed and its perhydro compound cannot be distinguished from the spectra of "natural farnesene" and 2 : 6 : 10-trimethyl-dodecane [2].

"Natural farnesene" was discovered in 1949 by Šorm, Mleziva, Arnold and Plíva [3] in oil of hops, but has since been shown in the laboratory of Professor Šorm to be a component of several essential oils, among others of oil of chamomille (*Matricaria chamomilla* L.), which botanically is closely related to scentless mayweed. Chemically the oil of chamomille is a terpenoid oil; the main constituents, besides »natural farnesene», being some cyclic sesquiterpenes and sesquiterpene alcohols [4, 5] chamazulene and its precursor chamazulenogen [6].

A spectral investigation of a trade sample of oil of chamomille (Polak and Schwarz) showed 13 % of matricaria ester. This content was so low that a contamination of the chamomille culture with scentless mayweed corresponding to this content seemed rather likely. So in the summer 1950 and 1951 we cultivated genuine *Matricaria chamomilla* L. The spectrographical investigation of this oil gave no indications of matricaria ester. As in contrast to the Polak and Schwarz sample the U.V.-absorption was very strong the oil was partitioned between petroleum ether and 92 % methanol, the hypophasic part distilled at 10^{-4}, and the U.V.-absorption measured for the fraction 50—60°, in which nearly all the matricaria ester would concentrate. This

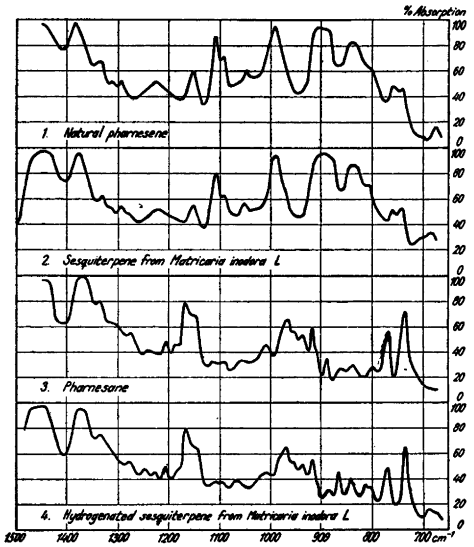


Fig. 1. Infrared spectra Perkin-Elmer infrared spectrometer model 12 B, 60° rock salt prism. All substances in liquid state at room temperature.

fraction had a very strong U.V.-absorption too, but without any resemblance to the known curve of matricaria ester. This fraction was chromatographed on activated alumina according to a procedure found suitable for the isolation of matricaria ester. This chromatography split up the fraction into different compounds, one of them spectroscopically identical with the unknown main component of the essential oil of *Matricaria discoidea* D.C., which may be related to "Composite-cumulen I" [7], but we were not able to obtain any fraction with a U.V.-spectrum corresponding to that of matricaria ester. So the chemical relations between chamomille and scentless mayweed seem restricted to the common occurrence of "natural farnesene".

"Natural farnesene" has the physical constants $n_D^{20} = 1.4899$ $d_4^{20} = 0{,}8363$ which gives $M_{D,\,obs.} = 70.58$, $M_{D,\,calc.}$ $C_{15}H_{24}$ $4/= = 69.54$, that is an exaltation of 1.04. The reason for the higher physical constants of the *Matricaria inodora* sesquiterpene might be either impurities or polymerisation. A contamination with cyclic sesquiterpenes would explain that the perhydro compound also has too high a density and refraction. To explain the hydrogenation number (3.12 mols H_2) a contamination by 30 % of a tricyclic sesquiterpene would be necessary. Although such a high proportion of cyclic sesquiterpenes is inconsistent with the U.V.- and infrared spectra, the optical activity of the scentless mayweed sesquiterpene $[\alpha]_D^0 = \div 15{,}5°$ strengthens the suggestion indicated by the molecular refraction of the inhomogeneity of this sesquiterpene fraction.

ACETYLENIC ESTERS

Elution of the chromatographic column with benzene gave an oil b.p. $50°/10^{-4}$ with U.V.-absorption close to that of the entire mother liquor, the matricaria ester concentration being about 60 %. The sharp absorption maxima below 2800 ÅU of these mother liquors are very close to the maxima found by Bruun, Haug and Sörensen [8] for lachnophyllol(= dec-2-ene-4:6-diyn-1-ol) viz. 2825, 2665, 2520 and 2395 ÅU. These maxima are characteristic of the unsaturated system

$$—C{\equiv}C—C{\equiv}C—CH{=}CH—$$

The analytical composition of the distilled oil was C 75.75, H 6.48 Calculated for matricaria ester $C_{11}H_{10}O_2$ C 75.84, H 5.79; for a dihydro matricaria ester $C_{11}H_{12}O_2$ C 74.97, H. 6.86.

Thus the most reasonable explanation is that the mother liquor is a mixture of a (liquid?) matricaria ester with its dihydro- compound having interrupted conjugation between the carbomethoxy group and the unsaturated chromophor.

Biosynthetically the most likely dihydromatricaria ester is the α,β-dihydro ester. This compound was therefore synthesized according to the Glaser coupling reaction, compare Christensen and Sörensen [9]. The 8-*trans*-α,β-dihydromatricaria ester was a solid with the low m.p. 15,5° C, the corresponding free acid showed m.p. 144°. The 8-*cis* isomer was a liquid, the 8-*cis* acid melted at 60—61,5° C. Both isomers have identical U.V.-absorption curves (within the limits of experimental error), the maxima lie, as would be expected, at

8-*cis*- α,β-dihydromatricaria ester	2810	2650	2514	2381	2275
8-*trans* » » » »	2814	2653	2510	2390	—

The possibility was thus strengthened that the main component of the scentless mayweed mother liquors was the 8-*cis*-α,β-dihydro ester. The isolation of this compound from natural essential oils was facilitated since it turned out that some composité oils were enriched in the compound. The spectrum so far has been found present in the oils from: *Amellus strigosus*, (Thunb.) Less., *A. strigosus* var *Wildenowii*, *Cephalophora aromatica* Schrad., *Felicia tenella* (L.) *Nees*. *Solidago sempérvirens* L.

Among these the root oil of *Amellus strigosus* var. *Wildenowii* turned out to be most homogeneous, compare Fig. 2, curve A. Saponification under nitrogen of the root oils gave colourless crystals, m.p. 61°—61,5°. The U.V.-spectrum substantiated, however, that the crystals were contaminated with

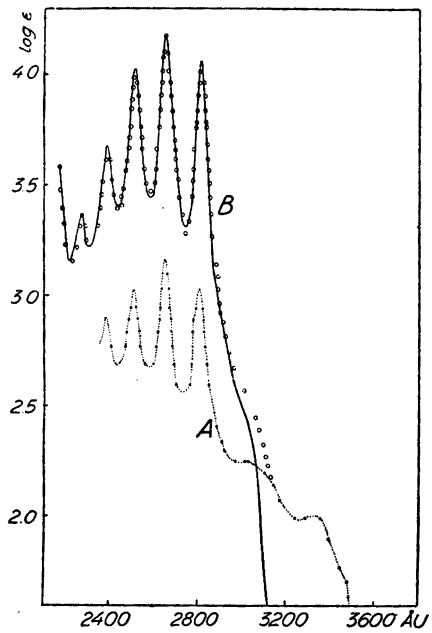


Fig. 2. Ultraviolet absorption of hexane solutions of:

Curve B. (———). *synthetic 8-cis-α,β-dihydromatricaria ester.*

» *B.* (○○○○). *8-cis-α,β-Dihydromatricaria acid from Amellus strigosus.*

» *A.* (▲···▲···). *Essential oil from the root of Amellus strigosus var. Wildenowii.*

some 19 % of a matricaria acid. The amounts of crystals were too small for further purifications. The crude essential oil from the flowers and from the leaves of *Amellus* showed U.V.-spectra very similar to each other and very like the spectra of the mother liquors from scentless mayweed. With this material, which was more abundant than the root oils, the isolation of 8-*cis*-α,β-dihydromatricaria acid could be realized. The laborious fractionation of these oils is described briefly in the experimental section. Besides demonstrating the presence of 8-*cis*-α,β-dihydromatricaria acid in nature this arduous experimentation was rewarded with the simultaneous isolation of 2-*cis*-8-*trans*-matricaria acid. 2-*cis* : 8-*trans*-Matricaria acid had been prepared earlier both by photoisomerization of 2-*cis* : 8-*cis*-matricaria ester and by total synthesis [10]. The demonstration of the occurrence of this stereoisomer in nature explains why some crude essential oils and some mother liquors which spectroscopically are indicated to have high concentrations of matricaria ester are without any tendency to crystallization. In contrast to the beautifully crystallizing 2-*cis* : 8-*cis*-ester, the 2-*cis* : 8-*trans*-ester m.p. + 2° is very soluble in all solvents and solidifies only when solvent-free and quite pure.

The separation of the *Amellus* oils led further to the discovery of a volatile polyene the U.V.-chromophor of which has an ethylenic fine structure spacing.

In this respect this volatile polyene resembles the »Centaur Y», a compound of unknown constitution found by Löfgren [11] in some extracts of different *Centaurea*-species. We are returning to the investigation of this substance later.

EXPERIMENTAL

Matricaria inodora flower oil mother liquors. The starting materials were two samples of combined mother liquors from the preparation of matricaria ester. In the 1940-experiments of Sörensen and Stene the mother liquors had been sealed under vacuum. The distillation of this material at 10^{-4} mm Hg gave 7.25 g boiling $< 75°$ (I). The second sample originated from the isolation of matricaria ester in September 1949 and weighed 12 g (II). Both samples were chromatographed on slightly activated alumina from petroleum ether solution. The column was washed with petroleum ether and eluted with petroleum ether with 20 % benzene, ether and ethyl alcohol. Only the petroleum ether washings and the benzene eluate contained appreciable amounts of material.

Sesquiterpene fractions. The petroleum ether washings were evaporated and distilled at 10^{-3} (air bath 35—40° C), I gave 1.2 g $[\alpha]_D^{20} = -15.5$, II 3.2 g $[\alpha]_D^{20} = -14°$.Analysis showed C 87.42, 87.64 and H 11.57, 11.36 so there must be some oxygen containing impurities present. The combined sesquiterpene fractions were dissolved in equal parts of petroleum ether and methanol and „entmischt" with water to 92 %, the procedure being repeated 3 times and the epiphasic part isolated and distilled.

$C_{15}H_{24}$ (204.3)	Calc.	C 88.16	H 11.84
	Found	» 88.22	» 11.57

Table 1. Dispersion of sesquiterpene.

$d_4^{20} = 0.8505$

λ	n_λ	R_λ	λ	n_λ	R_λ
6678.1	1.48841	69.22	5015.6	1.50089	70.72
5895.9	1.49283	69.75	4713.1	1.50491	71.20
5875.7	1.49300	69.77	4471.5	1.50875	71.66
5790.7	1.49359	69.85	4358.3	1.51082	71.90
5460.7	1.49617	70.16			

U.V.-absorption in hexane, compare Fig. 3, where the curve of Šorm *et al.*[3] for "natural farnesene" is given for comparison.

Infrared spectrum, compare Fig. 1.

Catalytic hydrogenation: 0.5789 g sesquiterpene in glacial acetic acid (catalyst 2 % Pd on $BaSO_4$) consumed 220 ml H_2, 742 mm, 24° C, that is 3.12 /=. The hydrogenation mixture was filtered, ether added and the acetic acid neutralized with sodium hydroxide, the ether solution washed with water, dried and distilled in vacuum at 0.5 mm (air bath 70° C).

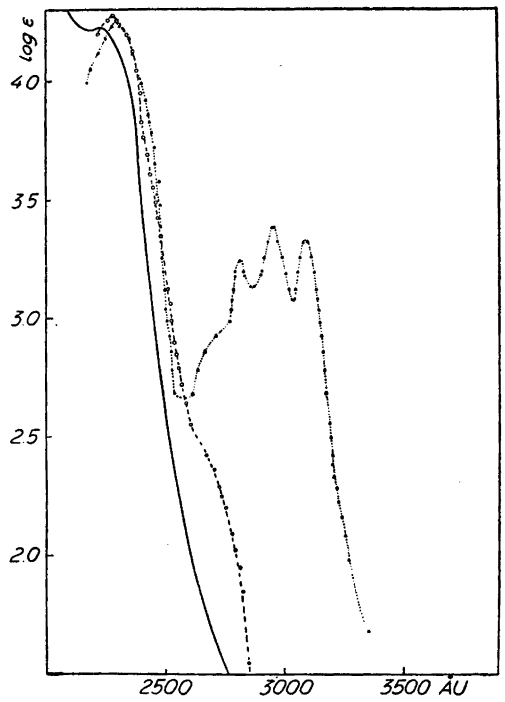


Fig. 3. Ultraviolet absorption of hexane solutions of:

——— *"Natural farnesene", see Šorm, Mleziva, Arnold & Plíva.*

-O--O- *Sesquiterpene from Matricaria inodora L.*

▲···▲··· *Polyene from the essential oil of Amellus.*

Table 2. Dispersion of perhydrogenated sesquiterpene.

$$d_4^{20} = 0.8029 \quad R_{\lambda,\,\text{calc}} = \frac{68.179 \cdot \lambda^2}{\lambda^2 \div 0.7948 \cdot 10^6}\,; \quad \lambda_0 = 891.5\ \text{ÅU}$$

λ	n_λ	$R_{\lambda,\,\text{obs.}}$	$R_{\lambda,\,\text{calc.}}$	λ	n_λ	$R_{\lambda,\,\text{obs.}}$	$R_{\lambda,\,\text{calc.}}$
6678.1	1.44294	69.41	69.41	4921.9	1.45105	70.51	70.49
5895.9	1.44562	69.77	69.77	4713.1	1.45267	70.73	70.71
5875.7	1.44572	69.79	69.79	4471.5	1.45472	71.01	71.00
5790.7	1.44610	69.84	69.83	4358.3	1.45583	71.16	71.16
5460.7	1.44761	70.05	70.05	4046.6	1.45903	71.59	71.66
5015.6	1.45039	70.42	70.40	—	—	—	—

The dispersion constant is in agreement with the standard value for saturated hydrocarbons, $\lambda_0 = 886$ ÅU, but the density and refraction are too high and the molecular refraction too low compared with 2 : 6 : 10-trimethyl-dodecane [3] : $d_4^{20} = 0.7767$, $n_D^{20} = 1.4360$, $M_D = 71.51$

Ester fraction: From the benzene eluates the solvent was removed in vacuum and the residue distilled at 50°/$10^{\div 4}$ mm. The distillate was dissolved in petroleum ether and

stored at +15°. Small amounts of matricaria ester crystallized and were removed; the mother liquor was distilled again. The U.V.-spectra then exhibited the following maxima:

I.	λ_{max}	3361	3137	2820	2650	2500	ÅU
	ε_{max}	7940	9886	11750	11600	17000	
II.	λ_{max}	3350	3125	2820	2654	2500	ÅU
	ε_{max}	6920	8730	10720	12450	14300	

These spectra correspond to mixture of matricaria ester and α,β-dihydromatricaria ester I 59 : 41, II 57 : 43.

	I. C 75.78	H 6.69	II. C 75.70	H 6.27
Matricaria ester $C_{11}H_{10}O_2$		Calc.	C 75.84	H 5.79
Dihydromatricaria ester $C_{11}H_{12}O_2$		»	» 74.97	» 6.86

Essential oils of some *Amellus* species

Amellus strigosus (Thunb.) Less, and *Amellus strigosus* var. *Wildenowii* were cultivated on open ground at Trondheim in the summer of 1951. The flowers and roots were distilled with steam separately, the leaves and stems were taken together. The following table gives the yields and apparent concentrations of acetylenic compounds in the essential oils.

Table 3.

Species	Part of plant	Essential oil		
		Yield %	Spectroscopically estimated concentrations in % of:	
			Matricaria ester	α,β-Dihydro-matricaria ester
A. strigosus (Thunb.) Less (Seed: Botanic Garden, Lund)	Root	0.2	20	15
» »	Flowers	5.8	19	15
» »	Rest	1.7	16	18
A. strigosus (Thunb.) Less. (Seed: Botanic Garden, Gent)	Root	0.2	7	84
» »	Flowers	3.8	16	24
» »	Rest	1.6	21	36
A. strigosus var. *Wildenowii* (Seed: Royal Botanic Gardens, Kew.)	Root	0.1	11	87
» » » » »	Flowers	2.9	24	18
» » » » »	Rest	1.3	20	20

Some of the essential oils of the flowers and of the stem and leaves crystallized at −12°. The semisolid mass was stirred with petroleum ether, filtered and the crystals recrystallized from petroleum ether to give colourless crystals of 2-*cis* : 8-*cis* matricaria ester identical in m.p. and U.V.-spectrum with an authentic sample. The mother liquors were distilled at 0.3 mm Hg, (air bath). A liquid amounting to 10—40 % was collected in the trap at room temperature. This liquid showed a weak but very characteristic U.V.-spectrum, compare Fig. 3. In contrast to all other essential oils from composité plants so far investigated in this laboratory this chromophor has ethylenic spacing:

$E^{1\%}_{1cm}$	67	78	57	550	
λ	3091	2959	2822	2300	ÅU
$\nu \cdot 10^{-12}$	970.5	1013.8	1063.1		
$\Delta\nu$ · »	43.3	49.3			

The fractions boiling between 40 and 90°, which contained the acetylenic esters, were then tested in a cooling bath (CO_2 + acetone). If the fraction solidified readily, it was dissolved in petroleum ether and left for crystallization at first at 0°, the mother liquors then at −12° and −30°. The remaining liquids were then diluted with petroleum ether and chromatographed on Floridin XXX using petroleum ether as solvent and mixtures of benzene and petroleum ether as eluants. The stability of these highly unsaturated compounds is better on Floridin XXX than on alumina; however, although the resolving power is inadequate with both of them, we have not been able to find better adsorbents. Repeated chromatography on Floridin XXX gave concentrates which spectroscopically was estimated to contain 82 % 8-*cis*-α,β-dihydromatricaria ester and 18 % matricaria ester. As essential oils or concentrates containing as much as 18 % 2-*cis* - 8-*cis* matricaria ester crystallize readily from hexane solution, whereas these concentrates deposited no crystals, it was obvious that some other stereoisomers of matricaria ester were present. Both the known stereoisomers [8], viz. 2-*cis*: 8-*trans* and 2-*trans*: 8-*trans* resemble in their U.V.-spectra 2-*cis*: 8-*cis* very much, but the inflexion at 3 600 ÅU is narrower and the first peak at 3 360 ÅU more pronounced in the first named. In actual fact these details were clearly shown by these *Amellus*-concentrates. As 2-*trans*: 8-*trans* esters are still more insoluble than 2-*cis*: 8-*cis*, this modification could be excluded. The 2-*cis:* 8-*trans* matricaria ester m.p. + 2° is readily soluble in petroleum ether, a crystallization of this ester from a solvent being so far unknown.

For this reason the chromatographic fractions with the highest matricaria peaks were saponified under nitrogen at 0° C and the free acids recrystallized several times from petroleum ether. The m.p. asymptotically approached the broad m.p. of 2-*cis:* 8-*trans* matricaria acid: 83—91° and Debye-Scherrer diagrammes, most kindly taken by Dr. H. Sörum, proved the identity.

The chromatographic fractions most enriched in the dihydromatricaria ester were saponified as well, and by repeated crystallizations from petroleum ether we succeeded in removing the 2-*cis:* 8-*trans* matricaria acid from the 8-*cis*-α,β-dihydroacid. Although the U.V.-spectrum of our purest preparation of the 8-*cis* dihydroacid has a broader "foot" on the long wave length side than the synthetic acid, the 5 sharp maxima and the extinction coefficients were in perfect agreement (compare Fig. 2). As the matricaria peak at 3 360 ÅU stands out markedly at concentrations down to ½ %, this contamination, in our best specimen, must be very low. The m.p. of this best fraction was 64.1—64.5 sharply, that is some 2—3° higher than found for the synthetic acid. The mixed m.p.

was intermediate (62—62.3°) and as a reinvestigation of the synthetic acid showed that this acid in part isomerizes to the corresponding *trans*-acid with depression of the m.p. for low concentrations of the *trans*-acid this difference in m.p. aught to be caused by a small contamination of trans acid in the synthetic preparation.

SUMMARY

Further investigations of the essential oil of scentless mayweed (*Matricaria inodora* L.) have demonstrated that this oil contains besides the main component 2-*cis*: 8-*cis* matricaria ester, the aliphatic sesquiterpene "natural farnesene", 2-*cis* : 8-*trans*-matricaria ester and 8-*cis*-α,β-dihydromatricaria ester. The isolation of the new acetylenic esters was facilitated through the high concentrations in which they occur in some essential oils of the genus *Amellus*. Spectroscopically the 8-*cis*-α,β-dihydromatricaria ester was further demonstrated to be present in *Felicia tenella* (L.) Nees in *Cephalophora aromatica* Schrad. and *Solidago sempervirens* L. Obviously these new acetylenic compounds are widely distributed amongst essential oils of plants belonging to the plant family of the compositae.

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Studies Related to Naturally Occurring Acetylene Compounds. X. The Synthesis of some Hydrogenated Relatives of Matricaria Ester

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From the essential oil of various plants belonging to the family of the Compositae the following closely related highly unsaturated derivatives of methyl caprate [1–3] amongst others have been isolated:

I. $H_3C-CH_2-CH_2-C\equiv C-C\equiv C-CH=CH-COOCH_3$ Lachnophyllum ester
II. $H_3C-CH=CH-C\equiv C-C\equiv C-CH=CH-COOCH_3$ Matricaria ester
III. $H_3C-CH=CH-C\equiv C-C\equiv C-C\equiv C-COOCH_3$ Dehydro matricaria ester

Further investigation in this plant family made it probable that this series might be extended in the hydrogenated direction (compare the preceeding paper of Stavholt-Baalsrud *et.al.* [4]).

For purpose of comparison the following new members of the hydrogenated series have been synthesized through oxidative coupling according to Glaser.

		m.p.	
		Methyl ester	Free acid
IV. $CH_3-CH=CH-C\equiv C-C\equiv C-CH_2-CH_2-COOCH_3$	*cis*	liq.	60.5°
	trans	15.5°	144°
V. $CH_3-CH_2-CH_2-C\equiv C-C\equiv C-CH_2-CH_2-COOCH_3$		liq.	92°
VI. $CH_3-CH_2-CH_2-CH_2-C\equiv C-C\equiv C-CH_2-COOCH_3$		»	53°

The preparations of IV to VI, and the unavoidable by-products, are given in the experimental part.

It turned out that the 8-*cis*-α,β-dihydromatricaria ester IV is identical with an ester rather widely distributed in nature (compare [4]).

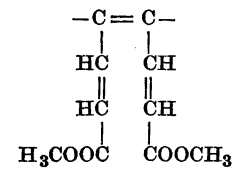
The 2 : 8-tetrahydro matricaria ester (V) and methyl deca-3 : 5-diyn-1-oate (VI) only show very weak selective absorption in U.V. below 2700 ÅU corresponding to dialkyl diacetylenes. The esters themselves will be very difficult to detect through the U.V. spectroscopy of natural essential oils, even when present in high concentrations as, at least most, composite oils contain small amounts of strong chromophors which will hide the spectrum of these esters completely. So far no indications have been found of the occurrence of V and VI in nature.

In the synthesis of VI by Glaser coupling dimethyl octa-3 : 5-diyn-1 : 8-dioate (VII)

$$H_3COOC-CH_2-C\equiv C-C\equiv C-CH_2-COOCH_3 \qquad \text{VII.}$$

results as a by-product in fair yield. This substance shows the interesting property of turning beautifully violet-red on standing. The coloration accelerates in light, but some crystals remain colourless, so obviously some catalysing impurities are co-responsible for the colour development. The red material is insoluble in organic solvents, unchanged VII is extracted by attempts of recrystallization. These secondary crystalline fractions repeat the curious colouration. A similar colouration was discovered by Castille [5] in 1939 with the acetylenic fatty acids from *Ongokea Klaineana* Pierre. The true nature of the component of the *Ongokea* acids responsible for their colouration is not known. Castille's formula for the main acid, which he on account of this colour development named erythrogenic acid, is — as was emphasized first by professor E. R. H. Jones [6] — obviously not in accordance with its spectral properties None of our synthetic or natural acetylenic compounds with an isolated diyne or enediyne grouping have shown this colouration phenomenon.

The red material has the unchanged composition $(C_5H_5O_2)_x$. This fact in connection with its extreme insolubility points to a polymeric nature. The beautiful colour necessitates conjugation in the polymer material. In analogy with the deeply coloured polyenes, which Eisler *et.al.*[7] recently have synthesized from cyclopentadiene, formula VIII, is put forward as a working hypothesis.



VIII.

Finally it should be mentioned that we have tried to synthesize methyl deca-2 : 4-diyn-1-oate through the coupling of *n*-hept-1-yne with propiolic acid

methyl ester. We were not able to isolate any amount of the desired asymmetric coupling product; besides tetradeca-6 : 8-diyne a colourless compound, m.p. 54,5°, was isolated in the same yield as when n-heptyne is omitted from the reaction. The elementary composition corresponds to $C_8H_8O_6$. Since dimethyl adipate is generated by catalytic hydrogenation with consumption of about 4 Mol H_2 this compound must originate by a Glaser coupling of the ester of propiolic acid with, formally, addition of water and one atom of oxygen.

Shortening the reaction time in the Glaser coupling gave a mixture of $C_8H_8O_6$ with a new compound $C_8H_8O_5$.

We are returning later to the investigation of the constitution of these compounds. The tendency of diacetylene dicarboxylic acid to add water might be one of the reasons for the difficulty which different investigators have met when trying to repeat the classical condensations of A. v. Bayer.

EXPERIMENTAL

α,β-Dihydromatricaria ester (IV). Pent-3-en-1-yne was prepared according to Eglinton and Whiting [8] and fractionationed as described in [9]. Methyl pent-4-yn-1-oate was prepared from tribromopropane and ethyl acetoacetate according to T. E. Gardner and W. H. Perkin jr. [10].

cis Isomer: 4 ml pent-3-en-1-yne b.r. 46—48° and 4 ml methyl pent-4-yn-1-oate were coupled under the experimental conditions worked out for the analogous synthesis of the matricaria esters. By the destillation at 10^{-4} mm Hg the hydrocarbon fraction passed into the trap, the desired dihydromatericaria ester was distilled over until the temperature reached 65° air bath and redistilled slowly, b.p. 50—53° Yield 1.1 g. The *cis* isomer is an oil m.p. $< -17°$.

$C_{11}H_{12}O_2$ (176.2) Calc. C 74.97 H 6.86
Found » 75.09, 74.82 » 6.80, 6.75
$d_4^{20} = 1.0017$ $n_D^{20} = 1.5450$ $M_D = 55.6$
$M_{D\ calc.}$ $C_{11}H_{12}O_2$ 2 $\equiv$, 1 $=$ = 50.14

Table 1. Dispersion of cis *α,β-dihydro-matricaria ester.*

$$R_{\lambda,\ calc.} = \frac{52.817 \cdot \lambda^2}{\lambda^2 - 1.7393 \cdot 10^6},\ (\lambda_0 = 1319\ \text{ÅU})$$

λ	n_λ^{20}	$R_{\lambda,\ obs.}$	$R_{\lambda,\ calc.}$
5895.9	1.54500	55.61	55.60
5790.7	1.54618	55.71	55.71
5460.7	1.55092	56.11	56.09
4358.3	1.57458	58.09	58.14

U.V.-absorption, in hexane, Fig. 1 curve 1.

$\varepsilon_{max.}$	11350	15140	11100	4900	2400	
$\lambda_{max.}$	2810	2650	2514	2381	2275	ÅU
$\nu_{max.} \cdot 10^{-12}$	1067.6	1132.1	1193.3	1260.0	1318.7	
$\Delta\nu$ » $\cdot 10^{-12}$	64.5	61.2	66.7	58.7		

Catalytic hydrogenation: 59 mg *cis*-ester was hydrogenated with Pt in alcoholic solution. Calc. 2 $\sqrt{=}$, 1 $\sqrt{-}$ 20°, 740 mm 41.3 ml. Found 41.2 ml H_2. The perhydroester = methyl caprate was distilled $n_D^{20} = 1.4288$, methyl caprate 1.4265. The hydrogenation product, which had the characteristic strawberry odour of methyl caprate, was saponified and the free capric acid isolated in the usual way; m.p. and mixed m.p. with authentic capric acid 31° C.

cis-α,β-Dihydromatricaria acid. The *cis* methyl ester was saponified under nitrogen as in (9) and recrystallized from petroleum ether, m.p. = 60.5°. Re-esterification with diazo-methane gave the liquid methyl ester with unchanged U.V.-spectrum.

trans-Isomer. 2 ml Pent-3-en-1-yne b.r. 52—53° and 3 ml methyl pent-4-yn-1-oate were coupled as above. The crude distillate, b.r. < 61° at 10^{-4} mm Hg (0.86 g), was recrystallized from petroleum ether as colourless leaflets, m.p. 15.5° C.

$C_{11}H_{12}O_2$ (176.2) Calc. C 74.97 H 6.86

Found » 74.76, 75.09, 75.11 » 6.67, 6.87, 6.68

$d_4^{20} = 0.9940$ $n_D^{20} = 1.5496$ $M_{D,\,obs.} = 56.14$ $M_{D,\,calc.}$ $C_{11}H_{12}O_2$ 2 $\sqrt{=}$, 1 $\sqrt{-}$ = 50.14

Table 2. Dispersion of trans-*α,β-dihydromatricaria ester.*

$$R_{\lambda,\,calc.} = \frac{53.545 \cdot \lambda^2}{\lambda^2 - 1.78 \cdot 10^6},\ (\lambda_0 = 1334\ \text{ÅU})$$

λ	n_λ^{20}	$R_{\lambda,\,obs.}$	$R_{\lambda,\,calc.}$
5895.9	1.54963	56.44	56.44
5790.7	1.55112	56.56	56.55
5460.7	1.55577	56.96	56.94
4358.3	1.58063	59.04	59.08

UV.-absorption in hexane. Fig. 1 curve 2.

$\varepsilon_{max.}$	17780	22650	16600	10720
$\lambda_{max.}$	2814	2653	2510	2390
$\nu_{max.} \cdot 10^{-12}$	1066.1	1130.8	1195.2	1255.2
$\Delta\nu_{max.} \cdot 10^{-12}$	64.7	64.4	60.0	

Catalytic hydrogenation: 81.45 mg *trans*-ester was hydrogenated with a Pt-catalyst in alcoholic solution. Calc. 18.5°, 738 mm 57.0 ml. Found 58.6 ml H_2, that is 5.14 $\sqrt{-}$. The perhydroester had the characteristic strawberry odour and showed $n_D^{20} = 1.4284$ (methyl caprate $n_D^{20} = 1.4265$). Saponification gave the free acid m.p. and mixed m.p. with authentic capric acid 31° C.

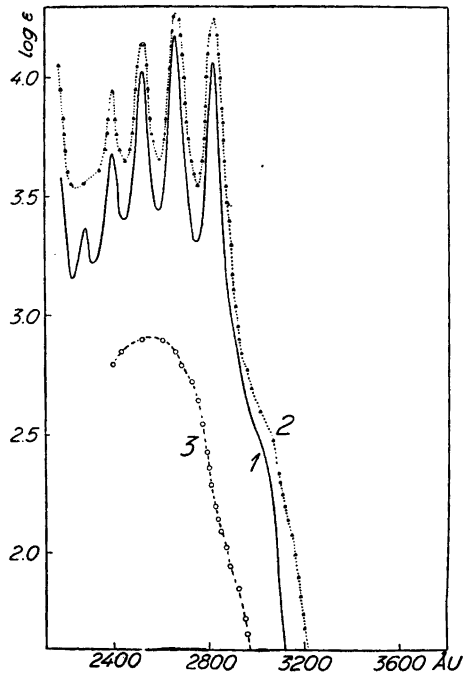


Fig. 1. Ultraviolet absorption of hexane solutions of:

Curve 1. 8-cis-α,β-dihydromatricaria ester.
» *2. 8-trans-α,β-dihydromatricaria ester.*
» *3. $C_8H_8O_6$ from propiolic acid methyl ester $E\,\frac{1\,\%}{1\,cm}$.*

trans-α,β-Dihydromatricaria acid. The *trans* ester was saponified cautiously under nitrogen according to the usual procedure. Crystallization from petroleum ether gave colourless prisms m.p. 144° (corr.).

Re-esterification with diazomethane gave the *trans* ester m.p. 15.5°. The U.V.-absorption spectrum of this regenerated ester has the data given above. In the U.V.-spectrum of the *trans* ester isolated primarily from the Glaser coupling a weak band occurred, maximum 3112 Å (log ε = 2.92). This impurity could not be eliminated through recrystallization. The four main maxima were in identical positions, the extinction coefficients of the regenerated ester lying some 10 % higher.

Dimethyl deca-4 : 6-diyne-1 : 10dioate [$(H_3COOC-CH_2-CH_2-C\equiv C-C\equiv C-CH_2-CH_2\text{-}COOCH_3)$.] The residues from the distillation of the *cis*- and *trans* dihydroesters above solidified on cooling and were recrystallized from petroleum ether, m.p. 37.5°.

$C_{12}H_{14}O_4$ (222.2)	Calc.	C 64.84	H 6.35
	Found	» 64.79, 64.62	» 6.21, 6.22

U.V.-absorption in hexane:

$\varepsilon_{max.}$	380	560	660	840
$\lambda_{max.}$	2814	2662	2515	2392
$\nu_{max.} \cdot 10^{-12}$	1066.1	1127.0	1192.8	1254.2
$\Delta\nu_{max.} \cdot 10^{-12}$	60.9	65.8	61.4	

Catalytic hydrogenation: 69.6 mg in alcoholic solution with a Pt-catalyst. $C_{12}H_{14}O_4$, 2 /≡ calc. 19°, 740 mm 30.8 ml. Found 30.4 ml H_2. The hydrogenation product was saponified, and the crystalline acid recrystallized from alcohol. M.p. and mixed m.p. with authentic sebacic acid, 134°.

Methyl deca-4 : 6-diyn-1-oate (= 2 : 8-tetrahydromatricaria ester (V)). 3.4 g Pent-1-yne prepared according to (11), and 3.6 g methyl pent-4-yn-1-oate prepared according to (12) were added to 112 g ammonium chloride, 70 g cuprous chloride and 2 ml 2 *N* ammonia in 250 ml water. The suspension was shaken mechanically for 2 hours without additional introduction of air. The reaction mixture was distilled at 10^{-4}, the hydrocarbon fraction passing into the trap. 1.73 g Tetrahydromatricaria ester distilled between 55—58° in the air-bath. Colourless liquid m.p. $< -12°$.

$C_{11}H_{14}O_2$ (178.2) Calc. C 74.11 H 7.92
Found » 73.7 » 7.8

$n_D^{20} = 1.5000$, $d_4^{20} = 0.9792$, $M_{D,\,obs.} = 53.87$, $M_{D,\,calc.}$ $C_{11}H_{14}O_2$, 2 /≡ = 50.65

U.V.-spectrum in hexane:

$\varepsilon_{max.}$	235	425	495
$\lambda_{max.}$	2530	2386	2256
$\nu_{max.} \cdot 10^{-12}$	1185.8	1257.3	1329.8
$\Delta\nu_{max.} \cdot 10^{-12}$	71.5	72.5	

Catalytic hydrogenation: 41.5 mg in alcoholic solution with PdO-catalyst, 19°, 760 mm. Calc. 2 /≡ 23.2 ml, found 21.7 ml H_2.

2 : 8-Tetrahydromatricaria acid. The methyl ester was saponified at 0° under nitrogen and worked up as above. The free acid was recrystallized from petroleum ether as colourless leaflets m.p. 92°.

Methyl deca-3 : 5-diyn-1-oate VI. *n*-Hex-1-yne was prepared according to (13). For the synthesis of methyl but-3-yn-1-oate ethylene oxide was reacted with sodium acetylide according to Henne and Greenlee [14] to give but-3-yn-1-ol, which was oxidized with chromic acid to but-3-yn-1-oic acid according to the procedure given by Heilbron, Jones and Sondheimer [15]. This acid was esterified with the calculated amount of diazomethane in anhydrous ether and the liquid ester distilled, b.p. 28° at 8 mm Hg. 4.1 g *n*-Hexyne and 4.9 g methyl-but-3-yn-1-oate were added to a solution of 112 g ammonium chloride, 70 g cuprous chloride and 2 ml. 2 *N* ammonia in 286 ml water and the suspension shaken in a flask for 1 hour mechanically, then one hour more by hand with connection to the air. The reaction mixture was distilled at 10^{-4} mm Hg and the fractions 50—70° and 90—100° C redistilled slowly. Methyl deca-3 : 5-diyn-1-oate distilled at 55°/10^{-4} mmHg as a liquid mp. $< -$ 18° C.

$C_{11}H_{14}O_2$ (178.2) Calc. C 74.11 H 7.92
Found » 73.9 » 7.93

$n_D^{20} = 1.4978$, $d_4^{20} = 0.9478$, $M_{D,\,obs.} = 55.09$, $M_{D,\,calc.}$ $C_{11}H_{14}O_2$, 2 /≡ = 50.65

Catalytic hydrogenation: PdO_2 catalyst, alcoholic solution, 60.7 mg 740 mm, 17.5° C. Calc. 33.4 ml, Found 33.8 ml H/, that is 2.02 /≡. —264.4 mg, 745 mm, 21.5° C. Calc. 146.0, Found 144 ml, that is 1.96 /≡. The hydrogenated ester was isolated in the usual way and distilled, $n_D^{20} = 1.4291$; methyl caprate 1.4265. The hydrogenated ester was saponified and the free acid isolated m.p. and mixed m.p. with authentic capric acid 31° C.

Deca-3 : 5-diyn-1-oic acid. The methyl ester was saponified under pure nitrogen at 0° C. The free acid formed colourless leaflets m.p. 53°.

Dimethyl octa-3 : 5-diyn-1 : 8-oate $H_3COOC-CH_2-C\equiv C-C\equiv C-CH_2-COOCH_3$. The fraction boiling range $90-100°/10^{-4}$ solidified and was recrystallized from petroleum ether. In the light about one half of the crystals turned a beautiful violet-red. The m.p. of both the colourless and the red crystals was 54° C.

$C_{10}H_{10}O_4$ (194.2) Calc. C 61.84 H 5.19
Found » 61.55, 62.05 » 5.06, 5.21

Catalytic hydrogenation: PdO_2, in alcoholic solution.

229.3 mg, 745 mm, $t = 19{,}5°$ C Calc. 2 / 116 ml, Found 114 ml
37.6 » 740 » $t = 20°$ C » 2 » 19.15 » » 19.6 »

The hydrogenation product was a colourless liquid m.p. = —6——5.5° C. Synthetic dimethyl suberate melted at — 6.5—— 6° C, mixed m.p. — 6—5.5° C.

$C_{10}H_{18}O_4$ (202.2) Calc. C 59.40 H 8.97
Found » 59.5 » 8.93

Glaser coupling of propiolic acid methyl ester. 4.2 g Propiolic acid methyl ester prepared according to [13,14] was added to a solution of 112 g ammonium chloride, 70 g cuprous chloride and 1 ml 2 *N* ammonia in 250 ml H_2O. The suspension was shaken mechanically for 1 hour and then stirred by a stream of air for 2 hours. The reaction mixture was worked up as usual and distilled at 10^{-3} mm Hg. The main fraction, boiling about 62° (air bath temperature), solidified and was recrystallized from 100 ml petroleum ether. Yield 0.8 g colourless needles m.p. 54.5° C.

$C_8H_8O_6$ (200.1) Calc. C 48.0 H 4.03
Found » 47.5, 47.7 » 3.47, 3.58

Ultra violet absorption, Fig. 1 curve 3.

Catalytic hydrogenation: PdO_2 in alcoholic solution.

39.4 mg, 745 mm, $t = 21°$ C Consumed 18.5 ml H_2, Calc. 4 MolH_2 19.4
40.4 » 745 » » » 18.9 » » » 4 » » 19.9

The hydrogenation product was not homogeneous. On distillation at 10^{-3} mm there passed over a main fraction at about 45° C as a colourless liquid,

$C_8H_{14}O_4$ (174.2) Calc. C 55.15 H 8.10
Found » 54.9, 55.6 » 8.06, 8.17

The hydrogenation product was saponified and the free acid isolated, m.p. 149°, authentic adipic acid 150.5°, mixed m.p. 150°.

Second fraction, b.r. $70-80/10^{-3}$, colourless liquid.

$C_8H_{14}O_5$ (190.2) Calc. C 50.52 H 7.42
Found » 50.3 » 7.13

Shortening of coupling time: Experimental conditions as above, but the aeration time cut down to 1 ½ hour. The reaction mixture was isolated as usual and distilled at 10^{-3} mm. The fraction of b.p. about 45° (air bath temperature) (450 mg) solidified at room temperature. It crystallized from petroleum ether, as colourless needles, m.p. 37—41° C.

$C_8H_8O_5$ (184.1) Calc. C 52.19 H 4.38
Found » 52.7, 52.7 » 4.04, 4.01

No selective absorption in U.V., but stepping out below 2 700 ÅU.

SUMMARY

The following unsaturated derivatives of methyl caprate has been synthesized by means of Glaser coupling of their acetylenic moieties:

α,β-Dihydromatricaria ester (IV) 8-*cis* and 8-*trans*
Methyl deca-4 : 6-diyn-1-oate (V)
Methyl deca-3 : 5-diyn-1-oate (VI)

So far only the 8-cis α,β-dihydromatricaria ester has been found in nature.

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Studies on the Hydrolysis of Metal Ions

III. The Hydrolysis of the Mercury (I) Ion, Hg_2^{2+}

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There are very few data in literature that might allow an estimation of the acidity of the ion Hg_2^{2+}. In 1904 Ley and Heimbucher [1] calculated $[H^+] = 3.42 \cdot 10^{-3}$ C from the velocity of sucrose inversion in 0.05 C $Hg_2(ClO_4)_2$ at 25° C, from which figure one may calculate $pk_{a1} = 3.6$, assuming Hg_2OH^+ to be the only product formed. Newbery [2] measured in 1936 the pH of mercury (I) perchlorate solutions with a glass electrode (temperature not stated), and from these data calculated values for pk_{a1} between 4.0 and 4.6.

For the sake of completeness it should be added that Guiter [3] measured the pH of mercury (I) nitrate solutions of various concentrations c, using a glass electrode. The accuracy seems to have been ± 0.1 or 0.05 pH units. From the variation of pH with log c he concluded that the predominant complex formed is $Hg_2OH(NO_3)_n^{-(n-1)}$ in the range 0.032–0.15 mC (n = 9 or 10), $(Hg_2OH)_n^{n+}$ in the range c = 0.15–65 mC (n = 2 or 3), and Hg_2OHNO_3 in the range 65–260 mC. Like many other of Guiter's results, this seems to conflict with the law of mass action. If the complexes with several nitrate groups, or those containing several Hg_2 groups, were to predominate even at low concentrations, their relative amounts would certainly be more likely to increase with increasing c.

There is one disturbing factor which does not seem to have been discussed in the earlier work quoted. Mercury (I) perchlorate or nitrate solutions as usually prepared generally contain about 1 % of mercury (II) ions, corresponding to the equilibrium $Hg^{2+} + Hg$ (l) $\rightleftharpoons Hg_2^{2+}$, the equilibrium constant of which is about 130[4,11]. Now Hg^{2+} is a rather strong acid ($pk_{a1} = 3.70$, $pk_{a2} = 2.60$, for 25° C and 0.5 C ClO_4^- as shown in part II), so that a large part of the observed acidity of mercury (I) solutions may be due to the mercury (II)

present. As will be shown below, this really accounts for the greater part of the observed acidity. However, there is a residual term due to mercury (I) acidity; but the acidity constant of mercury (I) is smaller than has previously been assumed.

The work on mercury (I) and mercury (II) was carried out parallel because the constants for one ion were needed for calculating those of the other. Actually, the work on mercury (I) was started before that on mercury (II).

Choice of method and experimental conditions. For the same reasons as in Part I it was decided to study the equilibria at 25.0° C, using solutions with $[ClO_4^-] = 0.5$ C.

From the analysis of our solutions we might calculate the total concentration a of mercury (I) and mercury (II), and H, the value the hydrogen ion concentration would have had, if the mercury ions were not hydrolysed. In addition it was thought desirable to measure both the actual concentrations $[Hg_2^{2+}]$ and $[H^+]$ $(= h)$.

The hydrogen ion concentration must be measured with a glass electrode, since both a hydrogen electrode and a quinhydrone electrode would have reduced the solutions, with the formation of metallic mercury.

The concentration of Hg_2^{2+} was measured with a mercury electrode. The same reference calomel electrode was used as in part II, CE $\|$ = Hg, Hg_2Cl_2 | 4 C NaCl | 0.5 C $NaClO_4$ |. Cells of the following types were thus used:

$-$CE $\|$ Hg^{2+}, Hg_2^{2+}, H^+ | glass electrode + (cell of type 1)

$-$CE $\|$ Hg^{2+}, Hg_2^{2+}, H^+ | Hg + (cell of type 3)

Cells of type 1 may be used for calculating the hydrogen ion concentration h in the same way as described in Part I. For cells of type 3 we have

$$E_3 = E_{03} + E_j + 29.58 \log [Hg_2^{2+}] \qquad (1)$$

Here E_{03} is a constant, which is at first unknown, and E_j was taken to be $E_j = -0.0972\ h$ mV (h in mC) from preliminary work of Biedermann and Sillén [6].

EXPERIMENTS

The *reagents* were prepared and analysed as described in Part I. The *apparatus* for titrating and measuring the emfs was also the same as in Part I. For cells of type 3, a mercury pool on the bottom of the titration vessel was used, into which a short platinum needle was dipped. The calomel electrode and salt bridge will be described below in some detail.

The *procedure* was the same as in Part I. First an acid-base titration was carried out with a glass electrode (cell of type 1), adding an alkaline solution T to an acid solution S_1. These solutions were free from mercury ions. Then a certain amount of a mercury (I) solution S_2 was added, mercury metal was introduced into the titration vessel, the platinum needle was inserted, and in the latter part of the titration both the CE-mercury cell (E_3) and the CE-glass electrode cell (E_1) were measured. A survey of the titrations is given in Table 1.

Table 1. Survey of the titrations.

		S_1		T	S_2			ml added		
No.	Symbol	a mC	mC H^+	mC OH^-	a mC	mC	H^+	S_1	T	S_2	$S_2 + T$
1	▲	2.00	40.00	40.06	4.00	10.46	40	39	79	27	+ 27
2	□	0.50	40.00	40.08	1.00	10.00	40	39	79	25	+ 25
3	◇	0.50	40.00	40.08	1.00	10.00	40	39	79	26	+ 26
4	△	0.50	40.00	40.05	1.00	10.00	40	39	79	25	+ 25
5	■	0.50	40.00	40.07	1.00	10.00	40	39	79	25.5	+ 25.5
6	○	0.20	40.00	40.05	0.60	10.07	39.5	38.5	39	5.8	+ 11.6
7	●	0.20	40.00	40.05	0.60	10.07	39.5	39	39.25	5.1	+ 10.2
8	◑	0.20	40.00	40.07	0.40	10.00	40	39	79	26.5	+ 26.5

Calomel electrode and salt bridge. Fig. 1 shows the calomel electrode and salt bridge with the stopcocks C_1—C_5, and the standard ground-glass joints J_1 and J_2. The apparatus is bent at right angles around the vertical lines through C_1 and C_3, so that seen from above it appears as on the lower right in Fig. 1. This is just to save space in our thermostats and has nothing to do with its function.

To the left of the three-way stopcock C_2 the apparatus is filled with 4 C NaCl, to the right of C_2 there is 0.5 C $NaClO_4$; this solution extends to the little bent capillary in the titration vessel to the right. The arrangement of the titration vessel has been described elsewhere [7]. The solution in it also contains 0.5 C ClO_4^-.

On the left in Fig. 1 is the electrode vessel with Hg and Hg_2Cl_2. A platinum needle dips into the mercury; there is a constriction at the lower end of the vessel in order to prevent aqueous solution from reaching the Pt needle at least with moderate movements of the electrode vessel.

In Fig. 1 the apparatus is shown as set up for a titration. The liquid junction at C_2 may be renewed at will by flushing alternately with NaCl and $NaClO_4$ solutions by suitable movements of C_1, C_3, and the three-way C_2. This was done before each titration, and sometimes in the middle of a titration. However, this operation never seemed to change the E of the cell, showing that the liquid junction potential at C_2 may be considered as constant.

Table 2 a. Data from titration no 3. Acid-base titration, initially 40.00 ml S_1 (40.00 mC H^+). T (40.08 mC OH^-) was added.

T ml	E_1 mV	$H = h$ mC	$E_{10} + E_d^*$ mV
9.00	+ 4.8	25.29	−78.2
12.00	+ 0.9	21.52	−77.9
17.00	− 5.1	16.11	−76.5
22.00	−13.4	11.58	−76.3
29.00	−28.2	6.343	−75.7
35.00	−49.9	2.629	−74.7
39.00	−92.5	0.4668	−72.9

Table 2 b. Mercury titration. Initially 79.00 ml S_2 (10.00 mC H^+, $a = 1.00$ mC) was added to the last solution from Table 2 a. For each volume of S_2, an equal volume of T was added.

T ml	H mC	E_1 mV	h mC	E_3 mV	δE mV
0.00	5.23	−32.8	5.21	428.20	
4.00	4.26	−37.8	4.25	428.00	
7.00	3.58	−42.0	3.60	428.10	0.05
12.00	2.56	−50.9	2.52	428.27	−0.01
16.02	1.81	−59.5	1.80	428.32	0.01
19.06	1.29	−68.0	1.29	428.42	−0.04
20.10	1.12	−71.9	1.10	428.31	0.08
21.00	0.98	−74.8	0.99	428.25	0.15
23.00	0.65	−84.6	0.67	428.23	0.21
24.00	0.51	−90.9	0.53	428.06	0.39
25.00	0.36	−98.9	0.38	427.87	0.59
26.00	0.21	−107.7	0.27	427.52	0.95

The emf was measured with C_5 open.

After a titration C_5 is closed, C_4 is opened, and C_2 is set so that $NaClO_4$ solution can be pressed out to the waste until its level reaches J_2. C_2 is closed, J_2 is disconnected, and a ground-glass stopper put into its she-joint. The apparatus to the left of J_2 is then kept in the thermostat. The part with C_4 and C_5 is emptied, washed, and dried.

At the onset of a new experiment, the tube to the right of C_5 is filled with $NaClO_4$ solution, which also extends about 1 cm to the left of C_5. C_5 is closed, the he-joint and she-joint of J_2 connected (the stopper having first been removed), and C_3 and C_4 opened so that the $NaClO_4$ solution fills the whole tube up to a few mm from C_4. Then C_3 and C_4 are closed.

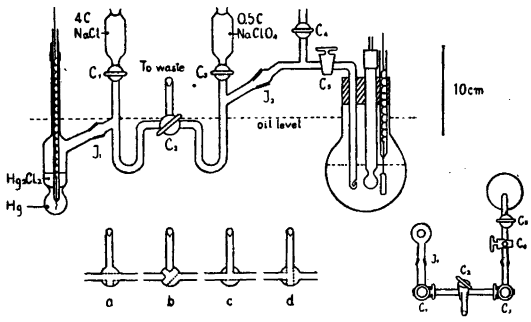


Fig. 1. Cell with calomel electrode, salt bridge and titration vessels. In the latter a glass electrode and a Pt electrode have been inserted. (Burettes, nitrogen inlet, etc. are not shown.) a-d stopcock C_2 in different positions. On the lower right: cell seen from above. For details see text.

When it is desired to clean the part between J_1 and J_2, J_1 may be disconnected and a stopper put into the she-part of J_1. The calomel electrode can be kept in the thermostat bath all the time.

This apparatus is easily handled and in comparison with that used previously has the advantage that all sorts of operations can be performed without taking the calomel electrode out of the thermostat bath. The level of the oil bath is indicated in Fig. 1.

CALCULATIONS

The whole effect to be measured was very small. The calculations were chiefly founded on the measurements with the mercury electrode, which were rather accurate. The glass electrode was used for measuring

$$[H^+] = h \tag{2}$$

However, the quantity $H-h$, the hydrogen ion concentration set free by the hydrolysis processes, could not be obtained accurately enough from the glass electrode and analytical data to be used in the calculations.

It has been shown by Jonsson, Qvarfort, and Sillén [4] that a Hg electrode in a solution containing Hg_2^{2+} and Hg^{2+} ions quickly acquires the potential corresponding to the final equilibrium between the solution and Hg metal. We may thus assume that this equilibrium had been established in the solution.

From Part I we take the equilibrium constants

$$Hg^{2+} + H_2O \rightleftharpoons HgOH^+ + H^+ \quad q_1 = [HgOH^+]\, h\, [Hg^{2+}]^{-1} = 0.20 \text{ mC} \tag{3}$$

$$Hg^{2+} + 2\, H_2O \rightleftharpoons Hg(OH)_2 + 2\, H^+ \quad q_2 = [Hg(OH)_2]\, h^2\, [Hg^{2+}]^{-1} = 0.50 \text{ mC}^2 \tag{4}$$

The work of Jonsson, Qvarfort and Sillén was carried out with $h = 10$ mC; with this acidity the hydrolysis of Hg_2^{2+} may be neglected, but a correction

should be made for that of Hg^{2+}. We take from their paper that at equilibrium for $[H^+] = 10$ mC

$$[Hg_2^{2+}][Hg^{II}]_{tot}^{-1} = 129.2 \pm 1.0 \tag{5}$$

Using (3), (4) and (9, Part I):

$$129.2 \pm 1.0 = [Hg_2^{2+}][Hg^{2+}]^{-1}(1 + q_1h^{-1} + q_2h^{-2})^{-1} = [Hg_2^{2+}][Hg^{2+}]^{-1} \cdot 1.025^{-1} \tag{6}$$

Thus we find the real equilibrium constant

$$Hg^{2+} + Hg(l) \rightleftharpoons Hg_2^{2+} \quad k_0 = [Hg_2^{2+}][Hg^{2+}]^{-1} = 132.4 \pm 1.0 \tag{7}$$

Since the hydrolysis of the Hg_2^{2+} ions is a very small effect, the best thing we can do is probably to make the simplest assumption, namely that the only product is Hg_2OH^+, so that the reaction is

$$Hg_2^{2+} + H_2O \rightleftharpoons Hg_2OH^+ + H^+ \quad k = [Hg_2OH^+]\, h\, [Hg_2^{2+}]^{-1} \tag{8}$$

From the analytical data we know the total mercury ion concentration a

$$a = [Hg_2^{2+}] + [Hg_2OH^+] + [Hg^{2+}] + [HgOH^+] + [Hg(OH)_2] \tag{9}$$

From the equilibrium conditions (3), (4), (7), and (8) we deduce

$$a = [Hg_2^{2+}]\ (1 + kh^{-1} + k_0^{-1}(1 + q_1h^{-1} + q_2h^{-2})) = [Hg_2^{2+}]\ (1 + \psi) \tag{10}$$

By introducing (10) we may transform the expression (2) for E_3

$$E_3 = E_{03} + E_j + 29.58\ \log\ a - 29.58\ \log\ (1 + \psi) \tag{11}$$

Now let us consider the fixed value $h' = 10^{0.5}$ mC $= 10^{-2.5}$ C and denote the values for E_3 and ψ at this point by E_3' and ψ'. Then we have

$$E_3' = E_{03} + 29.58\ \log\ a - 29.58\ \log\ (1 + \psi') + E_j' \tag{12}$$

We shall now define the quantity

$$\delta E = E_3' - E_j' - (E_3 - E_j) = E_3' - E_3 + 0.0972(h' - h) = \\ = 29.58\ [\log(1 + \psi) - \log(1 + \psi')] \tag{13}$$

Fig. 2. gives δE as a function of $\log h$. The experimental points have been calculated from the measured E_3 by means of (13). They are uncertain by several 0.01 mV depending on how E_3' is calculated. The curves have been calculated from the right member of (13) using the expression for ψ from (10)

$$\psi = kh^{-1} + k_0^{-1}\ (1 + q_1h^{-1} + q_2h^{-2}) \tag{14}$$

The values for q_1, q_2, and k_0 were taken from (3), (4), and (7), and different values for k were tried.

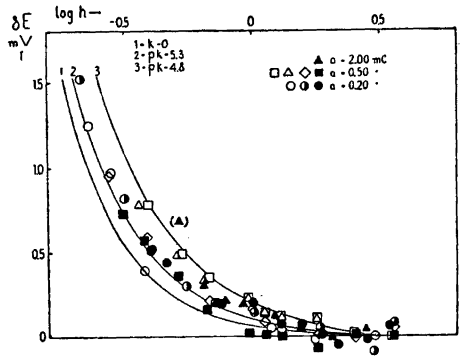


Fig. 2. δE in mV as a function of log h (h in mC). Experimental points: symbols see Table 1. Curves calculated for different values of k. Curve 1 gives the effect of Hg^{2+}, provided Hg_2^{2+} is not at all acid (k = 0).

As is seen from the curve $k = 0$ (no hydrolysis of Hg_2^{2+}), the greatest part of δE is explained by the hydrolysis of Hg^{2+}. However, the experimental points are consistently slightly above the curve for $k = 0$, thus showing that there is in addition a small hydrolysis of Hg_2^{2+}. Because of the smallness of the effect, the equilibrium cannot be studied very exactly. However, assuming that Hg_2OH^+ is the chief product, one may estimate the acid constant to be within the range

$$k = [Hg_2OH^+]\ h\ [Hg_2^{2+}]^{-1} = 10^{-5.0 \pm 0.3}\ C \qquad (15)$$

This value is smaller than those obtained in previous investigations [1,2].

One might argue that the hydrogen ion concentration has been measured in the bulk of the solution and thus does not correspond to the final equilibrium with mercury metal. However, much more weight has been put in the calculations on the values of E_3 (mercury electrode) than on those of E_1 (glass electrode). In Fig. 2, for instance, 1 mV in the mercury electrode scale corresponds to almost 40 mV in the glass electrode scale. Since the mercury (I) solution has previously been equilibrated with mercury, it cannot be far from the new equilibrium, so the error in h should be small.

STANDARD POTENTIALS

The work of our team on mercury complexes has hitherto been concerned with emfs and equilibria with solutions containing 0.5 C ClO_4^- at 25° C, and not with the constants for infinitely dilute solutions. However, we shall attempt a few remarks on the standard potentials. The standard potentials e_{10}^0 for $Hg_2^{2+} \mid Hg$ and e_{21}^0 for $Hg_2^{2+}, Hg^{2+} \mid Pt$ are related to the equilibrium constant K_0 for infinite dilution for the reaction

$$Hg^{2+} + Hg(l) \rightleftharpoons Hg_2^{2+}\quad K_0 = [Hg_2^{2+}][Hg^{2+}]^{-1} \qquad (16)$$

by the relation, for 25° C

$$e^0_{21} - e^0_{10} = 59.16 \log K_0 \qquad (17)$$

The accepted values for the standard potentials have been determined from emf measurements by extrapolation to infinite dilution, to eliminate the activity factors. However, with increasing dilution the hydrolysis of the ions causes an increasing error, which will be especially great for e^0_{21}, since Hg^{2+} is a rather strong acid. This error will tend to make e^0_{21} too low.

On the other hand, the constant K_0 contains the ratio of the activity factors of two dipositive ions which may be expected to be not far from unity even at considerable ionic strengths. Thus the concentration ratios measured at ionic strengths around 0.5 C may be good approximations for K_0, provided the solution is sufficiently acid.

We conclude that of the three quantities in (17), K_0 and e^0_{10} can be most reliably determined, whereas e^0_{21} should preferably be calculated from the other two than determined separately by the very uncertain extrapolation method.

For e^0_{10} Linhart's measurements [8] at 25° C have been recalculated by Lewis and Randall, and by Bray and Hershey [10]. They found + 798.6 mV, and + 797.5 ± 1.0 mV.

Let us assume

$$Hg_2^{2+} \mid Hg \qquad e^0_{10} = 798 \pm 1 \text{ mV} \qquad (18)$$

For K_0 we have Abel's measurements [11] which give the equilibrium ratio $[Hg_2^{2+}]_{tot}[Hg^{2+}]^{-1}_{tot} = 119.8$ at 25° C in about 0.3 C HNO_3. Now Hg^{2+} forms complexes with NO_3^- a little more readily than Hg_2^{2+}. Using Infeldt's and Sillén's values [12] for the equilibrium constants (although these are really valid for $[ClO_4^-] = 3$ C) we find that for 0.3 C NO_3^-, $[Hg^{2+}]_{tot} = [Hg^{2+}] \cdot 1.47_4$, and $[Hg_2^{2+}]_{tot} = [Hg_2^{2+}] \cdot 1.36$, which gives the ratio of the free ions $[Hg_2^{2+}]$ $[Hg^{2+}]^{-1} = 119.8 \cdot 1.47 \cdot 1.36^{-1} = 130$, which agrees well with the ratio 132.4 found in (7) for 0.5 C ClO_4^-. Since the activity factors should approximately cancel each other, we may assume for infinite dilution

$$Hg^{2+} + Hg(l) \rightleftharpoons Hg_2^{2+} \qquad K_0 = 130 \pm 10 \qquad (19)$$

thus from (17)

$$e^0_{21} - e^0_{10} = 125 \pm 2 \text{ mV} \qquad (20)$$

which gives, provided (18) is correct

$$Hg^{2+},\ Hg_2^{2+} \mid Pt \quad e^0_{21} = + 923 \pm 3 \text{ mV} \qquad (21)$$

SUMMARY

The hydrolysis of the mercury (I) ion Hg_2^{2+} has been studied at 25.0° C and $[ClO_4^-] = 0.5$ C. The acidity of mercury (I) salt solutions, which has previously been ascribed to the acidity of Hg_2^{2+}, has proved to be chiefly due to the acidity of Hg^{2+}, which is always present in small amounts and is a rather strong acid. A small residual effect must be ascribed to the acidity of Hg_2^{2+}; the pk_a is estimated at 5.0 ± 0.3, which means that Hg_2^{2+} is a weaker acid than has previously been assumed.

The standard potential e_{21}^0 for Hg^{2+}, $Hg_2^{2+} \mid Pt$ should preferably be calculated from measured values of the standard potential e_{10}^0 for $Hg_2^{2+} \mid Hg$ and the equilibrium constant K_0 for $Hg^{2+} + Hg(l) \rightleftharpoons Hg_2^{2+}$, than from direct measurements. Taking as best literature data $e_{10}^0 = 798 \pm 1$ mV and $K_0 = 130 \pm 10$ one finds $e_{21}^0 = 923 \pm 3$ mV, which is higher than the usually accepted value.

This is in good accord with the value 0.92 V given by Abegg, Auerbach, and Luther in 1911 [13] (they gave 0.80 V for e_{10}^0), but differs from later values: 905 mV [14] and 910 mV [15] obtained by an uncertain extrapolation of Popoff's values [14]. (Added in proof) Latimer ([14], 1952 edition) quotes $e_{10}^0 = 789$ mV from a New Zealand thesis of M. T. Christensen, which was not available to us. This would give $e_{21}^0 = 914$ mV.

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On the Reactions of the Aluminium Ion with Water

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The system Al-OH has attracted the interest of many scientists; especially the solid aluminium hydroxide phases and conversion from one to another, and colloid-chemical and technical questions connected with them. On the other hand, the equilibrium in the system Al-OH has been very little dealt with. The most important of the few works dealing with it is Brönsted and Volqvartz's [1] classical paper on acid dissociation of aquo ions. By measuring the hydrogen ion concentration by reaction kinetic methods in aluminium perchlorate solutions with different amounts of perchloric acid, they come to the conclusion that at 15° C the first stage of protolysis of the hexaquo-aluminium ion follows the formula:

$$Al(H_2O)_6^{3+} + H_2O \rightleftharpoons Al(H_2O)_5OH^{2+} + H_3O^+$$

and that the equilibrium is determined by the formula:

$$\frac{[H^+] \cdot [AlOH^{2+}]}{[Al^{3+}]} = 5.5 \cdot 10^{-6}$$

Nevertheless, the writers point out that the change in the equilibrium constant with the total concentration of aluminium was not found to take place as would be expected from the changes in the ionic strength.

Faucherre [2] thought he was able to show that the protolysis suggested by Brönsted and Volqvartz explained conditions in solutions where the total concentration of aluminium was less than $5 \cdot 10^{-3}$. For higher concentrations he proposed the following formula:

$$\frac{[(AlOH)_2^{4+}] \cdot [H^+]^2}{[Al^{3+}]^2} = k_1$$

Faucherre determined both k_1 and Brönsted's constant (k_2) at 20° C and at two different ionic strengths (I) with the following results:

I	k_1	k_2
0.60	$5.82 \cdot 10^{-9}$	$1.07 \cdot 10^{-6}$
0.12	$8.72 \cdot 10^{-9}$	$1.83 \cdot 10^{-6}$

The precipitation of aluminium hydroxide gel and its re-dissolution by the addition of alkali to aluminium salt solutions have been studied qualitatively by many writers. Semiquantitative measurements have been made by Treadwell and Bonn [3]. These workers titrated $AlCl_3$ solutions potentiometrically with NaOH, determining the pH with a hydrogen electrode. The opalescence and viscosity of the solutions were also investigated. The measurements show that the viscosity has a sharp maximum and the opalescence a flatter one near the point of equivalence. On the basis of their observations they suggested a possible mechanism for the reactions in question.

Finally, it should be mentioned that several determinations have been made of the solubility of different forms of aluminium hydroxide. The varying results indicate that the values obtained depend to a high degree on the experimental conditions.

Since, therefore, very little appears to be known of the equilibrium conditions in the system Al-OH, it would seem of interest to examine it more closely, particularly as the system is important both scientifically and technically.

EXPERIMENTAL METHODS

An examination of equilibria in complex systems is highly facilitated if it is possible to determine the concentrations both of the central group and the ligand. In the system Al-OH this is unfortunately impossible, as so far no satisfactory method has been found for determining $[Al^{3+}]$. (See, for instance, Berestneva and Kargin [4].) In several cases, however, relatively much data has been obtained on the equilibrium conditions in complex systems even when only ligand concentration measurements have been available. The present investigation which is based mainly on pH measurements, shows that this is also true of the system Al-OH.

The measurements and results given below were preceded by a preliminary investigation carried out at 25° C. The results of the latter indicated that some of the equilibria studied are established very slowly at this temperature. This occurs more rapidly at higher temperatures. For this reason, and bearing

in mind that some technical processes based on the equilibrium conditions in the system Al-OH take place at 40° C it seemed best to carry out the main part of the investigation at the latter temperature.

The measurements consist of determinations of the hydrogen ion concentration in solutions containing aluminium perchlorate, sodium hydroxide, and sodium perchlorate as ionic medium. These measurements are divided into three series and comprise the following concentration levels *:

		1	2	3
Total concentration of aluminium	$= [Al_t]$:	0.002209	0.01107	0.05541 C
Total concentration of sodium hydroxide	$= [NaOH_t]$:	$0-14 \cdot 10^{-3}$	$0-5 \cdot 10^{-2}$	$0-2 \cdot 10^{-1}\ C$

The ionic strength of all the solutions was kept at 2.02 C by addition of $NaClO_4$.

Within each series the hydrogen ion concentration was determined for different contents of $[NaOH_t]$ by measuring the EMF in cells of the following type:

$$Pt,\ H_2 \ \Big|\ NaClO_4\ 2\ C;\ \begin{matrix} Al(ClO_4)_3 & x\ C \\ NaOH & y\ C \end{matrix} \ \Big|\ NaClO_4\ 4\ C \ \Big|\ NaCl\ 0.1\ C \ \Big|\ Hg_2Cl_2, Hg$$

For this a Jensen potentiometer, type 10 A, was used with a Multiflex galvanometer, type MG 3, as the zero instrument. The error in values for the potential was less than $\pm$ 0.1 mV. The temperature, which was regulated by a paraffin oil thermostat enclosed in an air thermostat, always lay within the limits 40.0° $\pm$ 0.1° C. Hydrogen from cylinders was purified in the usual manner over copper at 455° C. It was then passed successively through an alkaline solution, sulphuric acid, a solution of 2.02 C $NaClO_4$ at 40° C, a vessel containing the test solution, and finally through another vessel containing test solution into which a platinized platinum electrode was inserted. The EMF values obtained were corrected in the usual manner for changes in the atmospheric pressure. The hydrogen ion concentration was calculated from the formula

$$[H^+] = [H^+_s] \cdot 10^{-16.094(E-E_s)}$$

where E is the EMF of the cell for the solution in question and E_s its EMF when the aluminium salt solution is replaced by a "standard acid", *i.e.* a

* All concentrations are given in C, *i.e.* mole per liter, at 40° C unless otherwise stated. When the ionic strength is defined by 2 C $NaClO_4$, measurements have shown the following relation to exist: $C_{40°} = C_{20°} \cdot 0.9888$.

solution of perchloric acid of known concentration together with $NaClO_4$ as ionic medium.

The concentrations of free and aluminium-bound hydroxyl ions were calculated from the expressions

$$[OH^-_{free}] = \frac{k_w}{[H^+_s]} \cdot 10^{+16.094(E-E_s)}$$

$$\text{and } [NaOH_t] - [H^+] - [H^+_a] = [OH^-_{free}] + [OH^-_{bound}]$$

where $[H^+_a]$ is the possible concentration of free acid in the aluminium salt solution to which we added sodium hydroxide, k_w is the ion product of water at 40° C and at the ionic strength in question. The k_w was determined by measuring the hydrogen ion concentration in solutions of NaOH of known strength. The results are given in Table 1.

Table 1. Determination of k_w at 40° C.

$$[NaOH] + [NaClO_4] = 2.02\ C$$

[NaOH]	$k_w \cdot 10^{14}$	pk_w
0.009848	3.034	13.518
.01970	3.049	13.516
.02954	3.049	13.516
.03939	3.046	13.516

In the following we shall use the value 13.52 for pk_w.

PREPARATIONS

The following preparations have been used:

1. $NaClO_4$. Various Swedish and foreign preparations of different qualities were purified by a suitable number (2—5) of recrystallizations from 70 % alcohol. The purified products contained < 0.01 % chloride and chlorate and approximately 0.0014 mole per cent alkali (probably bicarbonate). The solutions were standardized by running samples through a H^+-saturated ion exchange column and titrating the hydrogen ions set free in the filtrate.

2. $HClO_4$ solutions. These were prepared from perchloric acid (density 1.67) Merck pro analysi, and their concentrations were ascertained by the oxalate method.

3. NaOH solutions were prepared and kept in the usual way. The content of carbonate never exceeded 0.2 %.

4. $Al(ClO_4)_3$. For the preparation of this substance aluminium hydroxide was precipitated with NaOH from a hot water solution of $AlCl_3 \cdot 6\ H_2O$ (Merck pro analysi). The

precipitate was washed and then dissolved in perchloric acid. By repeated reprecipitation, washing and re-dissolution the last traces of chloride ion could be removed. The exceedingly hygroscopic substance was finally washed with chloroform to remove most of the free perchloric acid present. The preparation thus obtained was extremely pure. A very little sodium and some perchloric acid were the only impurities found. It would probably be very difficult to produce an absolutely neutral aluminium perchlorate and this is by no means necessary if we can determine the contents of the free acid in the preparation. Brönsted, for example, used an alkaline preparation which he obtained by letting $AlCl_3$ react with $AgClO_4$; the $AlCl_3$ contained as usual somewhat less than 3 Cl per Al. He could then calculate the "base" content from his pH measurements.

In the present case a stock solution was made from the preparation obtained. The Al concentration of this solution was found to be $0.449 \pm 0.001\ C$. Determinations of the total content of cation equivalents by means of the ion exchange method showed that the preparation contained 11—12 mole-% $HClO_4$. More exact values could be obtained by EMF measurements.

If we determine the hydrogen ion concentration $[H^+]$ in a series of solutions where $[Al_t]$ is kept constant and where the amount of free acid is gradually increased by additions of $[H_i^+]$ and if we plot

$$\frac{[H^+]-[H_i^+]}{[Al_t]} \text{ against } \frac{1}{[H^+]} \text{ and extrapolate to } \frac{1}{[H^+]} = 0, \textit{ i.e} \text{ an infinitely acid solution}$$

where all kinds of protolyses are suppressed, a value is obtained which should represent the number of moles free acid per mole $Al(ClO_4)_3$ in the stock solution. The results of such measurements are given in Table 2.

Table 2. Determination of free acid in the stock solution of $Al(ClO_4)_3$.

$[Al_t] = 0.01116\ C$; $t = 25°$ C

$[H^+]$ determined with a quinhydrone electrode

$[H_i^+]\ C \cdot 10^3$	$[H^+]\ C \cdot 10^3$	$\frac{1}{[H^+]}\ C^{-1} \cdot 10^{-3}$	$\frac{[H^+]-[H_i^+]}{[Al_t]}$
0.150	1.464	0.6831	0.1180
0.473	1.780	0.5618	0.1171
1.224	2.519	0.3970	0.1160
2.483	3.772	0.2651	0.1155
3.725	5.012	0.1995	0.1153

The extrapolation gives the value 0.115, *i.e.* 11.5 mole-%, which agrees well with the analytical result.

EXPERIMENTAL RESULTS

The experimental determination of the complexity curve for the system Al-OH was performed as follows: two solutions — one containing aluminium perchlorate and ionic medium, the other sodium hydroxide and ionic medium

— were pre-heated to approximately 40° C and well mixed, after which the electrode vessel was filled as quickly as possible with the mixture and the hydrogen gas current turned on. The EMF of the cell was then read at suitable time intervals until equilibrium was reached. When mixing we poured the alkali into the aluminium salt solution, when the complex formation number $\bar{n}$, *i.e.* in this case the average number of bound OH^--groups per aluminium atom, was less than 3. In the cases where $\bar{n}$ was greater than 3 the aluminium salt solution was poured into the alkali. In the range near $\bar{n} = 3$ two experiments were made, one in each way.

Table 3. Series 1. $[Al_t] = 2.209 \cdot 10^{-3}$ mole/l. Temp. 40° C.

No.	$(NaOH_t) \cdot 10^3$ mole/l	First value			Final value		
		t hours	pOH	$\bar{n}$	t hours	pOH	$\bar{n}$
1.1		0.50	10.016	0.033	1.33	10.015	0.033
2.1	0.277	0.58	9.686	0.082	1.55	9.685	0.082
3.1	0.366	0.45	9.632	0.115	18.03	9.648	0.117
4.1	0.554	0.53	9.543	0.189	20.22	9.615	0.198
5.1	0.752	0.37	9.493	0.273	21.70	9.600	0.285
6.1	1.088	0.47	9.467	0.423	19.30	9.573	0.434
7.1	1.978	0.62	9.433	0.823	20.25	9.498	0.829
8.1	2.472	0.75	9.367	1.041	20.27	9.467	1.049
9.1	3.589	0.41	9.262	1.540	20.33	9.384	1.548
10.1	4.687	0.40	9.031	2.027	20.30	9.250	2.036
11.1	5.310	0.37	8.918	2.305	19.95	9.066	2.310
12.1	5.781	0.41	8.782	2.515	19.40	8.845	2.517
13.1	6.427	0.35	6.502	2.799	19.60	7.995	2.801
14.1	6.650	0.37	6.056	2.900	20.77	6.887	2.900
15.1	6.758	0.32	5.708	2.948	9.80	5.842	2.948
16.1	6.872	0.35	5.470	2.999	45.76	4.895	2.995
17.1	6.872	0.40	5.576	3.000	43.92	5.228	2.998
18.1	6.981	0.45	5.345	3.048	21.12	4.610	3.039
19.1	7.189	0.47	5.104	3.141	19.87	4.364	3.125
20.1	7.812	0.23	4.942	3.421	96.23	3.888	3.368
21.1	8.453	0.27	4.477	3.702	40.10	3.606	3.605
22.1	8.658	0.40	4.313	3.787	44.88	3.511	3 670
23.1	8.905	0.55	4.183	3.892	84.42	3.371	3.729
24.1	10.18	0.28	3.017	4.063	2.65	3.016	4.061
25.1	10.96	0.36	2.766	4.076	2.56	2.766	4.077
26.1	13.81	0.40	2.333	4.042	5.15	2.328	4.020
27.1	14.04	0.40	2.313	4.047	1.74	2.311	4.039

Table 4. Series 2. $[Al_t] = 1.107 \cdot 10^{-2}$ mole/l. Temp. 40° C.

No.	$[NaOH_t].10^2$ mole/l	First value			Final value		
		t hours	pOH	$\bar{n}$	t hours	pOH	$\bar{n}$
1.2	0.1379	0.21	10.125	0.051	2.45	10.125	0.051
2.2	0.1822	0.24	10.018	0.083	2.17	10.016	0.083
3.2	0.2019	0.30	9.981	0.099	21.63	9.990	0.099
4.2	0.2265	0.30	9.942	0.119	21.38	9.969	0.120
5.2	0.2758	0.60	9.892	0.161	22.12	9.916	0.162
6.2	0.3742	0.35	9.799	0.245	21.85	9.858	0.248
7.2	0.5417	0.22	9.722	0.394	21.92	9.834	0.398
8.2	0.9848	0.45	9.669	0.792	21.60	9.776	0.796
9.2	1.231	0.35	9.587	1.013	19.98	9.756	1.018
10.2	1.231	0.48	9.591	1.013	19.43	9.747	1.017
11.2	1.792	0.34	9.527	1.519	20.14	9.699	1.523
12.2	2.340	0.31	9.360	2.010	10.76	9.532	2.013
13.2	2.344	0.37	9.366	2.014	45.00	9.558	2.017
14.2	2.620	0.57	9.209	2.261	43.63	9.384	2.253
15.2	2.890	0.38	8.908	2.502	22.00	9.135	2.504
16.2	2.895	0.20	8.864	2.508	93.58	9.229	2.510
17.2	3.166	0.47	7.663	2.750	47.55	8.607	2.751
18.2	3.209	0.37	6.972	2.789	71.64	8.379	2.789
19.2	3.320	0.30	5.971	2.889	53.10	7.798	2.889
20.2	3.333	0.74	6.073	2.901	25.50	6.909	2.901
21.2	3.375	0.47	5.332	2.938	70.37	6.392	2.939
22.2	3.403	0.33	5.103	2.963	49.67	5.978	2.964
23.2	3.431	0.28	4.941	2.988	67.76	4.588	2.987
24.2	3.431	0.24	4.989	2.988	68.65	4.925	2.988
25.2	3.442	0.30	4.929	2.893	69.20	4.505	2.997
26.2	3.442	0.30	4.907	2.998	68.57	4.559	2.997
27.2	3.486	0.27	4.739	3.037	90.42	4.297	3.034
28.2	3.699	0.38	4.063	3.223	19.50	3.302	3.186
29.2	3.955	0.30	3.869	3.451	65.62	2.807	3.322
30.2	4.029	0.38	3.985	3.521	22.13	2.716	3.355
31.2	4.473	0.87	3.326	3.888	23.40	2.448	3.609
32.2	4.826	0.33	2.630	4.038	22.03	2.558	4.000
33.2	4.911	0.34	2.545	4.070	23.44	2.491	4.035
34.2	4.944	0.22	2.437	4.026	19.70	2.440	4.028

The EMF was found to change in all cases in the course of time, quickly at the beginning of the experiments and then more slowly. In very acid and alkaline solutions the equilibrium was reached scarcely half an hour after the solution had been mixed ($t = 0$), or in as short time as when measurements

Table 5. Series 3. $[Al_t] = 5.541 \cdot 10^{-2}$ mole/l. Temp. 40° C.

No.	$[NaOH_t]$ mole/l	First value			Final value		
		t hours	pOH	$\bar{n}$	*t* hours	pOH	$\bar{n}$
1.3	0.01681	0.28	10.213	0.202	20.98	10.225	0.203
2.3	0.03283	0.26	10.036	0.488	46.15	10.180	0.491
3.3	0.08683	0.20	9.767	1.457	44.75	9.973	1.462
4.3	0.1412	0.28	9.223	2.438	43.80	9.485	2.439
5.3	0.1569	0.31	8.315	2.722	259.40	9.346	2.722
6.3	0.1703	0.27	5.014	2.963	91.40	6.589	2.963
7.3	0.1719	0.25	4.659	2.991	1.63	4.870	2.991

were carried out on the reference acid. We can, therefore, conclude that in a very acid or alkaline solution containing aluminium ion, the hydrogen ion concentration quickly reaches its equilibrium value. In other cases slow reactions occur which cause successive changes in the hydrogen ion concentration of the system. The measurements carried out are given in Tables 3, 4, and 5. In these tables the first values for pH and $\bar{n}$ indicate the state approximately half an hour after the preparation of the solution, *i.e.* at the moment when the hydrogen electrode might be supposed to give correct values; whereas the last pair of values will correspond, at least very nearly, to a state of equilibrium and has been calculated from the last read EMF, which did not appear to change with time. The data given in these tables are also to be found in Figs. 1, 2, and

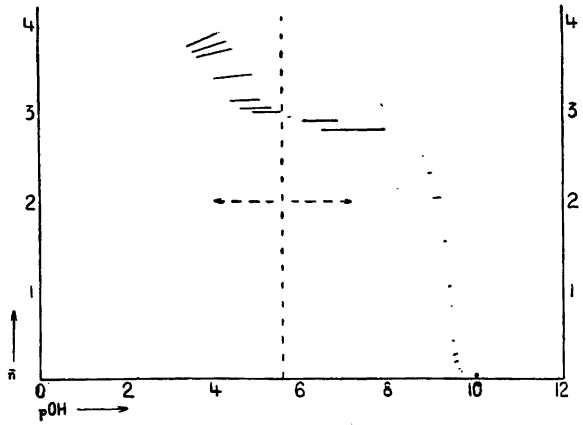


Fig. 1 (Table 3).

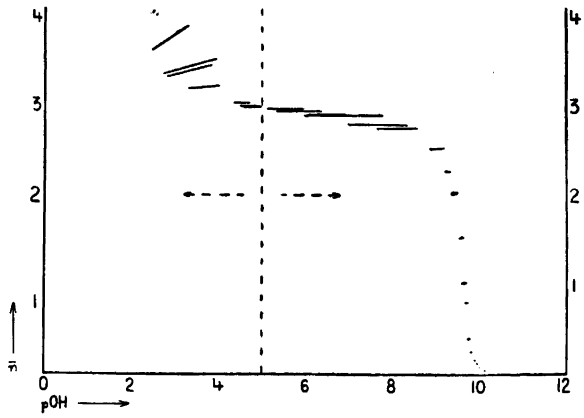


Fig. 2 (Table 4).

3. Here the first and last readings are joined by a line. The arrows mark the direction of the change. As will be seen this is reversed at $\bar{n} \sim 3$.

The way in which these changes take place in the hydrogen ion concentration was studied more closely. It was then found that for a given total concentration of aluminium salt the character of the process is mainly determined by the ratio $[NaOH_t]/[Al_t]$. The observations can be summarized in the following way:

1. If a small amount of alkali is added to an acid solution of aluminium perchlorate so that the solution still remains acid, $[OH^-]$ is independent of time (Table 6 a, Fig. 4 a).

2. If more NaOH is added to an aluminium perchlorate solution so that $[NaOH_t]/[Al_t]$ lies within 0.1—2.5, the system gets a higher OH^- ion concentration than the equilibrium value immediately after this addition. The

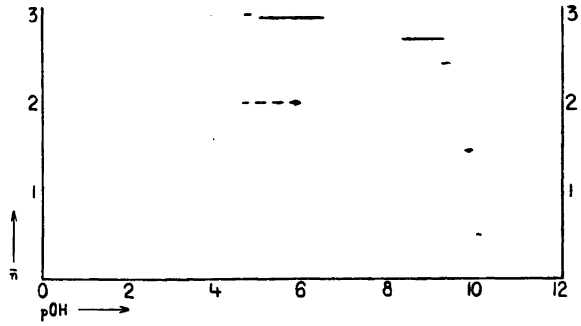


Fig. 3 (Table 5).

equilibrium is reached by a slow process during which $[OH^-]$ falls to its limiting value. This effect becomes more and more accentuated as the ratio $[NaOH_t]/[Al_t]$ is increased to ~ 2.5. (Table 6 b, Fig. 4 b.)

3. If NaOH is added to an aluminium perchlorate solution so that the ratio $[NaOH_t]/[Al_t]$ lies between 2.5 and 3, the solution in this case, too, will become relatively alkaline immediately after the addition. $[OH^-]$ falls, however, in a more complicated manner to its equilibrium value than in the earlier case, for after having decreased rather fast for a couple of hours it passes through a minimum and then through a maximum, before later continuing steadily to its limiting value. Such a process is illustrated by Table 6 c and Fig. 4 c. The minimum becomes more and more marked as the ratio $[NaOH_t]/[Al_t]$ approaches 3.

4. For $[NaOH_t]/[Al_t] \sim 3$ the change in the hydroxyl ion concentration is as shown in Tables 6 d and e and Figs. 4 d and e. In this case there is a very rapid fall in the concentration and then a slow rise. The tables and figures give two series of experiments. One when a NaOH solution was added to a solution of aluminium perchlorate (e), the other when an aluminium salt solution was added to the alkali (d). As will be seen the progress in this case is not appreciably affected by the way in which the solution was mixed.

5. In the range $\bar{n}$ greater than 3 the change in OH^- ion concentration with time is very characteristic. If an aluminium perchlorate solution is added to a sodium hydroxide solution the OH^- ion concentration of the latter falls instantaneously to a relatively low value, and then rises quickly for an hour or so immediately afterwards. After this the speed of reaction diminishes suddenly and the OH^- ion concentration continues to rise slowly to what appears to be the equilibrium value. The process is shown in Table 6 f and Fig. 4 f.

6. When NaOH and the Al salt solution are mixed so that $\bar{n}$ approaches 4 the OH^- ion concentration of the solution again becomes independent of time (Table 6 g and Fig. 4 g).

ANALYSIS OF THE TIME FACTOR IN THE SLOW REACTIONS

The general impression we get when we study the slow reactions described in the former section is that they are caused by a relatively complicated mechanism, where several effects are superimposed or occur successively. We can, however, distinguish a few simpler relations:

1. In highly acid and alkaline solutions the equilibrium is established very quickly;

Table

a(No. 3.2)		b(No. 16.2)		c(No. 17.2)				d(No.	
t hours	pOH	*t* hours	pOH	*t* hours	pOH	*t* hours	pOH	*t* hours	pOH
0.30	9.981	0.20	8.864	0.47	7.658	6.00	7.911	0.30	4.929
0.38	9.981	0.30	8.872	0.48	7.748	6.25	7.914	0.33	4.897
0.50	9.975	0.42	8.885	0.52	7.844	6.50	7.916	0.40	4.887
0.63	9.974	0.47	8.889	0.53	7.924	6.75	7.921	0.43	4.886
0.71	9.975	0.62	8.900	0.58	8.005	6.78	7.925	0.47	4.897
0.80	9.975	0.92	8.924	0.62	8.037	7.33	7.937	0.50	4.911
1.11	9.975	1.17	8.939	0.70	8.069	10.03	8.037	0.52	4.920
1.25	9.975	1.67	8.963	0.80	8.070	10.25	8.045	0.53	4.926
1.63	9.975	2.17	8.977	0.82	8.069	26.22	8.452	0.55	4.944
2.05	9.976	2.83	8.992	0.83	8.067	26.42	8.454	0.57	4.960
2.28	9.976	3.17	8.996	0.88	8.064	26.60	8.455	0.58	4.977
2.46	9.977	19.51	9.107	0.92	8.060	26.63	8.456	0.60	4.993
2.68	9.977	19.75	9.108	0.95	8.056	26.75	8.458	0.62	5.008
2.85	9.980	20.33	9.110	0.98	8.054	28.25	8.473	0.63	5.024
3.23	9.980	21.03	9.111	1.02	8.050	30.00	8.489	0.65	5.041
6.13	9.981	22.20	9.114	1.07	8.047	30.50	8.493	0.67	5.057
6.38	9.982	23.25	9.118	1.08	8.044	31.00	8.499	0.68	5.073
6.61	9.984	23.50	9.121	1.13	8.040	31.08	8.497	0.70	5.089
19.66	9.987	25.17	9.124	1.18	8.037	31.13	8.496	0.72	5.105
19.96	9.990	26.20	9.126	1.22	8.037	31.18	8.496	0.75	5.137
20.30	9.991	26.75	9.126	1.23	8.033	31.20	8.496	0.78	5.170
20.76	9.991	43.42	9.159	1.33	8.030	31.22	8.496	0.83	5.202
21.00	9.990	44.25	9.159	1.40	8.025	31.23	8.496	0.90	5.285
21.30	9.990	45.30	9.161	1.47	8.021	31.25	8.496	1.03	5.267
21.63	9.990	46.43	9.164	1.53	8.012	31.28	8.496	1.10	5.270
		48.19	9.167	1.65	8.002	31.32	8.497	1.13	5.270
		91.50	9.226	1.73	7.998	31.50	8.529	1.23	5.269
		91.92	9.226	1.78	7.995	35.75	8.594	1.27	5.268
		92.08	9.227	1.83	7.992	47.20	8.593	1.30	5.267
		92.37	9.227	1.88	7.990	47.55	8.598	1.35	5.264
		92.97	9.227	1.93	7.986			1.40	5.263
		93.58	9.229	1.98	7.984			1.45	5.259
				2.25	7.970			1.50	5.256
				2.50	7.960			1.57	5.252
				2.75	7.952			1.65	5.247
				3.00	7.944			1.82	5.237
				3.25	7.935			1.98	5.224
				3.50	7.927			2.15	5.212
				3.75	7.924			2.32	5.199
				4.00	7.919			2.48	5.186
				4.25	7.915			2.65	5.176
				4.50	7.913			2.82	5.166
				4.75	7.911			2.98	5.156
				5.00	7.909			3.15	5.147
				5.25	7.907			3.32	5.136
				5.50	7.907			3.48	5.127
				5.75	7.909			3.53	5.126

6.

25.2)		e(No. 26.2)				f(No. 29.2)		g(No. 33.2)	
t hours	pOH	*t* hours	pOH	*t* hours	pOH	*t* hours	pOH	*t* hours	pOH
5.48	5.034	0.30	4.907	5.25	5.081	0.30	3.869	0.34	2.545
5.65	5.028	0.32	4.892	5.50	5.072	0.35	3.761	0.47	2.514
5.82	5.022	0.34	4.876	5.75	5.064	0.44	3.537	0.92	2.490
5.98	5.015	0.37	4.871	6.00	5.056	0.52	3.422	3.09	2.494
6.15	5.013	0.39	4.871	6.50	5.041	0.69	3.301	3.17	2.491
6.32	5.013	0.44	4.876	7.00	5.027	0.77	3.270	3.34	2.493
6.65	5.011	0.47	4.883	7.50	5.015	1.09	3.182	3.45	2.491
6.82	5.009	0.49	4.891	8.72	4.986	1.24	3.161	3.67	2.493
7.15	5.006	0.54	4.891	10.10	4.956	4.10	3.032	3.84	2.491
9.65	4.977	0.57	4.926	10.54	4.818	17.80	2.813	4.04	2.493
9.82	4.970	0.60	4.939	18.80	4.817	18.35	2.813	4.22	2.491
9.98	4.964	0.64	4.956	18.99	4.814	18.87	2.803	4.59	2.493
19.65	4.887	0.65	4.973	19.50	4.808	19.20	2.803	4.84	2.491
19.82	4.886	0.67	4.987	20.00	4.800	19.49	2.801	5.04	2.493
20.15	4.883	0.70	5.004	20.50	4.794	19.87	2.798	5.20	2.491
20.57	4.879	0.72	5.021	21.00	4.788	20.52	2.794	5.59	2.492
20.90	4.875	0.74	5.036	21.50	4.782	21.24	2.793	5.97	2.491
21.15	4.863	0.75	5.052	22.00	4.776	22.44	2.781	6.09	2.492
21.50	4.858	0.77	5.068	22.50	4.768	64.77	2.806	22.50	2.490
21.82	4.848	0.80	5.100	23.02	4.765	65.10	2.806	22.67	2.490
22.15	4.838	0.82	5.117	23.57	4.757	65.62	2.807	22.87	2.490
22.48	4.827	0.84	5.133	24.00	4.754			23.09	2.490
22.82	4.818	0.87	5.148	24.50	4.747			23.30	2.491
23.15	4.808	0.90	5.181	25.24	4.738			23.44	2.491
23.48	4.799	0.94	5.197	25.50	4.736				
23.82	4.790	0.95	5.213	26.00	4.731				
24.23	4.780	1.00	5.246	26.50	4.724				
24.73	4.766	1.04	5.263	29.54	4.694				
25.87	4.740	1.09	5.277	30.00	4.690				
26.43	4.726	1.12	5.288	42.65	4.614				
27.40	4.707	1.19	5.295	43.00	4.612				
30.03	4.662	1.25	5.302	43.50	4.610				
32.27	4.629	1.32	5.306	44.00	4.607				
33.35	4.618	1.35	5.306	44.65	4.604				
45.30	4.545	1.39	5.303	45.00	4.602				
45.93	4.539	1.45	5.301	45.50	4.601				
46.43	4.539	1.49	5.299	46.00	4.598				
47.95	4.533	1.55	5.295	46.50	4.596				
50.40	4.527	1.69	5.295	47.50	4.593				
51.80	4.525	1.82	5.286	48.00	4.591				
67.65	4.511	2.00	5.275	50.00	4.585				
68.15	4.511	2.50	5.221	50.90	4.583				
68.50	4.510	3.07	5.182	66.79	4.557				
69.20	4.505	4.50	5.111	67.24	4.557				
		4.75	5.100	67.50	4.556				
		4.92	5.094	68.15	4.556				
		5.00	5.091	68.57	4.559				

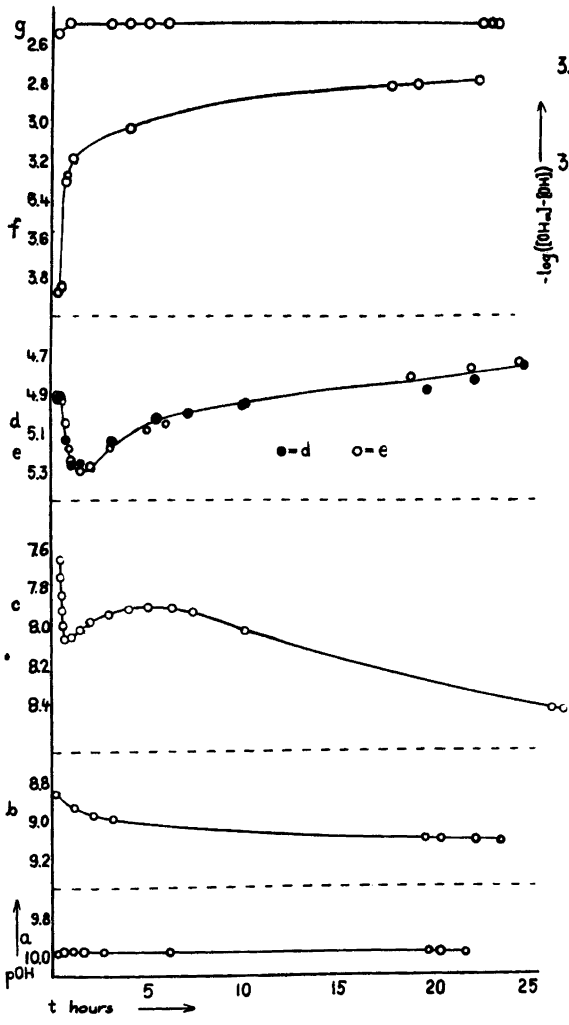


Fig. 4.

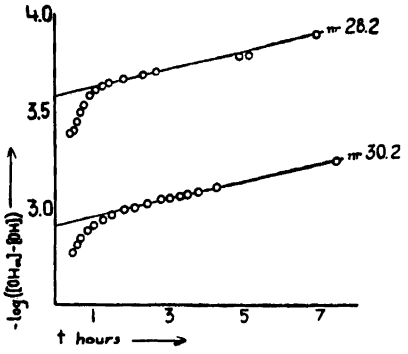


Fig. 5

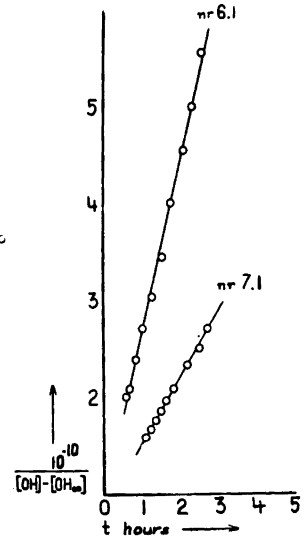


Fig. 6.

2. In moderately alkaline solutions ($3 < \bar{n} < 4$) equilibrium is established by a number of different processes, of which the final one appears, at least, to be of the first order and satisfies the following expression:

$$\log\ ([OH_\infty]-[OH]) = k' \cdot t + C'$$

where $[OH_\infty]$ is the final value, independent of time, of the OH ion concentration, and k' and C' are constants. An example of this relation is given in

Fig. 5, where both the curves appear to give the same value for the velocity constant. In other cases, however, different velocities have been found without obvious correlation with the experimental conditions (see Table 7).

Table 7. $\log ([OH_\infty] - [OH]) = k' \cdot t + C'$.

no.	k' hours^{-1}	no.	k' hours^{-1}
19.1	−0.034	27.2	−0.026
20.1	−0.009	28.2	−0.041
21.1	−0.047	29.2	−0.052
22.2	−0.035	30.2	−0.044
23.1	−0.014	31.2	−0.12

3. In solutions where $\bar{n}$ remains between 0 and 1.5 the equilibrium appears to be established by a reaction (or at least a partial reaction) of the second order. Thus the expression

$$\frac{1}{[OH]-[OH_\infty]} = k'' \cdot t + C''$$

is satisfied, as is shown by Fig. 6. In this case too the velocity constant varies with the experimental conditions in an indeterminable manner.

It is well-known that the addition of alkali to an aluminium salt solution gradually results in the formation of an aluminium hydroxide gel, that redissolves on larger additions of alkali. The solid phases formed during such reaction have been the subject of numerous examinations, chiefly by R. Fricke and his collaborators. From these and other investigations (see for instance Geiling and Glocker [5]) it has been concluded that aluminium ion reacts at suitable pH forming primarily a so-called α-gel with composition $Al(OH)_3$. Geiling and Glocker showed that this gel has an amorphous liquid-structure. α-gel ages rapidly, however, in a neutral or alkaline environment forming the ill-defined substance called β-gel. X-ray photographs of the latter (Geiling and Glocker [5]) show, however, both the liquid-interferences of the α-gel and diffuse böhmite lines. We may thus consider β-gel to be a product in which the highly disordered structure of the α-gel has begun to turn into the böhmite lattice. After some time, the length of time depending on the experimental conditions, the preparation changes into pure böhmite. In this case water is given off, as böhmite has the composition AlOOH. Böhmite is then very slowly through absorption of water turned into bayerite $Al(OH)_3$ and finally hydrargillite (also $Al(OH)_3$).

We were able to prove by means of X-ray powder photographs that solid crystalline phases appeared at an early stage in an alkaline environment. This was not the case, however, if the gel was kept in an acid solution, even if it were kept for as long as 2 months.

From this we are tempted to conclude that the first order reaction occurring in an alkaline environment is a direct manifestation of the aging process whilst the second order reaction in an acid environment is associated with the formation of α-gel or products which lead later to its formation. Therefore, the final values of pH and pOH in acid solutions will correspond to the (transient) equilibrium value in a system *where there are no crystalline phases*, while corresponding values in an alkaline environment are equivalent to an *equilibrium in a heterogeneous system where, at least, one phase possesses a well-defined lattice.*

Thus, it would be possible to examine the entire system Al-OH under similar equilibrium conditions if, within the alkaline range, we disposed of values corresponding to the state of equilibrium that would be established if the process of aging did not exist. If, however, as already assumed, this process of aging influences the pH of the solutions in the first order reaction observed in the alkaline range, such values can naturally be obtained by extrapolation of the reaction to $t = 0$. A calculation of this kind was made and will be described in the next section.

REACTION MECHANISM AND EQUILIBRIUM CONDITIONS IN THE SYSTEM Al-OH

The experimental material on which the considerations in this section is based is given in Table 8. The values are also plotted in Fig. 7. In this material, *which is assumed to correspond to the equilibrium conditions in the system Al-OH in the absence of crystalline phases*, we took for $\bar{n} < 3$ the final values from Tables 3, 4, and 5, while the pairs of values for $\bar{n} > 3$ were calculated as follows: log ($[OH_\infty] - [OH]$) was plotted against t. As already mentioned this function becomes linear for higher values of t (Fig. 5), which thus makes a linear extrapolation to $t = 0$ possible. In this way we obtain $[OH_\infty]-[OH_0]$ and therefore $[OH_0]$, *i.e.* the hydroxyl ion concentration which the system would have reached in the different cases at equilibrium, had the process of aging not existed. It is these values $[OH_0]$ which are used for the calculation of pOH and $\bar{n}$ in Table 8 and Fig. 7 for the points where $\bar{n} > 3$.

Despite numerous different trials we have not succeeded in finding an explanation of the connection found experimentally between $\bar{n}$ and pOH by using the usual expressions for homogeneous or heterogeneous complex functions. Furthermore, our measurements showed that aquo ion protolysis

Table 8.

$[Al_t] = 2.209 \cdot 10^{-3}$ mole/l			$[Al_t] = 1.107 \cdot 10^{-2}$ mole/l			$[Al_t] = 5.541 \cdot 10^{-2}$ mole/l		
No.	pOH	$\bar{n}$	No.	pOH	$\bar{n}$	No.	pOH	$\bar{n}$
1.1	10.015	0.033	1.2	10.125	0.051	1.3	10.225	0.203
2.1	9.685	0.082	2.2	10.016	0.083	2.3	10.180	0.491
3.1	9.648	0.117	3.2	9.990	0.099	3.3	9.973	1.462
4.1	9.615	0.198	4.2	9.969	0.120	4.3	9.485	2.439
5.1	9.600	0.285	5.2	9.916	0.162	5.3	9.346	2.722
6.1	9.573	0.434	6.2	9.858	0.248	6.3	6.589	2.963
7.1	9.498	0.829	7.2	9.834	0.398	7.3	4.870	2.991
8.1	9.467	1.049	8.2	9.776	0.796			
9.1	9.384	1.548	9.2	9.756	1.018			
10.1	9.250	2.036	10.2	9.747	1.017			
11.1	9.066	2.310	11.2	9.699	1.523			
12.1	8.845	2.517	12.2	9.532	2.013			
13.1	7.995	2.801	13.2	9.558	2.017			
14.1	6.887	2.900	14.2	9.384	2.253			
15.1	5.842	2.948	15.2	9.135	2.504			
16.1	4.895	2.995	16.2	9.229	2.510			
17.1	5.228	2.998	17.2	8.607	2.751			
18.1	4.979	3.05	18.2	8.379	2.789			
19.1	4.499	3.13	19.2	7.798	2.889			
20.1	4.226	3.40	20.2	6.909	2.901			
21.1	3.762	3.64	21.2	6.392	2.939			
22.1	3.733	3.73	22.2	5.978	2.964			
23.1	3.594	3.81	23.2	4.588	2.987			
24.1	3.016	4.061	24.2	4.925	2.988			
25.1	2.766	4.077	25.2	4.505	2.997			
26.1	2.328	4.020	26.2	4.559	2.997			
27.1	2.311	4.039	27.2	4.479	3.04			
			28.2	3.622	3.21			
			29.2	3.236	3.41			
			30.2	3.156	3.47			
			31.2	2.886	3.81			
			32.2	2.558	4.000			
			33.2	2.491	4.035			
			34.2	2.440	4.028			

cannot be the only existing reaction mechanism even in relatively acid solutions. These facts together with the observations described above on the kinetic conditions have finally led us to form certain hypotheses on the mechanism

for the reactions between aluminium ion and water. This reaction mechanism has been found, at least formally, to be somewhat different on the acid and the alkaline side of $\bar{n} = 3$. In the descripton below we thought it best to begin by explaining the conditions on the alkaline side, since these appear to be more perspicuous than is the case on the acid side.

$$\bar{n} > 3$$

The observations on the alkaline side of $\bar{n} = 3$ can be explained qualitatively if the following reaction mechanism is assumed:

1. In an alkaline environment, aluminium ion is protolysed instantaneously to $Al(OH)_4^-$. This manifests itself in a very rapid decrease in the pH of a sodium hydroxide solution into which aluminium ions have been introduced.

2. If the alkali excess is sufficiently large a solution is immediately obtained where practically speaking all the aluminium occurs as $Al(OH)_4^-$ (limiting complex) and where equilibrium prevails. In this case therefore a constant pH value is very rapidly reached. The viscosity must be low under these conditions, which was also the case in the measurements made by Treadwell and Bonn [3].

3. If a smaller excess of alkali is present ($3 < \bar{n} < 4$) the above-mentioned protolysis and simultaneous decrease in alkalinity are succeeded by the following reactions:

$$\begin{aligned}
&Al(OH)_4^- + Al(OH)_4^- \rightarrow Al_2(OH)_7^- + OH^- \\
&Al_2(OH)_7^- + Al(OH)_4^- \rightarrow Al_3(OH)_{10}^- + OH^- \\
&\quad\vdots \\
&Al_{n-1}(OH)_{3(n-1)+1}^- + Al(OH)_4^- \rightarrow Al_n(OH)_{3n+1}^- + OH^-
\end{aligned}$$

As is seen these reactions cause the formation of very large groups (ions) with the simultaneous increase of the pH of the solution. As n increases the probability for the ion in question to be neutralized by a cation (Na^+) forming α-gel presumably increases. This gel is, therefore, not to be considered as a definite solid phase.

4. The α-gel ages slowly under the formation of a crystalline phase. But the formation of a *solid phase* with the composition $Al(OH)_3$ (or AlOOH) in a system where $\bar{n}$ lies between 3 and 4 would imply that the ratio $[NaOH_t]/[Al_t]$ in *the solution* increased and thus the pH of the solution slowly rose.

The series of reactions given above under 3) are, at least formally, rather like the process of hydrolysis of Bi^{3+} ion examined by Granér and Sillén [6]. It would therefore seem probable that here, too, as in the case of bismuth hydrolysis, it would be possible to calculate the equilibrium from two equilibrium constants only. This calculation was performed in the following manner:

Instead of the reactions under 3) we will consider the reverse process, *i.e.* the transformation of α-gel into aluminate ion:

$$\vdots$$
$$Al_n(OH)^-_{3n+1} + H_2O \rightarrow Al_{n-1}(OH)^-_{3(n-1)+1} + Al(OH)^-_4 + H^+$$
$$\vdots$$
$$Al_3(OH)^-_{10} + H_2O \rightarrow Al_2(OH)^-_7 + Al(OH)^-_4 + H^+$$
$$Al_2(OH)^-_7 + H_2O \rightarrow Al(OH)^-_4 + Al(OH)^-_4 + H^+$$

For shortness we introduce the following symbols for concentrations:

$$[Al(OH)^-_4] = b \qquad [H^+] = h$$
$$[Al_2(OH)^-_7] = c''_2$$
$$[Al_3(OH)^-_{10}] = c''_3$$
$$\vdots$$
$$[Al_n(OH)^-_{3n+1}] = c''_n$$
$$\vdots$$

The following equilibrium equations are obtained:

$$\frac{c''_{n-1} \cdot b \cdot h}{c''_n} = k_{2,\,(n-1)}$$
$$\vdots$$
$$\frac{c''_2 \cdot b \cdot h}{c''_3} = k_{2,2}$$
$$\frac{b \cdot b \cdot h}{c''_2} = k_{2,1}$$

As in Granér and Sillén's [6] expressions we can approximately put $k_{2,2} = k_{2,3} \cdot \ldots = k_{2,\,(n-1)} = k_2$.

Further, if we put

$$k_{2,1} = k'_2, \quad \frac{b^2 \cdot h}{k'_2} = y \text{ and } \frac{b \cdot h}{k_2} = x$$

we obtain

$$[Al(OH)^-_4] = b$$
$$[Al_2(OH)^-_7] = c''_2 = y$$
$$[Al_3(OH)^-_{10}] = c''_3 = y \cdot x$$
$$\vdots$$
$$[Al_n(OH)^-_{3n+1}] = c''_n = yx^{n-2}$$

Using these expressions we now calculate $[Al_t]$ and the total concentration of hydroxyl ion bound to the aluminium, *i.e.* $\bar{n} \cdot [Al_t]$. Denoting $[Al_t]$ by a_t we get the following equations:

$$a_t = b + 2\ y + 3\ yx + 4\ yx^2 + \cdots + nyx^{n-2} + \cdots$$

$$\bar{n} \cdot a_t = 4\ b + 7\ y + 10\ yx + 13\ yx^2 + \cdots + (3\ n + 1)\ yx^{n-2} + \cdots$$

These give us:

$$a_t = b + y\ (2 + 3\ x + 4\ x^2 + \cdots + nx^{n-2} + \cdots)$$

$$\bar{n} \cdot a_t = 4\ b + y\ \{7 + (7 + 1 \cdot 3)x^1 + (7 + 2 \cdot 3)x^2 + \cdots + (7 + n \cdot 3)x^n + \cdots\}$$

Summation of the infinite series finally gives:

$$\left.\begin{aligned} a_t &= b + y\,\frac{2-x}{(1-x)^2} \\ \bar{n} \cdot a_t &= 4\ b + y\,\frac{7-4x}{(1-x)^2} \end{aligned}\right\} \tag{1}$$

where $a_t = [Al_t]$; $b = [Al(OH)_4^-]$; $y = \dfrac{b^2 \cdot h}{k_2'}$;

$x = \dfrac{b \cdot h}{k_2}$; och $h = [H^+]$

From this system of equations we obtain:

$$[Al(OH)_4^-] = \frac{2\bar{n} - 7}{\dfrac{[H^+]}{k_2}(\bar{n} - 4) + \dfrac{1}{[Al_t]}} \tag{2}$$

and

$$[H^+] \cdot [Al_t] = A_2^2\left\{B_2 + \frac{1}{A_2} \pm \sqrt{B_2\left(\frac{2}{A_2} + B_2\right)}\right\} \tag{3}$$

where $A_2 = \dfrac{k_2}{\bar{n} - 3}$ and $B_2 = \dfrac{2(\bar{n} - 3.5)^2}{k_2'(4 - \bar{n})}$

In this way we have obtained a relation between $[H^+]$ or $[OH^-]$ and $\bar{n}$. For $\bar{n} = 3.5$ we get immediately

$$[H^+]_{\bar{n}=3.5} \cdot [Al_t] = \left(\frac{k_2}{3.5 - 3}\right)^2 \cdot \frac{3.5 - 3}{k_2} = 2\ k_2 \tag{4}$$

or $$p[OH^-]_{\bar{n}=3.5} = \log \frac{2\,k_2}{k_w} - \log\,[Al_t] \qquad (5)$$

i.e. at $\bar{n} = 3.5$ the hydroxyl ion concentration of the solutions is directly proportional to the total concentration of aluminium. The calculation of the constant k_2 is, as is seen, very simple. For equation (4) gives us

$$k_2 = \tfrac{1}{2}\,[H^+]_{\bar{n}=3.5} \cdot [Al_t] \qquad (6)$$

k_2' can be calculated from equation (3), which we will now consider somewhat more closely. The following characteristics of the function are decisive here:

$\bar{n} \rightarrow$	symbol before the square root sign	$[H^+] \rightarrow$
4	+	∞
4	−	0
3.5	±	independent of the sign
3	+	∞
3	−	k_2'

In accordance with our assumption we apply, however (vide infra):

$$\lim\,[H^+]_{\bar{n}\rightarrow 3} = \infty$$

and $$\lim\,[H^+]_{\bar{n}\rightarrow 4} = 0$$

This implies the following choice of symbols before the square root sign

$$4 > \bar{n} > 3.5 : -$$
$$3.5 > \bar{n} > 3 : +$$

which leads to the function:

$$[H^+] \cdot [Al_t] = A_2^2 \left\{ \frac{1}{A_2} + B_2 + \frac{3.5 - \bar{n}}{|3.5 - \bar{n}|} \sqrt{\left(\frac{2}{A_2} + B_2\right) \cdot B_2} \right\} \qquad (7)$$

where $A_2 = \dfrac{k_2}{\bar{n} - 3}$ and $B_2 = \dfrac{2\,(\bar{n} - 3.5)^2}{k_2'\,(4 - \bar{n})}$

In calculating k_2 it is advantageous to use equation (6). In the present case we thereby obtain:

$$k_2 = 2.3_1 \cdot 10^{-13}$$

It is now necessary to find the value for k_2' which, when substituted in the equation (7), gives the best possible agreement with the experimental results. The values given in Table 9 have been calculated with $k_2' = 2 \cdot 10^{-11}$.

Table 9. $k_2' = 2 \cdot 10^{-11}$; $k_2 = 2.3_1 \cdot 10^{-13}$.

$\bar{n}$	pOH	
	$[Al_t] = 1.107 \cdot 10^{-2}$	$[Al_t] = 2.209 \cdot 10^{-3}$
3.01	5.28	5.98
3.10	3.96	4.66
3.30	3.41	4.11
3.50	3.14	3.84
3.70	2.95	3.65
3.90	2.76	3.46
3.99	2.40	3.10

The two curves shown in the alkaline range in Fig. 7 are based on these values. As is seen the agreement between these theoretical curves and the results of measurement is good, especially for the higher aluminium concentrations.

The reaction mechanism assumed in the foregoing paragraphs for the formation of α-gel from aluminate ion or for the break-down of this gel into aluminate ions necessitates the hydrogen ion concentration for $\bar{n} = 3$ being infinitely great. This, of course, is absurd and is due to the fact that no consideration has been paid to the break-down of the gel occurring in acid solution. In other words there are two processes, one of which manifests itself in the alkaline and the other in the acid range. At not too low total concentrations of aluminium, however, these processes assert themselves in pH ranges lying far from each other, which makes it possible to treat them separately. This implies a considerable simplification of the whole problem.

$$\bar{n} < 3$$

As already mentioned, the kinetic conditions in the range where $\bar{n} < 3$ are more difficult to grasp. On the other hand, we do not seem in this case to have to consider the effects of aging. Bearing in mind that there exists experimental support for the assumption that a primary rapid protolysis and a subsequent "protolytic association" take place in the alkaline range, it is reasonable to try to interprete the conditions within the acid range too in a similar manner. In other words in this case also we should like to assume a

primary protolysis with subsequent "protolytic association". But while we might suppose that the protolysis in the alkaline range could lead direct to the limiting complex $Al(OH)_4^-$, this is less likely in the acid range. Here, we could expect instead a primary formation of the ions $Al(OH)^{2+}$ and $Al(OH)_2^+$ with subsequent protolytic association in which these and Al^{3+} ions might take part. Since the adjustment of the equilibrium for the association is a slow process, it is conceivable that the primary protolysis in the range where $\bar{n}$ approaches 3 at equilibrium, goes so far that $Al(OH)_4^-$ ions are formed to a considerable extent. Owing to this, $\bar{n}$ can for a short time reach a value greater than 3, which must then cause a decided fall in the pH of the solution. As it approaches equilibrium, however, $\bar{n}$ diminishes to approximately 3 or a value somewhat lower than 3. This successive decrease in $\bar{n}$ must be followed by a corresponding increase in the pH of the solution. As already mentioned, such a change in pH (or pOH) with time has been observed. (See Figs. 4 c, d, and e.)

It might appear from the above that the reaction mechanism leading to the formation of α-gel from aluminium ion and water probably consists of a complicated interaction between or succession of several partial processes. To try to find out the nature of these partial processes with the present experimental material seemed to be hazardous, especially as data on aluminium ion concentrations are not available. We can, however, limit ourselves to finding out whether there exists any total reaction mechanism, consistent with the experimental data, which is capable of explaining the process from the initial stage, the aluminium ion, to the final stage α-gel, without giving the intermediate stages. The simplest possible total reaction mechanism fullfilling the conditions mentioned above seems to be the following:

$$Al^{3+} + Al^{3+} + 3\ H_2O \rightarrow Al_2(OH)_3^{3+} + 3\,H^+$$

$$Al_2(OH)_3^{3+} + Al^{3+} + 3\ H_2O \rightarrow Al_3(OH)_6^{3+} + 3\ H^+$$

$$\vdots$$

$$Al_{n-1}(OH)_{3(n-2)}^{3+} + Al^{3+} + 3\ H_2O \rightarrow Al_n(OH)_{3(n-1)}^{3+} + 3\ H^+$$

We introduce the following symbols for concentrations:

$$[Al^{3+}] = a \qquad [H^+] = h$$

$$[Al_2(OH)_3^{3+}] = c_2'$$

$$[Al_3(OH)_6^{3+}] = c_3'$$

$$\vdots$$

$$[Al_n(OH)_{3(n-1)}^{3+}] = c_n'$$

The following equilibrium equations are obtained:

$$\frac{c_2' \cdot h^3}{a^2} = k_{1,1}$$

$$\frac{c_3' \cdot h^3}{c_2' \cdot a} = k_{1,2}$$

$$\vdots$$

$$\frac{c_n' \cdot h^3}{c_{n-1}' \cdot a} = k_{1,(n-1)}$$

In the same way as before we put $k_{1,2} = k_{1,3} = \ldots\ldots = k_{1,(n-1)} = k_1$. Further, we put $k_{1,1} = k_1'$; $\frac{k_1' \cdot a^2}{h^3} = u$; $\frac{k_1 \cdot a}{h^3} = z$. Thus we get:

$$[Al^{3+}] = a$$

$$[Al_2(OH)_3^{3+}] = u$$

$$[Al_3(OH)_6^{3+}] = uz$$

$$\vdots$$

$$[Al_n(OH)_{3(n-1)}^{3+}] = uz^{n-2}$$

If $[Al_t]$ is denoted by a_t, we obtain in the same way as before:

$$a_t = a + 2\,u + 3\,uz + 4\,uz^2 + \ldots. + n \cdot uz^{n-2} + \ldots.$$

$$\bar{n} \cdot a_t = 3\,u + 6\,uz + 9\,uz^2 + 12\,uz^3 + \ldots. + 3(n-1)\,uz^{n-2} + \ldots.$$

Summation of the infinite series finally gives:

$$\left.\begin{aligned} a_t &= a + u\frac{2-z}{(1-z)^2} \\ \bar{n} \cdot a_t &= u\frac{3}{(1-z)^2} \end{aligned}\right\} \tag{8}$$

where $a_t = [Al_t]$; $a = [Al^{3+}]$; $u = \frac{k_1' \cdot a^2}{h^3}$; $z = \frac{k_1 \cdot a}{h^3}$ and $h = [H^+]$.

From this system of equations we obtain:

$$[Al^{3+}] = \frac{2\bar{n} - 3}{\bar{n}\frac{k_1}{[H^+]^3} - \frac{3}{[Al_t]}} \tag{9}$$

and in the same way as before:

$$\frac{[H^+]^3}{[Al_t]} = A_1 + B_1 + \frac{1.5 - \bar{n}}{|1.5 - \bar{n}|} \sqrt{B_1 (2 A_1 + B_1)} \qquad (10)$$

where $$A_1 = \frac{k_1}{3}(3 - \bar{n}) \text{ and } B_1 = \frac{k_1'}{6\bar{n}}(2\bar{n} - 3)^2$$

For $2\bar{n} = 3$ we have $B_1 = 0$ which gives:

$$\frac{([H^+]_{\bar{n}=\frac{3}{2}})^3}{[Al_t]} = \frac{k_1}{3}(3 - \tfrac{3}{2})$$

$$\therefore k_1 = \frac{2}{[Al_t]} \cdot ([H^+]_{\bar{n}=\frac{3}{2}})^3 \qquad (11)$$

The following value for the equilibrium constant was obtained from (11)

$$k_1 = 6 \cdot 10^{-10}$$

It was then necessary to find the value of k_1' which, when substituted together with this value of k_1 in equation (10), gives the best agreement with all the experimental data. Acceptable results were obtained for $k_1' = 5 \cdot 10^{-11}$, and are given in Table 10.

Table 10. $k_1' = 5 \cdot 10^{-11}$; $k_1 = 6 \cdot 10^{-10}$

$\bar{n}$	pOH		
	$[Al_t] = 55.41 \cdot 10^{-3}$	$[Al_t] = 11.07 \cdot 10^{-3}$	$[Al_t] = 2.209 \cdot 10^{-3}$
0.01	10.50	10.27	10.04
0.1	10.22	9.99	9.76
0.5	10.07	9.84	9.61
1.0	9.99	9.76	9.53
1.5	9.92	9.69	9.46
2.0	9.84	9.61	9.38
2.5	9.69	9.46	9.23
2.7	9.58	9.35	9.12
2.9	9.33	9.10	8.87
2.99	8.72	8.49	8.26

The values given in Table 10 are used for the three curves in the acid range in Fig. 7. As will be seen these curves lie slightly closer together than do the values obtained experimentally, which latter also approach $\bar{n} = 3$ more slowly than is predicted by the theory. This latter deviation, which is apparent in the range $2 < \bar{n} < 3$, may have several causes, for instance:

1. that rather large approximations were made both in the reaction mechanism and in the equilibrium constants;
2. that real equilibrium was not attained in the experiments;
3. that no consideration was paid to possible participation of the perchlorate ion in the equilibrium.

Alternative (3) deserves special attention. Several experiments (see for instance, Marion and Thomas [7]) show that the course of the titration curve when aluminium ion is titrated with NaOH or KOH can be strongly influenced by different anions. For instance, Cl^- can undoubtedly replace in some way a bound OH^-, and SO_4^{2-} to some extent two bound OH^-.

We cannot, therefore, disregard the possibility of ClO_4^- ions replacing bound OH^- ions at low OH^- ion concentrations, at least to a slight extent. As the concentration of sodium perchlorate was the same (2.02 C) in all experiments this replacement will diminish with increasing OH^- ion concentration. At pOH $\sim$ 6 it does not seem to influence $\bar{n}$ noticeably. On the other hand, at pOH $\sim$ 10 such an influence, even if great, would not make itself felt in the curves in Fig. 7 as $\bar{n}$ varies very strongly here with pOH. Thus the influence of the perchlorate ion which might depress the number of OH^- ions bound by aluminium, *i.e.* diminish the observed value of $\bar{n}$, will manifest itself most markedly in the region where pOH is still sufficiently high but $\bar{n}$ is not very sensitive to changes in pOH. In our case this region lies at pOH $\sim$ 8—9 and the discrepancy between the observed values and the theoretical curves is, as was to be expected, most pronounced here.

It can be seen from the above analysis that if we consider the formation of α-gel in acid and alkaline solutions as being independent phenomena each of the processes can be described by means of a pair of equilibrium constants. A quantity which does not appear in this connection is the solubility product of $Al(OH)_3$. This is probably because the above system has been considered in the absence of crystalline phases. Furthermore the α-gel contained in the system has been described as a series of macromolecules with varying numbers of aluminium atoms. It cannot possess a well-defined activity in this form and so *the concept of solubility product will not apply in this case.* The relatively

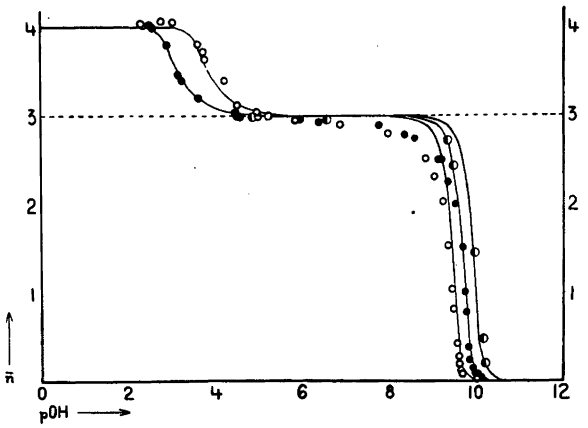


Fig. 7.

good agreement obtained between theory and experiment seems, at least to some extent, to support this view.

SUMMARY

1. In the absence of solid crystalline phases, the equilibrium conditions in the system Al-OH were studied by means of pH measurements.

2. Certain observations on kinetic reaction conditions led to the following assumptions on the reactions between aluminium ion and water:

$$Al^{3+} + Al^{3+} + 3\ H_2O \rightarrow Al_2(OH)_3^{3+} + 3\ H^+$$

$$Al_2(OH)_3^{3+} + Al^{3+} + 3\ H_2O \rightarrow Al_3(OH)_6^{3+} + 3\ H^+$$

$$\vdots$$

$$Al_{n-1}(OH)_{3(n-2)}^{3+} + Al^{3+} + 3\ H_2O \rightarrow Al_n(OH)_{3(n-1)}^{3+} + 3\ H^+$$

$$\vdots \quad \text{the iso-electric point}$$

$$Al_n(OH)_{3n+1}^{-} + H_2O \rightarrow Al_{(n-1)}(OH)_{3(n-1)+1}^{-} + Al(OH)_4^{-} + H^+$$

$$\vdots$$

$$Al_3(OH)_{10}^{-} + H_2O \rightarrow Al_2(OH)_7^{-} + Al(OH)_4^{-} + H^+$$

$$Al_2(OH)_7^{-} + H_2O \rightarrow Al(OH)_4^{-} + Al(OH)_4^{-} + H^+$$

3. The equilibrium conditions in the system are expressed by the following equations:

$$\frac{[Al_2(OH)_3^{3+}] \cdot [H^+]^3}{[Al^{3+}]^2} = k_1'$$

$$\frac{[Al_n(OH)^{3+}_{3(n-1)}] \cdot [H^+]^3}{[Al_{(n-1)}(OH)^{3+}_{3(n-2)}] \cdot [Al^{3+}]} = k_1$$

$$\frac{[Al_{(n-1)}(OH)^{-}_{3(n-1)+1}] \cdot [Al(OH)^-_4] \cdot [H^+]}{[Al_n(OH)^{-}_{3n+1}]} = k_2$$

$$\frac{[Al(OH)^-_4]^2 \cdot [H^+]}{[Al_2(OH)^-_7]} = k_2'$$

k_1 and k_2 are, as suggested by Granér and Sillén [6], assumed to hold good for all reasonable values of $n > 2$.

4. The above assumptions led to the following expression:

$$0 < \bar{n} < 3$$

$$[Al^{3+}] = \frac{2\,\bar{n} - 3}{\bar{n} \cdot \dfrac{k_1}{[H^+]^3} - \dfrac{3}{[Al_t]}}$$

$$\frac{[H^+]^3}{[Al_t]} = A_1 + B_1 + \frac{1.5 - \bar{n}}{|1.5 - \bar{n}|} \sqrt{B_1(2A_1 - B_1)}$$

where $$A_1 = \frac{k_1}{3}(3 - \bar{n}) \text{ and } B_1 = \frac{k_1'}{6}(2\bar{n} - 3)^2$$

$$3 < \bar{n} < 4$$

$$[Al(OH)^-_4] = \frac{2\bar{n} - 7}{\dfrac{[H^+]}{k_2}(\bar{n} - 4) + \dfrac{1}{[Al_t]}}$$

$$[H^+] \cdot [Al_t] = A_2^2 \left\{ \frac{1}{A_2} + B_2 + \frac{3.5 - \bar{n}}{|3.5 - \bar{n}|} \sqrt{B_2\left(\frac{2}{A_2} + B_2\right)} \right\}$$

where $$A_2 = \frac{k_2}{3 - \bar{n}} \text{ and } B_2 = \frac{2(\bar{n} - 3.5)^2}{k_2'(4 - \bar{n})}$$

In the above expression $[Al_t]$ denotes the total concentration of aluminium and $\bar{n}$ the average number of bound hydroxyl groups per aluminium atom.

5. At 40° C and in the presence of $NaClO_4$, 2 C as ionic medium, has acceptable agreement between theory and experiment been obtained with the following values for the equilibrium constants:

$$k_1' = 5 \cdot 10^{-11} \quad C^2$$
$$k_1 = 6 \cdot 10^{-10} \quad C^2$$
$$k_2' = 2 \cdot 10^{-11} \quad C^2$$
$$k_2 = 2.3 \cdot 10^{-13} \quad C^2$$

The deviations, which are greatest in the range, where $2 < \bar{n} < 3$, are discussed. In this connection it has been pointed out that ClO_4^- may possibly enter into the equilibrium.

6. It has been pointed out that *the concept of a solubility product cannot be applied* in the case of α-gel if, as is assumed, it consists of macromolecules of varying sizes.

I wish to offer my deepest thanks to Professor Arne Ölander for all the interest he has shown in my work and for the continual encouragement I received during this lengthy investigation. Likewise I want to thank Miss Åsa Bränd-Persson for valuable experimental help in the preliminary series of experiments at 25° C. Finally I wish to thank Mrs Stina Nord for her valuable assistance in the experiments.

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APPENDIX

In connection with the above calculations of the equilibrium conditions in a system where a hydroxide gel with amphoteric properties is formed, it may be of interest to derive corresponding expressions for the general case.

Assuming that the total concentration of the group M^{m+} in a water solution is M_t, the group will react with water in the following way:

$$M^{m+} + M^{m+} + mH_2O \rightarrow M_2(OH)_m^{m+} + mH^+$$

$$M_2(OH)_m^{m+} + M^{m+} + mH_2O \rightarrow M_3(OH)_{2m}^{m+} + mH^+$$

$$\vdots$$

$$M_{(n-1)}(OH)_{(n-2)m}^{m+} + M^{m+} + mH_2O \rightarrow M_n(OH)_{(n-1)m}^{m+} + mH^+$$

$$\vdots$$

(The iso-electric point)

$$M_n(OH)^{r-}_{n(q-r)+r} + rH_2O \rightarrow M_{(n-1)}(OH)^{r-}_{(n-1)(q-r)+r} + M(OH)^{r-}_q + rH^+$$
$$\vdots$$
$$M_3(OH)^{r-}_{3(q-r)+r} + rH_2O \rightarrow M_2(OH)^{r-}_{2(q-r)+r} + M(OH)^{r-}_q + rH^+$$
$$M_2(OH)^{r-}_{2(q-r)+r} + rH_2O \rightarrow M(OH)^{r-}_q + M(OH)^{r-}_q + rH^+$$

where m, q, and r are positive whole numbers connected by $m = q - r$. Let us assume that the equilibrium in the system is determined by the following expressions:

$$\frac{[M_2(OH)^{m+}_m] \cdot [H^+]^m}{[M^{m+}]^2} = k_{1,1}$$

$$\frac{[M_3(OH)^{m+}_{2m}] \cdot [H^+]^m}{[M_2(OH)^{m+}_m] \cdot [M^{m+}]} = k_{1,2}$$

$$\vdots$$

$$\frac{[M_n(OH)^{m+}_{(n-1)m}] \cdot [H^+]^m}{[M_{(n-1)}(OH)^{m+}_{(n-2)m}] \cdot [M^{m+}]} = k_{1,(n-1)}$$

$$\frac{[M_{(n-1)}(OH)^{r-}_{(n-1)(q-r)+r}] \cdot [M(OH)^{r-}_q] \cdot [H^+]^r}{[M_n(OH)^{r-}_{n(q-r)+r}]} = k_{2,(n-1)}$$

$$\vdots$$

$$\frac{[M_2(OH)^{r-}_{2(q-r)+r}] \cdot [M(OH)^{r-}_q] \cdot [H^+]^r}{[M_3(OH)^{r-}_{3(q-r)+r}]} = k_{2,2}$$

$$\frac{[M(OH)^{r-}_q]^2 \, [H^+]^r}{[M_2(OH)^{r-}_{2(q-r)+r}]} = k_{2,1}$$

Put $k_{1,1} = k'_1$ and $k_{2,2} = k'_2$.

According to Granér and Sillén [6] we can approximately put

$$k_{1,2} = k_{1,3} \ldots = k_{1,(n-1)} \ldots = k_1 \text{ and } \ldots k_{2(n-1)} = \ldots k_{2,3} = k_{2,2} = k_2$$

In the same way as before we first calculate $[M_t]$ and $\bar{n} \cdot [M_t]$:

$$0 < \bar{n} < m:$$
$$\left.\begin{aligned} [M_t] &= [M^{m+}] + y_1 \frac{2-x_1}{(1-x_1)^2} \\ \bar{n} \cdot [M_t] &= y_1 \frac{m}{(1-x_1)^2} \end{aligned}\right\} \quad (1)$$

where $x_1 = \dfrac{k_1 \cdot [M^{m+}]}{[H^+]^m}$ and $y_1 = \dfrac{k'_1 \cdot [M^{m+}]^2}{[H^+]^m}$

$m = q-r < \bar{n} < q$:

$$\left.\begin{aligned}[\mathrm{M_t}] &= [\mathrm{M(OH)_q^{r-}}] + y_2 \frac{2-x_2}{(1-x_2)^2} \\ \bar{n} \cdot [\mathrm{M_t}] &= q \cdot [\mathrm{M(OH)_q^{r-}}] + y_2 \frac{2q-r-qx_2}{(1-x_2)^2}\end{aligned}\right\} \quad (2)$$

where $x_2 = \dfrac{[\mathrm{M(OH)_q^{r-}}] \cdot [\mathrm{H^+}]^r}{k_2}$ and $y_2 = \dfrac{[\mathrm{M(OH)_q^{r-}}]^2 \cdot [\mathrm{H^+}]^r}{k_2'}$

From the equation systems (1) and (2) we obtain the following relations:

$0 < \bar{n} < m$:

$$[\mathrm{M^{m+}}] = \frac{2\ \bar{n}-m}{\bar{n}\dfrac{k_1}{[\mathrm{H^+}]^m} - \dfrac{m}{[\mathrm{M_t}]}} \quad (3)$$

$$k_1 = \frac{2}{[\mathrm{M_t}]} \left([\mathrm{H^+}]_{\bar{n}=\frac{m}{2}}\right)^m \quad (4)$$

and

$$\frac{[\mathrm{H^+}]^m}{[\mathrm{M_t}]} = A_1 + B_1 + \frac{\frac{m}{2}-\bar{n}}{\left|\frac{m}{2}-\bar{n}\right|} \sqrt{B_1(2A_1 + B_1)} \quad (5)$$

where $A_1 = \dfrac{k_1(m-\bar{n})}{m}$ and $B_1 = \dfrac{k_1'(2\bar{n}-m)^2}{2\bar{n} \cdot m}$

$m = q-r < \bar{n} < q$

$$[\mathrm{M(OH)_q^{r-}}] = \frac{2\ \bar{n}-(2q-r)}{(\bar{n}-q)\dfrac{[\mathrm{H^+}]^r}{k_2} + \dfrac{r}{[\mathrm{M_t}]}} \quad (6)$$

$$k_2 = \frac{[\mathrm{M_t}]}{2}\left([\mathrm{H^+}]_{\bar{n}=q-\frac{r}{2}}\right)^r \quad (7)$$

and

$$[\mathrm{H^+}]^r \cdot [\mathrm{M_t}] = A_2^2 \left\{B_2 + \frac{r}{A_2} + \frac{q-\frac{r}{2}-\bar{n}}{\left|q-\frac{r}{2}-\bar{n}\right|} \sqrt{B_2\left(\frac{2r}{A_2} + B_2\right)}\right\} \quad (8)$$

where $A_2 = \dfrac{k_2}{\bar{n}-q+r}$ and $B_2 = \dfrac{r\{2\bar{n}-(2q-r)\}^2}{2k_2'(q-\bar{n})}$

The significance of equations (5) and (8) can be expressed in the following manner:

If a substance M occurs with total concentration $[M_t]$ in a solution and if the ion M^{m+} reacts with water so as to build up or breakdown a macromolecule (hydroxide) gradually as would be the case with the above assumptions, then the expressions $\frac{[H^+]^m}{[M_t]}$ and $[H^+]^r \cdot [M_t]$ for the building-up and breaking-down, respectively, of the macromolecules depend only on the equilibrium constants and on $\bar{n}$ as the only variable. In these expressions m and r are numbers indicating how many hydrogen ions are liberated at each stage of the reaction, and $\bar{n}$ is the average number of OH^- groups bound per M atom.

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Action of Strong Acids on Acetylated Glycosides

X.* Synthesis of 4,6-β-Glucose α,β-Diglucoside Hendecaacetate

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In Part IX of this series the synthesis of a potential *limit dextrin*, 6-glucose α-maltoside, although in an impure state, was reported. The aim of the present work was to prepare another disaccharide of this type, 4,6-glucose α,α-diglucoside. The synthesis was, however, only carried out as far as 4,6-β-glucose α,β-diglucoside hendecaacetate, since the yield of this substance was so low that attempts to transform it into the α,α-diglucoside seemed to be without any prospect of success.

The synthesis followed the following scheme:

1. Phenyl β-maltoside heptaacetate was prepared in a yield of 45 % from β-maltose octaacetate and phenol, with *p*-toluene sulfonic acid as catalyst, using the fusion procedure of Helferich and Schmitz-Hillebrecht [1]. The substance melted at 154—155° ** and showed $[\alpha]_D^{20}$ + 42°. Fischer and Armstrong [2] report the melting point as 155—156° but give no value for the optical rotation.

From the mother liquors of the phenyl β-maltoside heptaacetate another substance of m.p. 181—182° and $[\alpha]_D^{20}$ + 164° was isolated in 5 % yield. This liberated phenol on acid hydrolysis and gave an analysis corresponding to a phenyl maltoside heptaacetate. From the high specific rotation it seemed probable that the substance was an α-maltoside and this was supported by the fact that it could also be prepared as the chief product by condensing β-maltose octaacetate and phenol with zinc chloride as condensing agent according to the general procedure for the preparation of phenyl α-glycosides [2]. Although it is generally supposed that a small amount of the α-glycoside is formed when a sugar acetate and a phenol are condensed in the presence of *p*-toluene sulfonic

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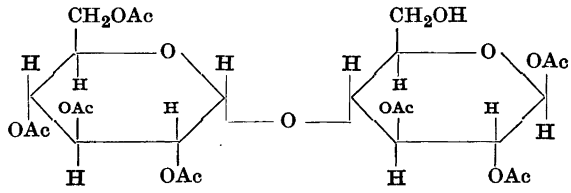
* Part IX, *Acta Chem. Scand.* **5** (1951) 665.

** All melting points uncorrected. All specific rotations in chloroform, $C = 2$.

acid [2], this appears to be the first occasion that such a product has been isolated. The phenyl α-maltoside heptaacetate has a higher melting point and a lower solubility than the β-maltoside, whereas the reverse is true for most pairs of glycosides, and this, together with the high crystallization tendency of the substance, undoubtedly, facilitated its isolation.

2. In one step the phenyl β-maltoside heptaacetate was deacetylated and degraded by alkali to 4-levoglucosan β-glucoside (maltosan), following in principle the methods of Montgomery, Richtmeyer and Hudson [3] for the synthesis of similar disaccharide derivatives. Subsequent acetylation gave a 72 % yield of maltosan hexaacetate, m.p. 182—183°, $[a]_D^{20}$ + 48°. Karrer and Kamienski [4] report the melting point as 184° but give no value for the optical rotation.

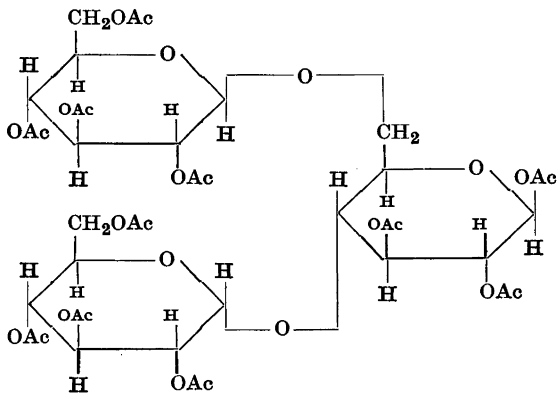
3. Zemplén and Csürös [5] have prepared 2,3,4-triacetyl glucosyl chloride by the action of titanium tetrachloride upon levoglucosan triacetate in chloroform. An analogous reaction was carried out with maltosan hexaacetate, followed by treatment of the reaction product with mercuric acetate in order to replace the chlorine atom by an acetoxyl group. From the reaction mixture β-maltose heptaacetate and unchanged starting material were obtained in yields of 25 % and 40 % respectively. The heptaacetate melted at 140—141° and showed $[a]_D^{20}$ + 65°. By acetylation with pyridine-acetic anhydride it yielded β-maltose octaacetate. It was easily tosylated and the tosyl group was readily replaced by iodine, which facts indicate the presence of a primary hydroxyl group in the heptaacetate. There are only two possible heptaacetates of maltose containing a primary hydroxyl group, and there is little doubt that the substance in question is 1,2,3,2′3′4′6′-β-maltose heptaacetate.



1,2,3,2′,3′,4′,6′-β-Maltose heptaacetate

4. The Koenigs-Knorr reaction, with the improvements of Reynolds and Evans [6], did not succeed very well with maltose heptaacetate and acetyl glucosyl bromide. Several attempts were made but the yield of 4,6,-β-glucose α,β-diglucoside hendecaacetate did not exceed 6 %.

The product melted at 181—182° and showed $[\alpha]_D^{20}$ + 47°. The reason for this unusually low yield is at present obscure, the usual reference to sterical hindrance might be justified. A similar effect perhaps operates in the reaction between acetyl glucosyl bromide and α,γ-dichlorohydrin where the yield is only 7 % by the Koenigs-Knorr reaction [7] and 14 % by the mercuric acetate method [8]. As only rather small amounts of the trisaccharide were available it was not practicable to attempt to transform it into the potential *limit dextrin*. Several branchedchain polysaccharides have been obtained from natural sources but this trisaccharide, as far as is known, is the only example of a synthetic trisaccharide where the "branch" is actually represented, *i.e.* one glucose unit is the bivalent aglucon of the two others. The substance and its synthesis are therefore of some interest.



4,6-β Glucose α,β-diglucoside hendecaacetate

EXPERIMENTAL

Phenyl β-maltoside heptaacetate. *p*-Toluenesulfonic acid (0.7 g) was dissolved in molten phenol (50 g) and β-maltose octaacetate (48 g) and acetic anhydride (2.5 ml) were added. The mixture was heated for 1 hour on the steam bath under water pump vacuum and whilst still hot dissolved in benzene (400 ml). The benzene solution was washed with water, several times with 2 *N* sodium hydroxide and again with water, dried over calcium chloride and concentrated under reduced pressure. The crystalline residue was recrystallized from methanol (120 ml). The yield of phenyl β-maltoside heptaacetate was 23 g (45 %). Its melting point, 152—153° could be raised to 154—155° by one further recrystallization, but the first material was sufficiently pure for the next step in the synthesis. The recrystallized substance showed $[\alpha]_D^{20}$ + 42°.

Phenyl α-maltoside heptaacetate. a. When the methanolic mother liquors from the first recrystallization mentioned above were kept at 0° overnight, crystals (3 g) of m.p. 168—169° were precipitated. After further recrystallizations from ethanol the substance

was obtained pure with m.p. 181—182° and $[\alpha]_D^{20}$ + 164°. When a small amount of the substance was heated in ethanolic sulfuric acid and the ethanol then evaporated a distinct smell of phenol was detected.

$C_{32}H_{40}O_{18}$ (712.7)	Calc.	C 53.9	H 5.66
	Found	» 53.8	» 5.68

b. A mixture of β-maltose octaacetate (10 g), phenol (9 g) and anhydrous zinc chloride (1.5 g) was stirred at 120° for 2 hours. Whilst still hot the mixture was dissolved in benzene (300 ml) and worked up as described above for the β-maltoside. After two recrystallizations from ethanol the product (4.9 g) was pure and melted at 181—182°, alone or in admixture with the specimen described above.

Maltosan hexaacetate. A mixture of phenyl β-maltoside heptaacetate (46 g) and 2.6 *N* aqueous potassium hydroxide (800 ml) was heated on the steam bath for 40 hours. After about half an hour the mixture had become homogenous. The rotation of the solution in a 2 dm tube changed from + 2.4° to + 4.5° during the first 24 hours and thereafter remained constant. The solution was neutralized with 4 *N* sulfuric acid and concentrated under reduced pressure. The distillation was interrupted twice and precipitated salts removed by filtration. The remaining sirup was dried overnight in vacuo over sulfuric acid and thereafter mixed with acetic anhydride (250 ml) and anhydrous sodium acetate (25 g) and heated on the steam bath for 2 hours. The mixture was poured into ice water (1 500 ml) and extracted with chloroform (2 × 200 ml). The light-brown extract was washed with sodium hydrogen carbonate solution, dried over calcium chloride and decolorized by filtration through a column of aluminium oxide. The chloroform solution was then evaporated under reduced pressure and the crystalline residue recrystallized from ethanol (250 ml). The yield of maltosan hexaacetate, m.p. 181—182°, was 27 g (72 %). By further recrystallizations the pure substance, m.p. 182—183°, $[\alpha]_D^{20}$ + 48°, could be obtained.

1,2,3,2',3',4',6'-β-Maltose heptaacetate. Maltosan hexaacetate (25 g) was dissolved in chloroform (300 ml) and ethanol (5 ml) and titanium tetrachloride (40 g) were added. The mixture was refluxed for 3 hours on a glycerol bath kept at 70°. When cold the yellow mixture was poured into ice water (1 000 ml). The chloroform solution was separated and washed with water (4 × 250 ml), then concentrated under reduced pressure at a bath temperature not exceeding 30°. The sirupy residue was dissolved in a solution of mercuric acetate (25 g) in acetic acid (250 ml). After 2 hours at room temperature the solution was poured into ice water (2 500 ml) and extracted with chloroform (2 × 500 ml). The chloroform solution was washed with sodium hydrogen carbonate solution and water, dried over calcium chloride and concentrated under reduced pressure. The residue was dissolved in hot ethanol (125 ml) and on cooling almost pure maltosan hexaacetate (8.0 g) of m.p. 178—179° precipitated. The mother liquors were concentrated to 50 ml and a second crop (1.7 g) of less pure starting material of m.p. 163—165° was recovered. The mother liquors from this crop were poured into water (450 ml) and kept overnight at 0°, when maltose heptaacetate (5.8 g), m.p. 135—137°, precipitated. From the aqueous residues a further amount (1.7 g) of maltose heptaacetate could be obtained. Further recrystallizations from ethanol-water (1 : 9) yielded the pure substance, m.p. 140—141°, $[\alpha]_D^{20}$ + 65°.

$C_{12}H_{15}O_{11}(OCCH_3)_7$ (636.5)	Calc.	$OCCH_3$ 47.3
	Found	» 47.6

A small amount of the heptaacetate was acetylated with acetic anhydride and pyridine. β-Maltose octaacetate, m.p. 157—158° alone or in admixture with an authentic specimen, was obtained in an almost quantitative yield.

6-Tosyl 1,2,3,2',3',4',6'-β-maltose heptaacetate. β-Maltose heptaacetate (250 mg) was dissolved in absolute pyridine (1 ml) and tosyl chloride (300 mg) was added. After 17 hours at room temperature the mixture was poured into water (15 ml). The tosyl derivative precipitated immediately and was filtered off, washed with water and dried in vacuo over sulfuric acid. The yield was 300 mg (96 %). After two recrystallizations from ethanol the product had a constant melting point of 140—141° and showed $[\alpha]_D^{20}$ + 50°.

$C_{33}H_{42}O_{20}S$ (790.7) Calc. S 4.03
Found » 3.88

6-Iodo 1,2,3,2',3',4',6'-β-maltose heptaacetate. The tosyl derivative (100 mg) and sodium iodide (100 mg) were dissolved in dry acetonylacetone (3 ml) and the solution was kept at 100°. Sodium *p*-toluene sulfonate was deposited after a few minutes. After 1.5 hours the mixture was poured into water (15 ml), whereupon the salt dissolved while the iodo compound precipitated out. The latter was collected on the centrifuge and recrystallized from 70 % ethanol. The substance (44 mg), which had m.p. 88—90° and $[\alpha]_D^{20}$ + 50° was not purified further.

$C_{26}H_{35}O_{17}I$ (746.5) Calc. I 17.0
Found » 16.2

4,6-β-Glucose α,β-diglucoside hendecaacetate. A mixture of maltose heptaacetate (6.36 g), silver oxide (3.0 g) and Drierite (5 g) in absolute chloroform (30 ml) was shaken mechanically for 1 hour with the exclusion of direct light. A solution of acetyl glucosyl bromide (4.11 g) and iodine (0.5 g) in absolute chloroform (30 ml) was then added in 4 portions at 15 minutes intervals. Shaking was continued for 29 hours, after which the solution was filtered through a layer of Celite, washed with dilute sodium thiosulfate solution and water, dried over calcium chloride and concentrated under reduced pressure. The residual sirup was crystallized from methanol (20 ml), giving a product (1.0 g) of m.p. 153—155°. After two further recrystallizations from the same solvent the substance (0.6 g) had a constant melting point of 181—182° and showed $[\alpha]_D^{20}$ + 47°.

$C_{40}H_{54}O_{27}$ (966.8) Calc. C 49.7 H 5.63
Found » 50.2 » 5.57

Correction to the text added in proof. We have observed that the substance believed above to be 4,6-β-glucose α,β-diglucoside hendecaacetate is actually maltosan hexaacetate, which gives approximately the same analyses and the presence of which is due to the fact that unpurified maltose heptaacetate was used in the Koenigs-Knorr synthesis. The real trisaccharide should be found in the non-crystalline material from the mother liquors. The discussion on the bad yield of the Koenigs-Knorr synthesis has thus no significance.

SUMMARY

1,2,3,2',3',4',6'-β-Maltose heptaacetate has been prepared by the action of titanium tetrachloride in chloroform-ethanol upon maltosan hexaacetate and subsequent treatment with mercuric acetate in acetic acid.

4,6-β-Glucose α,β-diglucoside has been prepared from this maltose heptaacetate and acetyl glucosyl bromide by the Koenigs-Knorr reaction.

The authors wish to thank *Statens Naturvetenskapliga Forskningsråd* for financial support.

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Action of Strong Acids on Acetylated Glycosides

XI. * Transglycosidation of Mannosides

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The investigation of the transglycosidation reaction in the hexose series has hitherto concerned glucosides and galactosides (Parts III [1] and VI [2] resp.). In the present paper a similar study with mannosides is reported. As the mannosides of simple alcohols, except methanol, as well as their acetates, are difficult to obtain in a crystalline state, the investigation has been rather limited. Crystalline ethyl β-mannopyranoside tetraacetate, m.p. 132—133°, was prepared in a low yield by treatment of mannose with ethanolic hydrogen chloride and subsequent acetylation. The crystalline substance has not previously been reported in the literature, although a sirup containing different ethyl mannosides has been prepared by Levene and Sobotka [3]. The identity of the product was evident from the similarity of its specific rotation (−49° in chloroform) to that of methyl β-mannopyranoside tetraacetate (−50° in the same solvent).

β-Mannose pentaacetate, in a mixture of sulfuric acid, acetic anhydride and acetic acid, was transformed into the equilibrium mixture of α- and β-acetate at a rate which was practically identical with that shown by β-glucose pentaacetate. Ethyl β-mannoside tetraacetate was treated similarly. The initially rapid change in optical roation which occurred indicated transglycosidation into the α-form, by analogy with the behaviour of other glycosides studied. This reaction proceeded 61 times faster than the isomerisation of β-glucose pentaacetate. The corresponding ratios for the ethyl β-glucoside and ethyl β-galactoside tetraacetates are 15 and 31 respectively. For the latter glycosides the rotation passed through a maximum and the final rotation was much lower than if α- and β-pentaacetates were the only products of acetolysis, indicating the formation of heptaacetate. For the mannoside, however, the

* Part X *Acta Chem. Scand.* 6 (1952) 941.

final rotation differed very little from the value calculated under the assumption that only pentaacetates were formed.

Methyl β-mannoside tetraacetate was transformed in a good yield into the α-mannoside by the action of titanium tetrachloride in chloroform.

EXPERIMENTAL

Ethyl β-mannoside tetraacetate. Mannose (25 g) in absolute ethanol (250 ml) containing hydrogen chloride (1.0 g) was boiled on the steam bath for one hour. When cold the solution was neutralized with silver carbonate, filtered through a layer of Celite and concentrated to a sirup. The sirup was refluxed with acetic anhydride (100 ml) and sodium acetate (10 g) for one hour. The solution was then poured into ice water (1 500 ml) and extracted with chloroform (3 × 100 ml). The extract was washed with sodium hydrogen carbonate solution and water, dried over calcium chloride and concentrated under reduced pressure. The residue was dissolved in hot methanol (100 ml) and on cooling semi-crystalline material (4.6 g) separated. Further recrystallizations from the same solvent yielded pure ethyl β-mannoside tetraacetate (1.3 g), m.p. 132–133° (uncorr.) $[\alpha]_D^{20}$ −49° (Chloroform, $C = 2$).

$C_{16}H_{24}O_{10}$ (376.4)	Calc.	C 51.1	H 6.42
	Found	» 50.8	» 6.36

Transglycosidation of methyl β-mannoside tetraacetate. Methyl β-mannoside tetraacetate (4.7 g) was dissolved in absolute chloroform (100 ml) and titanium tetrachloride (2.3 g) in chloroform (50 ml) was added. The mixture was refluxed for 5 hours on a glycerol bath, kept at 70°, and when cold poured into ice-water. The chloroform solution was washed with water several times, dried over calcium chloride and concentrated. The residue was dissolved in hot 50 % methanol and on cooling yielded the α-mannoside (3.0 g, 64 %), m.p. 62–63° alone or in admixture with an authentic specimen of methyl α-mannoside tetraacetate.

Kinetic determinations. The experimental conditions for the kinetical determinations were the same as in the preceding papers (compare Part III [1]). Sulfuric acid of concentration 0.03 C was used and the solvent was acetic anhydride-acetic acid, 10 : 3. The temperature was maintained at 20° C. The specific rotation of ethyl β-mannoside tetraacetate was initially − 48°, passed through a maximum of + 46° and finally decreased to about + 45° (all values calculated for ethyl mannoside tetraacetate).

SUMMARY

The transglycosidation reaction has been studied for some mannosides. Ethyl β-mannoside tetraacetate has been prepared in a pure crystalline state.

The authors wish to thank Statens Naturvetenskapliga Forskningsråd for financial support.

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Action of Strong Acids on Acetylated Glycosides

XII.* Transglycosidation of Arabinosides

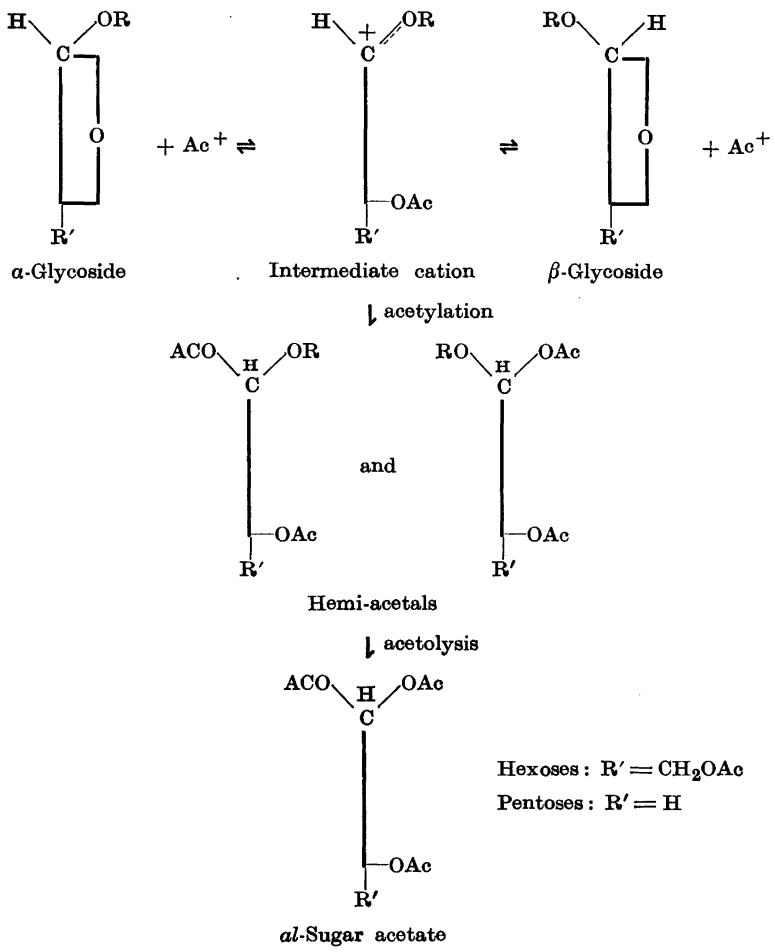
BENGT LINDBERG

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Montgomery, Hann and Hudson[1] have reported some interesting studies on the action of catalyzed acetylation mixtures upon the α and β methyl D-arabinopyranoside triacetates. From reaction mixtures containing strong catalysts (4 % sulfuric acid) they were able to isolate arabinose hexaacetate together with small amounts of β-arabinose tetraacetate. With weaker catalysts (0.16 % sulfuric acid or zinc chloride) the two arabinose methyl hemiacetal pentaacetates could be isolated from the reaction mixture. With sulfuric acid as catalyst it was also demonstrated that for the acetylated methyl α-arabinoside, transglycosidation into the β-form preceded the transformation into the *al*-sugar derivatives. The transglycosidation and the formation of an open-chain sugar acetate are in agreement with the results obtained for other sugars, as reported in the preceding papers of the series. The formation of the hemi-acetales is also consistent with the theory developed in Part III[2] for the analogous reactions with glucosides, as illustrated by the following.

(This scheme is analogous to that put forward in Part III, but is extended to include the acetylation of the intermediate cation as a separate step.) The hemi-acetal derivatives should accumulate in the reaction mixture only when the rate at which they are formed is higher than that of their acetolysis. It is reasonable to assume that the rate of acetolysis is little affected by the structure of the sugar. The formation of the hemi-acetales, however, passes through the same intermediate as the transglycosidation reaction, and structural relationships which favour one of the reactions should also favour the other. It seems therefore quite understable that these hemi-acetals have not been isolated in the glucose series, since transglycosidation reaction is about 50 times slower for glucosides than for arabinosides (see below).

* Part XI, *Acta Chem. Scand.* 6 (1952) 947.



The present investigation has been confined to a study of the transglycosidation reaction with arabinosides. Methyl glycosides are transglycosidated rather slowly compared to those of other aliphatic alcohols, and the side and consecutive reactions interfere considerably. For this reason ethyl and *iso*-propyl glycosides have been preferred in these investigations. In the arabinose series these glycosides, in their acetylated form have not previously been described in the literature. Ethyl α-L-arabinoside triacetate, m.p. 73—74° (uncorr.), $[\alpha]_D^{20} + 7°$ (chloroform, $C = 2$) was prepared by the mercuric acetate method. An attempt was also made to prepare the *iso*-propyl arabinoside, but the product wass non-crystalline. The transformation of α-L-arabinose tetraacetate to the equilibrium mixture of α- and β-forms and the transglycosidation of

ethyl α-L-arabinoside triacetate were studied kinetically in acetic anhydride-acetic acid, with sulfuric acid as catalyst. The reactions were 26 and 780 times faster respectively than the standard reaction, namely the transformation of β-glucose pentaacetate into the equilibrium mixture. The corresponding values for the sugar acetates and ethyl glycosides hitherto investigated are summarized in Table 1.

Table 1. Relative velocity constants for the trausformation of sugar acetates and ethyl glycopyranoside asetates into the equilibrium mixtures af α- and β-forms;

	Acetate	Ethyl glycoside
Glucose	1	15
Galactoce	2.5	31
Mannose	1	60
Xylose	30	1 120
Arabinose	26	780

The arabinose and xylose derivatives show very similar behaviour and are distinguished from the hexose derivatives by their high reactivity. The pentosides are also less stable than the hexosides in the acetylation mixture. While the former are inreversibly transformed into other products at a rate more than 100 times faster than that of the standard reaction, the corresponding rate for the latter is only about 0.1.

EXPERIMENTAL

Ethyl α-L-arabinoside triacetate. A solution of arabinose bromide triacetate (6.78 g) and mercuric acetate (3.03 g) in a mixture of absolute benzene (40 ml) and ethanol (16 ml) was refluxed for 15 minutes. After cooling, the solution was washed several times with water, dried over calcium chloride and concentrated under reduced pressure. The oily residue was crystallized from methanol, giving a product (4.2 g, 69 %) which melted at 67—69°. Two further recrystallizations from the same solvent yielded the pure substance, m.p. 73—74° and $[\alpha]_D^{20}$ + 7° (chloroform, $C = 2$)

$C_7H_{11}O_5$ $(OCCH)_3$ (304.3) Calc. Acetyl 42.4
Found » 42.0

Kinetic determinations. The experimental conditions for the kinetic determinations were the same as in the preceding papers (compare Part III [2]). For the transglycosidation runs sulfuric acid of approximate concentration 0.004 C was used in acetic anhydride-acetic acid, 10 : 3, at a temperature of 20°. The specific rotation of the ethyl α-L-arabinoside triacetate changed from the initial value of + 8° to the maximum value of + 97° and finally decreased to— 26° (all values calculated for ethyl arabinoside triacetate).

SUMMARY

The transglycosidation of ethyl α-L-arabinoside triacetate has been investigated and the mechanism of the reaction discussed.

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Short Communications

Further Investigations of Multimolecular Layers on Aqueous Surfaces

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Surface balance studies [1] of the multimolecular layers of isodextropimaric acid have shown that when the collapsed monolayer is compressed from a dimolecular to a trimolecular layer, a transition from the liquid to the solid state takes place. A further compression leads to a rapid increase in the surface pressure, after which the trilayer appears to collapse.

Also multimolecular layers of other substances undergo on compression changes similar in many respects to those described for isodextropimaric acid. Fig. 1 gives the surface pressure-area curves recorded with the surface balance for tetrahydroabietic (I), dihydroabietic (II) and isodextropimaric (III) acids. The curves for tetrahydroabietic and dihydroabietic acids apply to layers supported on an alkaline substrate (pH 9.1), the curve for isodextropimaric acid on diluted hydrochloric acid (pH 3.0). It is seen that the curves for tetrahydro- and dihydroabietic acids resemble that obtained for isodextropimaric acid in that they show a rapid increase of the surface pressure in the compression region *c-d* and their multilayers appear to collapse at *d*. As in the case of isodextropimaric acid the tetrahydroabietic acid layer is solid in appearance just before this collapse point. The first increase of the surface pressure appears to occur at *b*. Calculations [1] of the areas per molecule revealed that the isodextropimaric acid multilayers are dimolecular at point *b* and trimolecular at point *c*. The same appears to be the case with tetrahydro- and dihydroabietic acids as is evident from Fig. 1.

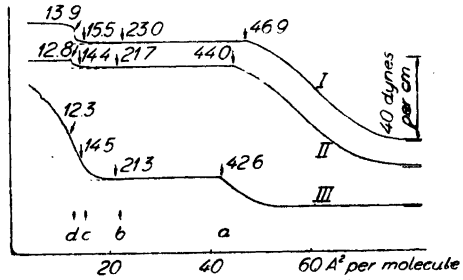


Fig. 1. Surface pressure-area curves of tetrahydroabietic (I), dihydroabietic (II), and isodextropimaric (III) acids. At point a: total collapse of the monolayer occurs, at b: the dilayer is completed, at c: the trilayer is completed, at d: the trilayer appears to collapse. Curves I and II have been taken on water containing 5 moles sodium chloride, $\frac{1}{100}$ moles sec. sodium phosphate per litre and sodium hydroxide to give pH 9.1; curve III has been recorded on dilute hydrochloric acid (pH 3.0). Temperature 20° C.

It seems that the observed phenomena cannot be explained by assuming that they are solely due to an increase in the thickness of the layers; the phenomena must be connected with marked changes in the inner structures of the layers. The relatively strong increase in pressure observed in the region *c-d*, which is specially marked in the case of isodextropimaric

acid, indicates a very stable arrangement of the molecules in the trilayer. The molecular arrangement shown in Fig. 2, B, which is similar in principle to those proposed for many built-up multilayers and micelles of association colloids, may explain the observed stability. This particular arrangement of the molecules in the trilayer is indicated by the fact that when multilayers of isodextropimaric acid containing different n-fatty acids are studied (mixtures containing 5, 10, and 20 mol per cent of the even *n*-fatty acids $C_{14}-C_{24}$ have been investigated [2]) the multilayer containing *n*-eicosanic acid ($C_{20}H_{40}O_2$) gives the most stable trimolecular layer, *i.e.* one that shows the highest surface pressure at point *d*. The length of the paraffin chain of the eicosanic acid molecule corresponds closely to the length of the hydrophobic parts of two isodextropimaric acid molecules, when built in the trilayer as shown in Fig. 2, B. When ionized molecules form the trilayer, as in the case of tetrahydroabietic and dihydroabietic acid on a substrate with a pH over 9, it is to be expected that water molecules and gegenions are incorporated between the hydrophilic groups of the trilayer. The presence of water molecules may explain the more liquid character of the multilayers on the alkaline substrate and also the fact that at higher temperatures (above 35° C) the trilayer arrangement is more stable on an acid substrate than on an alkaline one.

Fig. 2, A shows a possible arrangement of the molecules in the dilayer (at *b*), which takes into account the constancy of the surface pressure from *a* to *b*. The trilayer is formed from the dilayer by a gradual process on compression, which is indicated by the continuous increase in pressure from *b* to *c*.

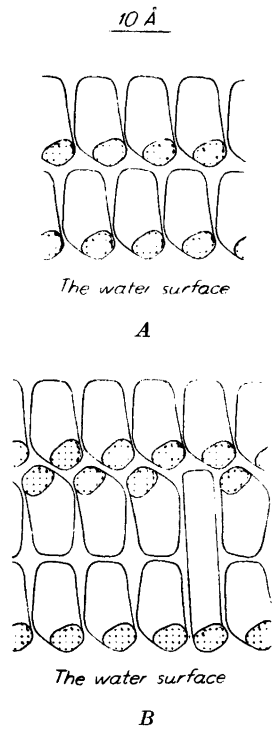


Fig. 2. Assumed molecular arrangement of the dilayer (A) and trilayer (B) of isodextropimaric acid. The carboxylic groups in the molecules are shaded. At the right, an eicosanic acid molecule is shown built in the trilayer.

It is possible that the study of the multilayers with the surface balance technique may throw light on problems in the field of the association colloids.

I am indebted to Professor Per Ekwall, the head of the Institute, for the opportunity of using the facilities of the Institute and for discussions.

1. Bruun, H. *Acta Chem. Scand.* **6** (1952).
2. Bruun, H. *To be published.*

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An Interpretation of the Effect of Ionization upon Monolayer Properties of Rosin Acids

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When the molecules in a monolayer undergo ionization their hydrophilic properties become stronger. It may be expected that this strengthening of the hydrophilic properties will affect the monolayer properties in a different manner depending on how strongly hydrophobic the molecules are. On aqueous substrates of low electrolyte concentration the following relationship may be expected to apply between the hydrophobic properties of the molecules and the properties, *e. g.* the stability and surface area, of the monolayers they form. In the case of weakly hydrophobic molecules in which the hydrophilic properties predominate after ionization it will be expected that the ionized molecules will not remain in the monolayer when it is compressed. If it is assumed that an equilibrium represented by the equation $\frac{[H_3O^+] \cdot A^-}{HA} = K$ applies to the ionization of an acid HA in the monolayer and that the ionized molecules do not remain in the monolayer on compression, it is to be expected that the curve Ia in Fig. 1 representing the decrease in the area A taken up by the spread substance with increasing pH of the substrate will be similar to the curve i in the same figure showing the variation of the degree of ionization, *i. e.* the ratio of ionized molecules (A^-) to the total number of molecules ($HA + A^-$) as a function of the pH of the substrate. When the ionized molecules do not remain in the monolayer, the stability of the monolayer as indicated by the collapse pressure π_K will not undergo change. The pressure π_K will hence remain constant

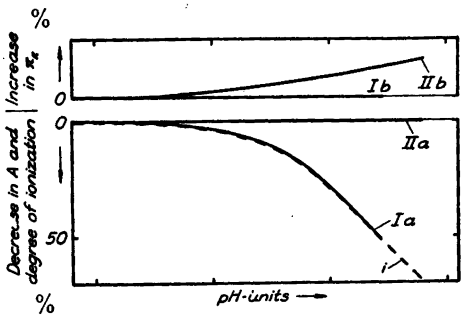


Fig. 1. The expected effect of ionization of a monolayer substance upon its monolayer properties on an aqueous substrate with a low electrolyte concentration when the molecules are I *weakly hydrophobic,* II *strongly hydrophobic. Curve a: Decrease in the surface area A of the compressed monolayer, b: Increase in the monolayer collapse pressure π_K. The dotted line i gives the variation of the degree of ionization.*

with increasing degree of ionization, Fig. 1, curve Ib.

In the other case in which the hydrophobic properties predominate even in spite of the ionization, the ionized molecules will be held in the monolayer even when it is compressed. The monolayer properties can be expected to vary in the following manner. The area A will not be altered with increasing ionization, Fig. 1, IIa. Factors such as changes in the tilt of the molecules in the surface layer and changes in the interaction between the molecules may, however, to some extent cause variations in A. The ionized molecules will be more strongly anchored in the surface of the substrate than the unionized molecules and this will result in higher values of π_K with increasing ionization, Fig. 1, IIb.

These two possibilities correspond to limiting conditions. If there exists a suitable balance between the hydrophobic and hydrophilic properties it will be expected that the curve representing the decrease in A will lie between the two curves *Ia* and *IIa*. π_K will increase to some extent, but will be smaller than in case IIb.

Fig. 2.

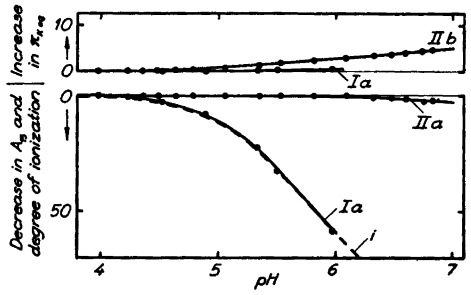
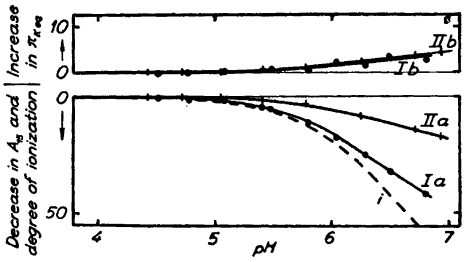


Fig. 2—3. The effect of ionization upon the properties of rosin acid monolayers. Fig. 2. I *dehydroabietic,* II *tetrahydroabietic acid. Fig. 3.* I *abietic,* II *dihydroabietic acid. Curves a: Decrease in the area* A_{15}, b: *Increase in the collapse pressure* π_{Keq}. *Curve i give the variation of the degree of ionization. 20° C. The pH of the aqueous substrate has been reduced first to pH 3.8 by adding hydrochloric acid, and then increased by adding sodium hydroxide solution.*

Fig. 3.



On the basis of the foregoing, if the strength of the hydrophobic properties of the molecules determines the effect of ionization upon the properties of the compressed monolayer, it is to be expected that in the case of molecules with weakly hydrophobic properties only the area A will undergo a change, and in the case of strongly hydrophobic molecules only the pressure π_K will be altered. In the case of molecules with intermediate hydrophobic properties in which there exists a balance between the hydrophobic and hydrophilic properties in the ionized state, both A and π_K will be affected.

In the rosin acid series, dehydroabietic, abietic, dihydroabietic, and tetrahydroabietic acid, the hydrophobic character of the molecules increases with decreasing number of double bonds in the molecule. The effect of ionization upon the monolayer properties of these acids on substrates with very low electrolyte concentrations is recently investigated [1] and is shown in Figs. 2—3. The curves for dehydroabietic acid, Fig. 2, I, indicate a marked decrease in A_{15} (the area at a surface pressure of 15 dynes per cm) and practically unaltered π_{Keq} (the equilibrium pressure after the collapse of the monolayer). The curve A_{15} almost exactly corresponds to the curve *i* for the degree of ionization calculated taking for *K* the value $10^{-5.84}$. At pH 5.97, a decrease in A_{15} of 58 per cent is accompanied by an increase in π_{Keq} of only 0.6 per cent. The effect of the ionization on the dehydroabietic acid monolayer thus corresponds very well with the curves I in Fig. 1, which were drawn for the case of weak hydrophobic properties of the molecule. The curves for tetrahydroabietic acid, Fig. 2, II, resemble the curves II in Fig. 1, which apply to strong hydrophobic properties of the monolayer substance. The increase in π_{Keq} is marked (5 per cent at pH 7.0), whereas the decrease in A_{15} is relatively insignificant (3—4 per cent). Fig. 3, I, for abietic acid and Fig. 3, II, for dihydroabietic acid indicate marked changes in both π_{Keq} and A_{15}. The increase in π_{Keq} is about 3 per cent for abietic acid at pH 6.81, the decrease in A_{15} 41.2 per cent. The corresponding percentages for dihydroabietic acid are 4 per cent and 17 per cent at pH 6.94. Abietic and dihydroabietic acids thus occupy an intermediate position with respect to the effect of ionization on the monolayer properties, which is to be expected since with respect to their hydrophobic properties they are situated between dehydroabietic and tetrahydroabietic acid.

The behavior of the rosin acids shows

clearly that the effect of ionization upon the monolayer properties is connected with their hydrophobic character. It is also evident that the strength of the hydrophobic properties largely determines the nature of the effect. Differences similar to those noted for the rosin acids may also be found among other groups of substances in which the hydrophobic properties undergo similar variations.

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1. Bruun, H. *Acta Chem. Scand.* **6** (1952) 494.

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Thiohydrazides and Thiohydrazones: A New Class of Antibacterial Substances

KAI ARNE JENSEN and CARL LUND JENSEN

Research Laboratory of A/S Ferrosan, Copenhagen, Denmark

In 1934 Jensen [1] prepared several co-ordination compounds of thiosemicarbazide and substituted thiosemicarbazides. Recently Jensen and Miquel [2] found that thiobenzhydrazide, $C_6H_5CSNHNH_2$, forms nickel complexes of the same type as are formed by the thiosemicarbazides. A close parallel appears to exist between tuberculostatic activity of the thiosemicarbazones and their ability to form co-ordination compounds with copper [3]. Since the complex compounds of thiosemicarbazides and thiohydrazides are similar we should expect that thiohydrazones would also possess antibacterial activity. A large number of thiohydrazides and thiohydrazones were therefore prepared and tested. Preliminary experiments showed that both classes of compounds were antibacterial as well as fungistatic, so we are now making a more detailed investigation of their activity.

The thiohydrazides were prepared by reaction of esters of dithioacids or the free dithioacids with hydrazine. The following new thiohydrazides were prepared:

Thiophenylacethydrazide. M.p. 71°.

$C_8H_{10}N_2S$ (166.2)
Calc. C 57.82 H 6.07 N 16.86 S 19.26
Found » 57.52 » 5.95 » 16.96 » 19.25

2-Hydroxythiobenzhydrazide. M.p. 102°.

$C_7H_8ON_2S$ (168.2)
Calc. C 50.00 H 4.80 N 16.66 S 19.07
Found » 49.98 » 4.85 » 16.95 » 18.92

4-Hydroxythiobenzhydrazide. M.p. 208°.

$C_7H_8ON_2S$ (168.2)
Calc. C 50.00 H 4.80 N 16.66
Found » 50.25 » 4.73 » 16.83

4-Methoxythiobenzhydrazide. M.p. 126°.

$C_8H_{10}ON_2S$ (182.2)
Calc. C 52.72 H 5.53 N 15.38 S 17.59
Found » 52.85 » 5.48 » 15.31 » 17.30

3-Methoxy-4-hydroxythiobenzhydrazide. M.p. 148°.

$C_8H_{10}O_2N_2S$ (198.2)
Calc. C 48.48 H 5.09 N 14.14
Found » 48.68 » 5.18 » 14.06

4-Acetamidothiobenzhydrazide. M.p. 234°.

$C_9H_{11}ON_3S$ (209.3)
Calc. C 51.67 H 5.30 N 20.09
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4-Dimethylaminothiobenzhydrazide. M.p. 170°.

$C_9H_{13}N_3S$ (195.3)
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2-Furanethiocarboxhydrazide. M.p. 135°.

$C_5H_6ON_2S$ (142.2)
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Calc. C 37.98 H 3.83 N 17.72 S 40.53
Found » 38.11 » 3.58 » 17.83 » 40.42

2-Pyrrolethiocarboxhydrazide. M.p. 122°.

$C_5H_7N_3S$ (141.2)
Calc. C 42.55 H 5.00 N 29.78 S 22.67
Found » 42.66 » 5.29 » 29.82 » 22.56

3-Indolethiocarboxhydrazide. M.p. 173°.

$C_9H_9N_3S$ (191.2)
Calc. C 56.54 H 4.75 N 21.98 S 16.77
Found » 56.97 » 5.02 » 22.16 » 16.85

A large number of thiohydrazones were prepared by condensation of the thiohydrazides with aldehydes, *e.g.* thiobenzhydrazones of salicylaldehyde (m.p. 155°), 2-methoxybenzaldehyde (m.p. 144°), anisaldehyde (m.p. 84°), 3-hydroxybenzaldehyde (m.p. 158°), 4-nitrobenzaldehyde (m.p. 109°), 4-dimethylaminobenzaldehyde (m.p. 125°), 4-acetamidobenzaldehyde (m.p. 170°), 4-succinylamidobenzaldehyde (m.p. 176° with decomposition), 1-naphthaldehyde (m.p. 126°), 2-naphthaldehyde (m.p. 132°), 2-quinolinecarboxaldehyde (m.p. 138°), 8-quinolinecarboxaldehyde (m.p. 159°), glucose (m.p. 194°); thiophenylacethydrazones of 2,4-dichlorobenzaldehyde (m.p. 152°), 4-nitrobenzaldehyde (m.p. 171°), 4-acetamidobenzaldehyde (m.p. 193°) phthaldehydic acid (m.p. 167°), 1-naphthaldehyde (m.p. 160°), furfural (m.p. 98°); 4-methoxythiobenzhydrazone of 4-acetamidobenzaldehyde (m.p. 172°); 2-furanethiocarboxhydrazones of furfural (m.p. 138°), 4-acetamidobenzaldehyde (m.p. 197°) *etc.* Some thioacylated hydrazides were also prepared in the same manner as benzoyl-thiobenzhydrazide (*cf.* Jensen and Miquel [2]), *e.g.* nicotinylthiobenzhydrazide (m.p. 142°) and *iso*nicotinyl-thiobenzhydrazide (m.p. 130°).

Further details will be given in a subsequent paper in this journal.

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3. Carl, E., and Marquardt, P. *Z. f. Naturforsch.* **4b** (1949) 280; Liebermeister, K. *ibid.* **5b** (1950) 79.

Received June 13, 1952.

On the Atomic Composition of the Mineral Belyankite

A. H. NIELSEN

Chemical Laboratory, "Kryolitselskabet Øresund A/S", Copenhagen, Denmark

The Russian mineralogist Dorfman has recently published a paper [1] on a new fluorine mineral, belyankite, from Central Kazakhstan. Amongst other things Dorfman has determined some optic constants, thermic data, and the specific weight (2.720) for this mineral. Further Dorfman gives x-ray data (S. S. Kvitka) and a chemical analysis (M. O. Stepan), giving the following weight percentages:

Table 1.

Al_2O_3	21.88
CaO	34.00
F	49.01
H_2O+	15.35
H_2O-	0.30
	120.54

On the basis of this analysis Dorfman assigns the following formula to belyankite: $Ca_2Al_3(F, OH)_{13} \cdot [H_2O]$.

If the analysis is recalculated according to the method usually employed for double fluorides (discounting H_2O^-, as Dorfman himself does), we get:

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If the analysis is recalculated according to the method usually employed for double fluorides (discounting H_2O^-, as Dorfman himself does), we get:

Table 2.

	1. Weight p.c.	2. Atomic quot.	3. Number of atoms and H_2O mol.	4. $Ca_3Al_2F_{12} \cdot 4\ H_2O$ weight p.c., calc.
Al	11.58	0.429	1.00	11.37
Ca	24.30	0.606	1.41	25.35
F	49.01	2.579	6.01	48.08
H_2O+	15.35	0.852	1.99	15.20
Total	100.24			100.00

From the values in column 3 we get the ideal formula: $Ca_3Al_2F_{12} \cdot 4\ H_2O$.

Already a glance at Dorfman's formula shows that it cannot possibly be correct, stating as it does that the percentage content of Ca and Al is about the same.

If we calculate the molecular weight from the figures in column 2, we get $M = 2 \cdot 233.5 = 467.0$.

By inserting Kvitka's cell dimensions, the specific weight and M we get:

$$Z = \frac{13.47 \cdot 8.46 \cdot 9.89 \cdot 2.720}{1.6604 \cdot 467.0} = 3.96$$

That is to say 4 formula units ($Ca_3Al_2F_{12} \cdot 4\ H_2O$) in the unit cell.

The present author has previously published a paper [2] in which amongst other things is described a "Ca-cryolite", produced by the wet method, with the formula $Ca_3Al_2F_{12} \cdot 3\ H_2O$, *i.e.* chemically closely allied to belyankite, with the same general properties (very readily fusible, easily dissolving in warm diluted hydrochloric acid). Bøgvad [3] has determined the specific weight for "Ca-cryolite" at 2.77 in good agreement with Dorfman's value 2.720.

1. Dorfman, M. D. *Doklady Akad. Nauk S.S.S.R.* **75** (1950) 851. Ref. *Chem. Abstr.* **45** (1951) 8943.
2. Nielsen, A. H. *Z. anorg. allg. Chem.* **232** (1937) 155. Ref. *Neues Jahrb. Mineral.* (1937) 595—6.
3. Bøgvad, *Z. anorg. allg. Chem.* **232** (1937) 156.

Received July 5, 1952.

Reaction of Organophosphorus Compounds with Dihydroxyphenyl Derivatives

KLAS-BERTIL AUGUSTINSSON

Institute of Organic Chemistry and Biochemistry, University of Stockholm, Stockholm, Sweden

From both a practical and theoretical point of view we are interested in the reaction mechanism of organophosphorus compounds. We know that these agents inhibit cholinesterases and that this most probably explains their toxic effects. We also know that the inhibitory reaction is a phosphorylation at the so-called esteratic site of the enzyme molecule.

We have tried to protect *in vitro* cholinesterase preparations against inactivation by various organophosphorus compounds. About 120 various compounds have been tested as protectors. The most promising results were obtained with some local anesthetics. It could be demonstrated, for instance, that procaine at a concentration (5×10^{-5} M) which has no measurable effect on the serum cholinesterase activity protects this enzyme against inactivation by Tabun. In the case of acetylcholinesterases from various sources (erythrocytes, nucleus caudatus, cobra venom) the protective effect is less, but a definite protection has been demonstrated in all cases [1].

The protective action has further been demonstrated when inactivator and protector are simultaneously mixed with the enzyme; in these instances inactivator and protector were mixed together about one hour before mixing with the enzyme. In the procaine experiments, the protective effect was the same, as in those experiments when the enzyme was incubated with the protector prior to the inactivator. With some other compounds tested, however, it was shown that Tabun and DFP react

Table 2.

	1. Weight p.c.	2. Atomic quot.	3. Number of atoms and H_2O mol.	4. $Ca_3Al_2F_{12} \cdot 4\ H_2O$ weight p.c., calc.
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F	49.01	2.579	6.01	48.08
H_2O+	15.35	0.852	1.99	15.20
Total	100.24			100.00

From the values in column 3 we get the ideal formula: $Ca_3Al_2F_{12} \cdot 4\ H_2O$.

Already a glance at Dorfman's formula shows that it cannot possibly be correct, stating as it does that the percentage content of Ca and Al is about the same.

If we calculate the molecular weight from the figures in column 2, we get $M = 2 \cdot 233.5 = 467.0$.

By inserting Kvitka's cell dimensions, the specific weight and M we get:

$$Z = \frac{13.47 \cdot 8.46 \cdot 9.89 \cdot 2.720}{1.6604 \cdot 467.0} = 3.96$$

That is to say 4 formula units ($Ca_3Al_2F_{12} \cdot 4\ H_2O$) in the unit cell.

The present author has previously published a paper [2] in which amongst other things is described a "Ca-cryolite", produced by the wet method, with the formula $Ca_3Al_2F_{12} \cdot 3\ H_2O$, *i.e.* chemically closely allied to belyankite, with the same general properties (very readily fusible, easily dissolving in warm diluted hydrochloric acid). Bøgvad [3] has determined the specific weight for "Ca-cryolite" at 2.77 in good agreement with Dorfman's value 2.720.

1. Dorfman, M. D. *Doklady Akad. Nauk S.S.S.R.* **75** (1950) 851. Ref. *Chem. Abstr.* **45** (1951) 8943.
2. Nielsen, A. H. *Z. anorg. allg. Chem.* **232** (1937) 155. Ref. *Neues Jahrb. Mineral.* (1937) 595—6.
3. Bøgvad, *Z. anorg. allg. Chem.* **232** (1937) 156.

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Reaction of Organophosphorus Compounds with Dihydroxyphenyl Derivatives

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From both a practical and theoretical point of view we are interested in the reaction mechanism of organophosphorus compounds. We know that these agents inhibit cholinesterases and that this most probably explains their toxic effects. We also know that the inhibitory reaction is a phosphorylation at the so-called esteratic site of the enzyme molecule.

We have tried to protect *in vitro* cholinesterase preparations against inactivation by various organophosphorus compounds. About 120 various compounds have been tested as protectors. The most promising results were obtained with some local anesthetics. It could be demonstrated, for instance, that procaine at a concentration (5×10^{-5} M) which has no measurable effect on the serum cholinesterase activity protects this enzyme against inactivation by Tabun. In the case of acetylcholinesterases from various sources (erythrocytes, nucleus caudatus, cobra venom) the protective effect is less, but a definite protection has been demonstrated in all cases [1].

The protective action has further been demonstrated when inactivator and protector are simultaneously mixed with the enzyme; in these instances inactivator and protector were mixed together about one hour before mixing with the enzyme. In the procaine experiments, the protective effect was the same, as in those experiments when the enzyme was incubated with the protector prior to the inactivator. With some other compounds tested, however, it was shown that Tabun and DFP react

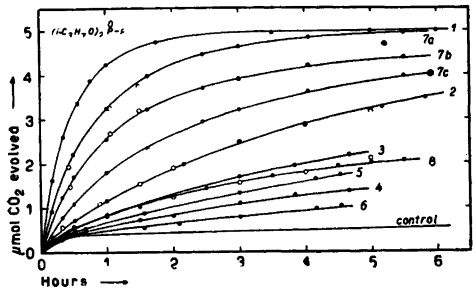


Fig. 1. Reaction of 5 µmol DFP with various compounds in 0.025 M $NaHCO_3$. pH 7.9. 25.0° C. Numbers in the following list refer to µmol, unless otherwise stated: 1) Pyrogallol 100; 2) Pyrocatechol 25 and DOPA 25; 3) Hydroquinone 100; 4) Resorcinol 100; 5) Phloroglucinol 100; 6) Phenol 100; 7a) Adrenaline 100; 7b) Adrenaline 50 and noradrenaline 50; 7c) Adrenaline 25; 8) Histamine 50 and cobra venom 19.2 mg.

with the "protector" to give a product less active as an inactivator of the enzyme. Pantocaine was the first compound tested which showed this effect.

The reaction between the "protector" and Tabun or DFP, however, was more pronounced with a series of di- and trihydroxyphenyl derivatives. For instance, it was shown that pyrocatechol reacts with DFP and Tabun. The inhibitory effect was decreased more the longer pyrocatechol was incubated with DFP or Tabun. When the reaction between DFP and pyrocatechol was tested in a bicarbonate solution in the Warburg apparatus, production of acid could be demonstrated. Pyrogallol gave a higher reaction rate with DFP. At that time a paper by Jandorf and co-workers [2] announced a similar observation. A great number of compounds have since been tested in this way in our laboratory, and some of the results are illustrated in Fig. 1.

Among the dihydroxyphenyl derivatives the most important ones are adrenaline and noradrenaline, which react with DFP in a bicarbonate solution with the evolution of CO_2. The reaction rate is the same for both adrenaline and noradrenaline. DOPA (3,4-dihydroxyphenyl-alanine) reacts with DFP at the same rate as pyrocatechol.

Like Jandorf and co-workers, we interpret this observation as due to phosphorylation of a phenolic group. With DFP, hydrofluoric acid is liberated and a diisopropylphosphate of the dihydroxyphenyl derivative is obtained. One molecule of DFP reacts with one molecule of the phenol. Even when the latter is 100 fold excess there is only this one to one relationship. The reaction is of second order or pseudo-first order.

Obviously one is immediately inclined to think of the reaction between DFP and cholinesterase as having a similar mechanism. If therefore a high concentration of cholinesterase is allowed to react with DFP the production of hydrofluoric acid should be detectable using the same technique. This was also the case when a relatively high concentration of cobra venom esterase reacted with DFP (Fig. 1).

The other organophosphorus compound which has been tested in detail in Sweden is Tabun. A comprehensive pharmacological study has been made by Holmstedt [3] and the physico-chemical properties of Tabun are reported by Larsson [4]. We have now demonstrated that Tabun also reacts

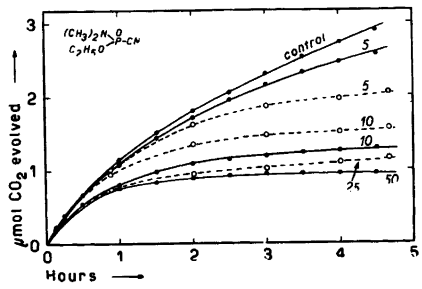


Fig 2. Reaction of Tabun with pyrogallol and adrenaline O in 0.025 M $NaHCO_3$. pH 7.9. 25.0° C. Numbers refer to µmol pyrogallol and adrenaline. Approximately 4 µmol Tabun.

with dihydroxyphenyl derivatives. The reaction with adrenaline is illustrated in Fig. 2. At pH 8 Tabun is hydrolysed yielding hydrocyanic acid and dimethylamido phosphoric acid [4]. The more adrenaline present the less acid is produced and we explain this by postulating a phosphorylation of adrenaline during the hydrolysis.

The inhibitory effects of the reaction products on cholinesterase have been tested. A concentration of DFP which is more than 1000 times higher than that required to give 50 % inhibition does not give any inhibition at all when the DFP has been incubated with adrenaline for 10 hours or more; the concentration of adrenaline was 20 times the concentration of DFP. Similar results were obtained with Tabun.

I am greatly indebted to Mrs Britta Tyrefors for technical assistance and for the skill and care which she has bestowed on the experiments.

1. Augustinsson, K.-B. *Acta Physiol. Scand.* In press.
2. Jandorf, B. J., Wagner-Jauregg, T., O' Neill, J. J., and Stolberg, M. A. *J. Am. Chem. Soc.* **74** (1952) 1521.
3. Holmstedt, B. *Acta Physiol. Shand.* **25** (1951) suppl. 90.
4. Larsson, L. *Acta Chem. Scand.* **6** (1952). In press.

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Diheterolevulosan III and IV

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The recent publication by Sattler *et al.*[1] prompts us to report the isolation of two new compounds which are probably isomerides of the previously known diheterolevulosans I and II. The substances were isolated from the unfermentable residue from hydrochloric acid treated fructose [2] by fractionating the mixture on a column of animal carbon, using the gradient elution technique [3] combined with fractional crystallisation. Both substances are non-reducing and difficult to hydrolyse, and on paper chromatograms they travel at about the same speed as diheterolevulosan II. The analytical data for one of the substances is consistent with its formulation as a diheterolevulosan ($C_{12}H_{20}O_{10}$). It has not been possible to analyse the second compound as yet owing to certain technical difficulties in the micro analytical department, but the periodic acid oxidation and the general properties of the substance indicate a diheterolevulosan type of structure.

Diheterolevulosan III, m.p. 279—281° ** (dec.), $[\alpha]_D^{20}$ −309° (water, c = 2), (Analysis: Found C, 44.5; H, 6.12. Calc. for $C_{12}H_{20}O_{10}$ C, 44.44; H, 6.18 %), consumes 4 moles of periodic acid and forms 2 moles of formic acid, and is thus probably an anomer of diheterolevulosan I. Acetate: M.p. 268—269° (sublimes), $[\alpha]_D^{20}$ − 199° (chloroform, c = 2). Analysis: C, 50.2; H, 5.67. Calc. for $C_{24}H_{32}O_{16}$ C, 50.0; H, 5.55 %.

"Diheterolevulosan IV", m.p. 240—242° (dec.), $[\alpha]_D^{20}$ −183° (water, c = 2), consumes 3 moles of periodic acid and forms 1 mole of formic acid. Acetate: M.p. 130—131°, $[\alpha]_D^{20}$ −159° (chloroform, c = 2).

A more detailed report will be published shortly in this journal.

Added in proof (29.7.52): Since this note was submitted for publication Wolfrom, Hilton and Binkley (*J. Am. Chem. Soc.* **74** (1952) 2867) have described a new di-D-fructose dianhydride "diheterolevulosan III" which appears to be identical with our "diheterolevulosan IV". To avoid confusion we propose that our names "diheterolevulosan III" and "diheterolevulosan IV" should be interchanged.

* Svenska Sockerfabriks AB Research Fellow 1951—1952.

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with dihydroxyphenyl derivatives. The reaction with adrenaline is illustrated in Fig. 2. At pH 8 Tabun is hydrolysed yielding hydrocyanic acid and dimethylamido phosphoric acid [4]. The more adrenaline present the less acid is produced and we explain this by postulating a phosphorylation of adrenaline during the hydrolysis.

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Diheterolevulosan III: Found C, 44.4; H, 6.14. Calc. for $C_{12}H_{20}O_{10}$ C, 44.4; H, 6.18 %. Acetate: Found C, 50.1; H, 5.63. Calc for $C_{24}H_{32}O_{16}$ C, 50.0; H, 5.55 %.

Hydrolysis of the methyl ether of diheterolevulosan IV (m.p. 122—122.5°, $[\alpha]_D^{20}$ — 243° (chloroform, $c = 2$) gave D-fructose 3,4,5-trimethylether (70 % yield) identified by conversion to D-arabonamide 2,3,4-trimethylether (80 % yield). This proves that diheterolevulosan IV is a di-D-fructopyranose 1,2′ 2,1′-dianhydride.

1. Sattler, L., Zerban, F. W., Clark, G. L. Chia-Chen-Chu, Albon, N., Gross, D., and De Whalley, H. C. S. *Ind. Eng. Chem.* **44** (1952) 1122.
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3. Tiselius, A. *Endeavour* **11** (1952) no. 41 p. 5.

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Note on the Electrolytic Methoxylation of Furan

NIELS CLAUSON-KAAS and ZDENĚK TYLE

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The electrolytic methoxylation of furan to 2,5-dimethoxy-2,5-dihydrofuran has previously been carried out by electrolyzing a methanolic solution of furan and ammonium bromide [1]. It has now been found, that dimethoxydihydrofuran is also formed, when the ammonium bromide is replaced by ammonium nitrate, sodium nitrate, sodium formate, boron fluoride etherate or sulfuric acid. The yield of dimethoxydihydrofuran (38—56 per cent) is somewhat lower than when ammonium bromide is used (86 per cent). These experiments indicate, that not only halogens, but also other oxidizing agents can oxidize furan in methanolic solution to dimethoxydihydrofuran.

EXPERIMENTAL

Solutions of ammonium nitrate (5.0 g), sodium nitrate (5.0 g), sodium formate (5.0 g), boron fluoride etherate (5.0 ml) and concentrated sulfuric acid (1.00 ml), in a mixture of methanol (250 ml) and furan (20.4 g = 0.30 mole) were electrolyzed as described previously [1]. 16.1 ampere hours (0.60 faraday) was passed through the cell. After electrolysis, the acid electrolytes were neutralized with a solution of sodium methoxide. The results of the electrolyses are summarized below.

The methanol was in each case distilled off through a Vigreux column and the residues filtered or centrifuged to remove the inorganic salts (washing with ether). The resulting brown liquids were then distilled.

Electrolyte	Time of electrolysis (hours)	Current (ampere)	Potential across the cell (volt)	Temperature in the cell (°C)	Colour of the electrolyte after electrolysis	g of sodium used for neutralization
NH_4NO_3	6.7	2.9—2.0	5.8—6.8	—10°	yellowish-brown	1.46
$NaNO_3$	6.8	2.6—0.9	6.2—7.4	—10° to —14°	reddish-brown	0
NaOOCH	8.3	3.1—0.6	7.0—8.8	—9° to —18°	yellowish-brown	0
BF_3	6.0	2.8—1.9	6.1—6.6	—10° to —13°	slightly yellow	2.30
H_2SO_4	8.3	2.3—1.8	6.0—7.0	—11° to —12°	yellowish-brown	0.95

Diheterolevulosan III: Found C, 44.4; H, 6.14. Calc. for $C_{12}H_{20}O_{10}$ C, 44.4; H, 6.18 %. Acetate: Found C, 50.1; H, 5.63. Calc for $C_{24}H_{32}O_{16}$ C, 50.0; H, 5.55 %.

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NaOOCH	8.3	3.1—0.6	7.0—8.8	—9° to —18°	yellowish-brown	0
BF_3	6.0	2.8—1.9	6.1—6.6	—10° to —13°	slightly yellow	2.30
H_2SO_4	8.3	2.3—1.8	6.0—7.0	—11° to —12°	yellowish-brown	0.95

Electrolyte	Fraction (g)	Distillation pressure mm	B.p.	n_D^{25}	Yield of dimethoxydihydrofuran
NH_4NO_3	1 (13.4)	14	49– 51°	1.4327	15.6 g (fractions 1 and 2)
–	2 (2.2)	–	51– 60°	1.4326	= 40 %.
–	3 (1.8)	–	68–130°	partly crystalline	
$NaNO_3$	1 (11.8)	14	52– 53°	1.4326	15.4 g (fractions 1 and 2)
–	2 (3.6)	–	53– 58°	1.4330	= 40 %.
–	3 (1.5)	–	60–125°	1.4407	
NaOOCH	1 (14.7)	11	50–56°	1.4329	14.7 g (fraction 1)
–	2 (1.0)	–	59–110°	1.4393	= 38 %.
BF_3	1 (19.6)	12	48– 52°	1.4321	21.7 g (fractions 1 and 2)
–	2 (2.1)	13	52– 60°	1.4320	= 56 %.
–	3 (5.2)	–	61–138°	1.4383	
H_2SO_4	1 (8.6)	13	48– 52°	1.4318	16.0 g (fractions 1 and 2)
–	2 (7.4)	–	52– 55°	1.4319	= 41 %.
–	3 (5.6)	14	54– 86°	1.4282	
–	4 (3.3)	–	88–156°	1.4382	

1. Clauson-Kaas, N. (to Kemisk Værk Køge A/S). Belg. patent 500356 (1951). Clauson-Kaas, N., Limborg, F. and Glens, K. *Acta Chem. Scand.* **6** (1952) 531.

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On the Existence of *a*-Glycol Groups in Lignin

BENGT O. LINDGREN and ULLA SAEDÉN

Department of Cellulose Technology and Wood Chemistry, Royal Institute of Technology, Stockholm, Sweden

Lignin appears to contain three types of sulphonatable groups [1], designated X, Z and B, and model experiments (treatment with sulphite solutions [2] and sulphide solutions [3]) have shown that the Z-groups may be *p*-alkoxybenzyl alcohol groups. Two phenylglycerol derivatives containing *p*-alkoxybenzyl alcohol groups, *viz.* veratrylglycerol (I) and its *β*-guaiacyl ether (II), have recently been studied by Adler *et al.* [4,5] Like other *p*-alkoxybenzyl alcohols these substances are converted by sulphite solutions into sulphonic acids (III resp. IV). The rate of the sulphonation of veratrylglycerol is of about the same order of magnitude as the rate at which the Z-groups in lignin are sulphonated. It was therefore of inter-

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–	3 (5.2)	–	61–138°	1.4383	
H_2SO_4	1 (8.6)	13	48– 52°	1.4318	16.0 g (fractions 1 and 2)
–	2 (7.4)	–	52– 55°	1.4319	= 41 %.
–	3 (5.6)	14	54– 86°	1.4282	
–	4 (3.3)	–	88–156°	1.4382	

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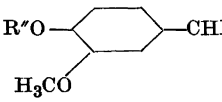
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est to investigate whether groups of structure V might be present in lignosulphonic acids.



I	R=H	R′=OH	R″=CH_3
II	R= o-(H_3CO)C_6H_4	R′=OH	R″=CH_3
III	R=H	R′=SO_3H	R″=CH_3
IV	R= o-(H_3CO)C_6H_4	R′=SO_3H	R″=CH_3
V	R=H	R′=SO_3H	R″=alkyl
VI	R=H	R′=OH	R″=alkyl

The structure V contains a terminal α-glycol group, and thus on periodate oxidation it should yield formaldehyde. Our experiments have shown, however, that no formaldehyde is formed when spruce lignosulphonic acid is treated with periodate at pH 3.5. This shows that the lignosulphonic acid does not contain any large number of groups such as V.

The di-*p*-toluenesulphonates of terminal α-glycol groupings, when heated with sodium iodide in acetone, react as follows (*cf.* experiments with erythritol [6], 1,2,3,4-dibenzylidene-D-sorbitol [7], glycol [8], and glyceraldehyde acetal [9])

$$-CH(OSO_2C_7H_7)-CH_2(OSO_2C_7H_7) + 2\,NaI \rightarrow -CH=CH_2 + I_2 + 2\,C_7H_7SO_3Na.$$

It has been found that the di-toluenesulphonate of safrol glycol [= 3-(3′,4′-methylenedioxyphenyl)-propane-1,2-diol] reacts in the same way.

Tosylated spruce lignosulphonic acids yield only small amounts of iodine – about one mol. of iodine for every 20 methoxyl groups, and this also indicates that lignosulphonic acids do not contain any considerable amount of terminal α-glycol groups.

Spruce lignin contains about one Z-group per 6 methoxyl groups [1], and thus the main part of the Z-groups may not be phenylpropane monomers containing a non-etherified glycerol side-chain (VI). Naturally these experiments do not exclude the possibility that the Z-groups are *p*-alkoxybenzyl alcohols with a structure other than VI.

EXPERIMENTAL. *Periodate oxidation of lignosulphonic acid.* Sodium periodate solution (25 ml, 0.2 N) was added to a solution of 2.5 g of a calcium lignosulphonate (Ca 11.7, S 5.4 and OCH_3 10.7 %) in 100 ml of an acetate buffer of pH 3.5, and the mixture was set aside at room temperature for 30 minutes. Lead nitrate solution (12.5 g/100 ml, 20 ml) was added to precipitate the iodate and periodate ions [10], and the solution was evaporated to dryness under reduced pressure in a stream of coal gas. The distillate was treated with dimedone in the usual way; no precipitate was obtained.

Corresponding oxidations at pH 6 and 7 (phosphate buffer solutions) gave small amounts of formaldehyde (0.01 mol. per OCH_3).

Tosylation of lignosulphonic acid. A mixture of 1 g of a low-sulphonated lignin [11] (S 3.95, OCH_3 13.6 %), 5 g of tosyl chloride, and 20 ml of pyridine was set aside for 92 hours, and the insoluble product (1.12 g: S 9.08, OCH_3 9.00 and Cl 1.68 %) was collected.

A mixture of this material (0.4 g), sodium iodide (1.5 g), and acetone (10 ml) was heated in a sealed tube at 100° for 17 hours. Water was added and the liberated iodine was titrated with thiosulphate. 1.30 Ml of 0.1011 N-thiosulphate was required, corresponding to 0.05 mol. of I_2 per OCH_3 group.

Safrole glycol ditoluenesulphonate. A mixture of safrole glycol (3 g), tosyl chloride (5.85 g), and pyridine (15 ml) was set aside at room temperature for 24 hours, then evaporated to dryness in a vacuum desiccator. Sodium bicarbonate solution was added to the residue and the mixture was extracted with chloroform. The chloroform solution was dried, filtered through a column of alumina and evaporated, yielding 2.85 g of a substance melting at 128° (yield: 39 %). The melting point was raised to 135° by recrystallization from benzene.

$C_{24}H_{24}O_8S_2$	Found	C 57.2	H 4.80	OCH_3	12.7
	Calc.	» 57.2	» 4.77	»	12.7

When this substance was heated with NaI and acetone in a sealed tube at 100° for 12 hours, toluenesulphonic acid (isolated as the benzylthiuronium salt) was obtained in a yield of *ca.* 95 % and I_2 (determined by titration with thiosulphate) in a yield of *ca.* 90 %.

This work have been supported by a grant from the Swedish Natural Science Research Council.

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A Preliminary X-Ray Investigation of Dipicrylamine and some of Its Salts

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The potassium salt of dipicrylamine, or 2,4,6 - 2′,4′,6′-hexanitrodiphenylamine, is almost insoluble in water, and it has been proposed to make use of this to manufacture potassium from sea-water [1]. The salt exists in at least two polymorphic forms, one red and one violet. These are distinguished by their different reactivity towards nitric acid, which is used to regenerate the amine from the salt [2]. It was therefore thought to be of interest to investigate the crystal structure of the two polymorphic forms. Besides, the dipicrylamine molecule presents in itself interesting stereochemical problems.

We have studied a number of dipicrylaminates by X-ray methods in order to find a compound suitable for complete structure determination. Single crystals of the substances were prepared by dissolving the calcium salt in water and precipitating the alkaline salts by adding the corresponding cations. The red form of the potassium salt was obtained by evaporating an aqueous solution of the salt at room temperature, the violet form by evaporation at 70° C [2]. X-ray oscillation and Weissenberg diagrams were taken, using CuKα and FeKα radiation (wave lengths 1.542 Å and 1.937 Å resp.), and the densities measured by the flotation method. The results are believed to be correct to about 1 %.

In the following the results of the measurements are given, the dipicrylamine anion being called *D* for short.

HD (dipicrylamine). The yellow, well-developed crystals have the shape of short prisms elongated parallel to *a*. Forms $\{010\}$, $\{210\}$, and $\{011\}$ were observed. The crystals are ortorhombic, with cell dimensions $a = 18.94$ Å, $b = 11.72$ Å, and $c = 7.37$ Å. Reflexions $h00$, $0k0$ and $00l$ are absent for h, k and l odd respectively and as no other systematic absences occur, the space group is $P2_12_12_1$. The density was found to be 1.77 g/cm [3] and there are four (calc. 3.97) molecules in the unit cell.

After this investigation was completed, McCrone [3] has published crystal data on HD. His findings are in agreement with the measurements reported above ($a = 11.75$Å, $b = 19.10$ Å, $c = 7.43$ Å; density 1.750 g/cm [3]; space group not given).

NaD. Red orthorhombic plates $\{001\}$, with $a = 17.61$ Å, $b = 11.77$ Å, and $c = 7.86$ Å. Density 1.89 g/cm^3 four (calc. 4.02) mols. in the unit cell. Space group $P2_12_12_1$.

KD, red form. Thick, rhombic plates, bounded by $\{11l\}$ and $\{001\}$, the latter form predominant. The *b* axis bisects the oblique angle. The crystals are monoclinic, with $a = 15.77$ Å, $b = 13.00$ Å, $c = 11.02$ Å, and $\beta = 130°$. Density 1.84

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KD, violet form. Plates $\{100\}$, elongated along the c axis. Monoclinic, with a = 15.12 Å, b = 13.70 Å, c = 9.15 Å, and β = 115°. Density 1.84 g/cm^3; four (calc. 4.00) mols. in the unit cell. Systematic absences and space group as for the red form, $P2_1/a$.

RbD. The crystals have the same shape as those of the red form of KD. They are monoclinic, with a = 15.97 Å, b = 13.22 Å, c = 11.02 Å, and β = 130°. Density 1.96 g/cm^3; four (calc. 4.03) mols. in the unit cell. The space group is $P2_1/a$. The $00l$ reflexions are very weak for odd values of l.

CsD. Monoclinic plates $\{001\}$, with a = 13.90 Å, b = 10.64 Å, c = 11.54 Å and β = 92°. Density 2.25 g/cm^3; four (calc. 4.05) mols. in the unit cell. The hkl reflexions are absent for $h + k$ odd, and the $h0l$ reflexions for l odd. Possible space groups are $C2/c$ and Cc, the latter being the most likely one, as the molecule probably is asymmetrical.

Discussion of results. It will be seen that the red and violet forms of KD have the same space groups and fairly similar unit cell dimensions. The development of crystal faces is, however, quite different in the two cases, as $\{001\}$ is predominant in the red form and $\{100\}$ in the violet form. This may possibly be connected with the difference in reactivity of the two forms towards nitric acid. Red KD is isomorphous with RbD. An attempt was made to solve the structure of the latter by Patterson and Fourier projections along the b axis. The vector map indicated x = 0.10 and z = 0.24 as possible co-ordinates for the Rb atom, but we did not succeed in interpreting the corresponding Fourier map.

Grison [4] has determined the structure of three polymorphic forms of *N*-picryl-*p*-iodoaniline, a compound which differs from HD in having no nitro groups in one of the benzene rings. Grison finds that the angle C-N-C at the amino-nitrogen is 138° and that the two benzene rings are rotated relative to one another through 65°. It appears likely that the corresponding angles in the dipicrylamine molecule are of the same order of magnitude, as it can be shown by means of a model that the introduction of nitro groups also in the other benzene ring may be compensated by suitable rotation of the ortho nitro groups.

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Polymorphism and Thermal Instability of 1$\varkappa$, 2$\varkappa$, 4$\varkappa$, 5$\varkappa$-Tetrabromocyclohexane

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Recently 1$\varkappa$, 2$\varkappa$, 4$\varkappa$, 5$\varkappa$-tetrachlorocyclohexane (m.p. 226° C) and 1$\varkappa$, 2$\varkappa$-dichloro-4$\varkappa$,5$\varkappa$-dibromocyclohexane (m.p. 240° C) have been prepared [1]. It was shown that both substances are dimorphous, corresponding modifications being isomorphous. The transition temperatures are 60—65° C and 90—95° C respectively. The tetrachloro-compound is stable above the melting point, but the dichloro-dibromo-compound, when heated above the melting point is transformed — at least to a large extent — into the stereoisomer of m.p. 171°

g/cm^3; four (calc. 4.03) mols. in the unit cell. The only systematic absences occur in the $h0l$ reflexions for h odd and in the $0k0$ reflexions for k odd, and the space group is therefore $P2_1/a$.

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Recently 1$\varkappa$, 2$\varkappa$, 4$\varkappa$, 5$\varkappa$-tetrachlorocyclohexane (m.p. 226° C) and 1$\varkappa$, 2$\varkappa$-dichloro-4$\varkappa$,5$\varkappa$-dibromocyclohexane (m.p. 240° C) have been prepared [1]. It was shown that both substances are dimorphous, corresponding modifications being isomorphous. The transition temperatures are 60—65° C and 90—95° C respectively. The tetrachloro-compound is stable above the melting point, but the dichloro-dibromo-compound, when heated above the melting point is transformed — at least to a large extent — into the stereoisomer of m.p. 171°

which has been shown to be the 1ε, 2ϑ-dichloro-4κ, 5κ-dibromo-compound. The melting point of the "four kappa" dichloro-dibromo-compound found on rapid heating is 240° C, but heating slowly considerably lower values may be obtained.

The crystals of the "four kappa" tetrabromo-compound described by Haak and Wibaut [2] are isomorphous with the high temperature (β) forms of the corresponding tetrachloro- and dichloro-dibromo-compounds. It would therefore seem possible that this substance is also dimorphous, the crystals obtained by Haak thus being the high temperature (β) modification. The fact that Haak and Wibaut reports a melting point of 218° C, a figure lower than those found for the two isomorphous crystals containing chlorine, indicates a thermo-instability similar or even more pronounced than that of the dichloro-dibromo-compound.

Professor Wibaut kindly placed some crystals of the tetrabromo-compound at our disposal. The melting point of these crystals turned out to depend markedly on the rate of heating. On rapid heating (using a heating table) a melting point of 255° C was observed, however. If the melt is allowed to solidify and heated again the melting point is 180° C. This finding would seem to indicate that a nearly hundred per cent transformation into the ε,ε,κ,κ-isomer (m.p. 187° C) has taken place. Actually on addition of a nearly equal amount of the 187° isomer the m.p. is *not* lowered, but raised to 183° C.

In order to obtain the low temperature (α) modification of the "four kappa" tetrabromocompound, crystals of the β modification were dissolved in ethyl acetate Some drops of this solution were isolated, seeded with a very small crystal of the α modification of the "four kappa" dichloro-dibromo-compound and the solvent evaporated at room temperature. One of the crystals thus obtained was used as seeding crystal for the main part of the solution. The crystals obtained at room temperature turned out to be isomorphous with the α modifications of the tetrachloro- and dichloro-dibromo-cyclohexanes (comp. Table 1). The crystals heated above 125° C undergo a transition into the β modification. It is difficult, however, to determine the transition point with accuracy (compare what has been said about the other α—β transitions [1].

When solutions of halogens are added to the 1,4-diene the yield of the higher melting ("four kappa") compound seems to be very poor in the case of bromine, but very high in the case of chlorine. Starting with the 1,2-dichlorocyclohexene (4) the yield of the corresponding dichloro-dibromocyclohexane seems to be intermediate between those found in the two other cases. It appears rather surprising that the "four kappa" compounds containing bromine are so easily transformed into the ε, ε, κ, κ-compounds as one would expect the energy of the *free* molecule to be smaller for the "four kappa" compounds. The mechanism of transformation would indeed

Table 1. Crystallographic data, melting points and approximate transition points of 1κ, 2κ, 4κ, 5κ-tetra-halogeno-cyclohexanes.

	a	*b*	*c*	β	M.p.	Transition point
α $C_6H_8Cl_4$	6.13	6.74	10.72	98°	226° C	60— 65° C
α $C_6H_8Cl_2Br_2$	6.29	6.93	10.81	98°	> 240° C	90— 95° C
α $C_6H_8Br_4$	6.40	7.03	10.80	98°	> 255° C	120—125° C
β $C_6H_8Cl_4$	6.40	11.41	6.20	110°	226° C	60— 65° C
β $C_6H_8Cl_2Br_2$	6.79	11.75	6.20	110°	> 240° C	90— 95° C
β $C_6H_8Br_4$	7.23	12.32	6.32	110.5°	> 255° C	120—125° C

be worth while studying. In the case of the dichloro-dibromocompound, however, we have observed the evolution of hydrogen bromide both when melting the solid substance and when heating the substance in carbon tetrachloride solution to 200° C.

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Oxidation of Thiol Groups by Catalase

ENZO BOERI* and ROGER K. BONNICHSEN

Biochemical Department Medical Nobel Institute, Stockholm, Sweden

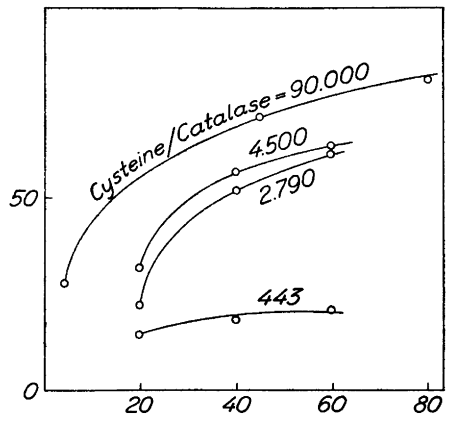


Fig. 1. Guinea-pig liver catalase. 20° C. Phosphate buffer M/100. Ordonates: percent decrease of the rate constant for the hydrogen peroxide decomposition as determined according to Bonnichsen, Chance and Theorell [6]. Abscissae: time in minutes of the incubation of the catalase solution with cysteine.

It is known that cytochrome-oxidase preparations in the presence of cytochrome-c and oxygen oxidize cysteine (Keilin [1]). We have found that the same reaction is effected by blood and liver catalase: oxygen is required, but neither cytochrome-c nor hydrogen peroxide.

The disappearance of the thiol groups was followed by titration of the thiol groups with the amperometric method of Kolthoff and Stricks [2] using a rotated platinum electrode, and the end product was identified by titrating the disulfide groups according to the same authors, after reduction with Na amalgam.

The rate of the catalytic action of catalase on cysteine oxidation is comparable to that of the system oxidase plus cytochrome-c: we obtained on a 10^{-3} *M* cysteine solution at pH 7 the same effect with 4.7×10^{-9} moles of catalase per ml as with a cytochrome-oxidase preparation whose Q_{02} was 212 per mg dry weight per hour (using 0.02 *M* succinate as substrate) with the addition of 1.25×10^{-8} moles of cytochrome-c per ml.

The catalytic effect of catalase on the oxidation of cysteine (and similarly on the oxidation of reduced glutathione and thioglycolic acid) is much higher than the hemin catalysis shown by Krebs [3]. No

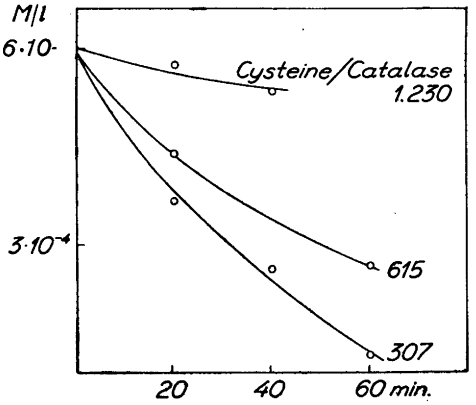


Fig. 2. Horse blood catalase. 20° C. Phosphate buffer M/100. Ordonates: cysteine concentration per liter: Abscissae: time in minutes.

* Rockefeller Fellow.

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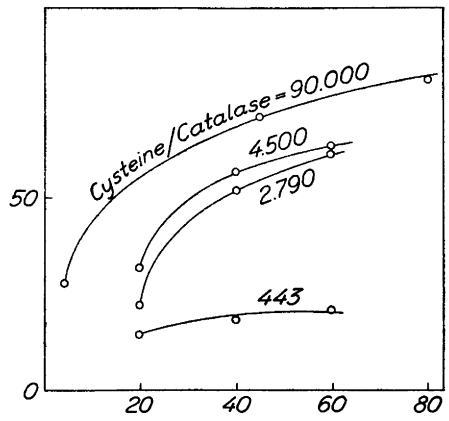


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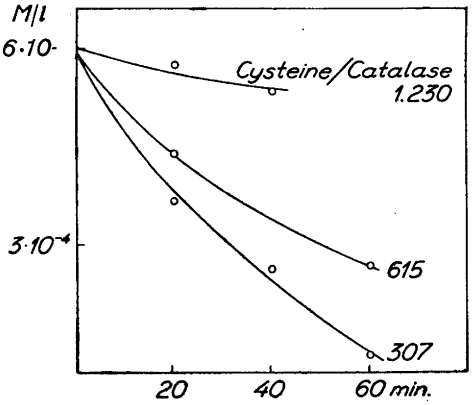


Fig. 2. Horse blood catalase. 20° C. Phosphate buffer M/100. Ordonates: cysteine concentration per liter: Abscissae: time in minutes.

* Rockefeller Fellow.

oxidation takes place if the catalase solution is boiled or if KCN is added in a concentration 1×10^{-3} *M*.

The rate of oxidation of cysteine in the presence of catalase is greatly influenced by the relative concentration of cysteine and catalase. At low ratios cysteine/catalase the rate of oxidation is high (see curves 2 and 3 in Fig. 1), about 9 moles of cysteine oxidized per mole of catalase per minute in the initial periode. At higher ratios cysteine/catalase, both the rate of oxidation of cysteine and the catalatic activity are inhibited. The former effect is seen in curve 1 of Fig. 1, where the rate reduced to 3 moles of cysteine oxidized per mole of catalase per minute. The latter effect has been known since Stern [4] and Waldschmidt-Leitz and collaborators [5], and appears clearly from Fig. 2.

A detailed report will shortly be published in this periodical, together with data on the spectroscopic changes of catalase in the presence of thiol groups.

1. Keilin D. *Proc. Roy. Soc.* **B 106** (1930) 418.
2. Kolthoff, I. M., and Stricks, W. *J. Am. Chem. Soc.* **72** (1950) 1953.
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Preparation of Cytochrome c with the Aid of Paper Electrophoresis *

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The electrophoresis of proteins on filter paper was introduced by Durrum [1], Cremer and Tiselius [2], Turba and Enenkel [3] and Wieland [4]. We have found this method to be very useful for the purification of cytochrome c.

An apparatus described by Köiw, Wallenius and Grönwall [5] was used. The paper, "Munktell 20 350 g", was supplied from Grycksbo Paper Mills, Sweden. The size of the paper strips was 29 × 9 cm. 0.05 molar glycine-NaOH was used for pH 9.87 and 0.05 molar phosphate for pH 7.0. The voltage was 50—150 volts giving a current between 2.5 and 4 mA. The experiments were performed at room temperature. About 100 mg lyophilized cytochrome c in 1.0 ml of the buffer was applied across the middle of the buffer-wet paper by the aid of a Linderström-Lang pipette.

Sometimes a certain spreading of the preparation occurred when pressing the glass-plates together; however, it disappeared some hours after the electrophoresis had been started. The experiments lasted between 20 and 25 hours. After the experiment was finished the cytochrome fractions were extracted from the paper with 0.1 % ammonia.

Preparations of cytochrome c from beef and chicken hearts were made according to Keilin and Hartree [6]. The iron content of these starting materials was 0.296 % and 0.264 % respectively.

In the glycine buffer of pH 9.87 the cytochrome moved towards the cathode as was to be expected from the results obtained by free moving boundary electrophoresis. A brown impurity appeared from beef heart cytochrome, a yellow one from chicken heart cytochrome, both moving towards the anode. After about 20 hours the cytochrome was fully separated from the coloured impurities and was cut apart and extracted with ammonia. At pH 9.87 it was observed after some few hours that the cytochrome fraction moving towards the cathode had a tendency of dividing into a faster moving fraction of red-violet colour and a slower moving fraction of red-brown

* Read before the XIII section at the 2nd international congress of biochemistry in Paris, July 1952.

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colour, which were identified as ferro- and ferricytochrome. Iron analyses were made on the combined cytochrome fractions. The highest value obtained for beef heart cytochrome was 0.45 %, for chicken heart cytochrome 0.40 %. At pH 7.0, practically no ferrocytochrome c appeared. When mixing equal parts of ferro- and ferricytochrome obtained from the paper electrophoresis at pH 9.87 and subjecting the mixture to paper electrophoresis at pH 7.0, ferri- and ferrocytochrome separated again but now the former moved faster than the latter towards the cathode. Furthermore the ferricytochrome fraction from the electrophoresis at pH 9.87 was dissolved in glycine buffer of pH 2.7 and oxygen bubbled through for half an hour in order to ascertain complete oxidation. After lyophilization part of the preparation was dissolved in a buffer of pH 7.0 and part in a buffer of pH 9.8. At pH 7.0 the spot seemed to move uniformly as ferricytochrome, at pH 9.8 it separated again as ferri- and ferrocytochrome. This tendency to spontaneous reduction of cytochrome c at pH 9 — 10 has often been observed in this institute. It is interesting to notice that it occurs even with these highly purified preparations.

The separation of ferro- and ferricytochrome c in electrophoresis was to be expected from the titration experiments of Theorell and Åkeson [7], who found that the oxidized form has one basic equivalent per molecule more than the reduced form around pH 7, whereas the reduced form is more basic at pH-values above pH 9.6. The tendency of cytochrome c to separate into a reduced and an oxidized fraction between pH 9 and 10 was also observed when purifying cytochrome c on ion exchange resin [8]. The observed spontaneous reduction is not caused by impurities in the paper since the same happens in a test tube.

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On the Complexity of Cadmium and Silver Sulphate

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A. SOME CHARACTERISTICS OF AQUEOUS SOLUTIONS OF 2–2-SULPHATES

In some recent papers aqueous solutions of sulphates of divalent metals have been studied. *E.g.* Fronaeus[1,2] and Näsänen[3] studied copper sulphate and Leden[4] cadmium sulphate. If the results of the measurements should be accounted for by assuming formation of complexes in the solutions, it turned up that it was necessary to presume polynuclear complexes to be formed. The complex systems thus appeared so intricate that they could not be completely unravelled. On the other hand it seems rather astonishing that the sulphate systems should be so complicated, as measurements on solutions of different 2–2-sulphates seem to indicate that the individual differences between them are rather small, and thus it seems natural to suppose that the conditions should be rather simple in all the solutions. Some examples of this remarkable uniformity are pointed out in the following.

The depression of the freezing points at increasing concentration of 2–2-sulphates is described by very similar functions for all these sulphates, as can be seen from the tables of Landolt-Börnstein, from which some values are interpolated in Table 1. Even at higher concentrations than those of the table the curves coincide remarkably well.

Table 1. Depression of the freezing point in some solutions of 2-2-sulphates.

Conc. *M*	Molal depression of the freezing point degree M^{-1}						
	$CdSO_4$	$CoSO_4$	$CuSO_4$	$FeSO_4$	$MgSO_4$	$NiSO_4$	$ZnSO_4$
0.02	2.57		2.50	2.57	2.62	2.57	2.58
0.1	2.22	2.19	2.13	2.22	2.26		2.28
0.2	2.03		1.93	2.01	2.07		2.08

Table 2. Osmotic coefficients according to Plake[5] in 0.3 M solutions of some 2-2-sulphates

	$CdSO_4$	$CuSO_4$	$MgSO_4$	$MnSO_4$	$NiSO_4$	$ZnSO_4$
OC_f:	0.53	0.48	0.54	0.55	—	0.54
OC_b:	0.41	0.44	0.47	0.42	0.43	0.44

Similarly Plake [5] has made a comparison of osmotic coefficients (OC), obtained from measurements of the elevation of the boiling point (OC_b) and the depression of the freezing point (OC_f), from which it is seen that the differences between solutions of varying 2-2-sulphates are surprisingly small. (See Table 2.)

Finally Güntelberg [6] has drawn the mean activity coefficients of $CdSO_4$, $MgSO_4$, $NiSO_4$, and $ZnSO_4$ as functions of the concentration and has obtained adjacent curves with much smaller individual variations than for solutions of representative 1-1-electrolytes. Also Hass and Jellineck [7] point out that the mean activity of $CdSO_4$ does not detach from sulphates such as $CaSO_4$ or $MgSO_4$ unlike other cadmium salts, *e.g.* the chloride, whose mean activity decreases much faster than the chlorides of calcium or magnesium at increasing concentrations.

In the following an attempt has been made to investigate the cadmium-sulphate system by determining the concentrations of free sulphate ions in it. This has been done by a new method, described in part C of the present paper. By using this method the complex formation between silver and sulphate ions must be known in the same ionic environment (3 C $NaClO_4$).

B. POTENTIOMETRIC MEASUREMENTS WITH SILVER ELECTRODES IN SOLUTIONS CONTAINING SILVER AND SULPHATE IONS IN A MEDIUM WITH A HIGH IONIC STRENGTH

The measurements were performed according to methods previously described [8,9], *i.e.* as potentiometric titrations with a silver-silver chloride electrode according to Brown [10]. The temperature of the thermostate was 25.16° C. The potentiometer was from N. C. Jensen, Copenhagen, type 10 A and the "multiflex" galvanometer type MG 2 from H. Tillquist, Stockholm, with a sensitivity of $2 \cdot 10^{-8}$ A/mm.

Stock solutions of silver and sodium perchlorate were prepared as previously described [9]. No foreign ions could be proved in the used sodium sulphate, *p.a.* The sulphate concentrations of the stock solutions were determined by precipitating with barium chloride.

As a reference electrode mercury in 3 C sodium perchlorate saturated with mercury (I) sulphate was used. The emfs measured were practically always reproducible within 0.1 mV.

Sodium perchlorate has been added to obtain as constant activity conditions as possible. It is doubtful, however, whether the solutions should be made so, that the ionic strength

$$I = \tfrac{1}{2}\sum c_i z_i^2 \qquad (1)$$

becomes constant, or if it is better to choose the ionic environment so, as to maintain the sodium ion concentration at a constant value. Therefore both the kinds of measure series have been performed. By comparing the two kinds of series it is also possible to find out how a change of the ionic strength affects the potential of the silver electrode (see below). By this comparison it must be kept in mind that the liquid junction potentials are different in the two cases. If this potential is calculated according to Henderson as is

previously described [9], it is found to be insignificant in the series with a constant ionic strength = 3 C. On the other hand a correction has been introduced for this potential in the series, where the sodium ion concentration is constant = 3 C (Table 4), where the emf E is given as the sum of two terms, the first of which being the emf directly measured and the second one thus being the correction for the diffusion potential. The notations in the tables and in the equations are the same as have previously [8,9] been used (see also (5), (6), and (7) in the present paper).

As long as $[SO_4^{2-}] < 0.6$ C all the values of $F_1(SO_4^{2-})$, obtained in Table 3, from the equation

$$F_1(SO_4^{2-}) = \frac{c_{Ag} - [Ag^+]}{[Ag][SO_4^{2-}]} \tag{2}$$

satisfy the equation of the straight line

$$F_1(SO_4^{2-}) = 1.7 + 1.9\ [SO_4^{2-}] \tag{3}$$

which means that the potentiometric measurements may be accounted for by assuming the complexes $AgSO_4^-$ and $Ag(SO_4)_2^{3-}$ to have been built up in the solutions. Their complexity constants are $\beta_1 = (1.7 \pm 0.05)\ C^{-1}$ and $\beta_2 = (1.9 \pm 0.1)\ C^{-2}$. No polynuclear complexes exist in the solutions, as $F_1(SO_4^{2-})$ is independet of c_{Ag}. For the theory, *vide ref.* 8.

The complex formation function or ligand number [4] $\bar{n}$ of the silver sulphate system is

$$\bar{n} = \frac{1.7[SO_4^{2-}] + 3.8[SO_4^{2-}]^2}{1 + 1.7[SO_2^{2-}] + 1.9[SO_4^{2-}]^2} \tag{4}$$

valid at a constant ionic strength of 3 C ($NaClO_4$). The function $\bar{n}/[SO_4^{2-}]$ will be used later on by the following study of the cadmium sulphate-system.

In Table 4 the corresponding measurements and calculations at a constant sodium ion concentration of 3 C are collected. $F_1(SO_4^{2-})$ is still represented by a straight line, from which the complexity constants $\beta_1 = (1.7 \pm 0.05)\ C^{-1}$ and $\beta_2 = (1.0 \pm 0.1)\ C^{-2}$ result.

In the solutions in Table 4 the ionic strength I, calculated from (1), increases for increasing sulphate concentrations, and thus can be conveniently written as $I = (3 + \Delta I)$ C. ΔI is given in mC in Table 4. By comparing the emfs E of the Tables 3 and 4 it is possible to calculate how much a quantitative change of the ionic strength, other circumstances unaltered, influences the potential of the silver electrode. In these tables E is a measure of the decrease of this potential, as the potential of the silver electrode decreases at increasing sulphate concentrations. The difference ΔE between the silver potentials at $I = 3$ C and $[Na^+] = 3$ C at the same sulphate concentrations is to be found

Table 3. Titrations with 1 C sodium sulphate on solutions of silver perchlorate containing sodium perchlorate to an ionic strength of 3 C.

c_{Ag} mC	c_{SO_4} mC	E mV	$[Ag^+]$ mC	$[SO_4^{2-}]$ mC */	$F_1(SO_4^{2-})$ C^{-1} (2)
3.33	0	0	3.33	0	
3.25	24.4	1.7	3.12	24.3	1.71
3.17	47.6	3.4	2.90	47.3	1.97
3.10	69.8	5.0	2.74	69.4	1.90
3.03	90.9	6.5	2.59	90.4	1.88
2.90	130.5	9.4	2.31	130	1.97
2.78	167	12.1	2.08	166	2.03
2.67	200	14.6	1.89	199	2.08
2.47	259	19.1	1.59	258	2.15
2.22	333	24.9	1.266	332	2.28
1.905	428	32.8	0.930	427	2.46
1.667	500	39.2	0.726	499	2.60
1.333	600	48.8	0.500	599	2.78
1.111	667	56.2	0.375	666	(2.95)
0.832	750	66.9	0.247	749	(3.16)
10.00	0	0	10	0	
9.76	24.4	1.7	9.36	24	1.56
9.52	47.6	3.4	8.69	46.8	2.04
9.30	69.8	5.1	8.20	68.7	1.95
9.09	90.9	6.6	7.74	89.5	1.95
8.69	130.5	9.4	6.94	129	1.96
8.33	167	12.1	6.25	165	2.02
8.00	200	14.6	5.67	198	2.08
7.41	259	19.1	4.76	256	2.18
6.67	333	24.8	3.81	330	2.27
5.71	428	32.7	2.81	425	2.43
5.00	500	39.1	2.19	497	2.58
4.44	556	44.3	1.79	553	2.68
4.00	600	48.9	1.49	597	2.82
33.3	0	0	33.3	0	
32.5	24.4	1.5	31.4	23.3	1.50
31.7	47.6	3.1	29.5	45.4	1.64
31.0	69.8	4.6	27.7	66.5	1.79
30.3	90.9	6.3	26.1	86.7	1.86
29.0	130.5	9.1	23.4	125	1.91
27.8	167	11.7	21.1	160	1.99
26.7	200	14.1	19.3	193	1.99
25.6	231	16.5	17.5	223	2.08

Table 3 contin.

c_{Ag} mC	c_{SO_4} mC	E mV	$[Ag^+]$ mC	$[SO_4^{2-}]$ mC */	$F_1(SO_4^{2-})$ C^{-1} (2)
24.7	259	18.7	16.1	250	2.14
22.2	333	24.7	12.7	323	2.32
19.05	428	32.5	9.40	418	2.46
16.7	500	38.9	7.33	490	2.73
14.8	556	44.3	5.96	547	2.70
13.3	600	48.9	4.98	592	2.82
100.0	0	0	100	0	
97.6	24.4	1.4	94.7	21.5	1.43
95.2	47.6	3.0	89.0	41.4	1.69
93.0	69.8	4.7	83.3	60.1	1.94
90.9	90.9	6.2	78.6	78.6	1.99

* $[SO_4^{2-}]$ has been computed from the equation $[SO_4^{2-}] = c_{SO_4} - \nu(c_{Ag} - [Ag^+])$, where $\nu = \dfrac{\beta_1 + 2\beta_2[SO_4^{2-}]}{\beta_1 + \beta_2[SO_4^{2-}]}$, *i.e.* $1 < \nu < 2$. *Cf.* 4, p. 16, and 8, p. 1321.

Table 4. Titrations with a solution containing 1 C sodium sulphate and 1 C sodium perchlorate on a solution containing 10 mC silver perchlorate and 2.99 C sodium perchlorate. Ionic strength 3 C + $\triangle I$ C.

c_{Ag} mC	c_{SO_4} mC	E mV	$[Ag^+]$ mC	$[SO_4^{2-}]$ mC */	$F_1(SO_4^{2-})$ C^{-1} (2)	ΔE mV	ΔI mC	$\frac{\Delta E}{\Delta I}$ mV/C
10.00	0	0	10.00	0			0	
9.76	24.4	1.6 + 0.0	9.40	24.0	1.60	0.1	20	(5.0)
9.52	47.6	3.2 + 0.1	8.79	46.9	1.77	0.1	50	(2.0)
9.30	69.8	4.7 + 0.1	8.30	68.8	1.75	0.3	70	4.3
9.09	90.9	6.1 + 0.2	7.83	89.6	1.80	0.3	90	3.3
8.69	130.5	8.7 + 0.2	7.07	129	1.78	0.5	130	3.8
8.33	167	11.2 + 0.3	6.39	165	1.96	0.6	170	3.5
8.00	200	13.5 + 0.3	5.85	198	1.86	0.8	200	4.0
7.41	259	17.6 + 0.4	4.96	256	1.93	1.1	260	4.2
6.67	333	22.9 + 0.6	4.01	330	2.01	1.3	330	3.9
5.71	428	30.1 + 0.7	3.02	425	2.10	1.9	430	4.4
5.00	500	35.8 + 0.8	2.43	497	2.21	2.5	500	5.0
4.00	600	44.7 + 0.9	1.70	597	2.26	3.3	600	(5.5)
							Mean:	4

* *Cf.* note under Table 3.

in Table 4. This difference seems to be about proportional to the increase ΔI of the ionic strength, as is seen from the rather constant values of $\frac{\Delta E}{\Delta I}$ in the last column of Table 4. As a mean of the increasing potential of the silver electrode at increasing ionic strength the value 4 mV/C is obtained. This value is to be used for some measurements with silver electrodes in the cadmium sulphate system, where the ionic strength could not be maintained quite constant.

C. THE USE OF A KNOWN COMPLEX STEP SYSTEM TO DETERMINE THE CONCENTRATION OF THE FREE LIGAND

If a metal ion M can form mononuclear complexes with a ligand A, it is valid that

$$c_M = [\mathrm{M}] + [\mathrm{MA}] + [\mathrm{MA}_2] + - - [\mathrm{MA}_N] = [\mathrm{M}] + \beta_1[\mathrm{M}][\mathrm{A}] + \\ + \beta_2[\mathrm{M}][\mathrm{A}]^2 + - - + \beta_N[\mathrm{M}][\mathrm{A}]^N \qquad 5)$$

and thus [Cf. 8]

$$\frac{c_M}{[\mathrm{M}]} = \mathrm{F(A)} = 1 + \beta_1[\mathrm{A}] + \beta_2[\mathrm{A}]^2 + - - + \beta_N[\mathrm{A}]^N \qquad (6)$$

where F(A) is a known function of [A] in a system with known complexity constants, *e.g.* the silver-sulphate system. In a solution, containing M and A, in which c_M is known but not [A], the latter concentration can be computed by measuring [M], *e.g.* by potentiometric methods. For this purpose it is only necessary to draw F(A) in a diagram. From the measured [M] the ordinate $\frac{c_M}{[\mathrm{M}]} = \mathrm{F(A)}$ is calculated and the corresponding abscissa [A] is then directly read in the diagram, drawn beforehand. If [M] has been determined with the aid of potentiometric measurements in a series of solutions with constant c_M, it may be simpler to draw E instead of F(A) in the diagram, as E, too, is a function of [A] only,

$$E = \frac{RT}{\mathrm{m}F} \ln \frac{c_M}{[\mathrm{M}]} \qquad (7)$$

and thus can be used in the same manner as F(A) to obtain [A] from the diagram.

As for the measurements with silver electrodes in solutions containing cadmium and a small amount of silver, it was only necessary to calculate how much sulphate that has been bound by the cadmium ions. In principle the measurements have thus been carried out in such a way that the potential of a silver electrode has been measured first in a series of solutions with known

sulphate concentrations, as is already described in Table 3, and then in the same solutions containing an amount of cadmium ions, constant in the whole series. Self-evidently both the series of solutions also contain small amounts of silver ions. At increasing sulphate ion concentrations the electrode potential decreases, in the first series, without cadmium, by E_0 and in the second one by E. The difference between E_0 and E is due to the cadmium ions having bound sulphate ions, so that the silver electrode becomes a little more noble in the solutions containing cadmium ($E_0 > E$). The decrease of the concentration of free sulphate ions according to the formation of cadmium sulphate complexes is called $\Delta[SO_4^{2-}]$. Thus $F(SO_4^{2-})$, *e.g.* $c_{Ag}/[Ag^+]$, is changed correspondingly, causing a change in the silver electrode potential. The measured potential change is corrected, to ΔE_{corr}, for the decrease of ionic strength, that is due to the complex formation between cadmium and silver ions. Thus it is valid

$$E_0 - E + corr = \Delta E_{corr} = \frac{RT}{F} \ln \frac{[Ag^+]}{[Ag^+]_0} \tag{8}$$

The index $_0$ denotes quantities in solutions free from cadmium, whereas quantities not indicated are used for the solutions containing cadmium. As the solutions are made so, that $c_{Ag_0} = c_{Ag}$, we have

$$\Delta E_{corr} = \frac{RT}{F} \ln \left(\frac{c_{Ag_0}}{[Ag^+]_0} : \frac{c_{Ag}}{[Ag^+]} \right) = \frac{RT}{F} \ln [F_0(SO_4^{2-}) : F(SO_4^{2-})]$$

$$\therefore \Delta E_{corr} = \frac{RT}{F} \Delta \ln F(SO_4^{2-}) \tag{9}$$

If ΔE_{corr} is sufficiently small, (9) can be written with good approximation

$$\Delta E_{corr} \simeq \frac{RT}{F} \cdot \frac{\Delta F(SO_4^{2-})}{F(SO_4^{2-})} \tag{10}$$

$$\text{But } \bar{n} = \frac{\sum_{n=1}^{N} n\beta_n [SO_4^{2-}]}{F(SO_4^{2-})} = \frac{[SO_4^{2-}] \cdot \frac{d[F(SO_4^{2-})]}{d[SO_4^{2-}]}}{F(SO_4^{2-})} \tag{11}$$

Thus (10) may be approximated to

$$\Delta E_{corr} \simeq \frac{RT}{F} \cdot \frac{\bar{n}}{[SO_4^{2-}]} \cdot \Delta [SO_4^{2-}] \tag{12}$$

$$\therefore \Delta [SO_4^{2-}] \simeq \frac{[SO_4^{2-}]}{\bar{n}} \cdot \frac{\Delta E_{corr}}{25.7} \tag{13}$$

where the numerical factor is valid at the temperature of the experiments, if ΔE_{corr} is inserted in mV. The practically usable range of (13), which is a variant of the well-known generalization of Bodländer's expression *, depends on the variation of the function $\frac{[SO_4^{2-}]}{\bar{n}}$. In our case this function passes a flat minimum at the actual range of concentration, so that (13) becomes sufficiently accurate. Random errors by measuring ΔE_{corr} will everywhere be predominant to the errors that are due to the approximations at deducing (13). $\Delta[SO_4^{2-}]$ has thus been computed by drawing the function

$$\frac{[SO_4^{2-}]}{\bar{n}} = \frac{1 + 1.7[SO_4^{2-}] + 1.9[SO_4^{2-}]^2}{1.7 + 3.8[SO_4^{2-}]} \qquad (14)$$

in a large scale diagram, where the value of $[SO_4^{2-}]/\bar{n}$ is read for the known value of $[SO_4^{2-}]_0$, in the solution free from cadmium. This value together with ΔE_{corr} is inserted in (13) and $\Delta[SO_4^{2-}]$ is obtained, *i.e.* the sulphate amount that has been bound as ligands by the cadmium ions present in the corresponding solution containing a known concentration of cadmium.

D. POTENTIOMETRIC MEASUREMENTS WITH CADMIUM AMALGAM

As for the methods of measuring with cadmium amalgam electrodes, *vide* [4]. Four series were performed with constant cadmium concentrations (3.33, 10, 33.3 and 100 mC) and sulphate concentrations ranging from 10 to 500 mC. Sodium perchlorate was added to a constant ionic strength = 3 C, disregarding the decrease of the ionic strength according to the complex formation. The results are summarized in Table 5, where analogously to the measurements with silver electrodes (Tables 3 and 4) E means the difference between the potential of the cadmium amalgam electrode in the solutions free from sulphate to be found at the top of Table 5 and the same potential in solutions containing sulphate. As is seen from the table, E is only slightly changed at the various values of c_{Cd}. If only mononuclear complexes should exist in the system, E would be a function of the free sulphate ion concentrations only, and as a considerable part of the sulphate is consumed in the complexes at the higher concentrations of cadmium, so that solutions with the same t o t a l concentration c_{SO_4} obtain very different concentrations, $[SO_4^{2-}]$, of f r e e sulphate ions, this fact should result in much lower values of E for the highest cadmium concentration than for the lowest one at the same c_{SO_4}. Thus the measurements cannot be explained by assuming only mononuclear complexes to exist in the solutions. (*Cf.* 4, p. 75, Fig. 6.)

At sufficiently small concentrations of cadmium the polynuclear complexes disappear and simultaneously $[SO_4^{2-}]$ approaches c_{SO_4}. In the limit case $c_{Cd} = 0$ and $[SO_4^{2-}] = c_{SO_4}$, and owing to the small variation of E at constant c_{SO_4} and changing c_{Cd} the extrapolation

* The generalized Bodländer equation has been used in several forms by Bjerrum [12], Fronaeus [1, p. 15], and Leden [4, p. 13; 11, p. 33; 13].

to this limit is easily done. Such extrapolated values $\lim_{c_{Cd}=0} E = E^0$ are also to be found in Table 5. From these the function $F^0(SO_4^{2-}) = 1 + \beta_1[SO_4^{2-}] + \beta_2[SO_4^{2-}]^2 + \beta_3[SO_4^{2-}]^3$ is calculated according to (15) (denotations according to [8])

$$E^0 = \frac{RT}{2F} \ln \lim_{c_{Cd}=0} \frac{c_{Cd}}{[Cd^{2+}]}) = \frac{RT}{2F} \ln F^0(SO_4^{2-}) \qquad (15)$$

Then the complexity constants can be computed as usual, *e.g.* by drawing

$$F_1^0(SO_3^{2-}) = \frac{F^0[SO_4^{2-}]-1}{[SO_4^{2-}]} \quad \text{and} \quad F_2^0(SO_4^{2-}) = \frac{F_1^0[SO_4^{2-}]-\beta_1}{[SO_4^{2-}]} \qquad (16)\ (17)$$

where $[SO_4^{2-}] = c_{SO_4}$ in this limit case. The result is $\beta_1 = (8 \pm 1)\ C^{-1}$, $\beta_2 = (10 \pm 10)\ C^{-2}$, and $\beta_3 = (110 \pm 10)\ C^{-3}$.

Table 5. The decrease of the potential of cadmium amalgam electrodes at increasing concentrations of sulphate but constant cadmium ion concentrations. Ionic strength = 3 C.

c_{SO_4} mC	c_{Cd} = 100 mC	c_{Cd} = 33.3 mC	c_{Cd} = 10 mC	c_{Cd} = 3.33 mC	$c_{Cd} = 0$		
	E mV	E mV	E mV	E mV	E^0 mV Extrapolation	$F^0(SO_4^{2-})$ (15)	$F_1^0(SO_4^{2-})$ C^{-1} (16)
0	0	0	0	0	0	1	
9.9	0.7	1.0	1.0	0.85	0.9	1.07	7.0
19.6	1.5	1.8	2.15	1.8	2.0	1.17	8.7
29.1	2.3	2.7	3.0	2.6	2.8	1.24	8.3
47.6	3.85	4.4	4.7	4.35	4.5	1.42	8.8
70.0	5.4	6.1	6.7	6.55	6.7	1.69	9.9
90.9	7.05	7.9	8.4	8.2	8.3	1.91	10.0
167	13.05	14.1	14.85	14.55	14.8	3.16	12.9
231	18.15	19.1	20.0	19.6	19.7	4.63	15.7
333	25.85	26.8	27.4	27.45	27.6	8.57	22.7
420	32.1	33	33.6	33.5	33.6	13.7	30.2
500	37.5	38.4	39.25	39.05	39.2	21.1	40.2
600	44.95	45.0	45.85	45.8	45.9	35.6	(57.7)
714	52.3	52.7	53.4	53.2	53.3	63.4	(87.4)

These complexity constants give a $F^0(SO_4^{2-})$-curve in good agreement to the experimental points, if $c_{SO_4} < 0.5$ C. (*Cf.* Fig. 1).

Hence it is possible to account for the potentiometric measurements with cadmium amalgam electrode at sufficiently small cadmium concentrations ($< \sim 5$ mC) by assuming that the complexes $CdSO_4$ and $Cd(SO_4)_3^{4-}$ are formed. If the intermediate complex $Cd(SO_4)_2^{2-}$ exists at all, it has such a narrow range of existence that its complexity constant β_2 cannot possibly be computed, but no doubt $\beta_2 < 20\ C^{-2}$. At higher concentrations of cadmium, > 5 mC, the results of the measurement cannot be accounted for by mononuclear complexes only.

E. DETERMINATION OF THE CONCENTRATIONS OF FREE SULPHATE IONS IN THE CADMIUM-SULPHATE SYSTEM BY THE AID OF THE SILVER-SULPHATE SYSTEM USING SILVER ELECTRODES

The measurements were still performed as potentiometric titrations with a silver-silver chloride electrode. To 20 ml solution with $c_{AgClO_4} = 0.01$ C and $c_{NaClO_4} = 2.99$ 1 C sodium sulphate solution was added from a burette. The result of this series is already accounted for in Table 3. The decrease of the potential of the silver electrode is also given in Table 6, here denoted E_0. Then the same titration was repeated but now with the same cadmium concentration both in the solution in the burette and in the electrode vessel. Three such series were performed with $c_{Cd} = 10$, 33.3 and 100 mC. The decrease of the silver electrode potential is in these cases denoted E in Table 6. In these measure series the cadmium content of the solutions thus was maintained at a constant value during a titration. The silver content of the solution was decreased on account of the dilution with the titration solution, but in all solutions with the same sulphate content, the silver concentrations were equal, too. Thus all the solutions with the same sulphate and silver contents are to be found at the same horizontal line in Table 6. ΔE_{corr} in Table 6 is given as the sum of two numbers, the first of which is $E_0 - E$, and the second one is a correction that must be brought about according to the decrease of the ionic strength following from the complex formation between cadmium and sulphate ions. This correction has been obtained by the aid of the cadmium amalgam potential in the solutions, *i.e.* by interpolation in Table 5. From the figures in column E the cadmium ion concentration $[Cd^{2+}]$ can be computed from Nernst's formula (7). The difference $c_{Cd} - [Cd^{2+}]$ then signifies how much cadmium has been bound in sulphate complexes. This complex formation is firstly described by the reactions:

$$Cd^{2+} + SO_4^{2-} \rightarrow CdSO_4,\quad Cd^{2+} + 2SO_4^{2-} \rightarrow Cd(SO_4)_2^{2-} \text{ and } Cd^{2+} + 3SO_4^{2-} \rightarrow Cd(SO_4)_3^{4-}$$

If the reacting concentration of cadmium is c, the decrease of ionic strength is apparently $4c$ by the first and second reaction and 0 at the third. Assuming a decrease of ionic strength equal to $4c$, we ought to obtain a m a x i m u m value of the correction. In Table 4 it has been calculated that the correction for decrease of ionic strength is 4mV/C, and from this figure and the assumed maximal decrease of ionic strength the second number in the column ΔE_{corr} has been obtained $= 4 \cdot 4c = 16(c_{Cd} - [Cd^{2+}])$ mV (concentrations in C). From the values of ΔE_{corr} the sulphate amount, $\Delta[SO_4^{2-}]$, bound in cadmium complexes has been computed from (13).

Table 6. Determination of $\Delta[SO_4^{2-}]$ and the ligand number $\bar{n}$ of the cadmium-sulphate system by measurements with a silver-silver chloride electrode.

$c_{Cd} = 0$ (*Cf.* Table 3)				$c_{Cd} = 10$ mC				$c_{Cd} = 33.3$ mC					$c_{Cd} = 100$ mC				
c_{Ag} mC	c_{SO_4} mC	$[SO_4^{2-}]_0$ mC	E_0 mV	E mV	ΔE_{corr} mV	c^* mC	$\Delta[SO_4^{2-}]$ mC (13)	E mV	ΔE_{corr} mV	c^* mC	$\Delta[SO_4^{2-}]$ mC (13)	$\bar{n}$ (18)	E mV	ΔE_{corr} mV	c^* mC	$\Delta[SO_4^{2-}]$ mC (13)	$\bar{n}$ (18)
10	0	0	0	0	0							□					+
9.76	24.4	24.0	1.7	1.7	0	1											
9.52	47.6	46.8	3.4	3.3	0.1+0.0	3	2	3.3	0.1+0.1	9	4	0.12	3.1	0.3+0.4	26	16	0.16
9.30	69.8	68.7	5.1	4.9	0.1+0.1	4	4										
9.09	90.9	89.5	6.6	6.5	0.1+0.1	5	4	6.3	0.3+0.2	15	11	0.33	6.0	0.6+0.6	35	26	0.26
8.69	130.5	129	9.4	9.3	0.1+0.1	6	4	9.0	0.4+0.3	19	16	0.48	8.6	0.8+0.7	42	33	0.33
8.33	167	165	12.1	12.0	0.1+0.1	7	4	11.6	0.5+0.3	22	18	0.54	11.1	1.0+1.0	64	45	0.45
8.00	200	198	14.6	14.5	0.1+0.1	7	4	14.3	0.3+0.4	24	16	0.48	13.5	1.1+1.1	70	49	0.49
7.41	259	256	19.1	19.0	0.0+0.2	8	4	18.7	0.4+0.4	27	18	0.54	17.8	1.3+1.3	79	59	0.59
6.67	333	330	24.8	24.8	0.0+0.2	9	5	24.3	0.5+0.5	29	23	0.69	23.4	1.4+1.4	87	65	0.65
5.71	428	425	32.7	32.7	0.0+0.2	9	5	32.0	0.7+0.5	30	29	0.87	31.0	1.7+1.5	92	76	0.76
5.00	500	497	39.1	39.1	0.0+0.2	10	5	38.1	1.0+0.5	31	33	(1.0)	37.2	1.9+1.5	95	83	0.83

* $c = c_{Cd} - [Cd^{2+}]$, where $[Cd^{2+}]$ has been obtained from the values of E in Table 5 from (7).

The result of this calculation shows that $\Delta[SO_4^{2-}]$ in all cases is less than $c_{Cd} - [Cd^{2+}]$, *e.g.* the quotient $\dfrac{\Delta[SO_4^{2-}]}{c_{Cd} - [Cd^{2+}]} = \nu < 1$. If the complexes between cadmium and sulphate ions were $CdSO_4$ and $Cd(SO_4)_3^{4-}$, the quotient ν would have to stay within the range $1 < \nu < 3$, and in the solution at the bottom of Table 6 ν ought to have reached a value of about 2.5. It is true that the relative error of $\Delta[SO_4^{2-}]$ might be rather considerable, particularly according to the uncertain correction for the decrease of ionic strength, but on the other hand it seems rather incredible that the error can be so large that only one third of the correct value should be obtained, *e.g.* an error of 200 %, all the more as the experimental values of ΔE_{corr} and thus of $\Delta[SO_4^{2-}]$ are already corrected maximally u p w a r d s.

Nor can the discrepancy be explained by the formation of polynuclear complexes. A value of $\nu < 1$ means that in such complexes, $Cd_m(SO_4)_n^{2(m-n)}$, m should on an average be greater than n, but that is contradicted by the generalized Bodländer equation used on the potentials of the cadmium amalgam electrodes in Table 5 (*cf.*[4, p. 75]) and the limit function $F^0(SO_4^{2-})$ also calculated in Table 5. A quantitative scrutiny of the results of Tables 5 and 6 gives as

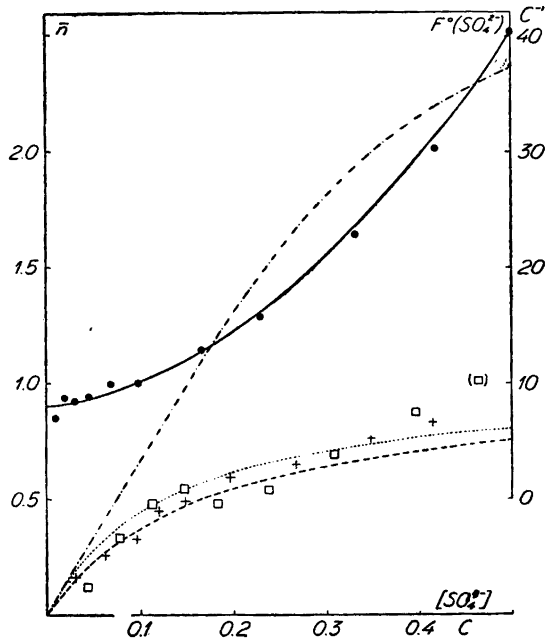


Fig. 1. The functions $\bar{n}$ and $F_1^0(SO_4^{2-})$ of the cadmium-sulphate system.

Solid curve: $F_1^0(SO_4^{2-}) = 8 + 10\,[SO_4^{2-}] + 110\,[SO_4^{2-}]^2$

● $F_1^0(SO_4^{2-}) = \dfrac{F^0(SO_4^{2-}) - 1}{c_{SO_4}}$, where $F^0(SO_4^{2-})$ is calculated from (15). *Cf.* Table 5.

Dashed-dotted curve: $\bar{n} = \dfrac{8[SO_4^{2-}] + 20[SO_4^{2-}]^2 + 330[SO_4^{2-}]^3}{1 + 8[SO_4^{2-}] + 10[SO_4^{2-}]^2 + 110[SO_4^{2-}]^3}$, three complexes provided

Dotted ($\beta_1 = 8$) and dashed ($\beta_1 = 6$) curves: $\bar{n} = \dfrac{\beta_1[SO_4^{2-}]}{1 + \beta_1[SO_4^{2-}]}$, one complex provided.

□ and + : $\bar{n} = \dfrac{\Delta[SO_4^{2-}]}{c_{Cd}}$ for $c_{Cd} = 33.3$ resp. 100 mC. *Cf.* Table 6.

result, that there cannot be found any complex system, which satisfies the measured values of b o t h the tables. Probably this negative result will remain, even though it were possible to correct the potentials in Table 5 for the decrease of ionic strength, a correction that cannot be done, however, as long as the complex system is not unravelled.

From the values of $\Delta[SO_4^{2-}]$ in Table 6 the formation function $\bar{n}$ for the cadmium-sulphate system, defined by (18), can be computed

$$n = \frac{\Delta[SO_4^{2-}]}{c_{Cd}} \tag{18}$$

This calculation has been made for the two highest cadmium concentrations in Table 6. In Fig. 1 the values obtained have been plotted together with the formation curve, which is arrived at by assuming only one complex to be built up in the solutions, *viz.* the uncharged compound $CdSO_4$, with a complexity constant $\beta_1 = 8\ C^{-1}$. As a comparison the complexity curve for $\beta_1 = 6\ C^{-1}$ has also been drawn in Fig. 1. Thus the curves represent the functions

$$\bar{n} = \frac{\beta_1[SO_4^{2-}]}{1 + \beta_1[SO_4^{2-}]} \tag{19}$$

for these two values of β_1. As is seen from Fig. 1 the experimental values are rather well gathered along a curve of this type. For the sake of comparison that $\bar{n}$-curve is also drawn in Fig. 1 that is obtained from the complexity constants, computed from the measurements with cadmium amalgam electrodes in Table 5.

The measurements of sulphate ion concentration with the silver electrode thus give the result that only one complex is formed in the solution, *viz.* the uncharged molecule $CdSO_4$, and that the complexity constant of the same is $\beta_1 = (7 \pm 2)\ C^{-1}$. This value coincides within the errors of experiments with the value of $\beta_1 = (8 \pm 1)\ C^{-1}$, computed from the measurements with cadmium amalgam electrodes.

F. DISCUSSION OF THE RESULTS OF THE MEASUREMENTS

The most reasonable explanation of the divergent results between the measurements with cadmium amalgam and silver electrodes seems to be the following one. By the calculation of the complexity constants from the measurements with cadmium amalgam electrodes, it has been assumed that the ionic environment can be regarded as unchanged when monovalent perchlorate ions are exchanged for divalent sulphate ions, so that the activity factor of the cadmium ion maintains a constant value during a titration. It is, however, rather probable that this consumption does not hold good. In solutions without a neutral salt two ions with opposite electrical charges are supposed to form associated ion pairs, an association starting at very small concentrations in aqueous solutions, if the ions are divalent (*vide* Bjerrum [14] or Güntelberg [6]). The decrease of activity factors at increasing concentrations is thus interpreted as a stabilization of the ion pair. Analogously the increase of the $F_1^0(SO_4^{2-})$-function, which has been formally interpreted as a formation of essentially the complex $Cd(SO_4)_3^{4-}$ in part D of this paper, is perhaps instead a measure of increasing stability of the associated ion pair $Cd^{2+}SO_4^{2-}$, so that the increase of $F_1^0(SO_4^{2-})$ has to be interpreted as an increase of the complexity "constant"

β_1 for this ion pair at increasing sulphate concentrations and not as a proceeding complex formation in a step system. The function $F_1^0(SO_4^{2-})$ is maintained at a constant value, as longs as $c_{SO_4} < \sim 60$ mC and at these small sulphate concentrations it ought thus to be possible to calculate with constant activity conditions in the solutions, if the cadmium content is also sufficiently small. This way of looking at the matter corresponds to the results of those authors (for literature, *vide* [4]), who have investigated cadmium sulphate solutions without adding a neutral salt and then have found a very strong complex formation ($\beta_1 \simeq 200$ C^{-1}). That would mean that there should appear a strong association already at much smaller concentrations of divalent ions in such solutions. It may also be observed that the authors (for literature, *vide* [4]), who have found the cadmium sulphate completely dissociated, calculate with activity factors in a manner which according to Bjerrum [14] (*cf.* Güntelberg [4, p. 26]) is tantamount to the assumption of associated ion pairs. In solutions of pure cadmium sulphate, not too concentrated, the activity factors of the two ions ought to be about equal, but in the solutions studied in this paper, where c_{SO_4} is mostly much greater than c_{Cd}, it is more probable that the activity factor of the cadmium ion is more influenced by the exchange of perchlorate for sulphate than the activity factor of the sulphate ion, as it is commonly supposed that an exchange of negative ions in an ionic environment influences more the activity factor of a positive ion than that of a negative one *. From this point of view the emfs in Table 6 are more to be considered as the result of a change of the activity factor of the cadmium ions caused by the increasing sulphate ion concentration than as the result of a complex formation. Hence it is also obvious, why the emf are mainly dependent on the t o t a l sulphate concentration c_{SO_4}, as the main part of the sulphate ions remain "free", even if the cadmium content of the solution is increased.

The proposed interpretation of the measurements of cadmium sulphate solutions diverges greatly from the common opinion of a complex system built up stepwise by successive complex compounds. This interpretation has been necessitated by the results of the measurements of sulphate ion concentration by the aid of the silver-sulphate system. Thus it seems convenient to discuss some objections that might be made to these measurements.

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* If the activity factors of the cadmium and sulphate ions were about equal, the function $\bar{n}$ of the cadmium-sulphate system ought to be described by the equation

$$\bar{n} = \frac{F_1^0(SO_4^{2-}) \cdot [SO_4^{2-}]}{1 + F_1^0(SO_4^{2-}) \cdot [SO_4^{2-}]} \qquad (20)$$

This curve is mainly of the same type as (18), but the experimental values of $\bar{n}$ in Table 6 do not agree so well with (20) as with (18).

Firstly, the influence of quantitative changes of the ionic strength owing to the formation of complexes (or ion pairs). The correction for this source of error is, it is true, uncertain, but as is already mentioned, it is not easy to see how the correction set about could be made greater, which were necessary, if the measurements should be able to result in complexes of the type $Cd(SO_4)_3^{4-}$.

It is rather more difficult to judge the influence on the potential of the silver electrode because of qualitative changes of the ionic environment, that are caused by the exchange of sodium for cadmium and sulphate for perchlorate. But this source of error ought to be outbalanced as the emf used in the calculation of $\Delta[SO_4^{2-}]$ in Table 6 is the difference between the potential of two silver electrodes, both of which are dipping in solutions, with the same total concentration of sulphate ions, which owing to their negative charges ought to have the greatest influence on the activity factor of the silver ion, and it does not seem very probable that an exchange of the positive ions cadmium for sodium should be able to affect the activity factor of the silver ion with an amount corresponding to a change of the electrode potential by several millivolts.

The emfs in Table 6 used for the calculations are very small, but parallel series demonstrate that they can practically always be reproduced with an accuracy of 0.1 mV, and this accuracy ought to be sufficient for the conclusions drawn.

Finally there is the possibility that complexes of the type $Ag_pCd_m(SO_4)_n^{p+2m-2n}$ are formed in the solutions. If this is the case it is not possible to make a simple computation of the complexes of the cadmium-sulphate system. But as there is no sign of polynuclear complexes in the silver-sulphate system and as, according to the opinion of the present author, the cadmium-sulphate system is most probably best described by assuming it to contain the ion pair $Cd^{2+}SO_4^{2-}$ as the only "complex", there are no strong arguments for assuming such complicated complexes to have been built up in sulphate solutions containing both silver and cadmium ions.

On the other hand there are many reasons that argue in favour of the result, obtained from the measurements with silver electrodes, *i.e.* that only one complex (associated ion pair) exists in solutions of 2—2-sulphates. Firstly the remarkable uniformity of the qualities of these electrolytes, already mentioned in the introduction of the present paper, which speaks in favour of complex compounds of the same sort in all solutions of 2—2-sulphates and thus suggests fairly simple conditions in all of them. The formation of associated ion pairs is mostly the result of electrostatic forces, which do not too much depend on individual qualities of the ions. The fact that the decrease

of the electrode potential in solutions of cadmium perchlorate, to which sulphate is added, becomes about the same independent of the initial cadmium ion concentration, suggests an activity influence from the sulphate ions rather than a complex formation. Finally some experiments with an anion exchange resin [15] also speak in favour of the absence of anionic complexes in the cadmium-sulphate system.

If this simplification of the complex chemistry of 2—2-sulphates is correct, it ought to be possible to describe their solutions mainly in terms of activity factors, and judging from the uniform conditions of the electrolytes it might be expected that the equations of a theory for these electrolytes will not need to contain too many parameters, not even in equations valid for rather concentrated solutions.

SUMMARY

1. Potentiometric measurements with silver electrodes in the silver-sulphate system can be accounted for by assuming that the complexes $AgSO_4^-$ and $Ag(SO_4)_2^{3-}$ are formed in the solutions. The complexity (concentration) constants are $\beta_1 = (1.7 \pm 0.05)\ C^{-1}$ and $\beta_2 = (1.9 \pm 0.1)\ C^{-2}$, if the ionic strength is maintained constant = 3 C ($NaClO_4$), and $\beta_1 = (1.7 \pm 0.05)\ C^{-1}$ and $\beta_2 = (1.0 \pm 0.1)\ C^{-2}$ if the concentration of sodium ions = 3 C.

2. The same measurements also give as a result that the potential of a silver electrode is increased by 4 mV/C, when the ionic strength, 3 C, is increased by adding more sodium perchlorate.

3. Potentiometric measurements with cadmium amalgam electrodes in the cadmium-sulphate system can be accounted for by assuming the complexes $CdSO_4$, ($Cd(SO_4)_2^{2-}$) and $Cd(SO_4)_3^{4-}$ to be formed, if the cadmium content of the solutions $<\sim 5$ mC. The complexity constants are computed to $\beta_1 = (8 \pm 1)\ C^{-1}$, $\beta_2 = (10 \pm 10)\ C^{-2}$, and $\beta_3 = (110 \pm 10)\ C^{-3}$. These results are obtained on the assumption that the ionic environment can be regarded as constant for all the solutions investigated. This assumption seems to be dubious judging from the following measurements. At cadmium contents > 5 mC the mononuclear complexes, just mentioned, are not sufficient to account for the measurements with cadmium amalgam electrodes.

4. A method of determining concentrations of the free ligand by the aid of a known complex step system is described.

5. This method has been used to determine the concentration of free sulphate ions in the cadmium-sulphate system by the aid of the known silver-sulphate system using silver electrodes. The result of this determination is, that only one complex compound exists in the cadmium-sulphate system,

viz. the uncharged molecule or associated ion pair $CdSO_4$. Its complexity constant is $\beta_1 = (7 \pm 2)\ C^{-1}$ in good agreement with the results from measurements with cadmium amalgam electrodes.

6. The nonagreement of 3 and 5 in this summary can be explained in the following way. The result from 3 has been obtained by assuming that the increase of the function $F_1^0(SO_4^{2-})$ is a consequence of a successive complex formation at increasing sulphate concentrations. But this increase might also be explained by a change of the activity coefficient of mainly the cadmium ion, when perchlorate is exchanged for sulphate. The activity conditions can only be regarded as constant, when the sum of cadmium and sulphate ion concentration $<$ 60 mC. Such a change of the activity factors can also be described as a formation of associated ion pairs, which are also formed at much lower concentration if the solutions contain no other ions than cadmium and sulphate.

The measurements with silver electrodes were carried out in the Department of Inorganic Chemistry at Chalmers' Institute of Technology in the summer of 1949. To the Head of the department at that time, Professor L.-G. Sillén, I am very much obliged for the readiness with which the facilities of his department were placed at my disposal. The measurements with cadmium amalgam electrodes were carried out in the Institute of Inorganic and Physical Chemistry at the University of Lund. I beg once more to express my gratitute to the Head of this Institute, Professor S. Bodforss, for the very kind interest he has always shown to my work.

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Microquantitative Determination of Calcium as Murexide Complex in the Presence of Magnesium

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Calcium changes the color of a murexide solution [1]. This property was utilised in the versenate titration for total hardness in water chemistry [2-4]. A thorough investigation of the reactions between metal ions and murexide was performed by Schwartzenbach and Gysling [5]. The direct use of the calcium murexide complexes for a spectrophotometric determination of calcium was first mentioned by Ostertag and Rinck in a short communication, where, however, little is said about their technic [6]. In a later paper the same authors give an account of the influence of other metal ions on the sensibility of their method and some details of their technic [7]. The purpose of our investigation is to enable the determination of microquantities of calcium in the presence of magnesium. In order to find the best conditions we investigated the influence of the wave-length of light on the sensibility and studied, moreover, the dependence of the color and stability of the test solutions on pH and the murexide concentration.

ABSORPTION CURVES

The wave-lengths corresponding to absorption maxima in solutions of murexide and solutions containing murexide and calcium and murexide and magnesium were determined. The results are summarized in the principal diagram in Fig. 1.

The point where the lines I and III intersect indicates the wave-length chosen for the determinations of calcium. Magnesium murexide complex and pure murexide absorb light of that wave-length to the same amount. In this way the magnesium complex does not interfere more than the normally established excess of murexide. Fortunately this wave-length (510 mμ) is

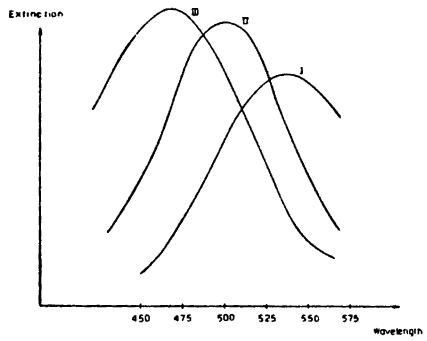


Fig. 1. Extinction as a function of the wave-length for

I. Murexide solution.
II. Calcium murexide complex.
III. Magnesium murexide complex.

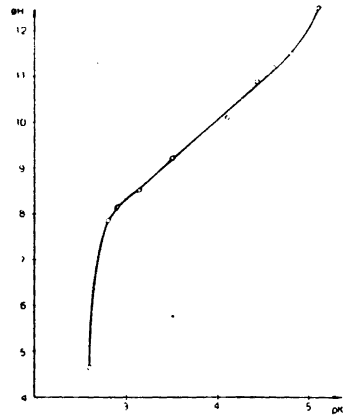


Fig. 2. pK calculated as a function of pH.

not too far from the absorption maximum of the calcium murexide complex. The accurate value of the wave-length giving maximum is, however, determined to be 500 mμ and in cases where interference from other ions can be neglected this point is preferable. Because of the low concentration of the test solutions and the relatively high equilibrium constant the excess of murexide necessary for complete formation of calcium complex is rather high. At calcium concentrations between $2.5 \cdot 10^{-6}$ and $7.5 \cdot 10^{-5}$ molar the murexide concentration was kept at $2 \cdot 10^{-4}$. This seems to be enough even when magnesium is present in concentrations of the same magnitude as calcium.

INFLUENCE OF pH

The amount of calcium bound to murexide may be calculated from the following formula

$$\frac{\text{Ca} \cdot \text{Mur}}{\text{Ca Mur}} = K$$

where Mur is murexide, protolysed or unprotolysed, and CaMur is the concentration of the calcium complexes. K is, however, no real constant but depends on pH according to Fig. 2.

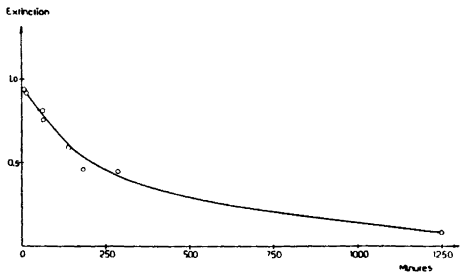


Fig. 3. The extinction as a function of time in a murexide solution at pH ≈ 11. The curve shows the decomposition of murexide.

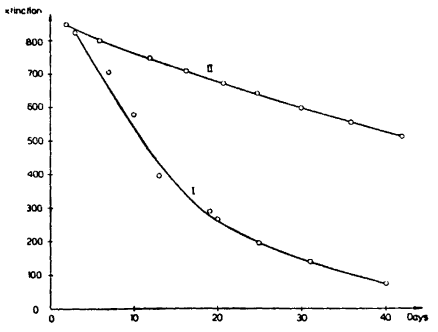


Fig. 4. The extinction of a murexide solution as a function of time at room temperature (I) and 5° C (II).

The diagram shows, that the higher pH the more of the calcium is bound to the murexide and consequently the sensibility of the determination will increase with pH. A counteracting effect is the breakdown of the murexide which increases with increasing pH. At pH about 11 the breakdown was found to be slow enough to permit accurate determinations. A suitable reagent to maintain this pH value is piperidine.

APPARATUS AND SOLUTIONS

The measurements were made by means of a Beckman Quartz spectrophotometer, type Du.

The murexide solution was prepared by saturating water during 24 hours and then filtering off insoluble impurities. This solution has to be stored in the refrigerator to decrease the rate of decomposition. The velocity of the decomposition at room temperature and 5° C is shown in Fig. 4.

The calcium solutions were prepared by dissolving calcium sulphate ($CaSO_4 \cdot 2H_2O$) in water thus obtaining a stock solution from which the very diluted solutions were made daily. Magnesium solutions were prepared from magnesium sulphate. Immediately before a reading two drops of piperidine (puriss) were added to 10 ml of the test solution.

ANALYSIS

0.5 ml of murexide solution and two drops of piperidine were added to 10 ml of a test solution containing from zero to three mg Ca/l. The results of the extinction readings are summarized in Fig. 5. The curve can be used for calibration of the method and thus enables determinations of unknown

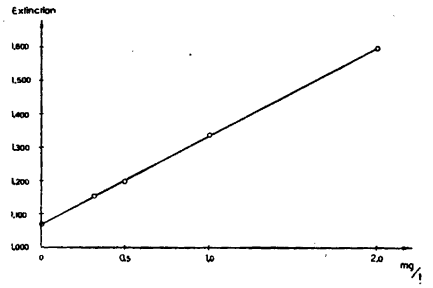


Fig. 5. Extinctions plotted against calcium concentrations. The extinction is determined in solutions containing murexide.

calcium concentrations. Because of the decomposition of the murexide solution a curve of this type has to be determined daily.

DISCUSSION

With the method described it is possible to determine calcium in water solutions, even in the presence of magnesium. In pure calcium solutions the error of the analysis is estimated to be $\pm$ 0.15 microgram. In the presence of magnesium the same value is about 0.5 microgram. The upper limit of 3 mg Ca/l. depends on the thickness of the cell (10 mm) and is necessary to avoid loss in accuracy because of too great light absorption. Using cells of different thicknesses it will be possible to extend the range of the analysis. Experiments have been made to test an analogous method for the determination of magnesium, but the equilibrium constant $K_{Mg} = \frac{Mg \cdot Mur}{Mg\ Mur}$ is too great to permit accurate values.

SUMMARY

A suitable wavelength for the spectrophotometric determination of the calcium murexide complex is determined. The stability of the reagents is studied and found to be satisfactory for analytical purposes. Influence of pH on the reaction between calcium and murexide and on the sensibility of the spectrophotometric determination is studied. The results show that microquantitative determination of calcium is possible by means of spectrophotometric determination of the calcium murexide complex also in the presence of magnesium.

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Synthetic Plant Hormones

III.* Sulphur Analogues of 2,4-Dichlorophenoxy- and 4-Chloro-2-methylphenoxy-acetic Acids

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The preparation of the sulphur analogues of 2,4-dichlorophenoxy- and 4-chloro-2-methylphenoxy-acetic acids, *viz.* 2,4-dichlorophenylmercapto-acetic acid (I) and 4-chloro-2-methyl-phenylmercaptoacetic acid (II), and the corresponding sulphinyl- and sulphonyl-acetic acids (III, IV respectively V, VI) was mentioned in part I of this series [1]. These compounds were prepared in order to study the influence of successive oxidation of the sulphur atom on the plant hormone activity of the acids, and the theoretical background of the investigation was discussed at that time, and it is the purpose of this communication to discuss the activity of the 4-chloro-2-methyl compounds, and to describe the preparative methods used for the whole series. The results of the testing of the 2,4-dichloro-compounds have been discussed elsewhere by Wilske and Burström [2]. A study on the activity of 2,4-dichlorophenyl-mercaptoacetic acid as well as some other substituted phenylmercaptoacetic acids has been published by Thompson, Swanson and Norman [3].



R	R	R
I R = Cl	III R = Cl	V R = Cl
II R = CH_3	IV R = CH_3	VI R = CH_3

* Part II. *Acta Chem. Scand.* **3** (1949) 904.

** Bönnelyche & Thuröe AB Research Fellow 1950–51.

The phenylmercaptoacetic acids, which have been synthesised earlier by other methods, were prepared by the general method of Kalle and Co [4] from the corresponding anilines by diazotisation, conversion to the thiophenols via the xanthates and finally coupling with chloroacetic acid in alkaline solution. The sulphinylacetic acids were obtained by oxidation of the mercaptoacetic acids with hydrogen peroxide in glacial acetic acid at room temperature, and oxidation with potassium permanganate in the presence of sodium carbonate gave the corresponding sulphonylacetic acids. When an attempt was made to oxidise 2,4-benzenesulphinylacetic acid with potassium permanganate at elevated temperatures the product obtained was methyl 2,4-dichlorophenylsulphone and not the expected sulphonylacetic acid.

EXPERIMENTAL *

2,4-Dichlorophenylmercaptoacetic acid. 2,4-Dichloroaniline (38 g) in ice cold dilute (1 : 6) hydrochloric acid (350 ml) was diazotised by the addition of sodium nitrite (17 g). When this solution was poured into a solution of potassium ethyl xanthate (46 g) and sodium carbonate (25 g) in water (160 ml) at 70° C, a red oil separated immediately. When the evolution of nitrogen had ceased, the mixture was cooled to room temperature, acidified with dilute sulphuric acid and extracted twice with ether. The ether extracts were combined and evaporated and the ethyl 2,4-dichlorophenyl xanthate was dissolved in a mixture of alcohol (75 g) and 35 % aqueous sodium hydroxide (46 g). The mixture was heated under reflux for four hours. Then a solution of chloroacetic acid (35 g) in 35 % aqueous sodium hydroxide (42 g) was added, and the mixture was boiled for a further four hours. The alcohol was distilled off, and the residual yellow material dissolved in water. The solution was filtered and acidified with hydrochloric acid. The yellowish crystalline precipitate (50 g, 90 %) was collected and recrystallised repeatedly from water and then formed colourless prisms.

M.p. 122—123° C.

$C_8H_6SO_2Cl_2$	Calc.	C 40.5	H 2.55	Cl 29.9	Equiv. weight	237.1
	Found	» 39.9	» 2.52	» 29.3	»	»	238

The synthesis of this acid has been reported in a patent [5], but no data were given of its physical properties.

4-Chloro-2-methylphenylmercaptoacetic acid. This acid was prepared analogously from 2-amino-5-chlorotoluene and after three recrystallisations from water melted at 127—128° C in agreement with value reported by Herz, Bauer, Steiger, Albrecht and Dereser [6]. The yield of crude product was 82 % of the theoretical.

$C_9H_9SO_2Cl$	Calc.	S 14.8	Cl 16.4
	Found	» 14.7	» 16.2

2,4-Dichlorobenzenesulphinylacetic acid. 2,4-Dichlorophenylmercaptoacetic acid (10 g) was dissolved in glacial acetic acid (40 ml), and 30 % hydrogen peroxide (12 g) was added. After a short time colourless crystalline material began to separate. The reaction mixture was kept at room temperature for about twelve hours, and the product (6 g) was collected

* All melting points uncorrected.

by filtration and repeatedly recrystallised from water, forming colourless needles of m.p. 145—146° C (decomp). Further material could be obtained by evaporation of the original mother liquor, the total yield of crude product being 89 % of the theoretical.

$C_8H_6SO_3Cl_2$	Calc.	C 38.0	H 2.39	Equiv. weight	253.1
	Found	» 37.8	» 2.40	»	»	252

4-Chloro-2-methylbenzenesulphinylacetic acid. 4-Chloro-2-methylphenylmercaptoacetic acid (4 g) was oxidised with 30 % hydrogen peroxide (4.8) in glacial acetic acid (15 ml) as described for the 2,4-dichlorocompound. The yield of crude product was 3.5 g (82 %) and after four crystallisations from water the compound had m.p. 128—129° C (decomp.).

$C_9H_9SO_3Cl$	Calc.	C 46.5	H 3.90	Equiv. weight	232.7
	Found	» 46.4	» 4.00	»	»	230

2,4-Dichlorobenzenesulphonylacetic acid. An aqueous solution of 2,4-dichlorophenylmercaptoacetic acid (3 g) and sodium carbonate (0.7 g) was treated at 30° C with a solution of potassium permanganate (4 g) in water. The solution was saturated with sulphur dioxide until the manganese sludge had dissolved. The sulphonylacetic acid separated as a colourless oil, that crystallised on stirring. Yield 1.8 g (56 %). Three recrystallisations from water yielded the pure product. M.p. 150—151° C.

$C_8H_6SO_4Cl_2$	Calc.	C 35.7	H 2.25	Equiv. weight	269.1
	Found	» 35.7	» 2.24	»	»	267

4-Chloro-2-methylbenzenesulphonylacetic acid. 4-Chloro-2-methylphenylmercaptoacetic acid was oxidised as described above for the 2,4-dichlorocompound. The yield of crude product from 2 g of 4-chloro-2-methylphenylmercaptoacetic acid was 1.5 g (65 %). The product was recrystallised three times from benzene and then melted at 147—148° C.

$C_9H_9SO_4Cl$	Calc.	C 43.5	H 3.66	Equiv. weight	248.7
	Found	» 43.6	» 3.65	»	»	247

Methyl-2,4-dichlorophenylsulphone. 2,4-Dichlorobenzenesulphinylacetic acid (2 g) and sodium carbonate (0.5 g) were dissolved in water and oxidised at 60° C with an aqueous solution of potassium permanganate (1.3 g). The manganese sludge was removed by filtration and the solution was evaporated almost to dryness. On cooling a neutral compound separated as long colourless needles (0.56 g) which after recrystallisation from water melted at 74—75° C.

$C_7H_6SO_2Cl_2$ (189.6)	Calc.	C 37.3	H 2.69
	Found	» 37.2	» 2.68

PHYSIOLOGICAL PART

The physiological activity of the acids was studied in simple growth tests using *Lepidium* seedlings.

Seeds were germinated in 20 cm Petri dishes. Two day old seedlings, selected for uniformity with roots *ca.* 10 mm long, were transferred to 7 cm petri dishes, containing five sheets of filter paper wetted by 5 ml of the test solutions, prepared by dissolving the acids in a nearly equivalent amount of potassium hydroxide, and adjusting the pH to 7. Each dish contained 10 seedlings and all experiments were run in quintuplicate. The dishes were placed in the dark at 20° C and the root lengths were recorded after two days. The mean error of the growth determinations amounted to ± 4 per cent.

The results are given in Table 1. For convenience the following abbreviations are used for the acids:

Table 1. Elongation of Lepidium roots in two days in solutions of (the given) acids and a phosphate buffer of pH 7. — Mean error ± 1 mm.

Concentration *M*	Acid tested				Phosphate buffer
	4-C-2-M	4-C-2-MS	4-C-2-MSO	4-C-2-MSO_2	
0	26.3	26.3	26.3	26.3	26.3
10^{-8}	30.0	—	—	—	—
10^{-7}	27.2	32.6	—	—	—
10^{-6}	15.6	24.4	25.7	29.6	—
10^{-5}	2.9 *	10.6	25.6	22.9	23.1
10^{-4}	0.1 **	0.9 *	13.1	28.2	23.6
10^{-3}	—	0 **	0.3 *	20.6	24.4
10^{-2}	—	—	—	10.4	13.8

* Typical "auxin-roots"; no elongation but swelling of the roots and excessive formation of root hairs.

** Roots dead.

4-chloro-2-methylphenoxyacetic acid	4-C-2-M
4-chloro-2-methylphenylmercaptoacetic acid	4-C-2-MS
4-chloro-2-methylbenzenesulphinylacetic acid	4-C-2-MSO
4-chloro-2-methylbenzenesulphonylacetic acid	4-C-2-MSO_2

A regularly decreasing growth retardation was obtained with increasing substitution from the phenoxy- to the sulphonylderivatives. In 10^{-5} *M* 4-C-2-M, 10^{-4} *M* 4-C-2-MS, and 10^{-3} *M* 4-C-2-MSO there appeared symptoms which regularly accompany an auxin excess, *i.e.* inhibited elongation, root swellings, and excessive formation of root hairs. No signs of this kind were encountered with 4-C-2-MSO_2, even in 10^{-2} *M* solution: the root elongation was certainly retarded but not much more than by a phosphate solution of the same strength. Thus it may be concluded that the sulphonyl derivative is lacking in specific growth inhibiting action.

The phenoxyacetic acid derivatives are all comparatively strong acids, and it was assumed by Wilske and Burström [2] that they might be regarded as completely dissociated, so that in comparing them the dissociation could be neglected. However, it has been found with other acids [7] that growth inhibition of this kind parallels the concentration of undissociated acid, even if this concentration is low. Whether this is due to the mode of action of the acids

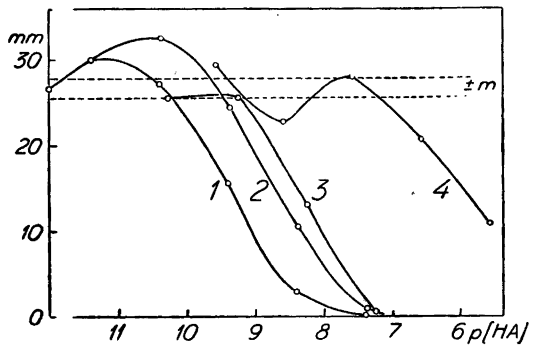


Fig. 1. The relationship between root elongation and concentration of undissociated acid for 1) 4-C-2-M, 2) 4-C-2-MS, 3) 4-C-2-MSO, and 4) 4-C-2-MSO$_2$. ± m denotes the mean error for the control roots.

on the root surface or to their permeation cannot be decided. It is, anyway, necessary to pay due attention to the dissociation, in evaluating the relative acitivity of the acids.

The dissociation constants for the acids in question were determined from the titration curves of the sulphinyl- and sulphonylacetic acid derivatives, and for the two less soluble compounds from pH-determinations of saturated solutions using a glass electrode. These determinations were carried out by Miss E. Welin-Berger, Botanical Laboratory, Lund, and the following (average) results were obtained:

Acid	p$K_{20°}$
4-C-2-M	3.62
4-C-2-MS	3.89
4-C-2-MSO	2.82
4-C-2-MSO$_2$	2.68

If root elongation is plotted as a function of the concentration of undissociated acid, the graph shown in Fig. 1 is obtained. There is still a difference between the phenoxy- and the thiophenoxy-compounds, which implies a marked decrease in activity if oxygen is substituted for sulphur, but the graphs for the thiophenoxy- and sulphinylacids fall close together. The most obvious conclusion is that no considerable decrease in the activity occurs until the sulphur atom is completely oxidised. — It must also be born in mind that the decreasing activity at the highest concentrations of the sulphonyl compound obviously does not depend specifically upon the acid.

The mean error for the control roots has also been plotted in the figure, and it shows that little significance can be attributed to the increase in root

lengths observed at the lowest concentrations of the phenoxy- and thiophenoxy-compounds. Much larger samples of seedlings would be required to reveal a statistically significant growth promotion by these acids.

The conclusion can be drawn that the results on the specific growth retardation give considerable supports to the hypothesis advanced by Erdtman [1], concerning the relationship between structure and activity. The agreement is much better than that obtained in a different test with the corresponding 2,4-dichlorophenylmercaptoacetic acid derivatives [2]. However, a confirmation of the theory requires that it could be shown to hold true for growth accelerating as well as growth inhibiting activity.

SUMMARY

The sulphur analogues of 2,4-dichlorophenoxy- and 4-chloro-2-methylphenoxy-acetic acid have been prepared and oxidised to the corresponding sulphinyl- and sulphonylacetic acids. The action of the 4-chloro-2-methyl-substituted acids upon *Lepidium* seedlings has been studied and compared with that of 4-chloro-2-methylphenoxyacetic acid. The mercapto- and the sulphinyl-acetic acid derivatives retard growth to a smaller extent than the phenoxyacetic acid derivative, whereas the sulphonylacetic acid derivative has no specific growth inhibiting activity.

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On the Microbiological Activity of a Plasma-peptide Fraction

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The present paper describes the microbiological activity of a peptide fraction prepared from calf's plasma by means of heat coagulation and precipitation with picric acid. The preparation is free from the plasma muco-protein recently described by Winzler *et al.*[1] and contains no free amino acids. Other characteristics will be published elsewhere.

MICROBIOLOGICAL PROCEDURES

The procedures followed for the cultures and inoculum have been described in previous papers [2,3]. Assays were carried out in 18 × 150 mm Pyrex culture tubes and in a total volume of 5 ml. The peptide solution, adjusted to pH 6.8, was added in volumes of 2.5 ml to 2.5 ml of the basal medium of Steele *et al.*[4] For *L. citrovorum* (8081) the addition of 0.4 ml of a concentrated liver preparation (Heptomin) to 100 ml of synthetic medium was used [5]. *L. lactis* (8000) was grown on the medium given by Shorb [6], and *L. leichmanii* was cultured on the medium described by Capps *et al.*[7]

RESULTS

The growth was followed simultaneously by photoelectric readings and by titrations. Typical results are given in Table 1 and 2. The figures in Table 1 clearly show the general growth stimulating effect of the peptide preparation. With the exceptions of *S.faecalis* R (8043) and *S.lactis* (9790) the other tested lactobacilli are more or less stimulated by the addition of the plasma peptides to the medium. For three microorganisms, *L.mesenteroides*, *L.fermenti* 36, and *L.arabinosus*, the effect is obvious only in the first 12 hours of incubation. For two of the other lactobacilli, *L.casei* and *L.delbrückii* 3, the effect is still observable after 72 hours of incubation. *L.mesenteroides* P-60, one of the microorganisms most commonly used for microbiological amino acid determination, is not stimulated by the presence of plasma peptides in the medium when an incubation time of 24 hours or longer is used.

Table 1. Growth of lactobacilli in synthetic media supplemented with two concentrations of a plasma-peptide preparation.

	Growth after*											
	12 hours			24 hours			48 hours			72 hours		
	control	1 mg peptide	0.1 mg peptide	control	1 mg peptide	0.1 mg peptide	control	1 mg peptide	0.1 mg peptide	control	1 mg peptide	0.1 mg peptide
L. citrovorum (8081)	10	28	21	20	37	31	26	41	34	40	58	48
L. citrovorum (8082)	5	28	17	125	160	148	210	203	195	200	190	195
L. casei (7469)	1	13	5	6	141	30	34	340	152	214	338	218
S. faecalis R (8043)	114	125	122	228	220	219	254	242	255	240	224	226
L. mesenteroides P 60 (8042)	90	181	167	280	288	292	310	320	320	300	318	317
L. fermenti 36 (9338)	9	56	42	123	154	130	196	180	170	194	180	165
S. lactis (9790)	150	164	150	228	236	235	242	250	245	220	232	222
L. delbrückii 5 (9595)	0	12	4	5	58	20	230	200	190	238	250	210
L. delbrückii 3	2	10	9	37	60	20	202	297	215	230	300	231
L. arabinosus (8014)	116	222	206	405	420	410	475	468	469	450	450	450

* Given as scale readings on the Klett-Summerson photoelectric colorimeter. The reading O corresponds to 100 per cent transmission and the values have been corrected for the blank values.

The effect on the lactic acid production is shown in Table 2. It is of considerable biological interest to note that the plasma peptide fraction has a stimulating effect both on growth and on lactic acid production after 72 hours of incubation. The most conspicuous results were obtained with *L.casei*, *L. delbrückii* 5, and *L.delbrückii* 3. The mechanism by which the lactic acid production is stimulated will be further investigated.

The growth stimulating effect recorded here both by photoelectric readings and titrations differs in some ways from that of the strepogenin effect found by Woolley [8]. He used *L.casei* and *S.lactis* as test organisms. Neither of the two types of *Streptoccus lactis* used in this investigation were stimulated by the addition of the plasma peptide fraction to the medium. With strepogenin present in the medium Woolley recorded a maximal growth effect after 18 hours of incubation. After 72 hours all tubes, whether supplemented or not, showed maximal growth and acid production. Similar results were obtained by the present author in microbiological tests with proteolysed liver and casein extracts [3]. When the plasma peptide fraction is added to the basal medium, however, the lactic acid production is stimulated even when an incubation time of 72 hours is used.

Table 2. Lactic acid production in synthetic media supplemented with two concentrations of a plasma peptide preparation.

Organism	ml of acid produced after 72 hours of incubation *		
	Control	1 mg peptide	0.1 mg peptide
L. citrovorum 8081)	1.4	2.4	1.8
L. citrovorum (8082)	5.8	6.4	5.8
L. casei (7469)	5.8	16.4	9.8
S. faecalis R (8043)	4.4	4.6	4.4
L. mesenteroides P 60 (8042)	14.8	16.8	15.4
L. fermenti 36 (9338)	6.8	8.6	7.4
S. lactis (9790)	5.0	6.0	5.2
L. delbrückii LD 5 (9595)	4.4	11.0	7.8
L. delbrückii LD 3	11.2	17.5	13.1
L. arabinosus (8014)	18.6	20.1	20.0

* Given as ml of 0.05 *N* NaOH to titrate 5 ml of final solution. The values are mean values from three tubes and have been corrected for the blank titrations.

With this difference in mind it was of special interest to investigate the growth effect of the plasma peptides on two other test organisms, *L.lactis* (8 000) and *L.leichmannii* (4797) used for determinations of vitamin B_{12}. Since Bird and Hoevet [9] have recently shown that this vitamin can be bound to a variety of proteins, the possibility could not be excluded that the peptide fraction contained some bound vitamin in an available form. To determine whether or not this was actually the case, experiments were carried out as parallels to those described in Table 1 and 2. The results are given in Table 3. From the figures indicating lactic acid production by the two microorganisms it seems probable that there is no available vitamin B_{12} present in the peptide preparation. The same conclusion can be drawn from the photoelectrice readings obtained with L.lactis. On the other hand, some of the results seem to suggest that the peptide fraction can have a synergistic effect with vitamin B_{12}. The growth figures obtained with *L.citrovorum* (8081) in Table 1 and 2 and in other experiments do not exclude the possibility that the stimulating effect of the peptide fraction to some extent may be caused by the citrovorum factors [10,3] or other unknown growth factors.

The question whether amino acids in peptide linkages can be utilized by lactobacilli or not has previously been investigated by means of synthetic peptides [11,12]. It was shown that all of the bacteria listed in Table 1—3 of the

Table 3. Growth of L. lactis (8000) and L. leichmannii (4797) on synthetic media supplemented with submaximal amounts of vitamin B_{12} and two concentrations of a plasma peptide preparation.

Organism	Growth after[1]							
	12 hours		24 hours		48 hours		72 hours	
	1 mg peptide	0.1 mg peptide	1 mg peptide	0.1 mg peptide	1 mg peptide	0.1 mg peptide	1 mg peptide	0.1 mg peptide
L. lactis + B_{12}[2])	1	2	3	5	2	3	84	84
L. lactis	4	1	6	5	5	6	40	40
L. leichmannii + B_{12}[2])	18	9	21	8	36	8	52	11
L. leichmannii	2	0	4	1	5	3	6	4
	Ml of acid produced in the same series[3]							
L. lactis + B_{12}[2])							2.4	2.6
L. lactis							0.4	0.4
L. leichmannii + B_{12}[2])							2.0	0.6
L. leichmannii							0.2	0

[1] Given as scale readings on the Klett-Summerson photoelectric colorimeter. The reading O corresponds to 100 per cent transmission and values have been corrected for blank values.

[2] Medium supplemented with 0.2 mμg of B_{12}. Maximal growth with 2.0 mμg of B_{12}.

[3] Given as ml of 0.05 *N* NaOH to titrate 5 ml of final medium. The values are the means from three tubes and have been corrected for blank values.

present investigation could utilize the leucine of several leucine containing tri- and dipeptides supplemented to the basal medium instead of the free amino acid. Virtanen and Nurmiko [13] recently demonstrated that *L.mesenteroides* P-60 could hydrolyze synthetic glycine and leucine peptides replacing these amino acids in the medium. How the peptides of deproteinized plasma behave in this respect has previously not been investigated. Henderson *et al.*[14] believe that the activity of such a proteinlike fraction is probably not available to lactobacilli since it is non-dialysable. From the methodological point of view it was of interest to analyze the availability of our peptide fraction.

L.mesenteroides P-60 is one of the lactobacilli most commonly used for microbiological amino acid determinations. The ability of that microorganism to use the hydrolyzed and unhydrolyzed peptide fraction supplemented to the basal medium instead of different free amino acids was investigated. Ten of the amino acids known by paperchromatography to be present in the hydrolysate were analyzed according to previously described methods. [15,16]

The experiments clearly showed that *L.mesenteroides* could not hydrolyze or utilize the amino acids of the plasma peptide when it replaced any of the ten free amino acids excluded from the synthetic medium. From the analytical data of the peptide hydrolysate it was calculated that the amino acids composition of the peptide fraction was the following

Arginine	10.9 %	Lysine	7.9 %
Glutamic acid	5.1 »	Methionine	0.8 »
Histidine	4.8 »	Phenylalanine	4.4 »
Isoleucine	4.0 »	Threonine	5.1 »
Leucine	8.0 »	Valine	5.2 »

The figures are not corrected for ash.

The results correspond to what is found in animal protein hydrolysates where the ten essential amino acids often correspond to about 50 per cent of the total amount. With regard to the discussed relationships to the glutamic acid-rich strepogenin the low glutamic acid content may be stressed. There is a comparatively high percentage of basic amino acids, *eg.* 23.6. In this respect the peptide fraction resembles the basic peptide, secretin, which is a heat stable polypeptide precipitated by picric acid and containing about 20 per cent of basic amino acids [17]. The possible requirements of basic peptides for the synthetis of nucleoproteins may also be mentioned in this connection.

Series of experiments were also carried out with *L.casei* and *L.delbrückii* 5 to determine if these microorganisms could utilize the plasma peptide when it replaced some amino acids excluded from the synthetic medium. As shown in Table 2 the lactic acid production of these two microorganisms was much more stimulated than in the case of *L.mesenteroides*. The results obtained with *L.casei* cultivated on the incomplete medium are given in Table 4. From a comparison with Table 2 it can be observed that the lactic acid production in the complete medium without peptide addition, is 5.8 ml as compared to 5.0—5.8 ml when the highest submaximal amount of the excluded amino acid, 40 μg, is added to the incomplete medium. The complete medium contains 310 μg of histidine, 6250 μg of leucine, and the same amount of valine per tube, and it is obvious that the tenfold increase of the submaximal amounts of amino acids does not effect the lactic acid production in the complete medium. Hence the effect of the peptides in the complete medium can not be due to the small amounts of free amino acids which the microorganisms possibly could set free from the peptide. The growth effect must in some way be connected with the action of the unhydrolyzed or partially split molecules.

The peptide fraction has some stimulating effect when added to the incomplete medium corresponding to 0.8—2.0 ml of acid per 0.1 mg of subs-

Table 4. Growth of L. casei in the medium of Steele et al[4] supplemented with the plasma peptide fractions instead of free amino acids. Incubation time 72 hours.

Amino acid omitted from the medium	Ml of acids produced with the following amounts of substances added per tube [1]					
	Free amino acid				Peptide	
Histidine	40 μg [2]	20	10	5	400 μg [3]	100
	5.0 ml	4.4	3.8	3.6	6.8	1.8
Leucine	40 μg [2]	20	10	5	400 μg	100
	5.8 ml	4.4	3.4	2.8	5.6	2.0
Valine	40 μg [2]	20	10	5	400 μg	100
	5.6 ml	3.8	2.6	1.8	2.2	0.8

[1] Given as ml 0.05 *N* NaOH to titrate 5 ml of final solution. The values are mean values from three tubes and have been corrected for blank titrations.

[2] The complete medium contains 310 μg of histidine, 6250 μg of leucine and 6250 μg of valine per 5 ml of final medium.

[3] 400 μg of peptide contains 19 μg of histidine, 32 μg of leucine, and 21 μg of valine.

tance. From Table 2 it can be observed that a similar amount added to the complete medium is accompanied by a lactic acid production which is from 5 to 10 times as large. From the figures in Table 4 it can not be definitely settled whether the effect of the peptide fraction in the incomplete medium depends on the action of the whole peptide molecules or is due to a cleavage to compensate for the excluded amino acid. However, it may be significant that the lactic acid produced by the peptides does not correspond to the amount which could be expected from the amino acid composition of the preparation. The most probable explanation of the low effect in the incomplete medium is that the excluded amino acids limit the growth.

The results obtained with *L.delbrückii* 5 were of the same qualitative nature as those obtained with *L.casei* recorded in Table 4. The conclusion drawn above that the growth stimulating effect must be ascribed to something else than the common amino acids present in the peptide molecules, is also substantiated by the experience gained in the tests with *L.delbrückii* 5.

The plasma peptide fraction is soluble in 80 per cent ethanol. Accordingly it may be present in "protein-free" amino acid extracts from plasma or organs prepared by this method. The presence of the peptides in such extracts may influence the microbiological amino acid determinations when *L.casei*, *L.delbrückii* 5 or LD 3 are used as tests organisms but not with *L.mesenteroides* P-60. Plasma filtrate deproteinized with picric acid or tungstic acid precipitation are free from the peptide fraction presently investigated.

SUMMARY

A plasma peptide fraction prepared from calf's plasma stimulates growth and lactic acid production of a series of lactobacilli, in some instances even when an incubation time of 72 hours is used. The fraction does not contain directly available vitamin B_{12}, but may contain citrovorum factors or other unknown growth factors. A microbiological amino acid analyses of the preparation shows a certain predominance of basic amino acids. The glutamic acid content is low.

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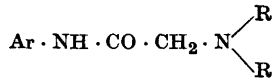
ACTA CHEMICA SCANDINAVICA 6 (1952) 1006–1009

Studies on Local Anaesthetics. VI [1–5]

NILS LÖFGREN and BERTIL TAKMAN

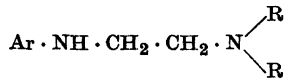
Institute of Organic Chemistry and Biochemistry, University of Stockholm, Stockholm, Sweden

In earlier investigations by Erdtman and Löfgren [6] and by Löfgren *et al.* [1–5], a fairly large number of compounds of the type



were synthesized and studied as to their local anaesthetic properties.

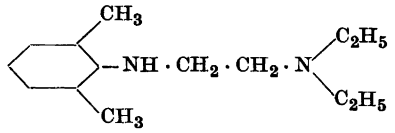
Here, investigations of three new compounds with the formula



I

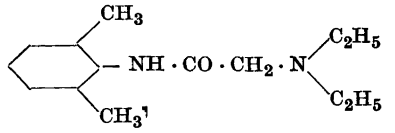
will be reported.

The compound

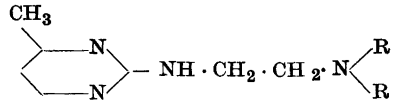


Ia

closely related to xylocaine [1, 2, 5]



was synthesized and studied pharmacologically. Further, by substituting 2-amino-4-methylpyrimidine in Ia for xylidine, two other diamines Ib and Ic were obtained:



Ib: R = methyl

Ic: R = ethyl

Attempts to synthesize N-(β-diethylaminoethyl)-2,6-dimethylaniline (Ia) were made by Löfgren and Widmark [4]; Löfgren [1] earlier showed N-(β-diethylaminoethyl)-aniline to possess local anaesthetic activity. Löfgren and Widmark synthesized N-(β-diethylaminoethyl)-2,6-dimethylformanilide and then tried to split off the formyl group. The compound, however, was so resistant that, despite boiling in strongly acid solution, the formyl group could not be split off.

We have now succeeded in preparing Ia by the reaction between two moles of 2,6-xylidine and one mole of β-diethylaminoethyl chloride, one mole of the former acting as proton acceptor. To separate the excess of xylidine we used a method based on the slight solubility of 2,6-xylidinium nitrate in water. For further purification a fractionated extraction with ether at suitable pH values was performed.

The compounds Ib and Ic were prepared by condensing 2-amino-4-methylpyrimidine with the dialkylaminoethyl chloride in question in the presence of sodamide. Some closely related pyrimidine derivatives have been prepared by Adams and Whitmore [7] and by Huttrer *et al.* [8]. None of these authors studied the anaesthetic properties of their compounds.

Compound Ia is active; on rabbit cornea it has, under the same conditions (pH 5.9), about half the duration of xylocaine and a much longer latency time. Its toxicity as determined from subcutaneous injections in white mice is approximately the same as that of xylocaine. Thus, the LD_{50} values for Ia and for xylocaine are 0.34 g/kg and 0.39 g/kg respectively. Ia is a strong irritant on the cornea and when injected subcutaneously it causes necrosis. Compounds Ib and Ic have only very slight activity. Further, all three compounds were tested for their spasmolytic and histaminolytic power. No appreciable effects were found.

EXPERIMENTAL *

The β-dialkylaminoethyl chlorides used were prepared by Slotta and Behnisch's method [9].

N-(β-Diethylaminoethyl)-2,6-dimethylaniline, $C_{14}H_{24}N_2$ (220.3). 0.297 mole (36.4g) of freshly distilled 2,6-dimethylaniline was heated to 180° in a two-necked flask equipped with a condenser and a dropping funnel. A freshly prepared absolutely dry solution of 0.150 mole (20.5 g) of β-diethylaminoethyl chloride in ether was added drop by drop through the funnel. The ether evaporated immediately. When all the ether solution had been added and the temperature began to rise, the condenser was arranged for refluxing and the mixture kept at 180° for 5 ½ hours. After cooling, the mixture was slowly poured into 60 ml of 6 *N* HNO_3 under vigorous stirring. The temperature was kept

* All our melting points are uncorrected.

below 12°. The xylidinium nitrate separated and was filtered off. NaOH-solution was added to the filtrate so that a pH value of 4.8 was obtained. The liquid was extracted once with ether, thus removing the residual excess of xylidine. The water phase was then made strongly alkaline and the liberated base thoroughly extracted with ether. After drying over $MgSO_4$, the ether was driven off. The remaining oil was then twice distilled under reduced pressure. The yield was 0.0437 mole (9.6 g; 29 %) of a colourless oil; b.p. 105°/0.6 mm; $n_D^{22} = 1.5151$.

Calc. C 76.3 H 10.98
Found » 76.2 » 10.81

2-(β-Dimethylaminoethylamino)-4-methylpyrimidine, $C_9H_{16}N_4$ (180.2). 100 ml of dry toluene and 0.308 mole (12 g) of sodamide were placed in a three-necked flask, fitted with a mechanical stirrer through a mercury seal, a dropping funnel and a condenser protected with a soda-lime tube. To this mixture a solution of 0.293 mole (32 g) of 2-amino-4-methylpyrimidine in 100 ml of dry toluene was added through the funnel. The flask was kept in an oil bath at 125° for 12 hours. After cooling 0.186 mole (20 g) of freshly prepared dry β-dimethylaminoethyl chloride was slowly added, the mixture heated to 125° for 18 hours, cooled and placed in a refrigerator overnight. The solid was filtered off and well washed with toluene. The toluene solution was thoroughly extracted with 3 *N* HCl. The combined aqueous extracts were purified by ether extraction, then made strongly alkaline and extracted sufficiently with ether. After drying over K_2CO_3 the ether was evaporated and the residue distilled under reduced pressure. Contaminating aminomethylpyrimidine sublimated. A slightly yellow fraction with a constant boiling point, 109—110°/2 mm, was obtained; yield 0.0993 mole (17 g; 51 %). The fraction was, however, not quite pure, as it contained some of the starting material, 2-amino-4-methylpyrimidine. The fraction was therefore dissolved in 200 ml of petroleum ether and the solution inocculated with some crystals of aminomethylpyrimidine. After storing in a refrigerator for three months the aminomethylpyrimidine had precipitated. The petroleum ether solution was filtered, the solvent evaporated and the residue twice distilled under reduced pressure. The compound was obtained as a colourless oil; b.p. 140.5—141°/14 mm; $n_D^{20} = 1.5317$.

Calc. C 60.0 H 8.95
Found » 59.7 » 8.85

Picrate, $C_{15}H_{19}N_7O_7$ (409.4). Small, yellow laths from ethanol-water; m.p. 186—189°.

Calc. C 44.0 H 4.68
Found » 44.2 » 4.48

2-(β-Diethylaminoethylamino)-4-methylpyrimidine, $C_{11}H_{20}N_4$ (208.3). A solution of 0.275 mole (30 g) of 2-amino-4-methylpyrimidine in 100 ml of dry toluene was added as in the preceeding preparation to a mixture of 0.274 mole (10.7 g) of sodamide and 100 ml of dry toluene. The mixture was kept at 100° for three hours, and then 0.138 mole (23.7 g) of freshly prepared dry β-diethylaminoethyl chloride was slowly added. The reaction mixture was kept at 100° for 24 hours. After cooling and filtering the toluene solution was washed with water. The toluene solution was dried over K_2CO_3 and the solvent evaporated. The residue was distilled under reduced pressure and a yellow fraction with a constant boiling point, 107°/0.9 mm., was obtained; yield 0.0634 mole (13.7 g; 46 %). To get a pure substance the same petroleum ether method was used as above. The purified compound was again distilled, and a colourless oil was obtained with b.p. 145.5—146°/12 mm. or 163—164°/21 mm; $n_D^{20} = 1.5217$.

Calc. C 63.4 H 9.68
Found » 63.2 » 9.56

Dipicrate, $C_{23}H_{26}N_{10}O_{14}$ (666.5). Thin, yellow needles from methanol-water; m.p. 170—172.5°.

Calc. C 41.4 H 3.93
Found » 41.6 » 3.97

SUMMARY

N-(β-Diethylaminoethyl)-2,6-dimethylaniline, closely related to xylocaine, 2-(β-dimethylaminoethylamino)-4-methylpyrimidine, and the corresponding diethylaminoethylamino compound have been synthesized and tested for their local anaesthetic, spasmolytic, and histaminolytic power.

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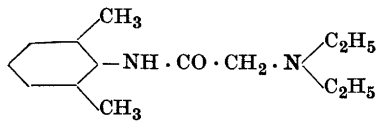
ACTA CHEMICA SCANDINAVICA 6 (1952) 1010–1015

Studies of Local Anaesthetics. VII [1–6]

NILS LÖFGREN and BERTIL TAKMAN

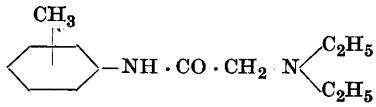
Institute of Organic Chemistry and Biochemistry, University of Stockholm, Stockholm, Sweden

It has already been shown that xylocaine



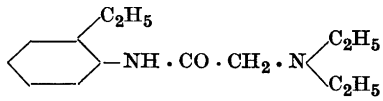
differs markedly from its five nuclear isomers, both in its physico-chemical and its local anaesthetic properties. The *o*-effect in xylocaine has been demonstrated by Löfgren [5] and by Fischer and Löfgren [7].

Of the next lower nuclear homologues of xylocaine, *i.e.* the three toluidides,



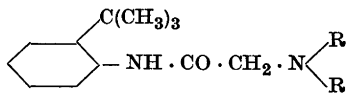
the one with the methyl group in the *o*-position seems to have a somewhat longer duration (tests performed on the tongue, *cf.* also Erdtman and Löfgren [8]), though the effect is not sufficient for the compound to be of use medically (it is also an irritant, *cf.* Löfgren [5]).

In this investigation we have synthesized and studied pharmacologically the *o*-ethyl compound



I

and two tertiary butyl derivatives



R = methyl: II

R = ethyl: III

To produce I commercial 2-nitro-ethylbenzene was reduced with iron and acetic acid, essentially by Löfgren's general method [1, 5]. (The amine has been obtained earlier [9] by reducing the nitro compound with iron and acetic acid, but with quite a different technique.) From 2-amino-ethylbenzene we prepared ω-chloro-2-ethylacetanilide with the aid of the acetate buffer method [1–5] and then I in the usual manner.

For the preparation of II and III we started with tert. butylbenzene. This hydrocarbon cannot be nitrated directly to *o*-nitro-tert. butylbenzene, as was found by du Toit Malherbe [10] and by Shoesmith and Mackie [11]. The latter authors prepared *o*-nitro-tert. butylbenzene by reducing the 4-nitro group in 2,4-dinitro-tert.butylbenzene with H_2S and eliminating the resulting amino group by diazotization in alcoholic solution. We used the same methods of synthesis with slight modifications. 2,4-dinitro-tert.butylbenzene was obtained by Shoesmith and Mackie by nitration of tert.butylbenzene with a nitric-sulphuric acid mixture at 60°, or by nitration of 4-nitro-tert.butylbenzene with nitric acid at 60°. We believe the description of their method must be incomplete, as we could not obtain the desired compound in spite of repeated experiments. After much experimental work we found an acceptable method for preparing 2,4-dinitro-tert.butylbenzene by nitrating 4-nitro-butylbenzene — easily obtained by Shoesmith and Mackie's method — at -10° in a nitric--sulphuric acid mixture, the yield being 86 %. We obtained *o*-amino-tert. butylbenzene from the nitro compound by reducing with iron and acetic acid (a modification of Löfgren's method being applied) and then II and III *via* the chloroacetyl derivative, prepared by the acetate method (*cf.* above); *o*-amino-tert.butylbenzene has been obtained previously [12] by reducing the nitro compound with tin and hydrochloric acid.

All three compounds are active when tested on rabbit cornea. They were compared with xylocaine under the same conditions (pH 6.0). I has about the same duration as xylocaine but a longer latency time. II gives only a very slight anaesthesia, whereas III has twice the duration of xylocaine and a longer latency time. III is weakly irritating to the eye. The toxicities were determined for I and III, the LD_{50} values as determined from subcutaneous injections in white mice being 0.93 g/kg and 1.52 g/kg respectively. The corresponding value for xylocaine is 0.39 g/kg. Hence, both compounds are less toxic than xylocaine.

I and III were tested for their efficiency on man; xylocaine was compared with I in block anaesthesia (finger anaesthesia), and with III in intercutaneous wheal. Both compounds showed a much shorter duration than xylocaine and, contrary to it, they were strong irritants. Further I, II,

and III were tested for their spasmolytic and histaminolytic power. No appreciable effects were found.

EXPERIMENTAL *

2-Amino-ethylbenzene, $C_8H_{11}N$ (121.2). A solution of 0.16 mole (24 g) of 2-nitro-tert. butylbenzene in 40 ml of ethanol was placed in a beaker fitted with a Hershberg stirrer. 1.33 moles (80 g) of glacial acetic acid were divided into three equal portions, one of which was mixed with the alcoholic solution. Under vigorous stirring 40 ml of water was added and then 0.54 g atom (30 g) of iron powder. The temperature began to rise immediately and was kept below 70° by adding crushed ice directly to the reaction mixture. The remaining two portions of the acetic acid were introduced after 15 and 30 minutes, respectively. When the temperature had dropped to 45° the mixture was heated to 85° for 35 minutes, vigorous stirring being maintained. Then 100 ml of water was added, and the oxide sludge centrifuged off and washed well with 80 ml of 1 *N* acetic acid (the centrifugated solutions protected against atmospheric oxygen by CO_2-current). The combined aqueous solutions were mixed with 60 g of Rochelle salt dissolved in 60 ml of water, and an excess of concd. ammonia. The liberated base was taken up in ether. After drying (K_2CO_3), the ether was evaporated and the residue distilled under reduced pressure; b.p. 97—100°/16 mm; (the recorded[9] b.p. is 210—211° at ordinary pressure); yield 0.12 mole (14.5 g; 75 %).

ω-Chloro-2-ethylacetanilide, $C_{10}H_{12}ClNO$ (197.7). 0.120 mole (14.5 g) of 2-aminoethylbenzene was dissolved in 94 ml of glacial acetic acid. After cooling to 10°, 0.132 mole (14.9 g) of chloroacetyl chloride was added rapidly. A solution of 40 g of AcONa · 3 H_2O in 170 ml of water was added quickly under vigorous shaking. After 35 minutes, shaking was discontinued. The precipitate was filtered off, well washed with water and dried in air. The yield was 0.101 mole (20.0 g; 84 %) of colourless crystals. The m.p. of this non-recrystallized product was 91—92°. Without further purification and elementary analysis, the product was used as the starting material for ω-diethylamino-2-ethylacetanilide.

ω-Diethylamino-2-ethylacetanilide, $C_{14}H_{22}N_2O$ (234.3). In a flask fitted with a reflux condenser protected with a soda-lime tube, a mixture of 0.097 mole (19 g) of ω-chloro-2-ethylacetanilide, 135 ml of dry benzene, and 0.26 mole (19 g) of diethylamine was boiled for six hours. The precipitated diethylammonium chloride was filtered off and washed with dry ether; yield 0.094 mole (10 g; 97 %). The combined benzene and ether solutions were extracted sufficiently with 3 *N* HCl. The aqueous solution was made alkaline with ammonia and the liberated base taken up in ether. The ethereal solution was dried over K_2CO_3, the solvent evaporated and the residue distilled under reduced pressure. A colourless oil boiling at 131—132°/0.25 mm was obtained; $n_D^{20} = 1.5269$.

Calcd.	C 71.8	H 9.46	Equiv.-weight:	Calcd.	234
Found	» 71.7	» 9.41	»	Found **	234

Picrate, $C_{20}H_{25}N_5O_8$ (463.4). Small, yellow laths from methanol-ethanol; m.p. 178—182°.

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* All our melting points are uncorrected.

** Titration of the base in 30 % ethanol with 0.1 *N* HCl, mixed indicator methylene blue — -methyl red.

The hydrochloride, the sulphate, and the nitrate are very hygroscopic.

2,4-Dinitro-tert.butylbenzene, $C_{10}H_{12}N_2O_4$ (224.2). 8.8 moles of nitric acid of sp. gr. 1.52 (556 g) and 5.6 moles of concd. sulphuric acid (556 g) were placed in a beaker provided with a mechanical stirrer and surrounded by a freezing mixture. 0.932 mole (167 g) of 4-nitro-tert.butylbenzene (prepared by Shoesmith and Mackie's method [11]) was added during two hours, so that the temperature of the reaction mixture was kept at $-10°$. Stirring was continued for another hour at the same temperature. The reaction mixture was poured on ice. The solid was filtered off, washed with 2 *N* NaOH solution and then with water. After drying, the product was recrystallized from petroleum ether. The compound appeared as small colourless crystals of m.p. 61—63°. (The recorded [11] m.p. is 61—62°.) The yield was 0.803 mole (180 g; 86 %).

4-Amino-2-nitro-tert.butylbenzene, $C_{10}H_{14}O_2N_2$ (194.2). A mixture of 0.714 mole (160 g) of 2,4-dinitro-tert.butylbenzene, 80 ml of ammonia (d = 0.88), and 610 ml of ethanol was placed in a beaker provided with a coil tube for cooling water. Under shaking, a rather slow gas-stream of H_2S was bubbled into the reaction mixture. By regulating the water flow of the cooling system the temperature was not allowed to exceed 25°. H_2S was let in until a sample of the mixture did not give an oily residue when boiled for some minutes with about four times its volume of 3 *N* HCl. The reaction mixture, diluted with water, was put into a refrigerator overnight. The solid was filtered off and repeatedly washed with ice-water. The slightly red filtrate was rejected. The solid was then heated under reflux with an excess of 3*N* HCl for five minutes and filtered hot from sulphur. When the hydrochloride commenced to crystallize, an excess of ammonia (d = 0.88) was immediately added. A solidifying oil separated from the now red liquid. After one hour in a refrigerator the nitroamine was sucked off, washed with diluted ammonia and dried on porous plates. After recrystallization from alcohol the yield was 0.554 mole (108 g; 78 %) of yellow plates; m.p. 45—50°. (The recorded [11] m.p. is 55°.)

2-Amino-tert.butylbenzene, $C_{10}H_{15}N$ (149.2). The starting material, 2-nitro-tert. butylbenzene, was prepared from 4-amino-2-nitro-tert.butylbenzene by Shoesmith and Mackie's method [11] without any modifications.

The amine was prepared by reducing 0.243 mole (43.5 g) of the nitro compound with 0.790 g atom (44.1 g) of iron and 1.88 moles (114 g) of glacial acetic acid in the same way as described for the preparation of 2-amino-ethylbenzene (*cf.* above). However, the amine base was so weak that it could not be extracted sufficiently from the iron oxide sludge in the usual manner with acetic acid, evidently because of its strong *o*-effect. The sludge was therefore dissolved in the minimum quantity of 4 *N* HCl, and, after filtering, the solution was mixed with 90 g of Rochelle salt dissolved in 90 ml of water and with an excess of concd. ammonia. The liberated base was extracted from the dark-coloured solution with ether. After drying (K_2CO_3), the solvent was evaporated and the residue distilled under reduced pressure; yield 0.171 mole (25.5 g; 70 %); b.p. 111—112°/18—19 mm. (The recorded [12] b.p. is 233—235° at ordinary pressure.) The acetyl derivative, synthesized in the usual manner with glacial acetic acid and acetic anhydride, was obtained as colourless needles with m.p. 162°. (The recorded [11] m.p. is 161°.)

ω-Chloro-2-tert.butylacetanilide, $C_{12}H_{16}ClNO$ (225.7). This compound was prepared in the same manner as described for ω-chloro-2-ethylacetanilide (*cf.* above). The yield of non-recrystallized product was 82 %. Recrystallized from *n*-butyl ether and from methanol-water the m.p. of the compound was 96—97°.

Calcd. C	63.6	H 7.15
Found »	63.6	» 7.25

ω-Dimethylamino-2-tert.butylacetanilide, $C_{14}H_{22}N_2O$ (234.3). In an iron autoclave a mixture of 0.044 mole (10 g) of ω-chloro-2-tert.butylacetanilide and a solution of 0.13 mole (6 g) of dimethylamine in 60 ml of dry benzene was heated at 100° for 6 ½ hours. After cooling, the solid was filtered off and washed with dry benzene. From the combined benzene solutions the solvent was evaporated and the residue extracted with hot 3 *N* HCl. After filtering, the solution was purified by shaking out with some ether and then mixed with 10 g of Rochelle salt dissolved in 15 ml of water. An excess of concd. ammonia was added and the liberated base taken up in ether. After drying with K_2CO_3, the solvent was evaporated and the residue distilled under reduced pressure. A faintly violet oil boiling at 141—142°/0.6 mm was obtained. In spite of repeated distillations the colour remained. The oil was therefore dissolved in dry ether and a dry ethereal solution of HCl added in some excess. The precipitated hydrochloride was filtered off, washed with dry ether and recrystallized from isobutyl alcohol — ethanol. From this salt, the free base was regenerated and again distilled; b.p. 136°/0.3 mm.; yield 0.037 mole (8.7 g; 84 %); $n_D^{20} = 1.5291$.

Calcd. C 71.8 H 9.46 Equiv.-weight: Calcd. 234
Found » 71.5 » 9.35 » » Found 234

Hydrochloride, $C_{14}H_{23}ClN_2O$ (270.8). Colourless leaflets from *n*-propanol-ethanol, easily soluble in water; m.p. 264—265° (decomp.).

Calcd. Cl 13.09
Found » 13.05 (Mohr)

ω-Diethylamino-2-tert.butylacetanilide, $C_{16}H_{26}N_2O$ (262.4). In a flask fitted with a reflux condenser protected with a soda-lime tube, a mixture of 0.044 mole (10 g) of ω-chloro-2-tert.butylacetanilide, 0.115 mole (8.4 g) of diethylamine, and about 50 ml of dry benzene was boiled for ten hours. The precipitated diethylammonium chloride (0.037 mole, 4.1 g, 84 %) was filtered off and washed with dry benzene. The combined benzene solutions were extracted sufficiently with 2 *N* hydrochloric acid. The aqueous solution was purified by extracting once with ether and then made alkaline with ammonia. The liberated base was isolated in the usual manner (*cf.* the preparation of ω-diethylamino-2-ethylacetanilide). A colourless oil boiling at 157°/0.7 mm. was obtained; $n_D^{20} = 1.5271$; yield 0.033 mole (8.6 g; 75 %).

Calcd. C 73.2 H 9.99 Equiv.-weight: 262
Found » 72.8 » 10.0 » » 261

Hydrochloride, $C_{16}H_{27}ClN_2O$ (298.8). Long, thin rods from isobutyl alcohol — butyl ether, easily soluble in water, ethanol, and propanol; m.p. 202—205°.

Calcd. Cl 11.86
Found » 11.88 (Mohr)

SUMMARY

ω-Diethylamino-2-ethylacetanilide, ω-dimethylamino-2-tert.butylacetanilide, and ω-diethylamino-2-tert.butylacetanilide have been tested for their local anaesthetic, spasmolytic, and histaminolytic power.

Acknowledgements are made to Dr. S. Wiedling of the Biological Department of A.B. Astra, Södertälje, for performing the pharmacological tests and to Dr. T. Gordh of the Caroline Hospital, Stockholm, who carried out the intercutaneous tests on man.

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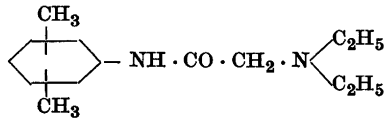
ACTA CHEMICA SCANDINAVICA 6 (1952) 1016–1019

Studies on Local Anaesthetics. VIII[1–7]

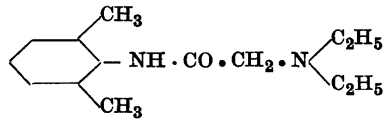
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It has been shown earlier [1, 2, 5] that of the six isomers,

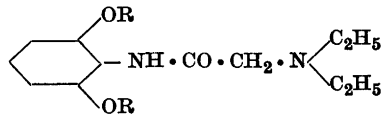


the one with the two methyl groups in the *o*-positions to the anilide nitrogen has outstanding physico-chemical and local anaesthetic properties. The *o*-effect in this compound, *i. e.* xylocaine



has been demonstrated by rather extensive measurements [5, 8].

Now, it seemed to us of interest to investigate such compounds in which the two methyl groups in xylocaine are replaced by other groups. In this work we have synthesized two alkoxy derivatives (I) and (II),



I: R = methyl

II: R = ethyl

and studied their pharmacological properties.

When tested on rabbit cornea, under the same conditions (pH 6.0), the methoxy compound I has a duration somewhat shorter than that of xylocaine; the ethoxy compound II shows about the same duration as that of xylocaine.

In latency time, both compounds are inferior to xylocaine. The methoxy derivative was also tested intercutaneously on man and found to have a much shorter duration than xylocaine. In this experiment the compound proved, unlike xylocaine, to be slightly irritating. The ethoxy compound is a strong irritant when tested subcutaneously on man. Both compounds are less toxic than xylocaine, the LD_{50} values as determined from subcutaneous injections in white mice, being 1.4 g/kg for the methoxy compound and 0.70 g/kg for the ethoxy compound. Further, the two compounds were tested for their spasmolytic and histaminolytic power. No appreciable effects were found.

EXPERIMENTAL *

2-Nitroresorcinol. This compound, required as starting material for both I and II, has been synthesized by Kauffmann and de Pay [9], and we followed their method. Their description, however, is rather incomplete, and we had to determine the reaction conditions. 44 g of finely powdered resorcinol (0.40 mole) was gradually added to 300 g of fuming sulfuric acid of sp. gr. 1.875 (2.89 moles H_2SO_4 + 0.204 mole SO_3) under vigorous stirring and the mixture then heated on a water bath for 30 min. After cooling, a mixture of 25.2 g of fuming nitric acid of sp. gr. 1.52 (0.40 mole) and 80 g of fuming sulfuric acid of sp. gr. 1.875 (0.77 mole H_2SO_4 + 0.054 mole SO_3) was cautiously added under vigorous stirring and external cooling at such a rate that the temperature did not exceed 15°. The suspension was agitated another hour, and steam distilled after addition of 290 ml of water. After 30 minutes, superheated steam (150°) was used. Yield 25 g (41 %) of almost pure product. M.p. after recrystallization from 50 % ethanol at 85°.

2,6-Dimethoxyaniline. This compound has previously [10] been synthesized by reduction of the corresponding nitro compound with tin and hydrochlorid acid, but we used catalytical reduction. 73 g of 2,6-dimethoxynitrobenzene (0.40 mole) was dissolved in 400 ml of hot ethanol. The solution was hydrogenated at 80° and a hydrogen pressure of 75 kg/cm^2 in the presence of Raney nickel (10 g, moist). After one hour no more hydrogen was consumed and after cooling and filtration the reaction mixture was evaporated to about 100 ml. This solution was cooled in ice and after standing overnight the crystals formed were collected. Yield 57 g (93 %). After recrystallization from light petroleum (b.p. 40—60°) they melted at 75.5—77°.

ω-Chloro-2,6-dimethoxyacetanilide, $C_{10}H_{13}ClNO_3$ (230.7). The compound was prepared according to Löfgren's general method [1,5]. 46 g of 2,6-dimethoxyaniline (0.32 mole) was dissolved in 255 ml of glacial acetic acid. After cooling to 10°, first 37 g of chloroacetyl chloride (0.38 mole) and then 99 g of sodium acetate (0.73 mole) in 445 ml of water were added under mechanical agitation. The resulting mixture was shaken for 30 minutes and the precipitate collected, washed with much water and after drying, recrystallized from ethanol (600 ml). Yield 52.4 g (75 %). M.p. 166—167°.

Calc. Cl 15.4
Found » 15.4

* All our melting points are uncorrected.

ω-Diethylamino-2,6-dimethoxyacetanilide, $C_{14}H_{22}N_2O_3$ (266.3). 22 g of the chloro compound (0.095 mole) and 27 ml of diethylamine (0.26 mole) were refluxed in benzene for five hours. The reaction mixture was filtered, benzene and excess of diethylamine evaporated and the residue dissolved in 3 *N* HCl (35 ml). After filtering the aqueous solution was extracted with ether (50 ml), made alkaline with concentrated ammonia and the liberated base sufficiently extracted with ether (3 × 50 ml). After drying, the combined ethereal extracts were evaporated and the residue distilled twice at a bath temperature of 200° and a pressure of 0.01 mm. Yield 18 g (68 %).

Calc. C 63.2 H 8.26
Found » 63.2 » 8.26

2,6-Diethoxyaniline. This has previously [10] been prepared in the same way as the corresponding methoxy compound. We reduced the nitro compound, prepared by Turner's method [10], with iron and acetic acid by Löfgren's method [1,5]. However, after separation of the ethereal layer and evaporating the solvent only a small residue was left, but the very weak base could be obtained by treating the iron oxide sludge with superheated steam in a yield of 46 % *.

After recrystallizing from light petroleum the m.p. was 57°. It could also be distilled *in vacuo;* b.p. 142–143°/13 mm.

ω-Chloro-2,6-diethoxyacetanilide, $C_{12}H_{16}ClNO_3$ (257.7). Prepared in the same way as the corresponding methoxy compound from 2,6-diethoxyaniline and chloroacetyl chloride in a yield of 89 %. M.p. after recrystallization from benzene was 129–130°.

Calc. C 55.9 H 6.26
Found » 55.7 » 6.28

ω-Diethylamino-2,6-diethoxyacetanilide, $C_{16}H_{26}N_2O_3$ (294.4). Prepared in the same way as the methoxy compound by refluxing ω-chloro-2,6-diethoxyacetanilide and diethylamine in dry benzene for six hours. Yield 66 %, b.p. 169°/0.6 mm, solidifying p. 32.5°; $n_D^{20} = 1.5174$ (supercooled substance).

Calc. C 65.3 H 8.90 Equiv. wt.: 294
Found » 64.8 » 8.92 » » 293 **

SUMMARY

ω-Diethylamino-2,6-dimethoxyacetanilide and the corresponding diethoxy compound have been synthesized and tested for their local anaesthetic, spasmolytic, and histaminolytic power.

Acknowledgements are made to Dr. S. Wiedling of the Biological Department of A.-B. Astra, Södertälje for performing the pharmacological tests and to Dr. T. Gordh of the Caroline Hospital, Stockholm who carried out the intercutaneous tests on man.

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* The reduction method has previously been used on a large number of nitro compounds and always given good yields. Turner does not mention the yield obtained.

** Titration of the base in 30 % ethanol with 0.1 *N* HCl, mixed indicator methylene blue-methyl red.

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Studies on Analgesics

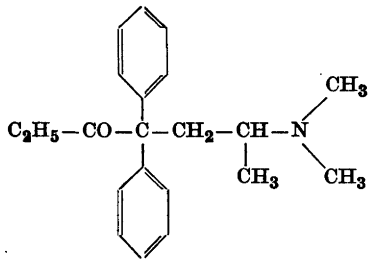
I. Synthesis of Ethyl 4-(N-Morpholinyl)-1,1-di-(α-thienyl)-butyrate and its Pharmacological Properties

NILS LÖFGREN and CLAËS TEGNÉR

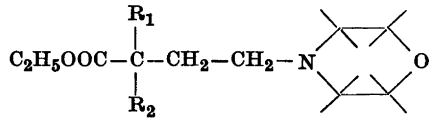
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Since the systematic work of Bockmühl and Erhardt[1] on amidone (I) and its derivatives, a number of other papers have been published on the synthesis of similar compounds. Speeter, Byrd, Cheney, and Binkley[2] prepared compound II, which has about the same analgetic effect as amidone and is ten times less toxic. Compound III, structurally very similar to II, was synthesized by Brown, Cook, and Heilbron[3], and proved to be four times as effective as pethidine.

In this investigation compound IV has been prepared.



I



II $R_1=R_2=$phenyl-
III $R_1=$phenyl-, $R_2=$thienyl-
IV $R_1=R_2=$thienyl-

Several attempts were made to obtain the starting material, di-(α-thienyl)-methane. This compound has already been described in the literature[4], but the yield never exceeded 10 %. Blicke and Burkhalter's method[5] (in which dithienylmethane was obtained as a by-product on preparing α-(chloromethyl)-

thiophene) was first tried, but though several attempts were made, no dithienylmethane could be isolated. The second method of synthesis was *via* the ketone, thienone. By letting α-thienyllithium react with carbon dioxide we obtained a yield of 67 % of the ketone. However, the compound desintegrated and polymerised on reduction.

The final synthesis of dithienylmethane was carried out in accordance with Haubein's observation [6] that no bibenzyl is formed when ArLi reacts with $PhCH_2Cl$. Thus, in the reaction between chloromethylthiophene and thienyllithium a 66 % yield of dithienylmethane was obtained. Ethyl 4-(N-morpholinyl)-1,1-di-(α-thienyl)butyrate was then made by the method used by Brown, Cook, and Heilbron [3] for preparing compound II. Hence the monopotassium derivative of dithienylmethane, obtained by the action of potassium in liquid ammonia, was carbethoxylated with diethyl carbonate. The ester thus prepared was condensed with N-(2-chloroethyl)morpholine in the presence of sodamide in dry toluene, yielding the ethyl 4-(N-morpholinyl)-1,1-di-(α-thienyl)butyrate (IV).

The compound has an analgetic effect somewhat less than that of pethidine. The toxicity is remarkable low($LD_{50} > 500$ mg/kg white mouse subcut.), morphine and pethidine being more toxic. The local anaestetic effect was compared with that of xylocaine. On rabbit cornea the compound has, under the same conditions (pH 3.7), a duration somewhat less than that of xylocaine and a much longer latency time. Further, the compound was tested for its spasmolytic and histaminolytic power. No appreciable effects were found.

EXPERIMENTAL *

Di-(α-thienyl)ketone (thienone). In a three-necked flask, equipped with a dropping funnel, a stirrer, a reflux condenser, and a gas inlet tube, was placed a mixture of 0.57 mole (48 g) of thiophene and 200 ml of absolute ether. A stream of dry nitrogen was used to expel the air. To the solution of thiophene was added 300 ml of a 1.35 *N* ethereal solution of butyllithium [7]. A vivid reaction took place immediately and the ether refluxed spontaneously. When all butyllithium had been added the solution was kept under gentle reflux for fifteen minutes, by means of a water bath. Then the stream of nitrogen gas was stopped, and carbon dioxide was allowed to bubble into the solution until test for organolithium compound [8] was negative (10 hours). A reddish solution and a white fine grained precipitate that had settled at the bottom were obtained. The content of the vessel was decomposed with water, and the ethereal layer separated and washed completely with 2 *N* sodium hydroxide solution (to remove thiophenecarboxylic acid). The ethereal layer was dried ($MgSO_4$) and the solvent evaporated. Greyish green needles

*) All our melting points are corrected.

separated; yield 25 g (67 %). On recrystallization from isopropyl ether a constant melting point of 90—90.5° was obtained.

Several attempts to reduce this compound with sodium and alcohol or by Wulff-Kishner's method failed.

α-(Chloromethyl)thiophene. The compound was made by the method given by Blicke and Burkhalter [5]. As a by-product in the synthesis they reported dithienylmethane. We therefore carried out the synthesis at first chiefly to obtain this hydrocarbon (*cf.* above). However, we could obtain no isolable amounts of dithienylmethane. Fortunately we were able to use the chief product, chloromethylthiophene, for the synthesis of dithienylmethane (*cf.* below). However, chloromethylthiophene distilled at 72.5—73.5°/18 mm contrary to Blicke *et al.*'s[4] statement that the boiling point is 81—82°/18 mm. We found the refractive index, n_D^{20}, to be 1.5638.

We also obtained a second fraction with the same boiling point as that reported by Blicke and Burkhalter [5]. They stated this fraction to be dithienylmethane with the correct m.p. 45—47°. After repeated recrystallizations from petroleum ether, we obtained the compound as small needles with m.p. 35—35.5°, which turned dark blue after a few days. On boiling with methanol the compound gave off hydrogen chloride. Further, our product was without smell, whereas dithienylmethane has a very characteristic odour. It is therefore evident that the second fraction could not be dithienylmethane.

Di-(α-thienyl)methane. As we could not find any useful method in the literature for preparing dithienylmethane we developed a synthesis using α-chloromethylthiophene. The apparatus used was the same as that previously described for α-thienylketone. To 150 ml of an ethereal solution of 0.37 mole (31 g) of thiophene was added 0.26 mole (400 ml of a 0.64 *N* solution of butyllithium) at such a rate as to cause gentle reflux. After 2 hours of heating on a water bath in order to complete the reaction the solution was allowed to cool. 0.32 mole (42 g) of chloromethylthiophene was then added slowly. The ethereal solution was boiled under reflux until test for organolithium compound [8] was negative (40 hours). The content of the vessel was decomposed with water and the ethereal layer separated, washed with water and dried ($MgSO_4$). After filtering, the ether was driven off and the residue distilled. The compound was obtained as a faintly yellow oil which solidified rapidly; b.p. 128—130°/11 mm.; yield 31 g (66 %). After recrystallization from petroleum ether the dithienylmethane appeared as colourless needles; m.p. 44—45°.

Ethyl di-α-thienylacetate, $C_{12}H_{12}O_2S_2$ (252.3). Ammonia gas was introduced through a potassium hydroxide drying tube into a three-necked flask of 500 ml, equipped with a dropping funnel, a Hershberg stirrer and a condenser for cooling with freezing mixture (trichloroethylene and solid carbon dioxide). The condenser was kept at −50° and the flask at −35°. When the flask contained 300 ml of liquid ammonia the cooling bath was taken away and 0.18 mole (7.2 g) of potassium cut into pieces was added for a period of half an hour. After one hour the blue colour had disappeared. The condenser with freezing mixture was replaced by an ordinary water cooled reflux condenser and 0.17 mole (30 g) of dithienylmethane in 375 ml of ether was added drop by drop for one and a half hours. On the addition the solution turned brown. When half the volume of the dithienylmethane solution had been added the reaction mixture was heated to remove ammonia. After the solution had reached room temperature 0.084 mole (9.9 g) of diethyl carbonate was added. The solution was boiled for 12 hours under reflux. It was then decomposed with 300 ml of diluted acetic acid (5 %). The ethereal layer was separated and washed with water. The solution was dried ($MgSO_4$) and the ether driven off, leaving a reddish brown

viscous oil (35 g). On fractionating *in vacuo* a fore-run of 15 g of dithienylmethane was collected. The fraction of ethyl dithienylacetate had a boiling point of 124—125°/0.6 mm.; $n_D^{20} = 1.5728$; m.p. ~ 7°; yield 12 g (28 %).

Calc.	C 57.1	H 4.80	S 25.4
Found	» 57.1	» 4.94	» 25.2

Ethyl 4-(N-morpholinyl)-1,1-di-(α-thienyl)butyrate, $C_{18}H_{23}NO_3S_2$ (365.5). To a 100 ml flask, equipped with a stirrer and a reflux condenser with a potassium hydroxide drying tube, was added 0.0284 mole (7.15 g) of the above mentioned ester, 0.0311 mole (4.65 g) of N-(2-chloroethyl)morpholine and 0.0310 mole (1.21 g) of sodamide suspended in 35 ml of dry toluene. On the addition of sodamide the solution turned brown, and on heating the colour deepened and ammonia was given off. The temperature of the mixture was kept at 120—130° for two hours, after which time the solution had turned light yellow. The toluene solution was shaken sufficiently with 2 *N* hydrochloric acid and from the combined extracts the base was liberated by addition of ammonia (1 : 1) and taken up in ether. After washing with water and drying (Na_2SO_4), the ether was driven off leaving a reddish brown oil (8.8 g), which was fractionated by distillation. The compound was obtained as a colourless oil; b.p. 164—164°/0.01 mm.; $n_D^{20} = 1.5676$; yield 5.85 g (57 %).

Calc.	C 59.1	H 6.34
Found	» 59.0	» 6.31

Nitrate, $C_{18}H_{24}N_2O_6S_2$ (428.5). Rhombic laths from propanol; m.p. 145—146°. The salt is slightly soluble in water.

Calc.	C 50.4	H 5.64
Found	» 50.3	» 5.60

SUMMARY

Ethyl 4-(N-morpholinyl)-1,1-di-(α-thienyl)butyrate was prepared and proved itself to have a distinctly analgetic effect and a remarkably low toxicity.

Acknowledgments are made to A.-B. Astra for financial support. We wish to express our sincere gratitude to Dr. S. Wiedling of the Biological Department of Astra and to Dr. L. Olsson of the Royal Veterinary College.

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On Polyenic and Polyynic Compounds in *Centaurea cyanus* L.

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It has already been shown [1] that at least two compounds, "Centaur X" and "Centaur Y", with highly characteristic absorption spectra in the ultraviolet, occur in Centaurea species (*C. cyanus* L., *C. jacea* L., and *C. scabiosa* L.). The compounds were found in the lipophilic solvent extracts of the herbs. Later, Stavholt and Sørensen [2] found a compound in *Artemisia vulgaris* L. with a spectrum almost identical with that of our CX-comp.*, *i.e.* they have the same chromophoric system. Even at an early stage in our investigations, we could not exclude the possibility of CX and CY spectra belonging to more than two compounds (*cf.* the earlier paper). We made many attempts to get a further fractionating. As the usual routine methods failed, we applied the countercurrent distribution principle.

A preparation, obtained by distillation in vacuo (115—165° / $5 \cdot 10^{-3}$ mm) of a chlorophyll free ethereal fraction (*cf.* the earlier paper), was distributed between cyclohexane and 90 % methanol, the cyclohexane being the mobile phase. The number of transfers was 49. The spectroscopic analysis showed the presence of a highly complicated component system. At least four CX and two CY components could be demonstrated.

The CX components formed two groups with strongly different partition coefficients ($K_{C_6H_{12}/MeOH}$), on the one hand CX_1 and CX_2 with $K = 0.14$ and 0.19 respectively; on the other CX_3 and CX_4 with $K = 5.1$ and 7.2 respectively. The two CY components have K values = 0.75 and 11. The CX compounds have, surprisingly enough, only two spectra, one belonging to CX_1 and CX_3 and the other to CX_2 and CX_4. The two CY compounds have identical spectra.

* C denotes Centaur.

From a preparative point of view, the experiment indicates that the countercurrent distribution method may be useful for an isolation of CY_1 and possibly of CY_2. but that the other compounds can be separated only with great difficulty.

It seems obvious to us that CX_1 and CX_3 belong to one group with the same chromophoric system, whereas CX_2 and CX_4 belong to another such group; CY_1 and CY_2 are also chromophorically identical compounds.

Table 1 shows λ_{max} of the absorption spectrum of Sørensen's CX compound compared with λ_{max} of our CX_1 or CX_3 compound.

Table 1. Comparison between the absorption bands of Centaur X_1 or X_3 (measurements in cyclohexane) and of Sørensen's Centaur X compound (measurements in hexane); λ_{max} in Å and ν_{max} in sec^{-1}.

λ_{max} of Sørensen's Centaur X (hexane)	3485,	3260,	3064,	2895,	2694,	2592,	—
λ_{max} of Centaur X_1 or X_3 (cyclohexane)	3500,	3270,	3070,	2905,	2705,	2600,	2555
$\nu_{max} \cdot 10^{-12}$	857,	917,	977,	1033,	1109,	1154,	1174
$\Delta\nu$		60	60	56			

As will be seen from Table 1 the compounds have almost the same spectrum. The main difference is that there is a very weak band at 2555 Å, which is absent in Sørensen's CX compound. The positions of the absorption maxima of the CX_2 or CX_4 compounds are shown in Table 2.

Table 2. Absorption bands of Centaur X_2 or X_4 (measurements in cyclohexane); λ_{max} in Å and ν_{max} in sec^{-1}.

λ_{max} of Centaur X_2 or X_4 (cyclohexane)	3395,	3170,	2985,	2450
$\nu_{max} \cdot 10^{-12}$	884,	946,	1005,	1224
$\Delta\nu$		62	59	

It may be mentioned that the absorption curve of Sørensen's CX compound shows small inflexions or perturbations at the same places as our CX_2 or CX_4 have their maxima. The value $\triangle \nu \sim 60 \cdot 10^{12}$ sec^{-1} occurring in all CX compounds indicates the presence of carbon-carbon tripple bonds. In a recent investigation Cook, Jones and Whiting [3] synthesized acetylenic compounds of the type

$$CH_3 - CH = CH - (C \equiv C)_n - CH = CH - CH_3$$

and measured their spectra. When $n = 3$ surprising agreement with Sørensen's and our values is obtained as is seen from Table 3, which gives the positions of λ_{max} for the synthetic compound.

Table 3. Absorption bands of compound $CH_3 - CH = CH - (C \equiv C)_3 - CH = CH - CH_3$ *according to C. Cook, E. Jones and M. Whiting (measurements in ethanol);* λ_{max} *in Å.*

λ_{max}	3485,	3250,	3055,	2890,	2690,	2590,	2450–2500 *,	1990

As is seen from Table 3, Cook *et al.* found a band at 1990 Å, which was not given by Sørensen or by us. In our case, we have not made measurements in this short-wave region.

It seems obvious that both CX_1 or CX_3 and Sørensen's CX compound have the sama chromophoric system as the synthetic compound

$$CH_3 - CH = CH - C \equiv C - C \equiv C - C \equiv C - CH = CH - CH_3$$

The two Y compounds CY_1 and CY_2 have identical spectra of quite another type, *cf.* Table 4 and Fig. 1.

Table 4. The absorption bands of Centaur Y_1 *or* Y_2 *(measurements in cyclohexane);* λ_{max} *in Å and* ν_{max} *in* sec^{-1}.

λ_{max} of Centaur Y_1 or Y_2 (cyclohexane)	3195,	3045,	2915,	2800 **
$\nu_{max} \cdot 10^{-12}$	939,	985,	1029,	1071
$\Delta\nu$		46	44	42

Hexatriene-1,3,5 [4], octatetraene-1,3,5,7 [5] and phytofluene [6], which are all polyenes with three, four, and five conjugated double bonds, respectively, have similar spectra. According to Hausser, Kuhn and Seitz [7] conjugated polyenes at −196° have two different values of $\Delta\nu$, $37.0 \cdot 10^{12}$ sec^{-1} and $47.1 \cdot 10^{12}$ sec^{-1}. At room temperature, however, the spectrum is more diffuse; $\Delta\nu$ then shows *one* intermediate value, often at about $44 \cdot 10^{12}$ sec^{-1}. It therefore seems evident to us that CY_1 or CY_2 contains conjugated double bonds.

For polyenes of the type

$$R - (CH = CH)_n - R$$

a simple quantitative relation *** exists between n and the position of the first band (that of the longest wave-length), λ_0:

$$\lambda_0 = k_1 \sqrt{n} + k_2 \qquad (1)$$

k_1 and k_2 are constants differing for the different types of homologous series.

* Inflexion.

** Inflexion point.

*** *Cf.* for instance, Eistert [8].

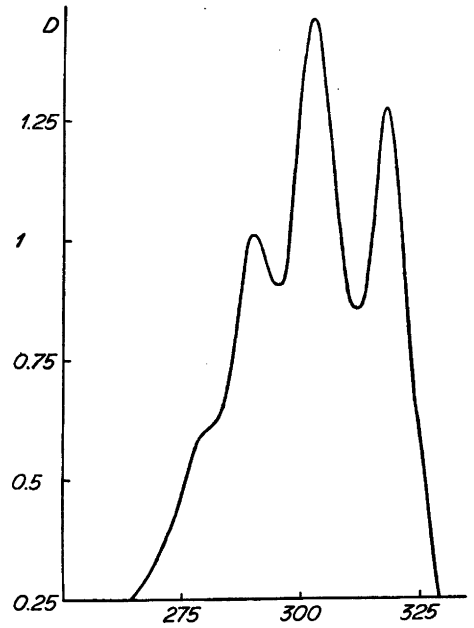


Fig. 1. The absorption spectrum of CY_1 *or* CY_2 *(measurements in cyclohexane); D = optical density.*

The first band of hexatriene [4] and octatetraene [5] are given in the literature at 2675 and 3040 Å respectively (measurements in cyclohexane). When inserting these values in (1) we will find $k_1 = 1362$ and $k_2 = 316$. From these values we are able to calculate λ_0 for decapentaene-1,3,5,7,9, equation (1) giving us $\lambda_0 = 3362$ Å. The first band of CY_1 or CY_2 has $\lambda_0 = 3195$ Å. We therefore assume that the compounds have a chromophoric system consisting of octatetraene with some bathochromic groups.

Values for some bathochromic groups, expressed as fractions of a new additional double bond (|=), are given by Kuhn and Grundmann [9]. For instance, a methyl group situated in the conjugated chain should be regarded as 1/4 |=.

In our case we may find the value of *one* new additional double bond by substracting the ν_0 value of decapentaene from that of octatetraene, giving us $\Delta\nu = 94.5 \cdot 10^{12}$ sec^{-1}. We may use this |= value in our calculations, not only for the first band but also for the others (*cf.* above). If the ν_{max} values of octatetraene are diminished by half the calculated |= value, the values obtained are in excellent agreement with the ν_{max} values of CY_1 or CY_2 found experimentally (*cf.* Table 5).

Table 5. The absorption bands of octatetraene (measurements in cyclohexane). Calculated band positions of dimethyloctatetraene compared with those of Centaur Y_1 or Y_2; λ_{max} in Å and ν_{max} in sec^{-1}.

λ_{max} of octatetraene (cyclohexane)	3040,	2905,	2780,	2678
$\nu_{max} \cdot 10^{-12}$	987,	1033,	1079,	1120
Decr. corresp. to two CH_3 groups $= 2 \cdot \frac{1}{4}\sqrt{\ }$	−47,	−47,	−47,	−47
Calc. $\nu_{max} \cdot 10^{-12}$ of $(Me)_2$-octatetraene	940,	986,	1032,	1073
Calc. λ_{max} of »	3191,	3042,	2907,	2796
Obs. λ_{max} of CY_1 or CY_2	3195,	3045,	2915,	2800

Hence our calculations indicate that CY_1 or CY_2 are octatetraene with 2 substituents, each equal spectroscopically to the methyl group. Though this result seems very plausible other possibilities cannot of course be excluded.

Decatetraene-2,4,6,8 (1,8-dimethyl-octatetraene-1,3,5,7) has been synthesized by Kuhn and Grundmann [10]. The first band of the CY compounds and of decatetraene have the same value, the other bands lie in the neighbourhood of each other though they do not agree very well (*cf.* Table 6).

Table 6. The absorption bands of Kuhn's decatetraene-2,4,6,8 (measurements in hexane); λ_{max} in Å and ν_{max} in sec^{-1}.

λ_{max} of Kuhn's decatetraene (hexane)	3200,	2970,	2830,	2720
$\nu_{max} \cdot 10^{-12}$	938,	1010,	1060,	1103
$\Delta\nu$		72	50	43

However, one of the $\Delta\nu$ values of the synthetic decatetraene seems to us rather peculiar ($72 \cdot 10^{12}$ sec^{-1}), and not characteristic of such a polyene. It is of interest to note that tetradecahexaene-2,4,6,8,10,12, synthesized by the same authors [10] does not show such a confusing $\Delta\nu$ value. (*Cf.* also for instance hexatriene, octatetraene and phytofluene, which show very constant and representative $\Delta\nu$ values.) The elementary analysis of the decatetraene is not quite acceptable, and the authors describe the compound as extraordinarily unstable, a fact that may perhaps have influenced the measurements.

It will be extremely difficult to isolate and characterize fully the CX and CY compounds present in the Centaurea species. CY_1 seems to us to be the only one of the compounds that can be isolated without too great difficulty. For this purpose a large amount of starting material will probably be necessary.

SUMMARY

It has been shown that *Centaurea cyanus* L. contains at least two compounds (Centaur X_1 and X_3) related to decadiene-1,9-triyne-3,5,7, and at least two compounds (Centaur Y_1 and Y_2) related to octatetraene-1,3,5,7.

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The Qualitative Separation of Purines, Pyrimidines, and Nucleosides by Paper Chromatography

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In recent yers much work has been done on paper chromatographic analysis of "nucleo" derivatives [1–7]. The separation into individual components of the mixtures of purines, pyrimidines, and nucleosides as they commonly occur in nature seems, however, to have received but little attention. Hotchkiss' work showed that water-saturated butanol can resolve certain combinations into their components. In connection with our attempts to isolate nebularine — an antibiotic active against *Mycobacteria* — it became necessary to carry out qualitative analyses of complicated systems containing purines, pyrimidines, and nucleosides. We therefore decided to try to separate qualitatively by means of paper chromatography, the 12 common derivatives, *i.e.* adenine, adenosine, guanine, guanosine, xanthine, hypoxanthine, uracil, uridine, cytosine, cytidine, thymine, and thymidine. We believe that almost any combination of these substances may be resolved into its components by our method.

EXPERIMENTAL

Since some purine and pyrimidine derivatives are weakly acid and others weakly basic, we chose solvent mixtures possessing both acid and basic characteristics. The spots on the paper were located by means of Holiday and Johnson's ultraviolet method [8] (*cf.* below). In addition to possessing good resolving power, the liquids must not absorb radiation in the wave-length region in question *. After many experiments we were able to select four different liquids which together fulfil the necessary conditions. We call them here the isobutyric acid, butanol-isobutyric acid, morpholine, and piperidine liquids.

* It might appear *a priori* possible to remove the liquid from the paper strip (by drying, washing) sufficiently for any possible absorption not to interfere with the analysis. Judging from our experiments, however, this does not seem to be easy in practice.

The compositions of the four mixtures are given below; the time in hours required for the liquid front to reach a distance of 49—50 cm from the starting line is also stated (h/49—50 cm). These figures refer to Whatman No. 4 filter paper strips and to a temperature of 22°C.

The isobut. acid liquid:

Isobutyric acid	400 ml
Water	208 ml
Ammonia, 25 %	0.4 ml

h/49—50 cm = 12 ± 2

The butanol-isobut. acid liquid:

n-Butanol	75 ml
Isobutyric acid	37.5 ml
Water	25 ml
Ammonia, 25 %	2.5 ml

h/49—50 cm = 13 ± 2

The morpholine liquid:

n-Butanol	90 ml
Morpholine	30 ml
Diethylene glycol	20 ml
Water	40 ml

h/49—50 cm = 22 ± 3

The piperidine liquid:

n-Butanol	80 ml
Piperidine	30 ml
Diethylene glycol	10 ml
Diethyl carbitol	20 ml
Water	40 ml

h/49—50 cm = 16 ± 2

The isobutyric acid liquid was used by Stjernholm [9] for paper chromatographic analysis of various "nucleo" derivatives. We used the morpholine liquid of Vischer and Chargaff [3], who used it to separate certain of the purine and pyrimidine bases (no nucleosides being reported). The commercial liquid components were purified by distillation, a fraction corresponding to a boiling-point interval of 2° being used as a rule; the diethylene glycol was distilled within the interval 130—134° at 14 mm Hg. In preparing the liquids, the individual components should be measured out very carefully. The butanol-isobutyric acid liquid undergoes certain changes with time, but if the mixture is kept in a refrigerator, no variation in the R_F values can be observed even after two months.

The method of descending paper strip chromatography was followed, a standardized procedure being applied. The paper used was Whatman No. 4 (available in 57 × 46.5 cm). The filter paper was cut into strips 57 cm long and 7 or 9 cm wide depending on the trough used. Two transverse lines were drawn with a pencil 50 and 60 mm, respectively, from one end of the strip. The line at 60 mm is the starting line, along which faint points were marked with a pencil, never closer than 2 cm to the edge of the strip (usually 2.3 cm). The solutions to be investigated were then deposited as spots on these points. On the narrower strips two or three such points were marked, on the broader ones three or four points.

The experiments were carried out in glass chambers placed in a room that could be kept at 22.0 ± 0.5° *. Each chamber consisted of a glass cylinder with a plane upper surface covered with a glass disk tightly joined to the edge of the cylinder by means of silicone grease. A little hole was bored in the glass disk and plugged with a cork. The glass trough could be filled through a funnel inserted in the hole. The trough was supported by a scaffolding of pyrex glass rods standing on the bottom of the chamber. The

* If the temperature varies more than that, the R_F values obtained will show larger errors than those stated here.

Table 1. R_F values at 22° C for purines, pyrimidines and nucleosides on Whatman No. 4 measurements for each

Solvent mixture	Adenine	Adenosine	Guanine	Guanosine	Xanthine
Butanol-isobut. acid liquid	0.56 ± 0.01 (4)	0.72 ± 0.02 (5)	0.39 ± 0.01 (4)	0.27 ± 0.02 (4)	0.34 ± 0.01 (4)
Isobut. acid liquid	0.83 ± 0.015 (4)	0.91 ± 0.02 (5)	0.70 ± 0.02 (5)	0.59 ± 0.03 (5)	0.60 ± 0.02 (5)
Morpholine liquid	0.64 ± 0.02 (3)	0.57 ± 0.01 (4)	0.36 ± 0.02 (4)	0.42 ± 0.01 (4)	0.34 ± 0.01 (4)
Piperidine liquid	0.56 ± 0.025 (5)	0.56 ± 0.03 (5)	0.23 ± 0.03 (5)	0.25 ± 0.03 (5)	0.24 ± 0.015 (4)

bottom was covered with the solvent mixture a sufficient length of time ahead of the test for the equilibrium liquid—vapour to be established. The bottom liquid may be used many times, provided care is taken not to keep the chamber unnecessarily exposed to the air.

The solutions to be investigated usually contained 5 mg/4 ml of each individual compound. These solutions were prepared by heating the substance in water at 100°, adding if necessary concentrated ammonia or dilute hydrochloric acid drop by drop from a micropipette *.

The R_F values of the individual compounds remain unchanged whether ammonia or hydrochloric acid is used, provided the routine treatment of the spots with ammonia gas is adhered to (*cf.* below).

* Of the twelve substances, only guanine requires the addition of hydrochloric acid; the others are easily dissolved in water or weak ammonia solution.

filter paper: mean values with maximal deviation; figures in brackets denote the number of individual compound.

Hypo-xanthine	Cytosine	Cytidine	Uracil	Uridine	Thymine	Thymidine
0.45 ± 0.01 (5)	0.60 ± 0.01 (4)	0.39 ± 0.015 (10)	0.50 ± 0.01 (3)	0.34 ± 0.025 (11)	0.63 ± 0.02 (6)	0.60 ± 0.01 (6)
0.69 ± 0.02 (5)	0.80 ± 0.02 (5)	0.73 ± 0.015 (10)	0.67 ± 0.02 (6)	0.60 ± 0.02 (11)	0.78 ± 0.01 (6)	0.75 ± 0.02 (6)
0.46 ± 0.02 (3)	0.57 ± 0.01 (3)	0.61 ± 0.02 (9)	0.62 ± 0.02 (3)	0.65 ± 0.03 (10)	0.81 ± 0.02 (6)	0.86 ± 0.01 (6)
0.37 ± 0.03 (5)	0.42 ± 0.01 (5)	0.44 ± 0.02 (5)	0.44 ± 0.03 (5)	0.35 ± 0.02 (5)	0.54 ± 0.03 (5)	0.54 ± 0.025 (5)

The solutions were applied with micropipettes to the filter strips at the points marked. The spots were allowed to spread to a diameter of about 7—9 mm, corresponding to a liquid volume of about 2—3 μl, *i. e.* each spot contained about 2.5—4 γ of each individual compound. The spots were then air-dried for 15 minutes. Ammonia gas was blown through a capillary tube at every spot for 15 seconds from either side. The paper was then allowed to stand for 20 minutes in air, before inserting it in the chamber. A glass rod about 6 mm in diameter was placed between the two pencilled lines, one of which is the starting line (*cf.* above), and the strip then folded over it. The strip was then placed in the *empty* trough in such a way that the line above the starting line lay over the inner edge of the trough. After the strips were inserted, the chamber was closed. One hour was allowed for equilibration of the paper with the liquid-vapour athmosphere, after which the solvent mixture was added to the trough through the funnel inserted in the hole in the glass disk. When the liquid front had reached about 1 cm from the lower edge of the strip (about 50 cm from the starting line), the experiment was interrupted. The strips

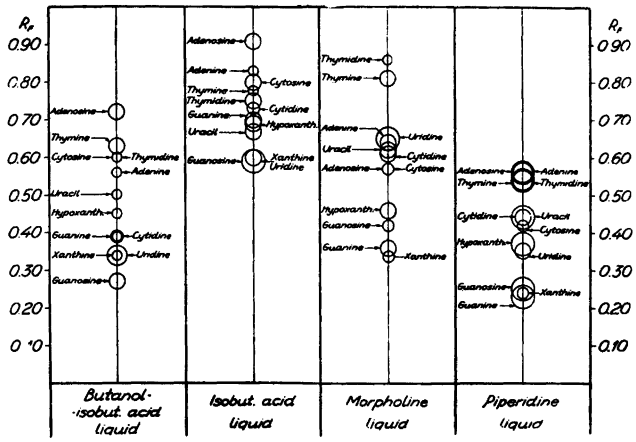


Fig. 1. Schematic presentation of R_F values at 22° C for purines, pyrimidines and nucleosides on Whatman No. 4 filter paper; each compound symbolized by a circle, the radius of which indicates maximal deviation from the mean value.

were taken out and the liquid front marked *. The strips were then dried in a suitable cabinet through which hot air from an ordinary hair dryer was blown for 25—30 minutes.

A masonite box 14 cm long and wide, and 60 cm high was used for the purpose. The strips were hung in this cabinet and hot air from an ordinary hair dryer, inserted through the lid, was blown in from above.

The dried strips were examined by Holiday and Johnson's method [8], *i. e.* the strips were exposed to a suitable ultraviolet lamp, the spots appearing as dark patches against the background of paper fluorescence ** .The boundaries of the dark patches were marked with a pencil. When applying 2.5—4 γ the dark patches are as a rule seen very distinctly. When using morpholine liquid (in which case the paper fluoresces rather strongly) some of the compounds are seen less distinctly. Therefore, it may be advisable when using this liquid for an untrained observer to double the quantity of substance in the spots; this change in the content of the spots does not change the R_F values.

RESULTS

The R_F value of each individual compound was determined by three or more measurements. The results are shown in Table 1, which gives the mean R_F values, the maximal deviation from the mean, and the number

* The solvent mixture in the trough may without detriment be returned to the original solution, care only being taken that the content of the trough is exposed to air for as short a time as possible. As previously mentioned, the bottom liquid is left in the *carefully closed* chamber; it may be used for a large number of subsequent tests.

** It was found that the argon-mercury lamp made by AB Luma Lamp, which gives a UV-radiation of almost exclusively 254 mμ, was excellent for the purpose.

of measurements. Fig. 1 gives a schematic diagram of the results; each substance is represented by a circle, the radius of which is an indication of the maximal deviation from the mean value.

When carrying out analyses of mixtures containing various mixtures of the twelve purine and pyrimidine derivatives, the R_F value of each individual compound does not change. Further, it is evident from these experiments that the components of almost any mixture of the twelve compounds can be separated and identified.

SUMMARY

By paper strip chromatography it has been possible to resolve and identify the components of almost any composited mixture of twelve natural purine and pyrimidine derivatives, *viz.* adenine, adenosine, guanine, guanosine, xanthine, hypoxanthine, cytosine, cytidine, uracil, uridine, thymine, and thymidine.

I wish to thank Assistant S. Olsson for very valuable help in the experiments.

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ACTA CHEMICA SCANDINAVICA 6 (1952) 1036–1040

Studies on Monoalkyl Carbonates

XIII. The Monoalkyl Carbonate of Allyl Alcohol

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The equilibrium conditions and the reaction mechanism for the formation and decomposition of various monoalkyl carbonates in aqueous medium, the most recent being $CH_2OH \cdot CH_2 \cdot CO_3^-$ and $CH_2Cl \cdot CH_2 \cdot CO_3^-$ [1], have been studied previously. The present investigation deals with the monoalkyl carbonate of allyl alcohol.

The allyl alcohol used was distilled with carbon tetrachloride [2] through a platinum wiregauze column [3]. The allyl alcohol thus obtained boiled at 96.7° C (751mmHg), $n_D^{20.0°} = 1.4132$. By a bromometric titration an equivalent weight of 58.28 was obtained: theoretically for $CH_2{:}CH \cdot CH_2OH$ 58.08. The above mentioned boiling point is corrected for calibration errors; stem corrections have been done.

No attempt was made to isolate the allyl carbonate as a substance. Where it has been used it was made by adding carbon dioxide to a basic solution of the alcohol, the carbon dioxide thus being converted partially to monoallyl carbonate and partially to ordinary carbonate.

As to the method of analysis we refer to previous investigations [1]. The data presented are corrected with regard to blank values, unless otherwise stated. "Uncorr." values are not corrected at all and "corr." values are corrected not only with regard to blank values but also with regard to the decomposition mentioned in the next section. The blank values amount to less then 1 unit of the percentage of monoalkyl carbonate in the equilibrium experiments where the concentration of monoalkyl carbonate + hydrocarbonate is 0.1—0.2 *M*. They amount to 5—6 units (in a single case up to 8 units) in the experiments where the concentration is about 0.02 *M*.

The experiments were carried out at 0° C. The velocity constants were calculated by means of Briggs' logarithms, the unit of time being the minute.

On the formation of allyl carbonate from carbon dioxide and allyl alcohol in basic solutions, and on the reaction

$$CH_2{:}CH{\cdot}CH_2O^- + CO_2 = CH_2{:}CH{\cdot}CH_2{\cdot}CO_3^-$$

The experiments were carried out in a 2 litre flask by vigorously shaking 500 ml of a basic solution of allyl alcohol with atmospheric air of which 250 ml were substituted by carbon dioxide. The solution was immediately analysed to determine the percentage of allyl carbonate. The results are listed in Table 1. Since allyl carbonate is very rapidly decomposed, the results are corrected not only for the blank values, but also for the decomposition taking place during the 6—7 minutes which elapse between the start of the shaking with carbon dioxide and the separation of the precipitate and the supernate by centrifugation. The corrections appear from the Table.

By the introduction of the experimental results in the expression

$$k' = \frac{\text{per cent monoalkyl carbonate}}{\text{per cent carbonate}} \cdot \frac{k_{CO_2 \cdot OH^-}}{c_{\text{alcohol}}}$$

the k' values presented in Table 1 are calculated.

k' is the velocity constant of the gross reaction:

$$CO_2 + CH_2{:}CH{\cdot}CH_2OH + OH^- = CH_2{:}CH{\cdot}CH_2{\cdot}CO_3^- + H_2O$$

and $k_{CO_2 \cdot OH^-}$ ($10^{4.39}$) is the velocity constant of the reaction:

$$CO_2 + OH^- = HCO_3^-$$

Table 1. Carbon dioxide in allyl alcohol + NaOH. 0°.

Initial solution		Absorbed CO_2 mol/liter	% alkyl-carbonate	% alkyl-carbonate (corr.)	k'	Mean of k'
c_{NaOH}	$c_{CH_2:CH\cdot CH_2OH}$					
0.50	1.00	0.0131	51.1	53.3	$10^{4.45}$	$10^{4.44}$
0.50	1.00	0.0209	50.1	52.1	$10^{4.43}$	
0.50	1.00	0.0123	51.4 50.8	53.8 53.2	$10^{4.46}$ $10^{4.45}$	
0.50	0.50	0.0211	33.2	35.2	$10^{4.43}$	
0.50	0.50	0.0195	33.4 32.1	35.7 34.4	$10^{4.44}$ $10^{4.41}$	

For the value of $k_{\text{allyl}\cdot CO_3^-}$, being the velocity constant of the reaction "$CH_2{:}CH{\cdot}CH_2{\cdot}CO_3^- = CH_2{:}CH{\cdot}CH_2O^- + CO_2$" we get 0.0059 when the value of K_{Eq} found in Table 2 is employed.

When k' and $k_{\text{alkyl}\cdot CO_3^-}$ for allyl alcohol is compared to the corresponding values for n-propyl alcohol [4], it can be seen that the introduction of the double bond influences the velocity constants significantly.

The equilibrium of the reactions

$$CH_2{:}CH{\cdot}CH_2{\cdot}CO_3^- + H_2O \rightleftharpoons HCO_3^- + CH_2{:}CH{\cdot}CH_2OH$$

The above equilibrium was established in aqueous solutions of allyl alcohol, potassium bicarbonate and sodium carbonate.

The contents of alkyl carbonate have been calculated as a percentage of HCO_3^- initially present; thus no attention was paid to carbonate.

In Table 2 are presented not only the "% allyl carbonate" corrected for blank values and the decomposition taking place until the precipitate has been separated from the supernate, but the uncorrected as well. This has been done in order to illustrate the accuracy that may be attached to the values found. From the experimental results the equilibrium constant of the reaction,

$$K_{Eq} = \frac{c_{CH_2:CH\cdot CH_2OH} \cdot c_{HCO_3^-} \cdot f}{c_{CH_2:CH\cdot CH_2\cdot CO_3^-} \cdot f}$$

may be calculated, the activity constants neutralizing each other.

Table 2. The solution of carbonate-alkylcarbonate in equilibrium. 0°.

Initial solution			% alkylcarbonate		K_{Eq}	Mean of K_{Eq}
$c_{CH_2:CH\cdot CH_2OH}$	c_{KHCO_3}	$c_{Na_2CO_3}$	uncorr.	corr.		
1.00	0.10	0.05	3.17	2.48[1]	$10^{1.59}$	$10^{1.61}$
1.00	0.20	0.10	2.87	2.44[2]	$10^{1.60}$	
0.50	0.10	0.05	1.78	1.16[3]	$10^{1.63}$	
0.50	0.20	0.10	1.47	1.15[4]	$10^{1.63}$	

[1] Mean of 4 determinations: 2.48, 2.51, 2.52, 2.41
[2] » » 4 » 2.44, 2.42, 2.39, 2.51
[3] » » 4 » 1.23, 1.16, 1.12, 1.13
[4] » » 4 » 1.13, 1.17, 1.18, 1.13

On the velocity of the reactions:

$$CH_2{:}CH{\cdot}CH_2{\cdot}CO^- + H_2O = HCO_3^- + CH_2{:}CH{\cdot}CH_2OH$$

Determinations have been made from the alkyl carbonate side only and in strongly basic medium, the alkyl carbonate thus being practically completely converted into carbonate.

The alkyl carbonate has been made by shaking the basic solution with CO_2 as stated in the introduction. The solution, therefore, contains ordinary carbonate, which, however, is of no importance in the present investigation.

In Table 3 are listed the experimental results of the decomposition of allyl carbonate. k_{Mono} is the value of $\frac{1}{t} \log \frac{a}{a-x}$.

Table 3. Monoalkyl carbonate in NaOH + alcohol. 0°.

Initial solution			Min.	% alkyl-carbonate left	k_{Mono}
$c_{alkylcarbonate}$	c_{NaOH}	c_{alcoho}			
0.010	0.50	1.00	0	100	—
			15	91.3	0.00262
			42	74.3	0.00307
			96	48.9	0.00323
			145	35.5	0.00311
			206	22.4	0.00315
			276	12.1	0.00332
			361	6.6	0.00326
			∞	(0)	—
				Mean:	0.0031
0.007	0.50	0.50	0	100	—
			7	93.7	0.00404
			16	86.5	0.00392
			27	77.8	0.00404
			47	67.1	0.00369
			86	42.1	0.00437
			142	21.8	0.00466
			204	11.6	0.00458
			∞	(0)	—
				Mean:	0.0042

The experiments are interpreted in a similar way to the one applied to the monoalkyl carbonates previously investigated. The decomposition takes place through the reactions:

1. $\text{alkyl} \cdot CO_3^- = \text{alkylate}^- + CO_2$
2. $CO_2 + OH^- = HCO_3^-$.

k_{Mono} may be calculated at $pa_H > 10$ by means of the following expression:

$$k_{\text{Mono}} = \frac{k' \cdot K_{Eq} \cdot \frac{K_{H_2O}}{K_{CO_2}}}{1 + k' \cdot \frac{c_{\text{alcohol}}}{k_{CO_2 \cdot OH^-}}}$$

the values of K_{H_2O} and K_{CO_2} being $10^{-14.93}$ and $10^{-6.65}$, respectively.

Upon introduction of the constants the equation for allyl alcohol is:

$$k_{\text{Mono}} = \frac{0.0059}{1 + 1.12 \cdot c_{\text{alcohol}}}$$

k_{Mono} for allyl carbonate in 1 *M* and 0.5 *M* solutions of allyl alcohol, $pa_H > 10$, will then be calculated to 0.0028 and 0.0038, respectively.

The experimental and calculated values agree well.

SUMMARY

The velocity constant of the reaction "CH_2:CH·$CH_2OH + OH^- + CO_2 = CH_2$:CH·$CH_2 \cdot CO_3^- + H_2O$" and the equilibrium constant for the reaction "CH_2:CH·$CH_2 \cdot CO_3^- + H_2O \rightleftharpoons CH_2$:CH·$CH_2OH + HCO_3^-$" have been determined. The velocity of the decomposition of CH_2:CH·$CH_2 \cdot CO_3^-$ in strongly basic medium was investigated and may be explained by assuming that the decomposition is a two-stage reaction, *viz.*

$$CH_2\text{:CH}\cdot CH_2 \cdot CO_3^- = CH_2\text{:CH}\cdot CH_2O^- + CO_2$$
$$CO_2 + OH^- = HCO_3^-$$

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Cholinesterase Activity Determined with an Electrometric Method

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The organo-phosphorus compounds which are used as insecticides or which are potential war gases are all potent cholinesterase inhibitors. Their toxic properties depend more or less on their cholinesterase inhibiting action. Work on these types of compounds requires a rapid and convenient method for determinations of esterase activity. Determinations according to the Warburg manometric technique are very accurate but require specially trained personel. It was felt, therefore, that an investigation of the method reported by Michel [1] would be of interest, the apparatus being a pH-meter.

The activity of a cholinesterase preparation is determined from the pH-change in a buffer solution to which acetylcholine and enzyme have been added. The principles of this method have been published by Michel [1] and our apparatus has been described by Tammelin and Löw [2] in a paper on the calibration of the electrometric method. In the papers mentioned above it has been shown that it is possible to obtain pH as a linear function of time. The purpose of the present paper is to give a detailed description of an electrometric method for esterase determinations, to show some simplifications of Michel's method, and to demonstrate the usefulness of the method for cholinesterase determinations.

Michel performed his measurements in the pH region between 8 and 6, in which range the activity of the cholinesterase varies with the pH level. The composition of the buffer solution counteracts this effect, but some deviation from the straight line remains owing to the pH change. In correcting this error, Michel used a correction factor arbitrarily set at 1.00 at pH 7.00. Since our apparatus permits reproducible registrations in a much smaller range of the pH scale, *i.e.* from 8 to 7.7, this correction can be disregarded, and moreover each test can be carried out in twenty minutes, thus saving

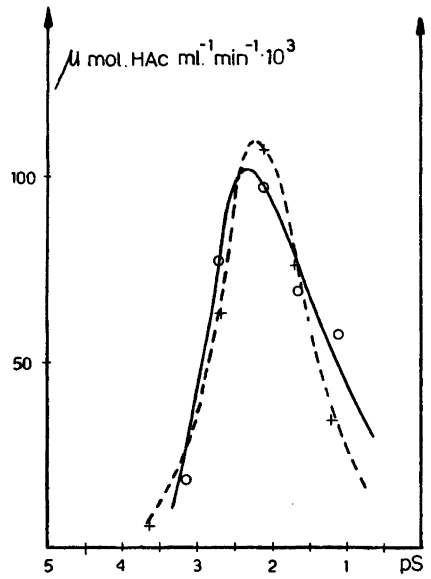


Fig. 1. Enzyme activity as function of pS. Electrometric method denoted by continuous line; manometric method by broken line.

time. The correction for non-enzymatic hydrolysis generally has only a negligible influence on the final value for the enzymatic activity.

The activity of an enzyme preparation was expressed in △ pH per unit of time by Michel, and the values from the manometric technique are originally expressed as b_{30} values according to Augustinsson [3], *i.e.* μl carbon dioxide evolved in 30 minutes from a bicarbonate solution. Both these ways of expressing the activity relate to secondary effects of the hydrolysis of acetylcholine and are incommensurable as absolute values. *A direct and commensurable measure of the activity is the velocity of the development of acetic acid in the solutions expressed in micromoles of acetic acid per ml per minute.* The amount of carbon dioxide obtained with the manometric technique can readily be converted to this measure, but the △ pH values are not directly convertible. If the electrometric apparatus is calibrated according to Tammelin and Löw [2] infusing a solution of acetic acid into the mixture of enzyme and buffer solution, the amount of acetic acid can be set against the slope of the straight line obtained on the recorder paper. The slope of the line is originally determined in cm/cm.

Apparatus and solutions. The apparatus is identical with that described by Tammelin and Löw [2] except for the use of a Micromax recorder. The recorder is connected to the valve potentiometer by a bridge making the total scale of the recorder paper correspond to about two pH units.

The reaction mixture in which the pH determinations were made had the following composition: 3.00 ml Michel's buffer solution, 2.34 ml redistilled water, 0.66 ml enzyme

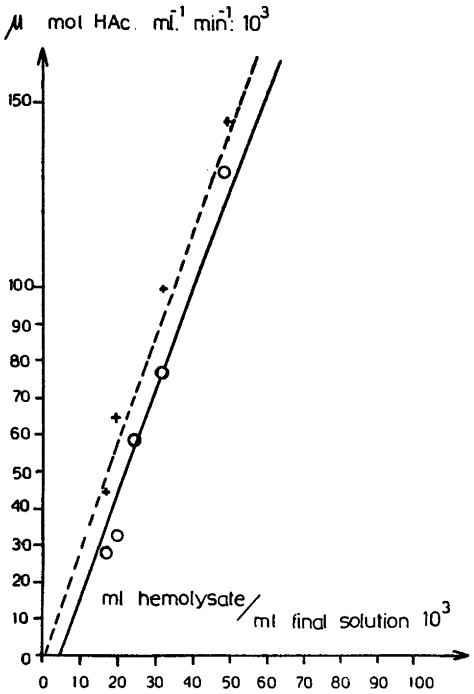


Fig. 2. Enzyme activity as function of hemolysate concentration. Electrometric method denoted by continuous line; manometric method by broken line.

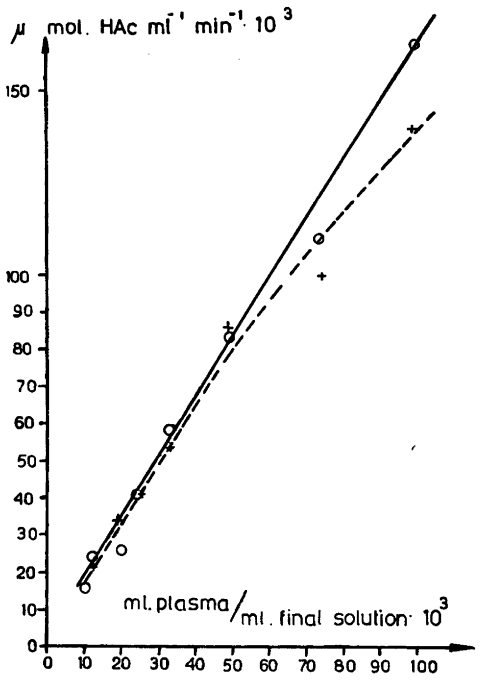


Fig. 3. Enzyme activity as function of plasma concentration. Electrometric method denoted by continuous line; manometric method by broken line.

solution, and 0.60 ml acetylcholine solution. For inhibitor tests the enzyme was suspended in the 2.34 ml of water and 0.66 ml of the inhibitor solution added.

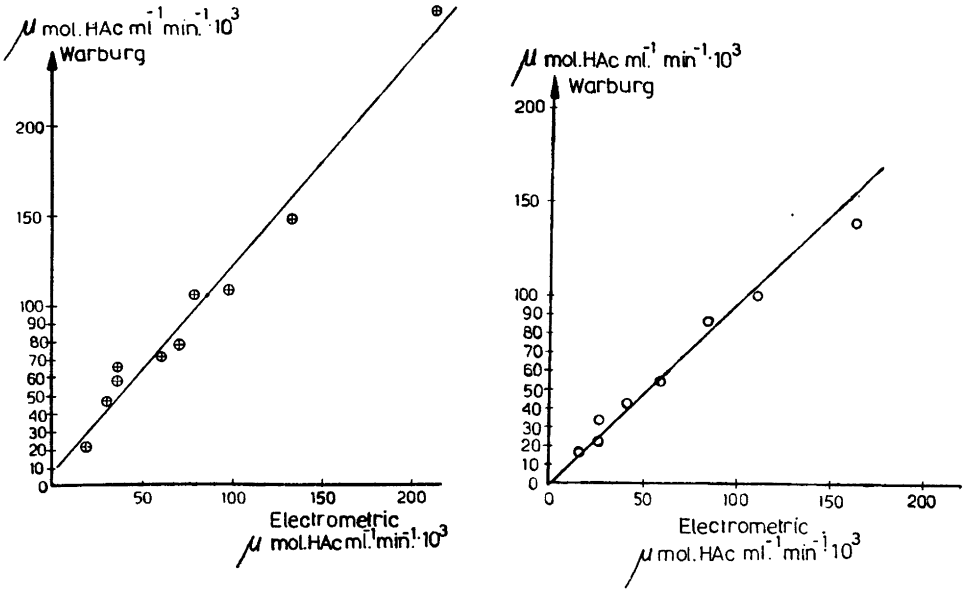
Michel's buffer solution has the following composition: 0.02 *M* sodium barbital (4.1236 g); 0.004 *M* KH_2PO_4 (0.5446 g); 0.60 *M* KCl (44.730 g); 28.0 ml 0.1 *M* HCl solution and water to 1 l.

The reaction mixture in the Warburg vessels has the following composition: 1.60 ml acetylcholine solution (0.100 g per 40 ml Ringer's solution) + 0.40 ml enzyme solution.

Or 1.60 ml acetylcholine solution + 0.20 ml enzyme solution + 0.20 ml inhibitor solution.

The enzyme solutions were prepared from heparinized centrifuged human blood. The supernatant plasma was used directly for the investigations. The red cells were washed four times with saline and then hemolyzed by addition of redistilled water to blood volume. The enzyme solutions were prepared from this stock solution immediately before use.

The substrate solution was made immediately before the experiments. In the electrometric method, 0.110 g acetylcholine iodide was dissolved in 5.00 ml redistilled water. In the Warburg manometric method, 0.100 g was dissolved in 40 ml Ringer's solution.



Figs. 4 and 5. The relation between manometric and electrometric values for the cholinesterase activity. Fig. 4 shows the erythrocyte values and Fig. 5 the plasma values.

Enzyme concentrations and acetylcholine concentrations are varied in the following, but the added volume is in each case consistent with the above composition. For the calibration we used a solution of 0.0504 M acetic acid.

Calibration and formulas. The purpose of the calibration was to elucidate the relation between the slope of the straight line obtained on the recorder paper expressed in cm/cm and the change in the concentration of acetic acid in the test solution. Therefore we immersed the electrode in a solution consisting of 3.00 ml Michel's buffer solution, 2.94 ml water and 0.66 ml plasma solution diluted to three times of the blood volume, or 0.66 ml erythrocyte hemolysate diluted to three times of the blood volume. The acetic acid solution was then infused by means of an Agla Micrometer syringe driven by a synchronous motor. The infusion time for 0.20 ml 0.0504 *M* acetic acid and the slopes of the lines obtained in the recorder were determined in respect of plasma and erythrocytes. Seventeen determinations were made on plasma solution and ten on erythrocyte solution. The mean value of the time readings is 12.7 ± 0.2 minutes for the infusion of 0.20 ml of the acetic acid, and for the corresponding "activity" in cm/cm, the plasma value is 0.38 ± 0.04 and the erythrocyte value 0.39 ± 0.03 cm/cm. There is no significant difference between these values, and the result of the determination is that a velocity of 0.20 ml 0.0504 *M* acetic acid solution in 12.7 minutes gives an "activity" which corresponds to 0.39 ± 0.04 cm/cm.

From this it follows that an activity measured in cm/cm, multiplied by the factor 0.31, gives the activity measured in micromoles of acetic acid per ml per minute. This conversion factor is limited to our special apparatus. However, one centimeter corresponds to 0.059 pH units and on the time axis to 0.98 minutes. The conversion factor

from pH per minute to micromoles of acetic acid per ml per minute is 5.15, and this factor is characteristic of the volume and composition of our reaction mixture. It may be used whenever an electrometric method is employed where time and pH will be determined under the conditions mentioned above. The conversion factors are subject to fairly substantial errors because of the errors in the determinations of infusing time and the obtained corresponding "activities". The errors are sufficiently small, however, to justify use of the factors, as is confirmed by a comparison of the electrometric and the manometric results (see Figs. 2 and 3). The conversion of b_{30} values to micromoles of acetic acid per ml per minute is done by using the factor $7.44.10^{-4}$.

EXPERIMENTAL

In order to find suitable conditions for the determination of the cholinesterase activity, the substrate concentration and the enzyme concentration were varied. According to Augustinsson [3], cholinesterase from erythrocytes shows optimum activity at a certain substrate concentration, since high concentrations give a complex of the enzyme with two molecules of the substrate. This complex cannot break down and yield acid and choline. The concentration of substrate which gives maximum activity is demonstrated by diagrams where the activity is plotted against the negative logarithm of the molar concentration of the substrate (pS). For comparison between electrometric and manometric methods, solutions equimolar in respect of substrate were used in the parallel experiments. The enzyme concentration was 0.033 ml erythrocyte hemolysate per ml of the final solution in the two methods.

Fig. 1 shows that a pS of 2.25 gives optimum activity. A concentration of $7.32.10^{-3}$ M will be obtained if solutions are prepared as recommended under Apparatus and solutions. The difference between the obtained value and the concentration used is negligible.

In order to show the relative activities at various enzyme concentrations, experiments were conducted with different dilutions of the hemolysate and plasma. The results are summarized in Figs. 2 and 3.

The manometric method results in somewhat higher activities in determinations of the specific cholinesterase from erythrocytes, but in the determinations of the non-specific cholinesterase from plasma the methods show the same activity until the manometric technique loses precision because of the high reaction velocity. The slope of the curves in Figs. 2 and 3 is the same for each method and hence the sensitivity of the methods is equal in spite of the higher values for erythrocytes in the manometric technique.

Values obtained by the manometric and the electrometric methods are plotted against each other, in Fig. 4 for erythrocytes and in Fig. 5 for plasma.

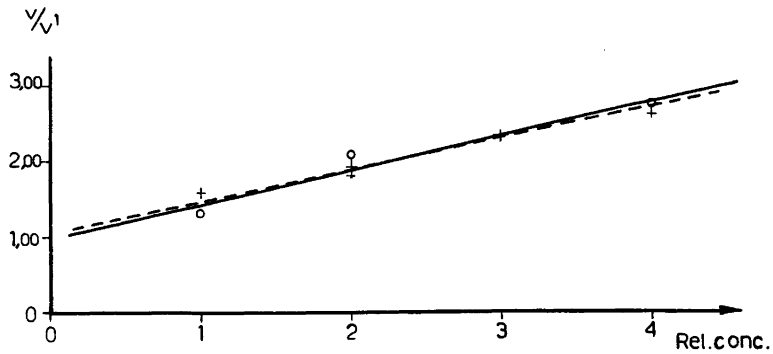


Fig. 6. Inhibition of erythrocyte cholinesterase as a function of the inhibitor concentration. v *is the reaction velocity without inhibitor,* v′ *reaction velocity in the presence of various inhibitor concentrations. Electrometric method denoted by continuous line, manometric method by broken line.*

The plotted values were obtained from the preceding experiments. Diagrams obtained in this way are useful when values of one kind are to be converted to the other.

The molar concentration of an inhibitor reducing the activity of an enzyme preparation by fifty per cent is called I_{50} and is a suitable constant to characterize an inhibitor [3]. In order to compare the two methods, a determination of the value of I_{50} of a preparation containing bis-dimethylamido-phosphoryl fluoride was done manometrically and electrometrically. Only relative values for the activity and the value in cm/cm were used in the electrometric method. These values are very accurate, since no conversion factor is necessary. The results are shown in Fig. 6.

DISCUSSION

The determination of the activity-pS curve shows that the same pS value gives optimum activity in the electrometric and the manometric method. Evidently, the pS value that corresponds to optimum activity is characteristic of the enzyme itself and independent of the buffer solution used. Essential for our comparison of the two methods is Strindberg's [4] demonstration, that potassium of the same concentration as in Michel's buffer may be substituted for the magnesium in the Ringer's solution without effect on the enzymatic activity.

A method for determining cholinesterase activity has to show a linear relation between activity and enzyme concentration within the range of measurements. The Warburg manometric technique shows a good relation,

and Figs. 2 and 3 demonstrate that the electrometric method also is satisfactory in this respect. At very low enzyme concentrations, both methods yield somewhat erroneous results. High concentrations cause a high reaction velocity, and in the Warburg technique the capacity of the manometer limits the measurable enzyme activity. The rapid movement of the manometer fluid makes accurate determinations impossible. The continuous registration in our electrometric method eliminates these disadvantages, and the method is therefore applicable to higher activities than the manometric technique. However, erythrocytes gave a deviation from the straight line at high enzyme concentrations in both methods, perhaps due to a high protein content. Plasma did not show this deviation when the electrometric method was used, but manometric values showed a deviation, presumably for reasons mentioned above.

The main purpose of this investigation was to find a rapid and convenient method for determinations of I_{50} values and studies of the blood of intoxicated animals. Since the results were satisfactory, an apparatus is under construction for simultaneous determinations of six enzyme preparations, thus reducing the time necessary for six determinations to about forty minutes.

SUMMARY

An electrometric method for the determination of cholinesterase activity is described. Values obtained with the electrometric method are compared with manometric values in determinations of enzyme activity as functions of substrate concentrations, erythrocyte concentrations, and plasma concentrations. The accuracy of the methods is discussed.

Acknowledgement: Our sincere thanks are due to the Director of this Laboratory Professor G. Ljunggren for the kind interest he has shown in this work.

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Studies on Quinones and Hydroquinones

II.* Monohalogen Derivatives of 2,5- and 2,6-Dimethoxyhydroquinone

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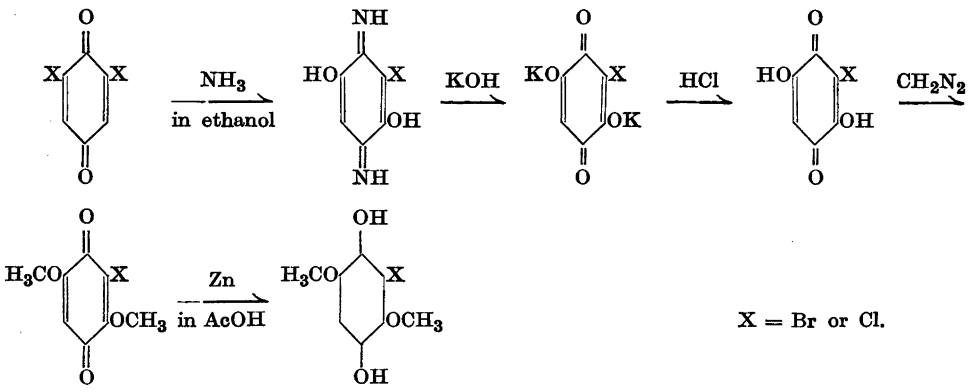
In a previous communication [1] it was reported that 3-bromo-2,6-dimethoxyhydroquinone, obtained by hydrolysis of its diacetate or by reduction of the corresponding quinone, is a very labile substance. It decomposed so easily into 2,6-dimethoxy quinone and hydrogen bromide, that it could not be obtained in the solid state (but only in solution). The decomposition was catalyzed by acids. This seems to be the first example of the reaction which is the reverse of the wellknown addition of hydrogen halides to quinones. In the present paper the synthesis and properties of some monohalogen dimethoxyhydroquinones is reported.

Reduction of 3-chloro 2,6-dimethoxyquinone with zinc in acetic acid gave the 3-chloro 2,6-dimethoxyhydroquinone which could be acetylated to the diacetate, identical with that obtained by reductive acetylation of the quinone, and could be oxidized to the quinone with ferric chloride. Thus, it is a fairly stable substance, but when dissolved in dilute hydrochloric acid it decomposed slowly in the cold, but very rapidly on warming, 2,6-dimethoxyquinone being precipitated. 3-Chloro 2,6-dimethoxyhydroquinone, therefore, provides a second example of a halogenated hydroquinone which decomposes into the hydrogen halide and the quinone.

The corresponding chloro and bromo derivatives with the methoxyl groups in the 2 and 5 positions were prepared by the series of reactions given in the scheme below.

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* Part I. On the Reactivity of 2,5- and 2,6-Dimethoxyquinone. Ref. 1.



The quinone, hydroquinone and hydroquinone diacetate could be interconverted by appropriate reactions, and the chloro and bromo hydroquinones were quite stable, even in boiling 2 *N* hydrochloric acid.

3-Bromo 2,6-dimethoxyquinone and the corresponding 2,5-derivative easily add hydrogen bromide and form the dibromo dimethoxyhydroquinones, as may have been anticipated, bearing in mind the observation of Aulin and Erdtman [2], that dibromo 2,6-dimethoxyhydroquinone was formed in good yield when 2,6-dimethoxy-quinone was treated with one mole of bromine in chloroform at room temperature. The two monobromo quinones are, however, recovered unchanged upon attempted Thiele acetylation.

The reason for the instability of the 3-chloro and 3-bromo 2,6-dimethoxyhydroquinones is at present obscure. The fact that the dihalogenated 2,6-dimethoxyhydroquinones and the monohalogenated 2,5-dimethoxyhydroquinones are quite stable makes it probable that a very special effect is operating in the labile compounds. The search for further labile halogenated hydroquinones is being continued in order to find a reasonable explanation of the unexpected behaviour of these substances.

EXPERIMENTAL

3-Chloro-2,6-dimethoxyhydroquinone. 3-Chloro 2,6-dimethoxyquinone (0.5 g) was reduced with zinc dust in boling acetic acid (10 ml). The solution was poured into water and extracted with ether and the extract was washed with sodium hydrogen carbonate solution, dried over calcium chloride and concentrated under reduced pressure. The residual brown oil was dissolved in hot benzene and on cooling colourless crystals (0.4) of the hydroquinone separated. Further recrystallizations from benzene yielded the pure substance, m.p. 98—99° *.

$C_8H_9O_4Cl$ (204.6) Calc. OCH_3 30.3 Found OCH_3 30.0

* All melting points uncorrected.

A small amount of the hydroquinone was dissolved in water and treated with ferric chloride. The yellow substance which precipitated was collected and recrystallized from ethanol. It melted at 146—147° either alone or in admixture with an authentic specimen of 3-chloro 2,6-dimethoxyquinone.

A small amount of 3-chloro 2,6-dimethoxyhydroquinone was dissolved in N hydrochloric acid. On warming a yellow, crystalline substance precipitated. This was collected, and recrystallized from acetic acid and then had m.p. 248—249°, undepressed on admixture with authentic 2,6-dimethoxyquinone.

3-Chloro 2,6-dimethoxyhydroquinone diacetate. The diacetate of 3-chloro 2,6-dimethoxyhydroquinone was prepared in good yield either by acetylation of the hydroquinone with acetic anhydride and pyridine or by reductive acetylation of the quinone with zinc dust and acetic acid. It was recrystallized from ethanol and melted at 111—112°.

$C_{12}H_{13}O_6$ Cl (288.7) Calc. OCH_3 21.5 Found OCH_3 21.6

3-Bromo-2,5-dihydroxyquinone diimide. Concentrated ethanolic ammonia (50 ml) was added slowly to a solution of 2,6-dibromoquinone (20 g) in ethanol (100 ml) at 60°, and the mixture was set aside at 0° overnight. The brown imide (6.5 g) was filtered off and recrystallized from acetic acid, forming brown crystals with a metallic lustre, which decomposed without melting at about 360°.

$C_6H_5O_2N_2Br$ (217.0) Calc. N 12.9 Found N 12.6

3-Bromo-2,6-dihydroxyquinone. The above imide (4 g) was dissolved in 20 % potassium hydroxide solution (25 ml) and the mixture was heated nearly to boiling. On cooling the potassium salt of the hydroxyquinone precipitated as dark red crystals, which were collected by filtration and dissolved in water. The aqueous solution was acidified with hydrochloric acid and extracted with ether, and the ether solution was dried over calium chloride and concentrated. The residue recrystallized from acetic acid, yielding the 3-bromo-2,5-dihydroxyquinone as brick-red crystals (1.6 g), m.p. 190° (decomp.).

$C_6H_3O_4Br$ (219.0) Calc. Br 36.5 Found Br 36.8

3-Bromo-2,5-dimethoxyhydroquinone. This substance was prepared in 80 % yield in the same way as the monochloro 2,6-dimethoxyhydroquinone. It was recrystallized from benzene- ligroin (1 : 1) and melted at 95—96°.

$C_8H_9O_4Br$ (249.1) Calc. OCH_3 24.9 Found OCH_3 24.9

The substance could be oxidized to the quinone with ferric chloride, as described for the 3-chloro-2,6-dimethoxyhydroquinone.

5-Bromo-2,5-dimethoxyhydroquinone diacetate. This substance was prepared by reductive acetylation of the quinone with acetyl bromide, with the elimination of bromine. It was also prepared from the hydroquinone by acetylation. When the substance was prepared by reductive acetylation, with zinc dust and acetic anhydride, 2,5-dimethoxyhydroquinone diacetate, m.p. 181—182°, was formed simultaneously in about 25 % yield. This side reaction did not occur when the quinone was first reduced in acetic acid solution, and then acetylated. The 3-bromo 2,5-dimethoxyhydroquinone diacetate was recrystallized from 70 % methanol and melted at 77—78°.

$C_{12}H_{13}O_6$ Br (333.1) Calc. OCH_3 18.6 Found OCH_3 18.4

3-Chloro-2,5-dimethoxyquinone. 3-Chloro-2,5-dihydroxyquinone (4 g) was methylated with diazomethane as described above for the bromoderivative. The orange-red substance (3.6 g) was recrystallized from ethanol and melted at 123—124°.

$C_8H_7O_4$ Cl (202.6) Calc. OCH_3 30.6 Found OCH_3 30.2

3-Chloro-2,5-dimethoxyhydroquinone. This substance, prepared in 90 % yield, in the same way as the other hydroquinones, was recrystallized from benzene-ligroin (1 : 1) and melted at 95—96°.

$C_8H_9O_4Cl$ (204.6) Calc. OCH_3 30.3 Found OCH_3 30.4

3-Chloro-2,5-dimethoxyhydroquinone diacetate. This substance, prepared from the hydroquinone by acetylation, or from the quinone by reductive acetylation with zinc and acetic anhydride, was recrystallized from 70 % methanol and melted at 70—71°.

$C_{12}H_{13}O_6Cl$ (288.7) Calc. OCH_3 21.5 Found OCH_3 21.6

SUMMARY

The 3-chloro and 3-bromo-2,5- and 2,6-dimethoxyhydroquinones, their acetates and the corresponding quinones have been prepared. Of the hydroquinones, the 2,6-dimethoxy derivatives decompose by an acid-catalyzed reaction into 2,6-dimethoxyquinone and the hydrogen halide, while the 2,5-dimethoxy derivatives and the dihalogen derivatives from both series are quite stable.

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Studies on the Chemistry of Lichens

II.* Umbilicin, an Arabitol Galactoside from *Umbilicaria pustulata* (L.) Hoffm.

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In connection with investigations on lichen acids the low-molecular carbohydrate constituents of some lichens have been studied. From *Umbilicaria pustulata* (L.) Hoffm. D-mannitol and another substance of m.p. 138—139° and showing $[\alpha]_D^{20}$ −81° (in water) were obtained. For the latter substance, which does not seem to be described in the literature, we propose the name umbilicin.

Umbilicin was easily soluble in water and had a low solubility in ethanol and moist acetone. It crystallized very slowly from supersaturated solutions; to obtain complete separation when the substance was not pure the solutions had to be kept several months, although one or two days were sufficient in the case of pure samples. Umbilicin did not reduce Fehlings solution. On the paper chromatogram it gave a single spot which lay just above that of mannitol and could be developed with lead tetraacetate [1]. After acid hydrolysis of the substance two spots were obtained, one of which could be developed with the usual sugar reagents (ammoniacal silver nitrate or aniline hydrogen phthalate) and was identical with the spot given by D-galactose on the same chromatograms. The second spot could not be developed with aniline hydrogen phthalate but did react with lead tetraacetate. After periodate oxidation on the paper, this spot could be developed with iodide-starch [1] but not with Schiff's reagent. This behaviour is typical of polyols, which give only formaldehyde and formic acid on periodate oxidation; the formaldehyde volatilises during

* Part I *Acta Chem. Scand.* 6 (1952) 818.

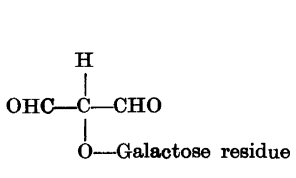
** Svenska Sockerfabriks A. B. Research Techn. 1950—1951.

the oxidation and thus no colour is produced with Schiff's reagent. The position of the second spot, much below that of mannitol, indicated that it was due to a pentitol.

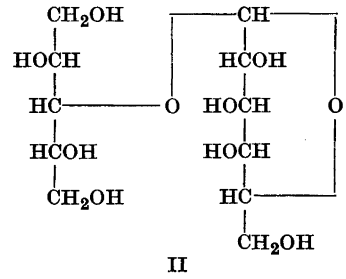
The two components of the hydrolysate were separated on a hydrocellulose column and isolated in a crystalline state. The fast-moving polyol was identified as D-arabitol. Its melting point, 98—100°, was not depressed on admixture with an authentic sample of D-arabitol, and the specific rotation in acidified ammonium molybdate solution was + 131°, agreeing with the value of —130° reported by Richtmyer and Hudson [2] for L-arabitol. The slow-moving sugar was identified as D-galactose by its specific rotation at equilibrium, + 76°, and by oxidation to mucic acid.

The hydrolysis experiments thus showed that umbilicin is a galactoside of D-arabitol, and analysis values for the substance and its acetate were consistent with the formula of an arabitol monogalactoside.

On periodate oxidation of umbilicin, 10 moles of oxidant were consumed, while one mole of galactose and one mole of arabitol should together consume only 9 moles. The high value observed can only be explained by the reaction proceeding through an intermediate containing an activated hydrogen atom; the latter is oxidized to an hydroxyl group, after which the whole molecule is degraded to one-carbon units. This abnormal type of periodate oxidation was first observed by Ahlborg [3] with maltose, and has been further studied by Huebner, Ames and Bublitz [4], who demonstrated that it occurs when a suitably activated hydrogen atom is present, *e.g.* in malonic acid. If the arabitol galactoside is a pyranoside, which is a very reasonable assumption, the third carbon atom of the arabitol must be glycosidically linked to the galactose in order to give an intermediate with an active hydrogen atom (I).



I



II

Further, the optical rotations of umbilicin and of its octaacetate ($[\alpha]_D^{20}$ —20° in chloroform) make it very probable that the substance is a β-galactoside. Even though it is impossible to calculate the contribution of the D-arabitol part of the molecule, it is very improbable that it would have a negative

value, high enough to more than equalise the high positive value of an α-galactoside. Thus the most probable structure of umbilicin would appear to be 3-D-arabitol β-D-galactopyranoside (II).

Umbilicaria pustulata is rather rich in carbohydrate constituents. In addition to D-mannitol and umbilicin it contains a polysaccharide, pustulin [5], composed of glucose units. Umbilicin seems to be the first glycoside isolated from a lichen and is of a type not frequently found in nature. A more careful investigation of the carbohydrate constituents of lichens is therefore justified from both biochemical and taxonomical points of view.

EXPERIMENTAL

Isolation of mannitol and umbilicin. Air-dried, ground *Umbilicaria pustulata* (500 g) (collected in the archipelago of Blekinge, Sweden) was continuously extracted with ether for about two days in order to remove the lichen acids. The extraction was then continued with acetone. After three hours the dark-brown acetone solution was discarded and the extraction continued with fresh acetone for 5 days. The extract deposited colourless crystals, which were separated and dissolved in water. Coloured impurities were removed by passing the aqueous solution through a small column of aluminium oxide. The acetone solution was concentrated to dryness, the residue shaken with water and the aqueous solution decolorized as before. The combined aqueous solutions were concentrated to dryness and the residue subjected to fractional crystallization from ethanol. By this procedure mannitol (1.5 g) was obtained in a pure state, m.p. 161—162° alone or on admixture with an authentic specimen, but it proved impossible to obtain the other component in a pure state by recrystallization alone. When most of the mannitol had been extracted, however, it occasionally happened that the umbilicin crystallized as hemispherical aggregates of stout prisms, which could be mechanically separated from the mannitol crystals and purified by further recrystallization. By tedious manipulation, 0.61 g of umbilicin, m.p. 136—137°, was finally obtained. (The total amount of umbilicin was certainly higher, the optical rotation of the combined aqueous solutions indicating a content of about 2.5 g.) Further recrystallizations yielded the pure substance, m.p. 138—139°, $[\alpha]_D^{20}$ −81° (water, c = 2).

$C_{11}H_{22}O_{10}$ (314)	Calc.	C	42.1	H	7.02
	Found	»	41.2	»	7.05

On periodate oxidation 1 mole of the substance consumed 10.2 moles of oxidant.

Umbilicin octaacetate. A small amount of umbilicin was acetylated with acetic anhydride and pyridine. The acetate was recrystallized from methanol, giving an almost quantitative yield of the octaacetate, m.p. 84—85°, $[\alpha]_D^{20}$ −20° (chloroform, c = 2).

$C_{11}H_{14}O_{10}$ $(OCCH_3)_8$ (650.6) Calc. CH_3CO 52.9 Found CH_3CO 53.0

Hydrolysis of umbilicin. Umbilicin (250 mg) was hydrolyzed with 0.2 *N* sulfuric acid (5 ml), at 100° for 16 hours, the solution freed from acid with barium carbonate and concentrated to a sirup which was then dissolved in butanol-ethanol, 1 : 1 (7 ml). This solution was introduced on to the top of a hydrocellulose column (8 cm^2 × 28 cm) saturated with butanol-ethanol-water (40 : 2 : 9), and eluted with the same solvent. The eluate was divided into fractions of 7 ml, which were examined for carbohydrates with the

reagents mentioned above. Fractions 47—72 contained the polyol, fractions 73 and 74 traces of both components and 75—150 the reducing sugar, although in fractions 110—150 only very small amounts were present.

Fractions 47—72 were combined, concentrated and crystallized from acetone-ethanol (1 : 1). D-arabitol (78 mg) of m.p. 98—100°, undepressed on admixture with an authentic specimen, was obtained. The specific rotation determined in acidified ammonium molybdate solution according to the method of Richtmyer and Hudson [2] was + 131° (c = 0.2).

Fractions 74—150 were combined, concentrated and crystallized from ethanol. After one recrystallization fairly pure D-galactose (47 mg), showing m.p. 148—150° and the final rotation $[\alpha]_D^{20}$ + 76° (water, c = 0.2), was obtained. The slight impurities in the galactose were probably due to the fact that the umbilicin used for the hydrolysis was not perfectly pure and that the impurities accumulated preferentially in the sugar fraction. The galactose was characterized by oxidation to mucic acid in the ordinary way.

SUMMARY

From the lichen *Umbilicaria pustulata* (L.) Hoffm. D-mannitol and a galactoside, umbilicin, have been isolated. Umbilicin has been shown to be 3-D-arabitol β-D-galactopyranoside, on the assumption that furanoside structures may be excluded.

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On the Modes of Reaction of Hydrogen Peroxide with some Remarks on the Determination of the Mechanism of a Chemical Reaction from its Kinetics

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It is shown that kinetical experiments prove that the sequence:

$$Fe^{+3} + HO_2^- \rightleftharpoons FeOOH^{+2}$$
$$FeOOH^{+2} \rightleftharpoons Fe^{+3} + \overset{*}{HO_2^-}$$
$$\overset{*}{HO_2^-} + H_2O_2 \rightarrow O_2 + H_2O + HO^-$$

where $\overset{*}{HO_2^-}$ is a reactive isomer of HO_2^-, is the correct expression for the mechanism of the ferric-catalyzed decomposition of hydrogen peroxide

It is further shown that tracer experiments exclude reversible reactions, in which basic ferric ions occur as reactants, as possible partial reactions of the abovementioned catalytic reaction.

Generally it is shown that among a finite number of possible so called kinetic integrals only one, $\varphi(x)$, which is denoted as the chronometric integral can obey the condition $t = \varphi(x)$ in the whole range of x measures the advancement of the reaction.

Haber and Weiss [1] in their renowned paper introduced the postulate that hydrogen peroxide "is never simultaneously attacked by two monovalent reagents nor by a bivalent reagent whether the material is oxidized to oxygen or reduced to water" [2].

In other words they deny the possibility that two electrons simultaneously be removed from or taken up by the molecule H_2O_2. The statement may also be expressed in the equivalent form that the reactions

$$O_2^{-2} \rightarrow O_2 + 2e \quad (A)$$

and

$$O_2^{-2} + 2e \rightarrow 2O^{-2} \quad (B)$$

do not occur. The postulate has been repeated by N. Uri [2] with the addendum that "this conclusion is clearly recognized to-day". This may be true, but the question is whether the recognition is justified. What we are going to discuss below is mainly reactions of the type (A).

There are very old observations, which strongly indicate that reactions which are of the reversed (A) type actually do occur. If e.g. solid silver is treated with oxygen dissolved in an alcaline cyanide solution the initial reaction is

$$2\ Ag + O_2 + 4CN^- \rightarrow 2\ Ag(CN)_2^- + O_2^{--}$$

or including the reaction of the strong base O_2^{--} with water

$$2\ Ag + O_2 + 4CN^- + 2H_2O \rightarrow 2\ Ag(CN)_2^- + H_2O_2 + 2HO^-$$

When the concentration of the peroxide has increased to some extent another reaction sets in, its stoichiometric equation being

$$2\ Ag^+ + H_2O_2 + 4CN^- \rightarrow 2\ Ag(CN)_2^- + 2HO^-$$

Recent experiments by Vibeke Lund [3] performed in this institute confirmed these results. Mrs. Lund took special case to investigate the stoichiometry at the beginning, when the concentration of peroxide is small. Under these circumstances the stoichiometry was exactly the one represented by the first of the above reaction equations. This can be explained in the simplest way by the assumption of one two-electron step [4], but the evidence is obviously not sufficient to exclude the two one-electron steps:

$$Ag + O_2 + 2CN^- \rightarrow Ag(CN_2^-) + O_2^-$$
$$Ag + O_2^- + 2CN^- \rightarrow Ag(CN)_2^- + O_2^{-2}$$

or the corresponding reactions including water:

$$Ag + O_2 + 2\,CN^- + H_2O \rightarrow Ag(CN)_2^- + HO_2 + HO^-$$
$$Ag + HO_2 + 2CN^- + H_2O \rightarrow Ag(CN)_2^- + H_2O_2 + HO^-$$

But we may with some confidence conclude from the experiments that the molecular oxygen is reduced to hydrogen-peroxide without breaking of the O—O bond.

This conclusion is substantiated by the tracer experiments of Cahill and Taube [5] and also by P. Baertschi [10] on reactions of the type (A). These authors conclusively proved that when hydrogen peroxide is transformed by oxydants

into molecular oxygen, O_2 is derived cleanly from the H_2O_2. Or in other words by reactions of the two types (+ A) and (−A)

$$O_2^{-2} \rightleftharpoons O_2 + 2e \quad (\pm A)$$

there is evidence to show that the O—O bond is not broken.

Some years before Haber made his statement kinetic and spectroscopic evidence strongly favoured the assumption of the radicals HO_2 and HO in gas-reactions and there are to-day hardly any experiments to disprove their intermediary existence in certain gas mixtures.

We may therefore with confidence accept them as intermediates in gas-reactions and it is quite a natural assumption that they also occur as intermediates in certain reactions especially in aqueous solutions. It is on this idea that the Haber-Weiss mechanism for the catalytic decomposition of hydrogen peroxide and its modifications are based. Curiously enough, at the time when Haber and Weiss started their work the kinetic investigations on the reaction were insufficient and, certainly on account of the tragic events which took place in Habers last days ending with his death in exile, their own experiments were not carried through to the end.

In 1946 I therefore invited V. Sten Andersen to procure the missing kinetic experiments to prove the correctness of the Haber-Weiss mechanism of the decomposition of hydrogen-peroxide with ferric ion as a catalyst. Much to my surprise it turned out however, that neither the Haber-Weiss mechanism in its original form nor a number of different modifications including as intermediates Fe^{++}, HO and HO_2 would lead to kinetic expressions in conformity with the kinetics found by Sten Andersen in his very accurate experiments. This forced us to look out for other possible mechanisms and the results are known from the papers by Sten Andersen [6,7] and one by Sten Andersen and myself [8]. In this the following mechanism has been arrived at:

$$Fe^{+3} + HO_2^- = FeOOH^{2+}$$

$$FeOOH^{+2} \rightleftharpoons Fe^{+3} + \overset{*}{HO_2^-}$$

$$\overset{*}{HO_2^-} + H_2O_2 \rightarrow O_2 + H_2O + HO^-$$

where $\overset{*}{HO_2^-}$ denotes a reactive isomer of the ordinary HO_2^--ion.

It must be emphasized that the mechanisms proposed in the papers cited are based directly on the experimental evidence and that the method always has been to follow the reaction till about 95 per cent decomposition. Of course the concentration of hydrogen-peroxide becomes very small at the end, but as well known it can nevertheless be determined with good relative accuracy

even at small concentrations by the simple device of replacing the ordinary burettes by microburettes for the later determinations, which procedure was adopted.

As can be seen from Sten Andersen's paper [6] the expression $\log (a/x) + A\ (1/x - 1/a)$ is a linear function of time in the whole interval from $x/a \sim 0$ to $x/a \sim 0.95$, and this is not true for other functions containing a limited number of constants. The deviations at the beginning of the experiment are certainly due to a certain relaxation-time during which the steady state is being established.

Considering that Sten Andersen's results were so unexpected it is no wonder that they have been criticized from different quarters. The criticisms follow two main lines. Some critics maintain that the mechanism for some preconceived reason is untenable. Considering that it turned out to be a necessary consequence of the experiments such objections can only be seriously discussed on the basis of new experimental evidence.

Others have tried to show that Sten Andersen's results can equally well be represented by other functions.

A typical example is found in a review by N. Uri [2]. He maintains that Sten Andersen's results (in [6] table 1) can be represented by the formula

$$\log\ (a-\varkappa)-\log(x-\varkappa) = k't$$

where $\varkappa = 1.77 \cdot 10^{-3}$. a in [6] Table 1 is $4.71 \cdot 10^{-2}$ M.

Now in the first place it is evident that this cannot be an exact expression as it would mean that x reaches the limit $\varkappa$ at $t \to \infty$. As however Uri does not pretend the formula to be more than an approximation it is of no use to stress this point.

It is more important that it can be shown that Uri's expression for values of x which are at least a few times greater than $\varkappa$ is simply an approximation to Sten Andersen's.

To start with, let us transform to natural logarithms thereby changing k' into $k = k'/0.4343$. We may then write the expression

$$\ln\ (a/x) + \ln(1-\varkappa/a)-\ln(1-\varkappa/x) = kt$$

Development of the two last logarithms yields

$$\ln(a/x) + \varkappa(\frac{1}{x} - \frac{1}{a}) + \frac{\varkappa^2}{2}\ (\frac{1}{x^2} - \frac{1}{a^2}) + \ldots = kt$$

Considering that Sten Andersen's last three values of x are respectively 4.02, 2.69 and 1.96 all multiplied by 10^{-3} it is thus no wonder that Uri has found that his expression conforms with Sten Andersen's results, when the

experiments at the lowest peroxide concentrations are omitted. This omission is however not justified by the inaccuracy of the experiments.

It is also questionable whether Uri's statement [2 p. 383] that the stationary values of ferrous ion and hydroxyl radical are nearly independent of the peroxide concentration, is correct. Unfortunately the mechanism assumed by Uri is not given in his paper and a sound discussion of this point is therefore excluded.

It may however be of interest to the reader to see how a mechanism of the Haber-Weiss type may be treated. Let us assume the following mechanism (H) which is similar to the one used by Barb, Baxendale, George and Hargrave [9] and which includes the reaction $Fe^{+2} + H_2O_2 \rightarrow FeOH^{+2} + HO$ advocated by Uri:

$$FeOH^{+2} + H_2O_2 \rightleftharpoons Fe^{+2} + H_2O + HO_2 \qquad \binom{01}{10}$$

$$Fe^{+2} + H_2O_2 \rightleftharpoons FeOH^{+2} + HO \qquad \binom{12}{21}$$

$$HO + H_2O_2 \rightleftharpoons HO_2 + H_2O \qquad \binom{23}{32}$$

$$HO_2 + FeOH^{+2} \rightarrow O_2 + H_2O + Fe^{+2} \qquad (31)$$

Replacing the symbols H_2O_2, Fe^{+2}, HO, HO_2 by X_0, X_1, X_2, X_3 respectively we may illustrate the situation by the following diagram (Fig. 1).

This is intended to mean that the intermediates X_1 and X_3 are formed simultaneously from the original stable molecules, $[FeOH^{+2}]$ being determined by the acidity of the solution and the total concentration of ferric salt. Furthermore X_1 disappears by the net reaction (12) and is formed by (31), X_2 disappears by the net reaction (23) and is formed by (12) and finally X_3 disappears by the net reaction (31) and is formed by (23). A stationary flow of intensity s in the direction 123 is obviously equivalent to the occurence of the overall reaction (of speed s)

$$2H_2O_2 \rightarrow O_2 + 2H_2O$$

The two flows o indicated must for stoichiometrical reasons be equal. Furthermore the conditions of stationarity are only fulfilled if the net flows s_{12}, s_{23} and s_{31} are all equal and the flow o is zero.

Calling the concentrations of X_1, X_2, X_3 respectively x_1, x_2, x_3 we thus get four equations

$$\begin{aligned} x_1 \cdot x_3 &= K_0[FeOH^{+2}][H_2O_2] = P \\ x_1 w_{12} - x_2 w_{21} &= s \\ x_2 w_{23} - x_3 w_{32} &= s \\ x_3 w_{31} &= s \end{aligned}$$

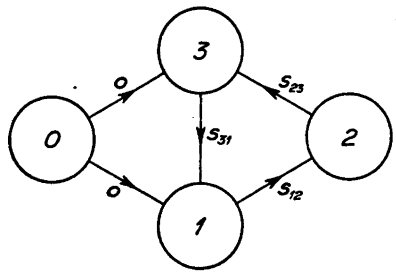


Fig. 1. The diagram shows the flows corresponding to the mechanism (H). For stoichiometric reasons $o_{01} = o_{03} = o$. *The steady state conditions furthermore require* $s_{12} = s_{23} = s_{31} = s$ *and* $o = o$.

The symbol w_{ij} is intended to mean the probability pro unit time that the intermediate X_i shall react with formation of the intermediate X_j.

Solution of the last three equations yields

$$\frac{x_1}{s} = \frac{1}{w_{12}} + \frac{w_{21}}{w_{12}}\frac{1}{w_{23}} + \frac{w_{21}}{w_{12}}\frac{w_{32}}{w_{23}}\frac{1}{w_{31}}$$

$$\frac{x_2}{s} = \frac{1}{w_{23}} + \frac{w_{32}}{w_{23}}\frac{1}{w_{31}}$$

$$\frac{x_3}{s} = \frac{1}{w_{31}}$$

and by combination with $x_1 x_3 = P$ we get

$$\frac{P}{s^2} = \frac{1}{w_{31}}\frac{1}{w_{12}}\left(1 + \frac{w_{21}}{w_{23}} + \frac{w_{21}}{w_{23}}\frac{w_{32}}{w_{31}}\right)$$

Now $w_{12} = k_{12}[H_2O_2]$, $w_{21} = k_{21}[FeOH^{+2}]$;
$w_{23} = k_{23}[H_2O_2]$; $w_{32} = k_{32}$
$w_{31} = k_{31}[FeOH^{+2}]$
$P = K_0[FeOH^{+2}][H_2O_2]$

which by insertion leads to

$$K_0 k_{12} k_{31}/s^2 = \frac{1}{[H_2O_2]^2}\frac{1}{[FeOH^{+2}]^2}\left[1 + \frac{k_{21}}{k_{23}}\frac{[FeOH^{+2}]}{[H_2O_2]} + \frac{k_{21}}{k_{23}}\frac{k_{32}}{k_{31}}\frac{1}{[H_2O_2]}\right]$$

The experiments indicate that the last member inside the brackets may be discarded, *i.e.* that k_{32}/k_{31} is effectively zero.

We may now set $[H_2O_2] = x$ and $-\frac{dx}{dt} = 2s$.

Furthermore in a definite experiment [$FeOH^{+2}$] is a constant. We thus arrive at a differential equation of the following form

$$-k\frac{dt}{dx} = \frac{1}{x}\sqrt{1+\frac{2\varkappa}{x}}$$

This can be integrated in finite terms, but to compare it with Sten Andersen's expression it is easier to note the fact that so long as $2\,\varkappa/x$ is small it approximates the form

$$-k\frac{dt}{dx} = \frac{1}{x} + \frac{\varkappa}{x^2} \text{ or}$$

$$kt = \ln(a/x) + \frac{\varkappa}{a}\left(\frac{a}{x} - 1\right)$$

To estimate the deviations we may use the better approximation

$$-k\frac{dt}{dx} = \frac{1}{x} + \frac{\varkappa}{x^2} - \frac{\varkappa^2}{2x^3} + \frac{\varkappa^3}{2x^4} \cdots\cdots$$

from which by integration

$$kt = \ln(a/x) + \frac{\varkappa}{a}\left(\frac{a}{x} - 1\right) - \frac{\varkappa^2}{4a^2}\left(\frac{a^2}{x^2} - 1\right) + \frac{\varkappa^3}{6a^3}\left(\frac{a^3}{x^3} - 1\right) + \ldots$$

This formula shows that the deviations from Sten Andersen's expression, which comprises only the first two members, become significant when $\varkappa/x$ approaches 1. As all Sten Andersen's experiments conform with his expression also when $\varkappa/x$ exceeds 1 this indicates that it is his formula and not the one derived above, which is the correct one.

This result has recently been confirmed by the afore-mentioned work by Cahill and Taube [5]. In the above reaction (12) the basic ion $FeOH^{+2}$ is formed from the peroxide. If now the peroxide oxygen is labelled by ^{18}O the basic ion also becomes labelled. In the back reaction (21) the peroxide is formed from the basic ion which certainly is in isotopic equilibrium with the solvent water. Therefore according to the mechanism (of the Haber-Weiss type) considered above a reversible isotopic exchange between the water and the peroxide must take place, but this would be in contradiction with Cahill and Taubes experiments on the ferric-ion catalyzed decomposition of hydrogen peroxide. To avoid misunderstandings it must be added that the water formed by the overall reaction: $2\,H_2O_2 \rightarrow 2\,H_2O + O_2$ must be labelled to the same extent as the hydrogen peroxide and the oxygen, but this effect is irreversible.

The tracer experiments show that the oxygen evolved has the same isotopic composition as the one of the original peroxide. The reverse experiment with

labelled water und unlabelled peroxide to yield unlabelled oxygen has not been performed by C. and T. so far as can be seen from their paper, but P. Baertschi has informed the author, that also in this case the isotopic composition of the oxygen evolved is independent of that of the solvent water[11,10].

Reverting to kinetics it can be proved that any mechanism leading to an expression, which is different from Sten Andersen's must be discarded. As the proof may be of interest also in other cases we shall try to present it in a general form.

We restrict our considerations to cases where one number (x) is sufficient to describe the advancement of the reaction.

Let us denote a function which has been derived by integration of the expression for the reciprocal steady state velocity derived from some reaction mechanism as a kinetic integral (belonging to the mechanism in question).

Any kinetic integral must obviously have one of the following two equivalent properties:

1) It approaches zero when x approaches a constant (a) and it approaches infinity, when x approaches zero or 2) It approaches zero when x approaches zero and it approaches infinity when x approaches a constant (a).

(In Sten Andersen's experiments x has been so defined that it is the condition in the form 1) which must be fulfilled).

If now a kinetic integral $\varphi\,(x)$ has been shown by experiments to obey the condition

$$t = \varphi\,(x)\ \ (a > x > 0)$$

where t is the time, we shall denote it as the chronometric integral (of the overall reaction in question).

Thus several but a finite number of different kinetic integrals may belong to one overall reaction. What we intend to prove is that only one of these can be the chronometric integral of that reaction.

The following statement must necessarily be true: Two different kinetic integrals containing the same limited number of adjustable constants may be made to coincide in a limited range of x by a suitable choice of the constants, but it is impossible to make them coincide in the complete range from a to zero.

If therefore a kinetic integral φ, containing only few adjustable constants has been proved by experiments to obey the condition $t = \varphi$ in the whole range of x, φ must be the chronometric integral of the reaction and no other satisfactory one can be found.

As now nearly any change in the mechanism, from which the chronometric integral has been derived, leads to a definite change in the form of the integral,

the mechanism from which it has been derived must be in nearly all cases the only one which is in agreement with the experiments.

It must be remembered that not all values of the constants are acceptable. In the first place the constants k_{ij} can never be negative. Furthermore, especially when their dependence on temperature is also investigated, it may turn out that they have impossibly large or small values. In such cases modifications of the mechanism must be looked for.

To quote an example: By extending his investigation of the catalytic decomposition of hydrogen-peroxide to different temperatures Sten Andersen [7] found for the mechanism (C):

$$\begin{aligned} &Fe^{+3} + HO_2^- \rightleftharpoons FeOOH^{+2} && \binom{12}{21} \\ &FeOOH^{+2} \rightleftharpoons FeOH^{+2} + O && \binom{25}{52} \\ &O + HO_2^- \rightarrow O_2 + HO^- && (50) \end{aligned}$$

that the ratio k_{50}/k_{52} was of the order of magnitude 10 [9] and was nearly independent of temperature. Now from our general knowledge of the numerical values of velocity-constants this result is extremely improbable. This compelled us to introduce the modified mechanism (D) (compare p. 1058):

$$\begin{aligned} &Fe^{+3} + HO_2^- \rightleftharpoons FeOOH^{+2} && \binom{12}{21} \\ &FeOOH^{+2} \rightleftharpoons Fe^{+3} + \overset{*}{HO_2^-} && \binom{25}{52} \\ &\overset{*}{HO_2^-} + H_2O_2 \rightarrow O_2 + H_2O + HO^- && (50) \end{aligned}$$

The assumption of mechanism (D) leads to a kinetic integral of exactly the same form as the one derived from mechanism (C) but now the ratio k_{50}/k_{52} becomes of the order of magnitude 1 and is still nearly independent of temperature, which is what we should expect from general knowledge.

To-day it may be added that the experiments by Cahill and Taube [5] definitely exclude the possibility (C) as the reaction

$$FeOOH^{+2} = FeOH^{+2} + O$$

and its reverse would cause a reversible isotopic exchange between hydrogen peroxide and the solvent water.

All the experiments thus go together to show that the mechanism (D) represents the way in which the ferric-catalyzed decomposition of hydrogen peroxide takes place.

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Titration of Pyrazolones with Perchloric Acid in Glacial Acetic Acid

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In 1927 Hall and Conant [1] showed that even very weak bases, *e.g.* amides, may be titrated in non-aqueous solvents, *e.g.* glacial acetic acid, with strong acids, *e.g.* perchloric acid, in the same solvent. Since then a considerable amount of work has been published. Recent summaries have been given by Tomicek and Heyrovsky [2], by Markunas and Riddick [3], and by Riddick [4].

Hall [5] and Tomiček and Heyrovsky [2] showed that by the potentiometrical titration it is possible to determine the pK_B-value of the base examined, as the $pK_{B(HAc)}$-values run parallel to the $pK_{B(H_2O)}$-values at all events for bases with $pK_{B(H_2O)}$-values between 9—10 and 13—14. Lemaire and Lucas [6] have given a theoretical survey of the potentiometrical determination of basic strength in non-aqueous solution, calculating the thermodynamical acid dissociation constant of the ion BH^+ from the apparent dissociation constant.

In previous studies on pyrazolones [7] we have shown that both 5- and 3-pyrazolones may be titrated potentiometrically in ethanolic solution with aqueous sodium hydroxide. From the titration curves it may be concluded that the 5-pyrazolones are stronger acids than the 3-pyrazolones. We have now determined the K_B-values for some 5- and 3-pyrazolones by titrating their glacial acetic acid-solutions with perchloric acid and have found that also as bases the 5-pyrazolones are stronger than the 3-pyrazolones.

The technique employed has been the following:

1 millimol of the pyrazolone is dissolved in 50 ml glacial acetic acid. The solution is titrated potentiometrically with a 0.1000 N solution of perchloric acid in glacial acetic acid, prepared as indicated by Markunas and Riddick [3], using a glass electrode, a saturated calomelelectrode as reference electrode, and a tube potentiometer for determining the potential between the electrodes. Hereby the $pK_{B(HAc)}$-values may be calculated from the potential measured by half-neutralisation of the pyrazolone.

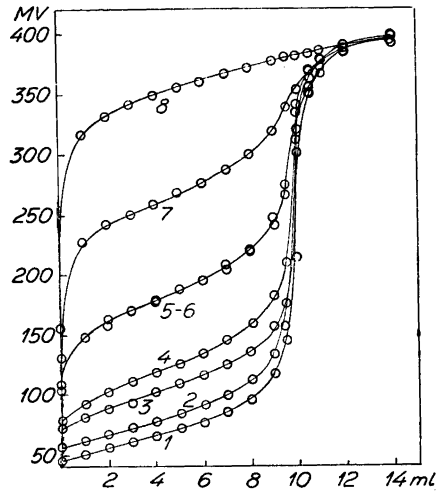


Fig. 1. Titration curves of different amines.

	pK_B
1. *Piperidine*	2.8
2. *Pyridine*	8.7
3. *p-Chloroaniline*	10.1
4. *m-Chloroaniline*	10.5_3
5. *m-Nitroaniline*	11.4
6. *o-Chloroaniline*	11.4_3
7. *p-Nitroaniline*	12.9
8. *o-Nitroaniline*	14.2

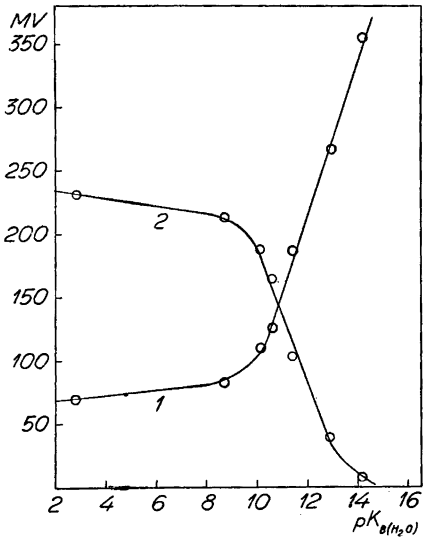


Fig. 2. Dependency of half neutralisation potentials (curve 1) and jump of potential 95 %—110 % neutralisation (curve 2) on $pK_{B(H_2O)}$.

In order to determine the curve indicating the relation between the $pK_{B(HAc)}$-values and the corresponding $pK_{B(H_2O)}$-values we titrated a series of weak bases with known $pK_{B(H_2O)}$-values. Fig. 1 shows the titration curves for 8 bases with $pK_{B(H_2O)}$-values from 2.8 to 14.2. Instead of calculating the $pK_{B(HAc)}$-values from the half-neutralisation potentials and plotting them against the $pK_{B(H_2O)}$-values we simply plotted the potentials measured after addition of 5 ml of the acid against the $pK_{B(H_2O)}$-values. This may be done because we are using constantly 1 millimol in 50 ml. If other concentrations are used a new series of curves has to be determined, the potential being dependent on the ionic strength of the solution. This has to be done also if the glass electrode is shifted out for another glass electrode.

Fig. 2 curve 1 shows the result. It is seen that both in the region pK_B 2—9 and in the region pK_B 10—14 there is a linear dependence between the half-

neutralisation potentials and the $pK_{B(H_2O)}$-values, but only in the last mentioned region is the slope of the line so that the $pK_{B(H_2O)}$-values may be determined with sufficient accuracy from a half-neutralisation potential found by titration.

In Fig. 2 curve 2 we have plotted the jump of potential from 95 % to 110 % of the theoretical amount of perchloric acid added. It is seen that this jump too is linearly dependent on the $pK_{B(H_2O)}$-value of the base titrated.

It is not strictly necessary to use exactly 1 millimol for the titration, but if other amounts of base are used the curve elaborated with 1 millimol titrations is not exactly valid. Fig. 3 shows the titration curves of 0.70 millimol, 1.00 millimol, and 1.38 millimol m-chloroaniline respectively. The potentials at half neutralisation are 129 MV, 126 MV, and 122 MV, to which correspond the $pK_{B(H_2O)}$-values 10.6, 10.5, and 10.4. The jumps of potential from 95 % to 110 % neutralisation are 166 MV, 168 MV, and 175 MV respectively, to which correspond the $pK_{B(H_2O)}$-values 10.5, 10.4, and 10.3, the value indicated in the litterature being 10.5_3.

We then titrated 1-phenyl-3-methyl-pyrazolone-5, 1-phenyl-3-methyl-4-ethyl-pyrazolone-5, 1,3-diphenyl-pyrazolone-5 and the 3 isomeric 3-pyrazolones. Table 1 summarises the half-neutralisation potentials and the $pK_{B(H_2O)}$ values.

Table 1. Potentiometrical titration of some pyrazolones.

Pyrazolone	Potential at 50 % neutralisation	$pK_{B(H_2O)}$
1-Phenyl-3-methyl-5	176 MV	11.3
1-Phenyl-3-methyl-4-ethyl-5	186 MV	11.5
1,3-Diphenyl-5	228 MV	12.2
1-Phenyl-5-methyl-3	226 MV	12.1
1-Phenyl-4-ethyl-5-methyl-3	236 MV	12.3
1,5-Diphenyl-3	273 MV	12.9

From these figures it is evident that the 5-pyrazolones are stronger bases than the 3-pyrazolones. As might be expected, the substitution of a phenyl group for a methyl group causes a weakening of the basic character of the substance. On the other hand, the substitution of an ethyl group for a hydrogen atom in position 4 has only a minor influence upon the pK_B-value.

Next we investigated the behaviour of a 4,4-dialkylsubstituted 5-pyrazolone when titrated with perchloric acid. We found that if such a substance has basic properties at all, the pK_B-value is at least > 15, as the potential measured

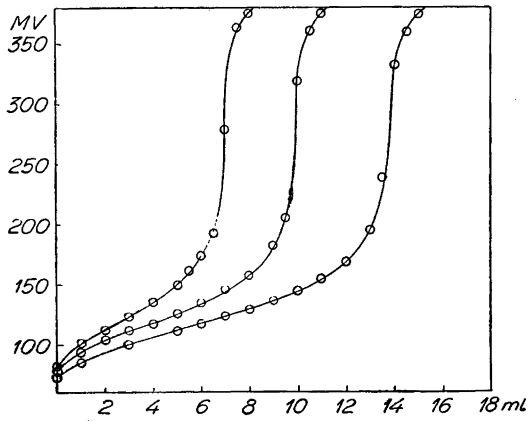
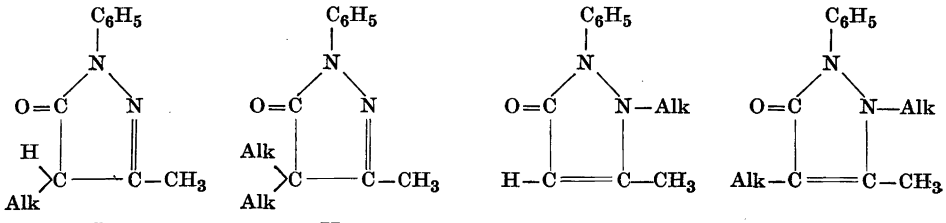


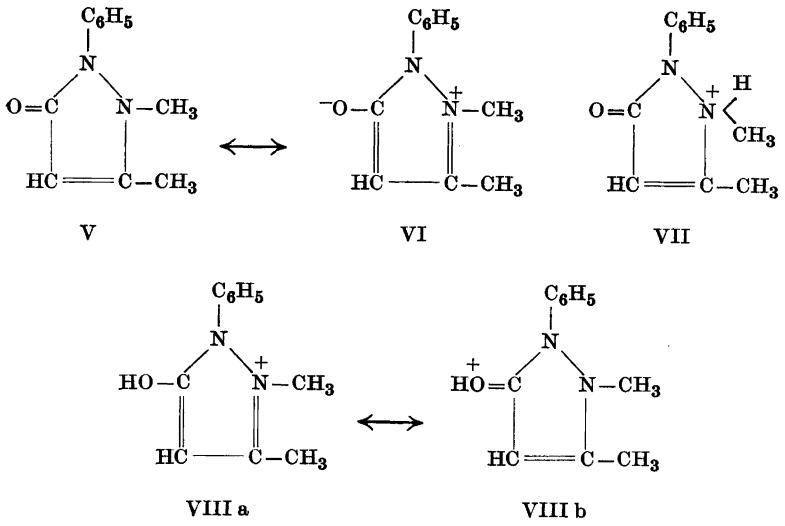
Fig. 3. Titration of 0.70 millimol, 1.00 millimol, and 1.38 millimol m-chloroaniline in 50 ml glacial acetic acid with 0.1000 N $HClO_4$.

after addition of 1—2 ml 0.1 *N* perchloric acid to 1 millimol of the pyrazolone was above 400 MV.

Hall [5] found that it is possible to titrate antipyrine, and we were able to confirm this. We then tried to titrate a 4-substituted antipyrine, *viz.* 4-isopropylantipyrine, which is a dialkylsubstituted 5-pyrazolone. We found it just as titratable as antipyrine itself. This means that it is not the substitution of 2 alkyl groups for 2 hydrogen atoms which abolishes the basic character of the 5-pyrazolone, but the positions in which the alkyl groups are placed determine whether the pyrazolone may be titrated or not.



Looking at the formulas I—IV one might draw the conclusion that the condition for titratability is that the pyrazolone is able to acquire an antipyrine structure. For antipyrine at least two mesomeric formulas V and VI are possible. To formula V corresponds the formulas VII or VIII b for the antipyrinium-



V VI VII

VIII a VIII b

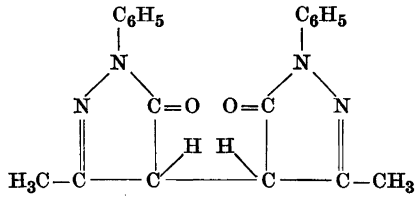
ion, to VI the formula VIII a, mesomeric with VIII b.

Jensen and Friediger [8] have shown that antipyrine has the abnormally high dipole moment of 5.48 D (3-methyl-pyrazolone-5 $\mu = 2.54$ D) and conclude from this that the contribution of formula VI to the constitution of antipyrine amounts to some 30 % (μ calculated for the phenol-betaine VI 18 D).

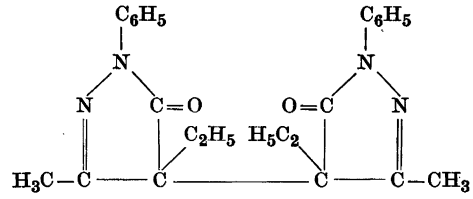
This being valid the untitrability of II is understandable as the condition for titratability seems to be the possibility of arranging conjugated double bonds in the nucleus, *i.e.* to establish a pyrazole structure VI in stead of the pyrazolone structure V, and with two substituents at C_4 this is impossible.

In order to try the theory of the necessity of an antipyrine-like structure for the titratability of a pyrazolone we investigated the titration of the following substances, the preparation of which will be described elsewhere.

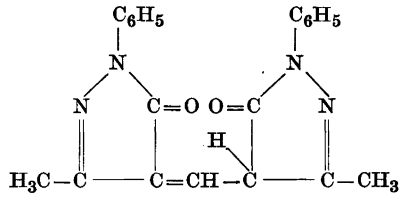
Of these substances X is untitratable. IX and XI are titratable, but IX claims two equivalents of perchloric acid pr. mol, XI only one. This is in accordance with the prediction. XII and XIII, on the other hand, are both titratable, and this cannot be explained if the formulas XII a and XIII a are valid. The two substances may be titrated as acids too, and this means that a displacement of a hydrogen atom from the cyclohexyl ring or from the methylene group in the side chain in position 4 to the carbonyl-oxygen (or to the nitrogen atom 2) is possible so that the formulas XII b and XIII b or the corresponding enol-betainstructures or the mesomeric antipyrine-structures are more likely than the pyrazolone formulas.



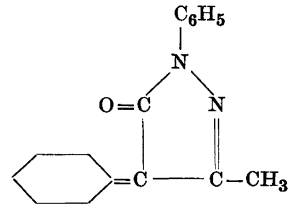
IX



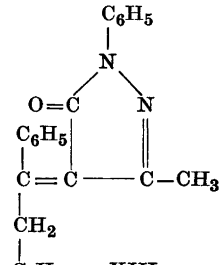
X



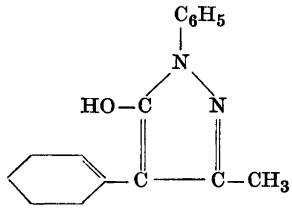
XI



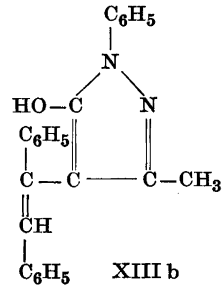
XII a



XIII a



XII b



XIII b

In table 2 we have summarised the half-neutralisation potentials and the $pK_{B(H_2O)}$-values for the pyrazolones investigated and not mentioned in Table 1.

Table 2. Potentiometrical titration of some pyrazolones.

Pyrazolone	Potential at 50% neutralisation	$pK_{B(H_2O)}$
Antipyrine	169 MV	11.2
Isopropylantipyrine	189 MV	11.6
X	> 410 MV	>15
XI	one nucleus 312 MV	13.8
	the other —	> 15
XII	173 MV	11.3
XIII	230 MV	12.2

SUMMARY

It has been shown that pyrazolones may be titrated as bases with perchloric acid in glacial acetic acid. A condition for the titrability of the pyrazolones seems to be that they are able to establish a pyrazole structure by moving a hydrogen from C_4 to N_2 or to O at C_5. From the potentiometrical titration curves of the pyrazolones their $pK_{B(H_2O)}$-values may be estimated.

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Studies on Carbamates

VIII. The Carbamates of Benzylamine, Piperidine and Aniline

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1. The equilibrium conditions and reaction mechanism of the formation and decomposition in aqueous medium of ammonium carbamate and of the carbamates formed by amines, the most recent being *n*-propylamine and *iso*-propylamine [1], and a few amino acids [2] have been studied in previous investigations.

The present investigation deals with the corresponding conditions with regard to the carbamates formed by benzylamine, piperidine and aniline. The conditions have been shown to be analogous to those of the carbamates previously investigated, only differing somewhat quantitatively. The experimental methods likewise being the same, we refer to previous investigations [1-3] for the detailed information concerning method, theory, significance of constants *etc.* It should be noted, however, that "Am" means amine and "AmH^+" aminium ion. $c_{carbamate}$ signifies the concentration of $C_6H_5CH_2NHCOO^-$, $C_5H_{10}NCOO^-$ or $C_6H_5NHCOO^-$, and $c_{AmHcarbamate}$ signifies the concentration of $C_6H_5CH_2NHCOONH_3CH_2C_6H_5$ or $C_5H_{10}NCOONH_2C_5H_{10}$.

2. The following preparations were used in the experiments:

Benzylamine and piperidine (both from Fischer Scientific Co.) were purified by distillation through a wiregauze column with 55 sets of platinum plates [4], provided with a sodalime tube to prevent the admission of carbon dioxide from the atmosphere. For the experiments with benzylamine we used a fraction with a boiling point 184.5–184.8° C (759 mm Hg), $n_D^{20.0°} = 1.5439$, and by acidimetric titration with 0.1 *N* HCl we obtained a result corresponding to a

* The co-operation of this author is made possible by grants from *Det teknisk-videnskabelige Forskningsråd.*

content of 99.4 % $C_6H_5CH_2NH_2$. For the experiments with piperidine we used a fraction with a boiling point 106.2—106.4° C (757 mm Hg), $n_D^{20.0°} = 1.4537$, and by titration with 0.1 *N* HCl we obtained a result corresponding to a content of 99.7 % $C_5H_{10}NH$.

Aniline (puriss. Merck) was distilled from zinc dust in an ordinary distiller. The fraction used had a boiling point of 184.4° C (760 mm Hg), $n_D^{20.0°} = 1.5860$, and gave a result corresponding to a percentage of 100.4 by a bromometric titration, according to Vaubel [5].

The applied phenyl isocyanate was an Eastman Kodak Company product.

The above-mentioned boiling points are corrected for calibration errors; stem corrections have been done.

3. The carbamates of benzylamine and piperidine were prepared partly in solution by leading a deficit of carbon dioxide to aqueous solutions of amines, practically all of the carbon dioxide thus being converted to carbamate "$CO_2 + 2C_6H_5CH_2NH_2 = C_6H_5CH_2NHCOONH_3CH_2C_6H_5$" and "$CO_2 + 2C_5H_{10}NH = C_5H_{10}NCOONH_2C_5H_{10}$", and partly as substances. The carbamate of aniline was only prepared in solution.

Solutions of the carbonates of benzylamine and piperidine were prepared by mixing equivalent amounts of solutions of benzylammonium chloride (piperidinium chloride, respectively) and sodium carbonate "$2C_6H_5CH_2NH_3Cl + Na_2CO_3 = (C_6H_5CH_2NH_3)_2CO_3 + 2$ NaCl" and "$2C_5H_{10}NH_2Cl + Na_2CO_3 = (C_5H_{10}NH_2)_2CO_3 + 2$NaCl", *i.e.* the resulting solutions contain some sodium chloride, but this is insignificant in the present investigation.

a) The carbamate of benzylamine, $C_6H_5CH_2NHCOONH_3CH_2C_6H_5$, was prepared mainly according to Fichter and Becker [6]. 10 g benzylamine were gradually dissolved in 100 g anhydrous ether — added in portions of about 1 g. After each addition the solution was saturated with dry carbon dioxide, whereby a microcrystalline precipitate of the carbamate was formed. The precipitate was collected on a filter and after the evaporation of the ether it was placed in an exsiccator over concentrated sulphuric acid. It should be noted, that the precipitate turns gelatinous, if all the benzylamine is dissolved in the ether at the beginning. With regard to the solubility of the carbamate it should be stated, that 100 g of the mother liquor contains amine corresponding to approx. 0.5 g carbamate. 82.7 % af amine (theoretically 83.0 %) was found by titration of the carbamate with 0.1 *N* HCl, and by the method of analysis, stated under 4, 17.4 % of carbon dioxide in the form of carbamate (theoretically 17.0 %) was found.

b) An attempt was made to prepare the carbamate of piperidine by the same method as that used in the preparation of the carbamate of benzylamine, but no precipitate appeared by saturation of the etheric piperidine solution

with carbon dioxide. If on the other hand a solution of piperidine in ether containing a little water is saturated with carbon dioxide, a precipitate of carbamate at once appears. 17 g piperidine and 1.8 g water, *i.e.* half a mole of water per mole of piperidine, were dissolved in about 150 g anhydrous ether, and by saturation of the solution with carbon dioxide a distinctly crystalline precipitate of carbamate was formed. With regard to the solubility of the carbamate it should be stated that 100 g of the mother liquor contains amine corresponding to approx. 1 g carbamate. 73.1 % of amine was found by titration of the carbamate with 0.1 *N* HCl, and by the method of analysis, stated under 4, 18.8 % of carbon dioxide in the form of carbamate was found. This corresponds with the compound $C_5H_{10}NCOONH_2C_5H_{10},H_2O$, *i.e.* with a mole of crystal water, the theoretical contents of which are 73.3 % of amine and 18.9 % of carbon dioxide.

The carbamate of piperidine — as well as the carbamate of benzylamine — can, at least for some time, be stored in closed bottles in an exsiccator with concentrated sulphuric acid.

c) Solutions of the carbamate of aniline are prepared according to Mohr [7]. 0.04 mole of phenyl isocyanate is shaken for twenty minutes with 1 litre of approx. 0.24 *N* NaOH, whereby all the phenyl isocyanate is dissolved and converted to $C_6H_5NHCOO^-$, *viz.* "$C_6H_5NCO + OH^- = C_6H_5NHCOO^-$". After the twenty minutes all smell of phenyl isocyanate has disappeared, and no phenyl isocyanate can be extracted by ether. The solution remains limpid by the addition of barium chloride showing that no carbonate is formed — or at any rate to an extremely small extent.

4. The method of analysis was as in previous investigations precipitation with barium chloride, *i.e.* barium chloride is added to 100 ml of the solution, if necessary previously made alkaline by the addition of sodium hydroxide. In this way the carbonate is precipitated, whilst the carbamate remains dissolved. The precipitate and the supernate are separated as quickly as possible by centrifugation. If the supernate is left standing or is heated, the carbamate decomposes forming a precipitate of barium carbonate, the amount of which can be determined by titration with 0.1 *N* HCl.

Since the solutions containing only carbonate, and no carbamate, upon precipitation with barium chloride yield a supernate precipitating a small amount of barium carbonate, it was found necessary to carry out blank experiments.

All of the data presented in later tables are corrected, unless otherwise stated, for the blank values, *viz.* about 3 units of the percentage.

5. All of the experiments were done at 18° C, and the velocity constants were calculated by means of Briggs' logarithms, the unit of time being the

minute. As in previous investigations the activity coefficient f for a monovalent ion was calculated from the expression of Bjerrum: $-\log f = 0.3\ ^3\sqrt{c_{ion}}$.

6. Hantzsch and Sebaldt [8] have found the basic dissociation constant for benzylamine at 0° C to be $10^{-4.82}$ and at 25° C $10^{-4.71}$, the latter being in good agreement with Bredigs [9] corrected value of $10^{-4.70}$. By interpolation the value for the basic dissociation constant of benzylamine is calculated to $10^{-4.74}$ at 18° C. Hantzsch and Sebaldt [10] for piperidine have the values $10^{-2.95}$ and $10^{-2.94}$ at 15° C and 20° C, respectively. At 25° C they have the value $10^{-2.92}$, and this one is in good agreement with Bredigs [9] corrected value of $10^{-2.92}$. The value $10^{-2.95}$ may here be used for 18° C.

It should be noted, that the basic dissociation constants for benzylamine and piperidine are called "K_{Am}".

The basic dissociation constant for aniline is for 18° C approx. $10^{-9.3}$. The exact value is insignificant in the present investigation, all the solutions in the experiments being so basic, that practically all the aniline is present as $C_6H_5NH_2$.

For the value of K_{H_2O} at 18° C $10^{-14.22}$ has been used.

On the reaction "amine + carbon dioxide ⇌ carbamic acid"

500 ml of an aqueous solution containing both amine and sodium hydroxide, were placed in a 2 litre flask. In the gas phase above this solution approximately 250 ml of carbon dioxide were substituted for an equal amount of atmospheric air, and the flask was shaken vigorously for about 2 minutes to obtain absorption of the carbon dioxide. The carbon dioxide reacts partly with the amine "carbon dioxide + amine → carbamic acid", partly with the hydroxyl ion "carbon dioxide + hydroxyl ion → bicarbonate ion".

The mixture was immediately analysed, see Table 1, where "% carbamate" indicates how many per cent of the carbon dioxide absorbed have been converted to carbamate. In the case of benzylamine and piperidine considerable amounts of carbamate are formed, while only small, but undoubtedly measurable amounts are formed in the case of aniline. In order to illustrate how much importance may be attached to the determinations as to aniline the amount of 0.1 N HCl used in the experiments as well as in the blank experiments is presented as a footnote of the table.

In Table 1 are also listed the values of $k_{CO_2 \cdot Am}$ calculated from the experiments, where $k_{CO_2 \cdot Am}$ is the velocity constant o f the reaction "carbon dioxide + amine → carbamic acid".

To get a better determination of $k_{CO_2 \cdot Am}$ for aniline we made another experiment in a fa r less basic solution than the ones listed in Table 1. The solution

Table 1. Carbon dioxide in amine + NaOH. 18°.

	Initial solution		Absorb. mol CO_2/litre	% carbamate	Final solution		Mean		$k_{CO_2\cdot Am}$	
	c_{NaOH}	c_{Am}			c_{NaOH}	c_{Am}	c_{NaOH}	c_{Am}		Mean
$C_6H_5CH_2NH_2$	0.20	0.10	0.0195	37	0.17	0.09	0.18	0.10	$10^{5.06}$	$10^{5.06}$
	0.10	0.10	0.0176	54	0.07	0.09	0.09	0.10	$10^{5.04}$	
	0.10	0.05	0.0178	39	0.07	0.04	0.08	0.05	$10^{5.08}$	
$C_5H_{10}NH$	0.20	0.10	0.0189	79 [1]	0.18	0.09	0.19	0.09	$10^{5.89}$	$10^{5.89}$
	0.20	0.05	0.0175	65 [2]	0.18	0.04	0.19	0.04	$10^{5.91}$	
	0.20	0.05	0.0197	63 [3]	0.17	0.04	0.19	0.04	$10^{5.88}$	
$C_6H_5NH_2$	0.08	0.22	0.0202	5.6 [4]	0.04	0.22	0.06	0.22	$10^{3.23}$	[9]
	0.05	0.24	0.0189	4.6 [5]	0.01	0.24	0.03	0.24	$10^{2.80}$	
	0.10	0.10	0.0212	3.1 [6]	0.06	0.10	0.08	0.10	$10^{3.42}$	
	0.10	0.12	0.0210	2.5 [7]	0.06	0.12	0.08	0.12	$10^{3.26}$	
	0.10	0.12	0.0200	3.6 [8]	0.06	0.12	0.08	0.12	$10^{3.42}$	

[1], [2], [3] found by the analysis: 77.2, 62.7, 60.3 respectively.

Since the piperidine-carbamate is comparatively rapidly decomposed, it was necessary — by means of the velocity constants determined in section IV — to correct the analytically determined percentage of carbamate for the decomposition taken place during the 7 to 9 minutes passing between the start of the shaking with carbon dioxide and the separation of precipitate and supernate by centrifugation. Similar corrections are done in Tables 2,3 and 4. The correction amounts at most 4 units of the percentage.

	Blank experiments	Corrected data
[4]	3.18 ml 0.1 *N* HCl	0.93 ml 0.1 *N* HCl	2.25 ml 0.1 *N* HCl
[5]	2.74 » » » »	0.99 » » » »	1.75 » » » »
[6]	2.06 » » » »	0.76 » » » »	1.30 » » » »
[7]	1.96 » » » »	0.91 » » » »	1.05 » » » »
[8]	2.08 » » » »	0.65 » » » »	1.43 » » » »

[9] See in the end of this chapter the value $10^{2.28}$, which is more accurate and, therefore, used for the calculations in Table 5.

contained 0.2 *M* aniline, approx. 0.11 *M* triethylamine and approx. 0.09 *M* triethylaminium chloride. It should be mentioned that tertiary amines do not react with carbon dioxide. The pa_H of the buffer was measured by means of a glass electrode and found to be 11.07, corresponding to a c_{OH^-} of $10^{-3.02}$; the addition of carbon dioxide is calculated to decrease the c_{OH^-} to $10^{-3.16}$, *i.e.* the mean value of c_{OH^-} being $10^{-3.09}$. In the course of 1 minute atmospheric air containing 10 % of carbon dioxide was lead into the solution in the form of fine bobbles under vigorous stirring. Immediately hereafter the solution was made strongly basic and analysed. 0.013 moles of carbon dioxide was absorbed per litre, 31 % as carbamate corresponding to a $k_{CO_2\cdot Am} = 10^{2.28}$.

The equilibrium "carbamate ⇌ carbonate"

For all three amines experiments have been done from the carbonate side as well as from the carbamate side, see Table 2. In the case of benzylamine and piperidine the pa_H of the solutions was fixed at about 9.5 and 11, respectively, by means of aminiumion-amine-buffers of the amines concerned. In the case of aniline, the pa_H in two experiments is fixed at about 10—11 by means of bicarbonate-carbonate-buffers. In the case of benzylamine, a considerable amount of carbamate was found in the equilibrium, while only small, but undoubtedly measurable amounts were found in the case of piperidine and aniline.

Furthermore, in Table 2 is presented the calculated constitution of the equilibrium. For information on the method of calculation concerning benzylamine and piperidine we refer to a previous investigation [1]; in the case of aniline the following equations are valid:

Table 2. The solution of carbonate-carbamate in equilibrium. 18°.

	Initial solution								Equilibrium					
	c_{Am}	c_{AmH^+}	$c_{(AmH)_2CO_3}$	$c_{AmH\ carbamate}$	$c_{HCO_3^-}$	$c_{CO_3^{--}}$	$c_{carbamate}$	% carbamate	c_{AmH^+}	c_{Am}	$c_{carbamate}$	$c_{HCO_3^-}$	K_{Eq}	Mean
$C_6H_5CH_2NH_2$	0.10	0.10	0.02					86[2]	0.121	0.102	0.0173	0.0021	$10^{-1.90}$	$10^{-1.91}$
	0.02	0.05	0.01					64[3]	0.060	0.023	0.0066	0.0033	$10^{-1.93}$	
	0.05	0.10		0.02				80[4]	0.121	0.056	0.0158	0.0035	$10^{-1.90}$	
$C_5H_{10}NH$	0.05	0.10		0.02				7.8[5]	0.137	0.0526	0.0016	0.0025	$10^{-1.08}$	$10^{-1.09}$
	0.05	0.10	0.02					7.4[6]	0.136	0.0523	0.0015	0.0023	$10^{-1.09}$	
$C_6H_5NH_2$	0.10				0.05	0.10	0.02	12[7]	0	0.117	0.0023	0.0664	$10^{0.53}$	$10^{0.55}$[10]
	0.10				0.05	0.10		2.7[8]	0	0.099	0.0013	0.0480	$10^{0.56}$	
	0.12[1]						0.02	1.9[9]	0	0.144	0.0004	0.0159	$10^{0.76}$	

[1] pa_H = approx. 10
[2] Mean of 3 determinations: 86.5, 86.3, 86.4.
[3] » » » » 64.4, 64.2, 63.9.
[4] » » » » 79.0, 80.0, 79.8.
[5] » » 2 » 7.58, 8.01.
[6] » » 4 » 7.93, 7.81, 6.62, 7.23.
[7] » » » » 11.3, 11.4, 11.5, 11.8.
[8] Percentage of bicarbonate converted into carbamate.
Mean of 3 determinations: 2.68, 2.73, 2.75.
[9] » » 4 » 2.27, 1.80, 1.69, 1.66.
[10] The value of $10^{0.76}$ is not taken into consideration in the calculation of the mean-value. The concentration of carbamate in this experiment has been very small and the determination consequently being encumbered with especially great error.

$c_{Am} = c_{Am}$ (column 2 of the Table) + $c_{carbamate}$ (column 8 of the Table) − $c_{carbamate}$.

$c_{HCO_3^-} = c_{HCO_3^-}$ (column 6 of the Table) + $c_{carbamate}$ (column 8 of the Table) − $c_{carbamate}$.

The solutions initially containing carbamate were, in the case of benzylamine and piperidine, prepared by dissolving the preparations of carbamates, see 3a and 3b, in the aminiumion-amine-buffers. In the case of aniline, phenyl isocyanate was shaken with a solution of sodium hydroxide for twenty minutes and then — in two experiments — an adequate amount of potassium bicarbonate and a solution of aniline were added and — in a third experiment — a solution of aniline and hydrochloric acid until $pa_H = 10.3$. No aniline was allowed to be added before all of the phenyl isocyanate was converted into carbamate ion, since aniline and phenyl isocyanate react with each other forming diphenylurea.

The equilibrium constants K_{Eq} of the reaction carbamate$^-$ "$+ H_2O \rightleftharpoons HCO_3^- +$ amine" were also calculated.

The velocity of the conversion "carbamate ⇌ carbonate"

Experiments on velocity in weakly basic medium have, in the case of benzylamine, been done from the carbonate side, and in the case of piperidine and aniline from the carbamate side. The pa_H of the solutions was in the case of benzylamine and piperidine fixed by means of aminiumion-amine-buffers of the amines concerned, in the case of aniline by means of bicarbonate-carbonate-buffer. In the experiments from the carbamate side the solution was prepared, in the case of piperidine, by dissolving the preparation of carbamate, see 3b, in the aminiumion-amine-buffer. In the case of aniline, phenyl isocyanate was shaken with a solution of sodium hydroxide for twenty minutes, to which was added an adequate amount of potassium bicarbonate together with a solution of aniline. In Table 3 are presented the experiments on velocity in these weakly basic solutions, where a measurable equilibrium is established between carbamate and carbonate.

In Table 4 are presented those experiments, which have been carried out in a medium containing sodium hydroxide; in these solutions carbamate is converted practically completely to carbonate. The solutions were, in the case of benzylamine and piperidine, prepared partly by dissolving carbon dioxide in a pure solution of amine and subsequently adding sodium hydroxide and partly by dissolving the preparations of carbamate, see 3a and 3b, in the solutions containing amine and sodium hydroxide. In the case of aniline,

Table 3. Velocity constants for the process "carbamate ⇌ carbonate". p_{aH} = approx. 9—11. 18°.

	Initial solution							p_{aH}	Min.	% carbamate	$k_{amate} + k_{onate}$
	c_{Am}	c_{AmH^+}	$c_{(Am)_2CO_3}$	$c_{AmH\ carbamate}$	$c_{HCO_3^-}$	$c_{CO_3^{--}}$	$c_{carbamate}$				
$C_6H_5CH_2NH_2$	0.10	0.10	0.02					approx. 9.6	40	14.6	0.00200
									80	30.1	0.00233
									95	33.0	0.00220
									124	40.1	0.00218
									160	46.7	0.00211
									261	63.4	0.00220
									∞	86.4	Mean: 0.0022
											k_{amate}: 0.0003
											k_{onate}: 0.0019
	0.02	0.05	0.01					approx. 9.2	44	18.2	0.00331
									65	26.9	0.00364
									121	40.6	0.00360
									312	58.7	0.00343
									∞	64.2	Mean: 0.0035
											k_{amate}: 0.0013
											k_{onate}: 0.0022
$C_5H_{10}NH$	0.05	0.10		0.02				approx. 11.0	0	100	
									57	72.8	0.00266
									134	51.0	0.00246
									219	35.0	0.00242
									312	23.3	0.00248
									∞	7.8	Mean: 0.0025
											k_{amate}: 0.0023
											k_{onate}: 0.0002
$C_6H_5NH_2$	0.10				0.05	0.10	0.02	approx. 10.6	0	100	
									12	30.0	0.0563
									25	17.2	0.0477
									43	12.5	0.0452
									∞	11.5	Mean: 0.050
											k_{amate}: 0.049
											k_{onate}: 0.00066

phenyl isocyanate was shaken with a solution of sodium hydroxide for twenty minutes to which was added water or a solution of aniline.

In addition to the experimental data presented in Table 3 and 4 are listed the velocity constants calculated from the experiments. k_{amate} is the velocity constant for the conversion "carbamate → carbonate" and k_{onate} the one for the reversed reaction. k_{amate} are, in the experiments in strong basic medium, the values of $\frac{1}{t} \log \frac{a}{a-x}$. In weakly basic solution $k_{amate} + k_{onate}$ are the values of $\frac{1}{t} \log \frac{K \cdot a}{K \cdot a - (1+K)x}$, the expression for a process which is mono-

Table 4. Velocity constants for the process "carbamate → carbonate". pa_H = approx. 13. 18°.

	Initial solution			Min.	% carbamate left	k_{amate}
	$c_{carbamate}$	c_{NaOH}	c_{Am}			
$C_6H_5CH_2NH_2$	0.019 (CO_2 in solution of amine)	0.10	0.08	0	100	
				218	94.4	0.000114
				633	83.6	0.000123
				1402	68.6	0.000117
				4060	33.8	0.000116
				5985	20.9	0.000114
				∞	(0)	Mean: 0.00012
	0.020 (preparation of carbamate)	0.10	0.08	0	100	
				748	83.1	0.000108
				1835	60.8	0.000118
				5681	22.3	0.000115
				7531	13.5	0.000116
				∞	(0.8)	Mean: 0.00011
$C_5H_{10}NH$	0.017 (CO_2 in solution of amine)	0.11	0.08	0	100	
				45	90.3	0.00098
				117	75.7	0.00103
				270	53.4	0.00101
				384	41.3	0.00100
				1319	4.8	0.00100
				∞	(0)	Mean: 0.0010
	0.020 (preparation of carbamate)	0.10	0.08	0	100	
				94	79.1	0.00108
				210	59.1	0.00109
				278	50.6	0.00107
				347	43.6	0.00104
				680	21.0	0.00100
				745	17.7	0.00101
				∞	(0)	Mean: 0.0011

This Table is continued.

molecular from both sides and which does not run to completion. The constant K signifies the relation between the equilibrium values of the concentrations of carbamate and carbonate. The sum "$k_{amate} + k_{onate}$" is approximately constant within each experiment. By means of

Table 4 continued.

	0.020 (preparation of carbamate)	0.11	0	0	100		
				150	91.7		0.000249
				462	78.0		0.000233
				1078	55.9		0.000235
				1347	48.1		0.000236
				1594	41.9		0.000237
				2561	24.1		0.000241
				3015	18.4		0.000244
				∞	(0)	Mean:	0.00024
$C_6H_5NH_2$	0.020 (preparation of carbamate)	0.11	0.12	0	100		
				150	91.6		0.000253
				463	78.2		0.000231
				1079	56.1		0.000233
				1348	48.6		0.000232
				1592	42.0		0.000237
				2562	24.2		0.000241
				3015	17.8		0.000249
				∞	(0)	Mean:	0.00024
	0.043 (preparation of carbamate)	0.20	0	0	100		
				1340	58.2		0.000175
				2500	34.7		0.000184
				3880	21.1		0.000174
				∞	(0)	Mean:	0.00018

$\frac{k_{\text{onate}}}{k_{\text{amate}}} = \frac{\text{equilibrium value of } c_{\text{carbamate}}}{\text{equilibrium value of } c_{\text{carbonate}}} = K$, the values of k_{amate} and k_{onate} are obtained.

The experiments can be interpreted in a way similar to the one used by carbamates previously examined allowing a margin for the ones regarding aniline. The conversion is a two-stage reaction

$$\text{carbamate} = \text{amine} + CO_2$$
$$CO_2 = \text{carbonate}$$

and it is possible to calculate in advance the velocity constants.

The conversion of the carbon dioxide into carbonate and the reciprocal conversion take place through the two reactions:

$$H_2O + CO_2 \rightleftharpoons H_2CO_3 \quad (1)$$
$$OH^- + CO_2 \rightleftharpoons HCO_3^- \quad (2)$$

If pa_H of the solution is $>$ about 10, the conversion takes place through reaction (2) only. This is the case with all the solutions containing piperidine or aniline, together with those containing benzylamine and sodium hydroxide and k_{amate} and k_{onate} may be calculated from the expression:

$$k^{11}_{onate} = \frac{k_{HCO_3^-}}{1 + \frac{a_{H+}}{K_{H_2CO_3}} \cdot f + \frac{K_{HCO_3^-}}{a_{H+}} \cdot \frac{1}{f}}$$

$$k^{3}_{amate} = \frac{k_{CO_2 \cdot Am} \cdot K_{Eq} \cdot K_{H_2O} \cdot \frac{1}{K_{CO_2}}}{c_{OH^-} + \frac{k_{CO_2 \cdot Am}}{k_{CO_2 \cdot OH^-}} \cdot c_{Am}}$$

If pa_H of the solution is $<$ about 10, process (1) must also be taken into consideration. In this case k_{onate} and k_{amate} have to be calculated from the expressions previously deduced [12].

For the conversion of carbonate we have:

$$\frac{dc_{carbonate}}{dt} = - \frac{k_{H_2CO_3}}{1 + \frac{K_{H_2CO_3}}{a_{H+}} \cdot \frac{1}{f} \cdot (1 + \frac{K_{HCO_3^-}}{a_{H+}} \cdot \frac{1}{f})} \cdot c_{carbonate} = - k_a \cdot c_{carbonate}$$

$$\frac{dc_{carbonate}}{dt} = - \frac{k_{HCO_3^-}}{1 + \frac{a_{H+}}{K_{H_2CO_3}} \cdot f + \frac{K_{HCO_3^-}}{a_{H+}} \cdot \frac{1}{f}} \cdot c_{carbonate} = - k_b \cdot c_{carbonate}$$

Since both processes take place simultaneously, we have:

$$\frac{dc_{carbonate}}{dt} = - (k_a + k_b) \cdot c_{carbonate} = - k_{onate} \cdot c_{carbonate}.$$

For the conversion of carbamate we have:

$$\frac{dc_{carbamate}}{dt} = - (k_{CO_2} + k_{CO_2 \cdot OH^-} \cdot c_{OH^-}) \cdot c_{CO_2} = k_{carbon\ dioxide} \cdot c_{CO_2} =$$
$$- k_{carbon\ dioxide} \cdot c_{carbamate} \cdot \alpha_{CO_2} = - k_{amate} \cdot c_{carbamate}.$$

α_{CO_2} indicates the part of the carbamate being dissociated into CO_2, and it is calculated by means of

$$\frac{c^2_{Am} \cdot c_{CO_2}}{c_{AmH^+} \cdot c_{carbamate} \cdot f^2} = \frac{K_{H_2O} \cdot K_{Eq}}{K_{Am} \cdot K_{CO_2}}$$

i.e.

$$k_{\text{amate}} = (k_{CO_2} + k_{CO_2} \cdot {}_{OH^-} \cdot c_{OH^-}) \frac{K_{Eq} \cdot \frac{1}{K_{CO_2}}}{c_{Am}} \cdot a_{H^+} \cdot f$$

K_{CO_2} ($10^{-6.52}$) is the equilibrium constant for the process "$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$" $K_{H_2CO_3}$ ($10^{-3.7}$) the one for the process "$H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$" and $K_{HCO_3^-}$ ($10^{-10.3}$) the equilibrium constant for the process "$HCO_3^- \rightleftharpoons H^+ + CO_3^{--}$".

k_{CO_2} (0.66) is the velocity constant for process (1) from left to right and $k_{H_2CO_3}$ (426) the one for process (1) from right to left. $k_{CO_2 \cdot OH^-}$ ($10^{5.02}$) is the velocity constant for process (2) from left to right and $k_{HCO_3^-}$ ($10^{-2.74}$) is the one for process (2) from right to left.

Table 5 contains a survey of the experimental and calculated values of k_{amate} and k_{onate}. Considering the nature of the conditions the agreement may be described as satisfactory in the case of benzylamine and piperidine. In the case of aniline, the calculated values of k_{amate} are not so good, but when estimating the results it should be taken into consideration that the determinations of $k_{CO_2 \cdot Am}$ as well as the one of K_{Eq} in this case may be encumbered with especially great errors.

Table 5. Velocity constants, experimental and calculated.

	Initial solution								k_{amate}		k_{onate}	
	c_{Am}	c_{AmH^+}	$c_{(AmH)_2CO_3}$	c_{NaOH}	$c_{\text{carbamate}}$	$c_{\text{AmH carbamate}}$	$c_{HCO_3^-}$	$c_{CO_3^{--}}$	exp.	calc.	exp.	calc.
$C_6H_5CH_2NH_2$	0.10	0.10	0.02						0.00030	0.00028	0.0019	0.0017
	0.02	0.05	0.01						0.0013	0.0014	0.0022	0.0025
	0.08			0.10	0.019				0.00012	0.00015		
	0.08			0.10	0.020				0.00011	0.00015		
$C_5H_{10}NH$	0.05	0.10				0.020			0.0023	0.0033	0.00020	0.00022
	0.08			0.11	0.017				0.0010	0.0016		
	0.08			0.10	0.020				0.0011	0.0017		
$C_6H_5NH_2$	0.10				0.020		0.05	0.10	0.049	0.03	0.00066	0.00066
	0			0.11	0.020				0.00024	0.00014		
	0.12			0.11	0.020				0.00024	0.00014		
	0			0.20	0.043				0.00018	0.00008		

SUMMARY

The velocity constants of the reaction "amine + CO_2 = carbamic acid" and the equilibrium constant for the reaction "carbamate$^-$ + H_2O = HCO_3^- + amine" have been determined. The velocity of the decomposition of carbamate ion in basic medium was investigated and may be explained for piperidine and benzylamine in assuming that the decomposition is a two-stage reaction, *viz.*

carbamate = amine + carbon dioxide
carbon dioxide = carbonate

In the case of aniline there is a relatively great difference between the experimental and calculated velocity constants, not excluding that the decomposition also here is the same two-stage reaction, because the experiments, on which the calculations are based, in this case may be encumbered with particularly great errors.

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Studies on Carbamates

IX. The Carbamate of Allylamine

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The equilibrium conditions and reaction mechanism of the formation and decomposition in aqueous medium of the carbamate formed by allylamine have been studied. As the experimental and theoretical conditions are analogous to those of the previous carbamates, we find that, on the whole, it is sufficient here to state the experimental data and the calculated constants, referring for further information to an earlier paper [1].

The allylamine (Eastman Kodak Company) used was distilled through a platinum wiregauze column [2]. The allylamine thus obtained boiled at 53.3°–53.5° C (748 mm Hg) and had a $n_D^{22.2°} = 1.4193$ according to the value of Brühl [3]. A molecular weight of 56.8 and 57.1 was obtained by a bromometric titration and an acidimetric titration, respectively; the theoretical value is 57.09. The above mentioned boiling point is corrected for calibration errors; stem correction has been done.

The carbamate of allylamine, $CH_2 : CH \cdot CH_2 \cdot NH \cdot COONH_3 \cdot CH_2 \cdot CH : CH_2$, in solid form was prepared by treating a solution containing 10 % amine in anhydrous ether with dry carbon dioxide. The precipitate which

Table 1. Carbon dioxide in allylamine + NaOH. 18°.

Initial solution		Absorbed CO_2 mol/litre	% carbamate	Final solution		Mean		$k_{CO_2 \cdot Am}$	
c_{NaOH}	c_{Am}			c_{NaOH}	c_{Am}	c_{NaOH}	c_{Am}		Mean
0.20	0.10	0.0179	37	0.17	0.09	0.19	0.10	$10^{5.07}$	
0.10	0.10	0.0193	54	0.07	0.09	0.09	0.09	$10^{5.07}$	$10^{5.06}$
0.20	0.10	0.0200	35	0.17	0.09	0.18	0.10	$10^{5.03}$	
0.20	0.20	0.0191	54	0.17	0.19	0.19	0.19	$10^{5.08}$	

Table 2. The solution of carbonate-carbamate in equilibrium. 18°.

Initial solution				% carbamate	Equilibrium				K_{Eq}	
c_{Am}	c_{AmH+}	$c_{(AmH)_2CO_3}$	$c_{carbamate}$		c_{Am}	c_{AmH+}	$c_{carbamate}$	$c_{HCO_3^-}$		Mean
0.05	0.05	0.02		68 [1]	0.054	0.072	0.0137	0.0041	$10^{-1.79}$	
0.02	0.05	0.01		58 [2]	0.024	0.061	0.0058	0.0034	$10^{-1.86}$	$10^{-1.81}$
0.04	0.06	0.02		66 [3]	0.044	0.082	0.0132	0.0049	$10^{-1.78}$	
0.04	0.06		0.02	71 [4]	0.044	0.080	0.0121	0.0044	$10^{-1.80}$	

[1] Mean of 4 determinations: 68.1, 68.3, 68.4, 68.9
[2] » » 4 » 58.7, 57.4, 57.8, 57.2
[3] » » 4 » 66.6, 65.7, 67.2, 64.7
[4] » » 4 » 71.3, 72.1, 69.2, 69.1

Table 3. Velocity constants for the process "carbamate ⇌ carbonate". 18°. pa_H approx. 9.8.

Initial solution			Min.	% carbamate	$k_{amate} + k_{onate}$	
	c_{AmH+}	c_{Am}				
			40	11.7		0.00203
			85	21.0		0.00187
			120	27.6		0.00187
0.020 *M* $(AmH)_2CO_3$	0.05	0.05	260	45.2		0.00181
			∞	68.4	Mean:	0.00190
					k_{amate}:	0.0006
					k_{onate}:	0.0013
			40	14.8		0.00322
			85	25.9		0.00304
			120	33.5		0.00314
0.010 *M* $(AmH)_2CO_3$	0.05	0.02	160	38.9		0.00303
			242	48.0		0.00320
			∞	57.7	Mean:	0.00313
					k_{amate}:	0.0013
					k_{onate}:	0.0017
			40	11.0		0.00197
			80	21.2		0.00211
			120	29.1		0.00210
0.020 *M* $(AmH)_2CO_3$	0.06	0.04	166	34.1		0.00190
			270	49.0		0.00217
			∞	66.0	Mean:	0.00205
					k_{amate}:	0.0007
					k_{onate}:	0.0014

Table 4. Velocity constants for the process "carbamate → carbonate". 18°. pa_H = approx. 13.

Initial solution			Min.	% carbamate left	k_{amate}
$c_{carbamate}$	c_{NaOH}	c_{Am}			
			0	100	
0.020			154	94.9	0.000147
(CO_2			407	87.1	0.000148
in solution	0.08	0.08	1428	62.5	0.000143
of amine)			4293	23.1	0.000148
			8623	4.6	0.000155
			∞	(0)	Mean: 0.00015
			0	100	
			221	93.3	0.000138
			420	86.6	0.000149
0.019			1400	61.5	0.000151
(preparation	0.08	0.08	3065	33.1	0.000157
of carbamate)			5960	12.2	0.000153
			8630	4.9	0.000152
			∞	(0)	Mean: 0.00015
			0	100	
			231	91.6	0.000166
			422	84.8	0.000170
0.022			1573	56.0	0.000160
(preparation	0.08	0.08	3094	32.2	0.000159
of carbamate)			5838	12.6	0.000154
			8948	4.0	0.000156
			∞	(0)	Mean: 0.00016

Table 5. Velocity constants, experimental and calculated.

Initial solution					k_{amate}		k_{onate}	
$c_{(AmH)_2CO_3}$	$c_{carbamate}$	c_{AmH^+}	c_{Am}	c_{NaOH}	exp.	calc.	exp.	calc.
0.02		0.05	0.05		0.00060	0.00067	0.0013	0.0013
0.01		0.05	0.02		0.0013	0.0018	0.0017	0.0018
0.02		0.06	0.04		0.00070	0.00087	0.0014	0.0015
	0.02		0.08	0.08	0.00015	0.00021		
	0.02		0.08	0.08	0.00015	0.00021		
	0.02		0.08	0.08	0.00016	0.00021		

appeared was separated by filtration and dried over concentrated sulphuric acid. We found 72.2 % allylamine (theoretically 72.18 %) by titration with 0.1 *N* HCl of the carbamate. We found 27.6 % (theoretically 27.82 %) by determination of carbon dioxide in the carbamate.

The basic dissociation constant K_{Am} at 18° C of allylamine was fixed at $10^{-4.38}$, the value of which is calculated on the basis of the values at 0°, 25° and 40° C of Hantzsch and Sebaldt [4]. It should be noted that the value at 25° C of Hantzsch and Sebaldt agrees well with the corrected value of Bredig [5].

As the experiments are carried out analogously to those of the *n*-propylamine, and as the addition and equilibrium constants of the two amines have approximately the same quantitative values, it would seem, that the double bond in allylamine has no influence on the conditions of addition and equilibrium.

SUMMARY

The velocity constant of the reaction "$CH_2 : CH \cdot CH_2NH_2 + CO_2 = CH_2 \cdot CH \cdot CH_2NHCOOH$" and the equilibrium constant for the reaction "$CH_2 : CH \cdot CH_2NHCOO^- + H_2O = HCO_3^- + CH_2 : CH \cdot CH_2NH_2$" have been determined. The velocity of the decomposition of $CH_2 : CH \cdot CH_2NHCOO^-$ in basic medium was investigated and may be explained in assuming that the decomposition is a two-stage reaction, *viz.*

carbamate = allyl amine + carbon dioxide
carbon dioxide = carbonate

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On Hydrogen Peroxide Decomposition

The Influence of Small Amounts of Cupric Salt on the Ferric Salt Catalysis

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It has for a long time been a well-known fact that cupric ions, although they themselves have only a slight influence on the decomposition of hydrogen peroxide under acid conditions, are able to accelerate this process considerably when a ferric salt is used as a catalyst.

Already in 1923 Bohnson and Robertson [1] have tried to account for this problem. They made a series of kinetic experiments on the decomposition of hydrogen peroxide in acid solution in the presence of varying quantities of cupric and ferric salts; but as they did not succeed in calculating velocity constants for the individual experiments, the conclusions they were able to draw on the basis of their experimental results could only be of a semi-quantitative nature. They suggest the following mechanism for the reaction:

Hydrogen peroxide oxidizes the ferric ion into an unstable intermediate, FeO_3, which is able to interact with more hydrogen peroxide evolving oxygen; but this intermediate should also be able together with cupric ions to form another unstable intermediate, CuO_2, which will interact more rapidly with hydrogen peroxide to form oxygen.

The present work is an attempt at a somewhat more thorough elucidation of the problem, or in other words, an attempt at drawing up a more complete scheme for the reaction.

EXPERIMENTAL TECHNIQUE AND REAGENTS

A number of velocity experiments have been made at 25° C. Nitric acid was in all cases added to the reaction mixture to produce a concentration of 0.01 Mol/l, while varying quantities of cupric and ferric nitrate were added. The experimental technique was the same as the one employed for the author's work on the ferric salt catalysis [2] and the reagents used were of the same purity. It should further be mentioned that the progress of all reactions have been followed until a degree of decomposition of about 95 %.

MATHEMATICAL TREATMENT OF THE EXPERIMENTS AND SURVEY OF EXPERIMENTAL RESULTS

The author found that a mathematical treatment of the velocity experiments could neither be based on the expressions for monomolecular nor for bimolecular reactions, but that a similar rate expression to the one used for the reaction catalysed by ferric salt could be applied, *viz.:*

$$t = \frac{1}{B} \log \frac{a}{x} + \frac{A}{B}\left(\frac{1}{x} - \frac{1}{a}\right) \qquad (1)$$

where a is the initial concentration of hydrogen peroxide, and x the concentration at the time t; A and B are constants which can be calculated graphically, confer page 4 of the paper quoted [2].

Table 1 gives the results from one of the experiments. A comparison between the observed t-values in column 1 and the values of column 8 which

Table 1. Experiment 106. Decomposition of hydrogen peroxide at 25° C in solution which is 0.003 Molar as to ferric nitrate, 0.00005 Molar as to cupric nitrate and 0.01 Molar as to nitric acid. The experiment has been calculated according to equation (1). The constants A and B have been calculated graphically. $A = 3.30 \cdot 10^{-3}$, $B = 2.84 \cdot 10^{-2}$. The t-values stated in column 8 have been calculated on the basis of equation 1 and the constants A and B.

1	2	3	4	5	6	7	8
t min. obs.	x	$\log \frac{a}{x}$	$\frac{1}{x}$	$\frac{1}{x} - \frac{1}{a}$	$A\left(\frac{1}{x} - \frac{1}{a}\right)$	(3)+(6)	t min. calc.
0.00	0.06388	—	15.65	—	—	—	0.00
2.10	0.05636	0.0544	17.53	1.88	0.0062	0.0606	2.13
5.00	0.04800	0.1242	20.83	5.18	0.0171	0.1413	4.98
10.00	0.03643	0.2439	27.47	11.82	0.0391	0.2831	9.97
15.00	0.02776	0.3618	35.97	20.32	0.0671	0.4289	15.10
20.00	0.02164	0.4701	46.30	30.65	0.1012	0.5713	20.12
25.00	0.01713	0.5716	58.48	42.83	0.1414	0.7130	25.11
30.00	0.01365	0.6703	72.99	57.34	0.1892	0.8595	30.26
35.00	0.01118	0.7570	89.29	73.64	0.2430	1.0000	35.21
43.00	0.008384	0.8820	119.33	103.68	0.3421	1.2241	43.10
50.00	0.006694	0.9797	149.48	133.83	0.4417	1.4214	50.05
60.00	0.005066	1.1008	197.24	181.59	0.5993	1.7001	59.86
71.00	0.003951	1.2087	253.16	237.51	0.8313	1.9925	70.16
80.00	0.003263	1.2918	306.75	291.16	0.9606	2.2524	79.31
90.00	0.002683	1.3768	373.13	357.48	1.1796	2.5564	90.01
100.00	0.002298	1.4440	434.78	419.13	1.3831	2.8271	99.55
105.00	0.002122	1.4786	471.70	456.05	1.5050	2.9836	105.06

are calculated on the basis of the figures found for A and B and equation (1) shows close agreement even when the reaction has proceeded to a great extent.

Values for A and B have been calculated from each experiment. With a view to the subsequent discussion values have been calculated for the two coefficients A_1 and A_2 of the differentiated velocity expression:

$$-\frac{dt}{dx} = A_1 \frac{1}{x} + A_2 \frac{1}{x^2} \qquad (2)$$

where $A_1 = 0.4343/B$ and $A_2 = A/B$; the values found for A_1 and A_2 are listed in Table 2.

Figs. 1 and 2 show how A_1 and A_2 vary with the concentrations of cupric salt and ferric salt. It appears from the figures that A_1 and A_2 are both func-

Table 2. Survey of the values found for A_1 and A_2.

$c_{Fe} \cdot 10^3$ \ $c_{Cu} \cdot 10^5$		0.00	1.00	2.00	5.00	10.00	20.00	40.00	100.00
1.00	A_1	96.50	55.68	43.43	34.47	24.68	21.50	19.13	—
	A_2	0.089	0.141	0.180	0.238	0.244	0.248	0.264	—
2.00	A_1	48.25	32.41	26.64	20.11	17.10	14.38	13.44	—
	A_2	0.084	0.112	0.123	0.144	0.157	0.166	0.176	—
3.00	A_1	32.18	23.48	19.48	15.29	13.26	11.73	10.24	9.87
	A_2	0.082	0.097	0.112	0.116	0.123	0.135	0.142	0.152
4.00	A_1	24.15	18.56	15.11	13.22	11.31	10.75	8.81	9.40
	A_2	0.085	0.094	0.087	0.106	0.113	0.114	0.117	0.119
5.00	A_1	19.56	15.42	13.90	10.81	—	—	—	8.40
	A_2	0.086	0.085	0.091	0.100	—	—	—	0.106

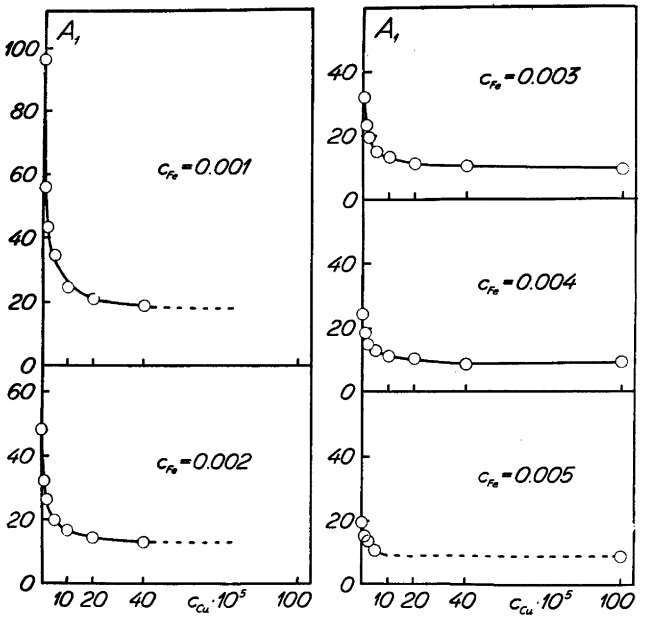


Fig. 1. Relation between A_1 and the cupric salt concentration at various ferric salt concentrations.

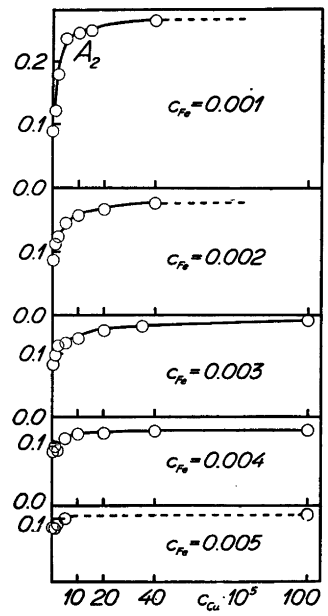


Fig. 2. Relation between A_2 and the cupric salt concentration at various ferric salt concentrations.

tions of the ferric salt and cupric salt concentrations; qualitatively they may be described as follows:

$A_1 = A_1\ (c_{Fe}, c_{Cu})$: Decreases towards a limiting value with increasing cupric salt concentration. Decreases with increasing ferric salt concentration. (3)

$A_2 = A_2\ (c_{Fe}, c_{Cu})$: Increases towards a limiting value with increasing cupric salt concentration. Decreases with increasing ferric salt concentration. When the cupric salt concentration is zero, A_2 is independent of the ferric salt concentration. (4)

When the values for A_1 and A_2 for the cupric salt concentration zero are denoted by $A_{1(0)}$ and $A_{2(0)}$ (3) and (4) can be written:

$$A_1 = A_1\ (c_{Fe}, c_{Cu}) = A_{1(0)}\ (1 - \Delta_1) \tag{3a}$$

$$A_2 = A_2\ (c_{Fe}, c_{Cu}) = A_{2(0)}\ (1 + \Delta_2) \tag{4a}$$

DERIVATION OF THE MECHANISM OF THE REACTION

In the following it has been endeavoured to arrive at a mechanism with a velocity expression which agrees with the experimental results, which means that it must be of the following type

$$-\frac{dt}{dx} = A_1 \frac{1}{x} + A_2 \frac{1}{x^2} \qquad (2)$$

where the coefficients A_1 and A_2, as stated in the preceding section, equations (3) and (4), must be functions of both the ferric salt and the cupric salt concentrations.

As the velocity expression which applies to the hydrogen peroxide decomposition catalysed by ferric salt also applies to this reaction, there can be no reasonable doubt that the mathematical treatment of the scheme which it is our aim to find should be based upon the scheme for the former reaction.

The ferric salt catalysis has been dealt with in previous investigations [2,3] and [4]. These investigations resulted in the suggestion of the following scheme for the reaction: *

$$\begin{array}{lcll} Fe^{+++} + HO_2^- & \rightleftharpoons & FeOOH^{++} & 12 \\ FeOOH^{++} & \rightleftharpoons & Fe^{+++} + \overset{*}{HO}_2^- & 25 \\ \overset{*}{HO}_2^- + H_2O_2 & \rightarrow & HO^- + O_2 + H_2O & 50 \end{array} \qquad \text{III}$$

($\overset{*}{HO}_2^-$ being the symbol of an activated HO_2^- ion.)

Three possible ways in which the cupric ions can interfere in the ferric salt catalysis are illustrated in Fig. 3: the cupric ions may catalyse either reaction 12, reaction 25, or reaction 50.

On the assumption that a steady state is obtained immediately after the start of the reaction a velocity expression has been calculated for each of the mechanisms suggested; the calculations, which were carried out according to the lines suggested by Christiansen [6], showed that the second way is the only possible one, while the first and the third must be discarded; that is, the cupric

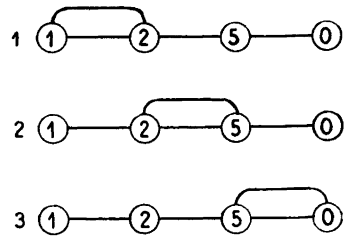


Fig. 3. Diagrammatic representation of three possible ways in which the cupric ions can interfere in the ferric salt catalysis.

* Abel [4] has criticized my investigations; I intend to deal with his theory later on.

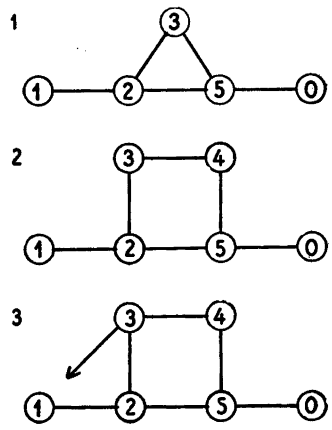


Fig. 4. Diagrammatic representation of the reaction schemes no. IV, V and VI.

ions must catalyse reaction 25 of scheme III; but the question is now how this catalysis is brought about.

It is an obvious assumption that the cupric ions will react with the $FeOOH^{++}$-ions to form a new intermediary product, perhaps $CuOOH^{+}$-ions, which must be considered to be able to decompose more rapidly than the $FeOOH^{++}$-ions.

The mechanism will then be as III, supplemented with the two reactions:

IV

$FeOOH^{++} + Cu^{++}$	$\rightleftharpoons$	$Fe^{+++} + CuOOH^{+}$	23
$CuOOH^{+}$	$\rightleftharpoons$	$Cu^{++} + \overset{*}{H}O_2^{-}$	35

The corresponding diagrammatic representation of the reaction is given in Fig. 4,1.

A calculation yielded a velocity expression which was in conformity with (2), but not with (3) and (4).

There is, however, another possibility, namely the one illustrated in Fig. 4,2 in which the reaction from 3 to 5 has been divided up into two steps 34 and 45. The reaction scheme will then be as follows — the new intermediate being denoted by X_4:

V

$Fe^{+++} + HO_2^{-}$	$\rightleftharpoons$	$FeOOH^{++}$	12
$FeOOH^{++}$	$\rightleftharpoons$	$Fe^{+++} + \overset{*}{H}O_2^{-}$	25
$FeOOH^{++} + Cu^{++}$	$\rightleftharpoons$	$Fe^{+++} + CuOOH^{+}$	23
$CuOOH^{+}$	$\rightleftharpoons$	$Cu^{++} + X_4$	34
X_4	$\rightleftharpoons$	$\overset{*}{H}O_2^{-}$	45
$\overset{*}{H}O_2^{-} + H_2O_2$	$\rightarrow$	$O_2 + OH^{-} + H_2O$	50

Unfortunately the velocity expression which could be calculated on the basis of this mechanism proved not to be quite up to the expectations. It fulfils the conditions of (2) and (3), but not the conditions stated in (4) as A_2 will not be a function of the ferric salt or cupric salt concentration, but merely a constant identical with the one found for the pure ferric catalyses.

This deviation need not result in an absolute rejection of the mechanism proposed — perhaps it merely discloses a flaw in the mechanism; upon further consideration it will be seen that it seems to be possible to suggest a mechanism giving a velocity expression which better than the one calculated from V has the required properties, by adding another reaction to V. This reaction is given below and denoted by 31.

The final reaction scheme will then be as follows:

$Fe^{+++} + HO_2^-$	$\rightleftharpoons$	$FeOOH^{++}$	12	
$FeOOH^{++}$	$\rightleftharpoons$	$Fe^{+++} + \overset{*}{H}O_2^-$	25	
$FeOOH^{++} + Cu^{++}$	$\rightleftharpoons$	$Fe^{+++} + CuOOH^+$	23	
$CuOOH^+$	$\rightleftharpoons$	$Cu^{++} + X_4$	34	VI
X_4	$\rightleftharpoons$	$\overset{*}{H}O_2^-$	45	
$\overset{*}{H}O_2^- + H_2O_2$	$\rightarrow$	$O_2 + OH^- + H_2O$	50	
$CuOOH^+$	$\rightarrow$	$Cu^{++} + HO_2^-$	31	

besides which we have the equilibria:

H_2O_2	$\rightleftharpoons$	$H^+ + HO_2^-$
$Fe^{+++} + H_2O$	$\rightleftharpoons$	$FeOH^{++} + H^+$

Fig. 4,3 illustrates the diagrammatic representation of the mechanism.

CALCULATION OF THE VELOCITY EXPRESSION FOR THE REACTION SCHEME NO. VI

In the following the calculation of the velocity expression for reaction scheme No.VI will be accounted for in detail.

The following symbols have been used in the calculations:

$x_1 = c_{Fe^{+++}}$, $x_2 = c_{FeOOH^{++}}$, $x_3 = c_{CuOOH^+}$, $x_4 = c_{X_4}$ and $x_5 = c_{\overset{*}{H}O_2^-}$.

The velocity of reaction is denoted by s; s without a subscript denotes the over-all velocity, $s = dO_2/dt$, while s with a subscript denotes the resulting velocity of one of the partial reactions, *e.g.* the resulting velocity of reaction 25 is denoted by s_{25}. The reaction probability is denoted by w,

which thus in the case of a reaction of the 1. order merely represents a velocity constant, and in the case of a reaction of the 2.order represents a velocity constant multiplied by a concentration; *e.g.* it may be mentioned that $w_{34} = k_{34}$, and $w_{23} = k_{23} \cdot c_{Cu++}$.

With this use of symbols we have *e.g.* $x_2 w_{23}$ = the velocity of the reaction from 2 to 3 and $s_{23} = x_2 w_{23} - x_3 w_{32}$ = the resulting velocity of reaction 23.

The calculations have been carried through according to the lines suggested by Christiansen [6] and on the following assumptions:

1) Reaction 12 is an equilibrium. This assumption appears extremely reasonable, if only because the reaction is one in which a complex is formed. The equilibrium has, as already mentioned in a previous paper, been investigated by Evans and coworkers [7], who have determined its equilibrium constant. This assumption leads to the equation:

$$x_1 w_{12} = x_2 w_{21} \tag{5}$$

2) The concentrations of the unstable intermediates $CuOOH^+$, X_4 and HO_2^- are stationary practically from the start of the reaction, *i.e.* the conditions of the stationary state are fulfilled as regards these intermediates. This assumption leads directly (see Fig. 4, 3) to the following system of equations:

$$s = s_{50} = s_{25} + s_{45} = s_{25} + s_{34} = s_{25} + s_{23} - s_{31}$$

or, expressed in greater detail:

$$s = x_2(w_{23} + w_{25}) - x_3(w_{32} + w_{31}) - x_5 w_{52} \tag{6}$$
$$s = x_2 w_{25} + x_3 w_{34} - x_4 w_{43} - x_5 w_{52} \tag{7}$$
$$s = x_2 w_{25} + x_4 w_{45} - x_5(w_{52} + w_{54}) \tag{8}$$
$$s = x_5 w_{50} \tag{9}$$

Dividing equations (6), (7), (8) and (9) by s, we find:

$$1 = \frac{x_2}{s}(w_{23} + w_{25}) - \frac{x_3}{s}(w_{32} + w_{31}) - \frac{x_5}{s} w_{52}$$
$$1 = \frac{x_2}{s} w_{25} + \frac{x_3}{s} w_{34} - \frac{x_4}{s} w_{43} - \frac{x_5}{s} w_{52}$$
$$1 = \frac{x_2}{s} w_{25} + \frac{x_4}{s} w_{45} - \frac{x_5}{s}(w_{52} + w_{54})$$
$$1 = \frac{x_5}{s} w_{50}$$

From these equations it is possible to calculate x_2/s; the two determinants required for the calculation are in the following denoted D_0 and Dx_2:

$$D_0 = \begin{vmatrix} w_{25}+w_{23} & -(w_{32}+w_{31}) & 0 & -w_{52} \\ w_{25} & w_{34} & -w_{43} & -w_{52} \\ w_{25} & 0 & w_{45} & -(w_{52}+w_{54}) \\ 0 & 0 & 0 & w_{50} \end{vmatrix}$$

$$= w_{50}w_{25}\,(w_{34}w_{45} + w_{32}w_{45} + w_{31}w_{45} + w_{32}w_{43} + w_{31}w_{43} + w_{23}w_{34}w_{45}/w_{25}); \quad (10)$$

$$Dx_2 = \begin{vmatrix} 1 & -(w_{32}+w_{31}) & 0 & -w_{52} \\ 1 & w_{34} & -w_{43} & -w_{52} \\ 1 & 0 & w_{45} & -(w_{52}+w_{54}) \\ 1 & 0 & 0 & w_{50} \end{vmatrix}$$

$$= w_{50}\,(w_{34}w_{45} + w_{32}w_{45} + w_{31}w_{45} + w_{32}w_{43} + w_{31}w_{43}) + w_{52}(w_{32}w_{43} + w_{31}w_{43} + w_{32}w_{45} + w_{31}w_{45} + w_{34}w_{45} + w_{54}w_{43}w_{32}/w_{52} + w_{54}w_{43}w_{31}/w_{52}) \quad (11)$$

Hence we have

$$x_2/s = D_{x_2}/D_0$$

or, when applying equation (5)

$$1/s = w_{21}D_{x_2}/x_1w_{12}D_0; \quad (12)$$

If we apply the principle of microscopical reversibility (proposed for the first time by Wegscheider (1902) and later on treated by others *c. f.* Christiansen in the paper already quoted) on the cyclic reaction 23452 — see the diagrammatic representation of the reaction in Fig. 4,3 — we have

$$w_{54}w_{43}w_{32}/w_{52} = w_{23}w_{34}w_{45}/w_{25} \quad (13)$$

Substituting this expression in (11) we find that (12) can be written:

$$\frac{1}{s} = \frac{w_{21}}{x_1w_{12}w_{25}}\left(1 - \frac{w_{23}w_{34}w_{45}/w_{25}}{w_{34}w_{45}+w_{32}w_{45}+w_{32}w_{43}+w_{23}w_{34}w_{45}/w_{25}+w_{31}(w_{43}+w_{45})}\right)$$

$$+\frac{w_{21}w_{52}}{x_1w_{12}w_{25}w_{50}}\left(1+\frac{w_{31}w_{54}w_{43}/w_{52}}{w_{34}w_{45}+w_{32}w_{45}+w_{32}w_{43}+w_{23}w_{34}w_{45}/w_{25}+w_{31}(w_{43}+w_{45})}\right); \quad (14)$$

reducing the two fractions in the brackets by $w_{23}w_{34}w_{45}/w_{25}$ and again using (13) we have:

$$\frac{1}{s} = \frac{w_{21}}{x_1w_{12}w_{25}}\left(1-\frac{1}{N}\right) + \frac{w_{21}w_{52}}{w_{12}w_{25}w_{50}}\left(1+\frac{w_{31}}{w_{32}N}\right) \quad (15)$$

where: $$N = \frac{w_{32}w_{25}}{w_{34}w_{23}} + \frac{w_{25}}{w_{23}} + \frac{w_{52}}{w_{54}} + 1 + \frac{w_{31}w_{52}}{w_{32}w_{54}}\left(1+\frac{w_{45}}{w_{43}}\right); \quad (16)$$

substituting the values of the w-symbols and setting:

$$s = dO_2/dt = -dH_2O_2/2dt;\ c^{\dagger}_{HO_2^-} = K_{H_2O_2} \cdot c_{H_2O_2}/c_{H^+} \text{ and } k_{12}/k_{21} = K_{12} \cdot$$

in the expression thus obtained we have:

$$-\frac{dt}{dH_2O_2} = \frac{c_{H^+}}{2k_{25}K_{12}K_{H_2O_2}c_{Fe^{+++}}}\left(1-\frac{1}{N}\right)\frac{1}{c_{H_2O_2}}$$

$$+\frac{k_{52}c_{H^+}}{2k_{25}k_{50}K_{12}K_{H_2O_2}}\left(1+\frac{k_{31}}{k_{32}c_{Fe^{+++}}}\frac{1}{N}\right)\frac{1}{c^2_{H_2O_2}}; \tag{17}$$

where $N = \frac{k_{32}k_{25}}{k_{34}k_{23}} \cdot \frac{c_{Fe^{+++}}}{c_{Cu^{++}}} + \frac{k_{25}}{k_{23}} \cdot \frac{1}{c_{Cu^{++}}} + \frac{k_{52}c_{Fe^{+++}}}{k_{54}} + 1 +$

$$\frac{k_{31}k_{52}}{k_{32}k_{54}}\left(1+\frac{k_{45}}{k_{43}c_{Cu^{++}}}\right) \tag{18}$$

For a constant hydrogen ion concentration N may be written:

$$N = N_0 = \frac{k'_{32}k_{25}}{k_{34}k_{23}}\frac{c_{Fe}}{c_{Cu}} + \frac{k_{25}}{k_{23}c_{Cu}} + \frac{k'_{52}c_{Fe}}{k_{54}} + 1 + \frac{k_{31}k'_{52}}{k'_{32}k_{54}}\left(1+\frac{k_{45}}{k_{43}c_{Cu}}\right) \tag{18a}$$

where c_{Fe} and c_{Cu} denotes the total ferric salt and cupric salt concentration.

THE VELOCITY EXPRESSION (17) IN COMPARISON WITH THE EXPERIMENTAL RESULTS

In a previous section (p. 1091) it has been shown that the cupric-ferric salt catalysed hydrogen peroxide decomposition proceeds according to the following velocity expression:

$$-dt/dH_2O_2 = A_1/c_{H_2O_2} + A_2/c^2_{H_2O_2} \tag{2}$$

in which A_1 and A_2 can be written:

$$A_1 = A_1(c_{Fe}, c_{Cu}) = A_{1(0)}(1-\Delta_1) \text{ and } A_2 = A_2(c_{Fe}, c_{Cu}) = A_{2(0)}(1+\Delta_2); \quad \text{(3a) and (4a)}$$

The two functions are qualitatively described on p. 1093 (3) and (4).

The velocity expression (17) for the reaction scheme VI has been calculated in the preceding section.

(17) can be written:

$$-dt/dH_2O_2 = A'_1/c_{H_2O_2} + A'_2/c^2_{H_2O_2} \tag{19}$$

Table 3. Survey of the values found for Δ_1, Δ_2 and Δ_2/Δ_1.

$c_{Cu} \cdot 10^5$		1.00	2.00	5.00	10.00	20.00	40.00	100.00
$c_{Fe}=0.001$	Δ_1	0.423	0.550	0.642	0.744	0.777	0.802	—
	Δ_2	0.585	1.022	1.674	1.742	1.787	1.966	—
	Δ_2/Δ_1	1.38	1.86	2.61	2.34	2.30	2.45	—
$c_{Fe}=0.002$	Δ_1	0.329	0.448	0.583	0.646	0.702	0.721	—
	Δ_2	0.333	0.464	0.714	0.869	0.976	1.095	—
	Δ_2/Δ_1	1.10	1.06	1.22	1.35	1.39	1.52	—
$c_{Fe}=0.003$	Δ_1	0.270	0.395	0.525	0.588	0.635	0.682	0.693
	Δ_2	0.182	0.366	0.414	0.500	0.646	0.732	0.854
	Δ_2/Δ_1	0.674	0.927	0.789	0.850	1.017	1.073	1.232
$c_{Fe}=0.004$	Δ_1	0.231	0.374	0.454	0.531	0.555	0.635	0.611
	Δ_2	0.119	(0.024)	0.247	0.329	0.341	0.376	0.400
	Δ_2/Δ_1	0.515	(0.064)	0.544	0.620	0.614	0.592	0.655
$c_{Fe}=0.005$	Δ_1	0.212	0.290	0.448	—	—	—	0.571
	Δ_2	(0.00)	0.058	0.163	—	—	—	0.233
	Δ_2/Δ_1	(0.00)	0.500	0.364	—	—	—	0.245

When the cupric salt concentration is zero $1/N_0$ (18a) is equal to zero; therefore, for a constant hydrogen ion concentration the coefficients A_1' and A_2' can be written:

$$A_1' = A_{1(0)}\ (1 + 1/N_0) \text{ and } A_2' = A_{2(0)}\ (1 + k_{31}/k_{32}' c_{Fe} N_0) \qquad (20) \text{ and } (21)$$

in which $A_{1(0)}$ and $A_{2(0)}$ mean the A_1 and A_2 when the cupric salt concentration is zero.

Now it is possible to test whether the proposed reaction mechanism VI is in agreement with the experiments. If so (19) must be in agreement with (2), which is the case in so far as the two expressions for $-\mathrm{d}t/\mathrm{d}H_2O_2$ are dependent in the same way on the hydrogen peroxide concentration.

Furthermore, the dependence of (20) and (21) on the cupric- and ferric salt concentrations must agree with the experimental results; this is the case when the values found for Δ_1 and Δ_2 in (3a) and (4a) fit into the functions:

$$\Delta_1^{-1} = N_0 \qquad (22)$$

$$\Delta_2 = k_{31}/k_{32}' c_{Fe} N_0 \qquad (23)$$

$$\Delta_2/\Delta_1 = k_{31}/k_{32}' c_{Fe} \qquad (24)$$

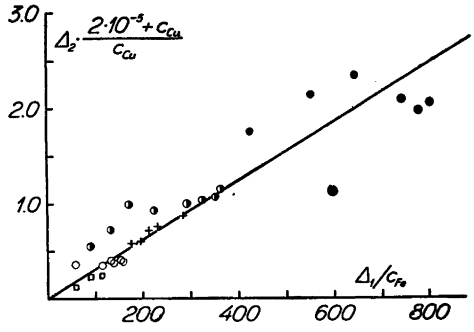


Fig. 5. $\Delta_2\,(2 \cdot 10^{-5} + c_{Cu})\,/c_{Cu}$ *plotted against* Δ_1/c_{Fe}.

● $c_{Fe} = 0.001$
◑ $c_{Fe} = 0.002$
\+ $c_{Fe} = 0.003$
○ $c_{Fe} = 0.004$
□ $c_{Fe} = 0.005$

The values for Δ_1 and Δ_2 which has been calculated from the observed values according to (3a) and (4a) are given in Table 3 together with the values for Δ_2/Δ_1. From the table appears that Δ_2/Δ_1 with a reasonable amount of accuracy may be considered to be inversely proportional to the ferric salt concentration, which is in agreement with (24); but it also appears that Δ_2/Δ_1 depends on the cupric salt concentration, which conflicts with (24). However, the discrepancy may be eliminated by substituting the reaction 31 in VI by the following sequence:

$$CuOOH^+ \rightleftharpoons X_{3'}, \qquad 33'$$
$$Cu^{++} + X_{3'} \rightarrow 2Cu^{++} + HO_2^- \qquad 3'1$$

Reaction 33′ is assumed to proceed spontaneously whereas 3′1 has to be catalysed by cupric ions.

This assumption necessitates some change in the calculated velocity expression (17). In the calculation of this s_{31} is supposed to be equal to $x_3 w_{31}$

or $$x_3/s_{31} = 1/w_{31} = 1/k_{31}$$

whereas the sequence by application of the usual calculation method gives:

$$x_3/s_{31} = 1/w_{33'} + w_{3'3}/w_{33'}w_{3'1} = 1/k_{33'} + k_{3'3}/k_{33'}k_{3'1}c_{Cu}{}^{++}.$$

If, in the original velocity expression (17) $1/k_{31}$ is substituted by $1/k_{33'} + k_{3'3}/k_{33'}k_{3'1}c_{Cu}$, (24) will be as follows:

$$\frac{\Delta_2}{\Delta_1} = \frac{k_{33'}}{k'_{32}c_{Fe}} \cdot \frac{c_{Cu}}{c_{Cu} + k_{3'3}/k_{3'1}} \qquad (24a)$$

or

$$\frac{c_{Cu} + k_{3'3}/k_{3'1}}{c_{Cu}} \Delta_2 = \frac{k_{33'}}{k'_{32}c_{Fe}} \Delta_1 \qquad (24b)$$

The validity of (24b) is thus a necessary condition for the correctness of the modified reaction scheme. This condition seems to be fulfilled as the left side of (24b), using $k_{3'3}/k_{3'1} = 2 \cdot 10^{-5}$, with a reasonable amount of accuracy is proportional to Δ_1/c_{Fe}. This is shown in Fig. 5. The slope of the straight line in the figure gives

$$k_{33'}/k'_{32} = 3.1 \cdot 10^{-3}.$$

If $1/k_{31}$ is substituted by $1/k_{33'} + k_{3'3}/k_{33'}k_{3'1}c_{Cu}$ in (18a), (22) will be as follows:

$$\Delta_1^{-1} = \frac{k'_{32}k_{25}}{k_{34}k_{23}} \cdot \frac{c_{Fe}}{c_{Cu}} + \frac{k_{25}}{k_{23}} \cdot \frac{1}{c_{Cu}} + \frac{k'_{52}}{k_{54}} c_{Fe} + 1 + \frac{k_{33'}}{k'_{32}} \cdot \frac{k'_{52}}{k_{54}} \cdot \frac{c_{Cu} + k_{45}/k_{43}}{c_{Cu} + k_{3'3}/k_{3'1}} \quad (22a)$$

By introducing γ, δ and ξ for the ratios k'_{52}/k_{54}, $k'_{32}k_{25}/k_{34}k_{23}$ and k_{25}/k_{23}, respectively, and using the values found above for $k_{3'3}/k_{3'1}$ and $k_{33'}/k'_{32}$, (22a) can be written:

$$\Delta_1^{-1} - 3.1 \cdot 10^{-3} \cdot \gamma \cdot \frac{c_{Cu} + \delta/\gamma}{c_{Cu} + 2 \cdot 10^{-5}} = (\delta c_{Fe} + \xi) \frac{1}{c_{Cu}} + \gamma c_{Fe} + \varepsilon; \quad (22b)$$

or

$$\Delta_1^{-1} - P = \alpha/c_{Cu} + \beta. \quad (22c)$$

The ratio k_{45}/k_{43} has been written as δ/γ as it is equal to $k'_{32}k_{25}k_{54}/k'_{52}k_{34}k_{23}$ according to the principle of microscopical reversibility (13), and ε has been written instead of one.

We now see that a second necessary condition for the correctness of the modified reaction scheme is, that we are able to calculate such values for δ and γ so that for a constant ferric salt concentration the left side of (22b) can be depicted as a linear function of $1/c_{Cu}$, one for each ferric salt concentration. Furthermore the straight lines representing these functions must have a common point of intersection, because their slopes $\alpha = \delta c_{Fe} + \xi$ and their intersepts $\beta = \gamma c_{Fe} + \varepsilon$ are linear functions of the same variable.

By iteration γ and δ can be calculated to:

$$\gamma = 120, \qquad \delta = 5.2 \cdot 10^{-3}, \qquad \delta/\gamma = 4.6 \cdot 10^{-5}.$$

As a proof of the correctness of this statement the values of $\Delta_1^{-1} - P$ are plotted against $1/c_{Cu}$ in Fig. 6.

From the slope of the straight lines and the co-ordinates for their intersection can be calculated:

$$\gamma = 120, \quad \delta = 5.2 \cdot 10^{-3}, \quad \xi = 0.36 \cdot 10^{-5} \text{ and } \varepsilon = 0.73.$$

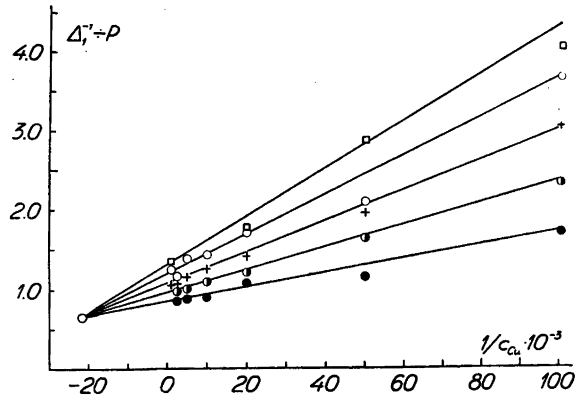


Fig. 6. $\Delta_1^{-1} - P$ *in equation (22c) plotted against* $1/c_{Cu}$.

● $c_{Fe} = 0.001$ ○ $c_{Fe} = 0.004$
◑ $c_{Fe} = 0.002$ □ $c_{Fe} = 0.005$
\+ $c_{Fe} = 0.003$

It appears that these values for γ and δ are identical with the values used above. ε has been calculated to 0.73; as it in (22b) has been written instead of one, the calculations of course ought to have given this value. It has been impossible to find any explanation for this discrepancy, but it is not improbable that it is due to errors in the experimental results, for even small errors in the values for A_1 and A_2 or $A_{1(0)}$ and $A_{2(0)}$ may cause rather considerable errors in the calculations.

The calculations have given values for the following ratios between velocity constants:

$$k_{3'3}/k_{3'1} = 2 \cdot 10^{-5}; \quad k_{33'}/k'_{32} = 3.1 \cdot 10^{-3}; \quad k'_{52}/k_{54} = \gamma = 120$$

$$k_{25}/k_{23} = \xi = 0.36 \cdot 10^{-5}; \quad k_{45}/k_{43} = \delta/\gamma = 4.3 \cdot 10^{-5}; \quad k'_{32}/k_{34} = \delta/\xi = 1.44 \cdot 10^{3}$$

DISCUSSION

As will appear from the preceding section, it has been possible to show that scheme VI when reaction 31 is regarded as two consecutive reactions 33′ and 3′1 corresponds to a velocity expression which in the whole is satisfied by the experimental results. In the following this result will be discussed.

When considering the scheme suggested for the reaction, the question arises whether this scheme is the only applicable one, *i.e.* the scheme which is best satisfied by the experimental results. To this question the reply may be given

that several other schemes have been carefully considered, but they have all had to be discarded because of conspicuous discrepancies between the corresponding velocity expressions and the experimental results. It is of course a fact that the value of the suggested scheme is entirely dependent on the correctness of the expression for the velocity of the overall reaction (1) used for the mathematical treatment of the kinetic experiments, but there can hardly be any doubt that this expression is correct, as it reproduces the experimental values so closely, even when the reaction has run nearly to completion.

As it will have appeared from the above, reaction 3'1

$$X_3 + Cu^{++} \rightarrow 2\,Cu^{++} + HO_2^- \qquad 3'1$$

has been treated as an irreversible reaction. It should be emphasized that this is necessary in order to make the calculated velocity expression agree with the experimental results. However, it involves the following difficulty: The reactions 12, 23, 33' and 3'1 together constitute a cyclic reaction sequence proceding in the direction 1233', but this apparently conflicts with the principle of the microscopical reversibility according to which the stationary condition of such a cyclic reaction is characterized by the over all velocity being zero. It has proved difficult to find an explanation of this discrepancy; it may perhaps be explained by assuming the cupric ions to occur in two different forms, perhaps two forms of different spins.

SUMMARY

It has been shown that

1. the hydrogen peroxide decomposition catalysed by cupric and ferric salt complies with the same velocity expression as the one calculated for the decomposition (equation (2)) catalysed by ferric salt,

2. the constants A_1 and A_2 of equation (2) are both dependent on the ferric salt and cupric salt concentrations (equations (3) and (4)).

On the basis of the experimental results a mechanism for the reaction has been suggested (scheme no. VI where reaction 31 is to be considered as two partial reactions 33' and 3'1) and this mechanism has been discussed.

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On the Szilard-Chalmers Reaction with Potassium Bromate

Preparation of ^{82}Br, Hot-atom Chemistry, and Radiation Chemistry

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In a previous paper [1] the preparation of an ion-exchanger for the separation of halide and halate ions was described. The purpose of this paper is to investigate the use of this ion-exchanger in the preparation of ^{82}Br having a high specific radioactivity.

Irradiation of bromine in the nuclear reactor of the A.E.R.E.**, Harwell, yields a product of specific radioactivity between 22 and 60 mc/gram [2]. For some purposes it is desirable to increase the specific radioactivity particularly because the half life of ^{82}Br is short (36 hours). The problems associated with the Szilard-Chalmers reaction used for the concentration of ^{82}Br are the following:

1. The development of a method for the separation of bromide and bromate suitable for further use of ^{82}Br, when the radioactivities involved are of the order 100 to 1000 mc.
2. The decomposition of the bromate target during irradiation in the reactor.
3. The retention of bromates irradiated in the reactor.

1. THE SEPARATION OF BROMIDE AND BROMATE

For the separation the ion-exchanger described in the previous paper [1] is used. The properties of the material are: capacity $= 4 \times 10^{-5}$ equiv./cm^3 bed volume, volume fraction of liquid in the column ≈ 60 %, possible flow rate $= 0.6$ ml per minute per cm^2 for halide concentrations of 0.01 to 0.02 M.

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* Operated under the auspices of the Swedish Atomic Energy Committee.

** A.E.R.E. = Atomic Energy Research Establishment.

For purification of the eluate a small column containing the cation-exchanger Dowex 50 in hydrogen form is used.

For the checking of the operation of the column it is considered unsuitable to measure the radioactivity of the eluate. If quantities of 100 to 1 000 mc are handled, very thick radiation shields have to be used between the column and the instrument for the measurement of the radioactivity. This would require long tubes between the column and the outlet of the system. As will be shown later, this would lead to a very bad separation between bromide and the iodide used for the elution.

The eluate contains small amounts of silver ions of concentration depending on the composition of the eluate. The operation of the column may thus be checked by measurement of the silver ion concentration in the eluate. By use of the arrangement shown in Fig. 1 the E.M.F. of the cell

$$- \mathrm{Hg_2Cl_2, Hg} \;\big|\; \mathrm{KCl\ (sat.)} \;\big\|\; \mathrm{Ag^+\ in\ eluate} \;\big|\; \mathrm{Ag}$$

can be measured. If we neglect the activity coefficients, the E.M.F. is determined by:

$$E = e^0_{\mathrm{Ag}} - e_{\mathrm{calomel}} + \frac{RT}{F}\ln[\mathrm{Ag^+}]_{\mathrm{eluate}} \qquad (1)$$

i. e. at 25° C,

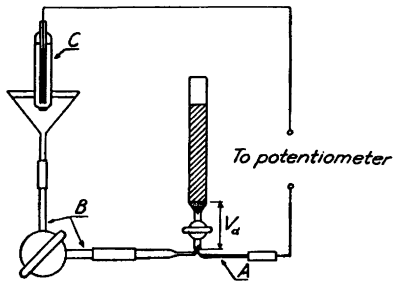
$$E = 553 + 59.21\log[\mathrm{Ag^+}]_{\mathrm{eluate}} \qquad (2)$$

where E is expressed in mV.

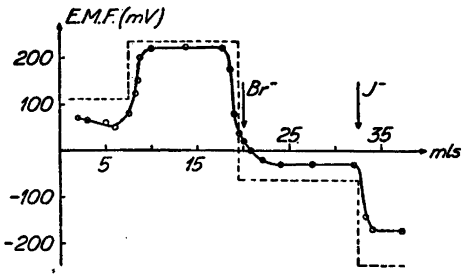
The appearance of bromate has no influence on the silver ion concentration in the eluate which before the halide ions appear is determined by the hydroxyl ion concentration. However, if the ion exchange column is treated prior to the separation with a solution of hydroxyl ion concentration different from that of the solution to be separated, the appearance of the bromate fraction can be approximately observed. When the bromide fraction appears in the eluate, the measured E.M.F. must suddenly drop due to the decrease in silver ion concentration. When the bromide fraction is finished, the E.M.F. must once more suddenly drop and the pure bromide fraction can thus be observed in the eluate.

Experimental

Experiment 1: A solution 0.1 *M* in potassium bromate and 0.01 *M* in sodium bromide was separated using the apparatus shown in Fig. 1. Bromide was eluted by 0.01 *M*



1. Apparatus for measurement of $[Ag^+]$ in the eluate, A = silverelectrode, B = salt bridge containing 1.5 M ammonium nitrate solution, C = saturated calomel electrode.



2. Measured E.M.F. as a function of the volume of the eluate. — — — = calculated values.

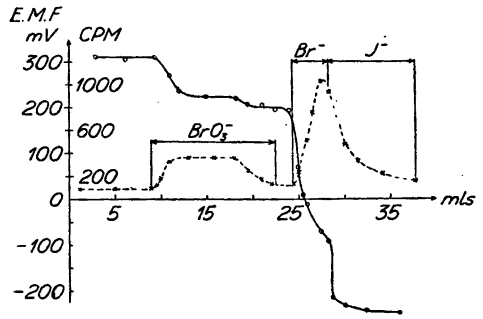
sodium iodide solution. The E.M.F. measured during the separation is shown in figure 2 together with the calculated values. Before the separation the column was treated with a solution 1 *M* in sodium hydroxide.

Experiment 2: In order to demonstrate the importance of having short tubes, an experiment was performed in which the column was connected to a liquid flow counter by a rubber tube of about 15 cm in length. A solution equal to the one of experiment 1 and containing ^{82}Br as bromide and as bromate was treated. The silver ion concentration of the solution was measured immediately after it left the counter and compared to the radioactivity measured by the counter. In this case the column was treated before the separation with pure water. The results are shown in Fig. 3.

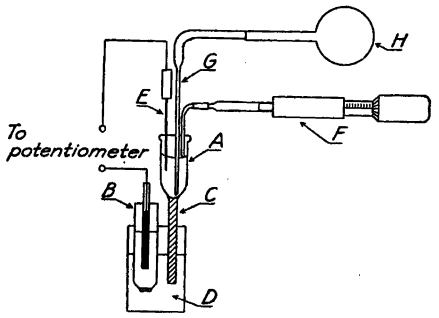
The yield of pure bromide in the separation is determined by the length of the bromide band on the column and by the volume in the column between the end of the ion-exchanger and the outlet of the system. This volume is called the "dead volume" (V_d) and is indicated in Fig. 1.

If the boundary between bromide and iodide on the column were absolutely sharp the length of the bromide band could have any value. This is, however, not the case. There is always a small overlapping due to irregularities in the exchanger bed. The bromide in this fraction is not pure and cannot be used. The magnitude of this overlapping, in the experience of the author, is about 2 to 4 mm for areas of 0.2 to 1 cm^2. Hence if the loss due to the diffuse region between iodide and bromide is to be kept below 10 to 20 %, a reasonable figure, the bromide band has to be at least 2 cm long.

The yield of the separation excluding the loss due to the diffuse section is determined by the dead volume. A comparison between the Figs. 2 and 3 shows that a considerable mixing of iodide and bromide through diffusion takes place after they leave the ion-exchanger bed. Hence, as soon as the



3. Measured E.M.F. ——— and radioactivity — — — as a function of the volume of the eluate.



4. Microtitration apparatus for the determination of bromide.

iodide band has left the ion-exchanger, the bromide fraction has to be cut off. The yield can thus be calculated from the equation

$$\eta = 1 - \frac{[I^-]\ V}{l'' A(C + \alpha\ [I^-])} - \frac{0{,}4}{l''} \qquad (4)$$

where η = yield, l'' length of bromide band, C = capacity of ion exchanger, A = area of the column, and $[I^-]$ = concentration of the solution used for the elution and α = volume fraction of liquid.

For a calculation of the optimum conditions for the irradiation, separation, and yield, the decomposition during the irradiation and the retention must be known. The investigation of these factors is described in the following sections.

2. RADIATION CHEMISTRY AND HOT-ATOM CHEMISTRY

The best compound for the irradiation is potassium bromate. Ammonium bromate is not stable and sodium bromate would yield a very high disturbing radioactivity from sodium, which would increase the difficulties with the radiation shield.

Experimental

Decomposition: 500 mg of potassium bromate containing less than 0.01 % of bromide were irradiated in the pile of A.E.R.E. for five days, with a neutron flux of about 10^{11} $cm^{-2}sec^{-1}$. After the irradiation the preparation had a brownish colour, indicating that some decomposition had taken place. The potassium bromate was dissolved in 10 ml of 0.1 *M* sodium hydroxide solution and diluted to 50 ml. The bromide content of this solution was determined by potentiometric titration of 1 ml with 0.1 *M* silver nitrate solution using a micro titration apparatus as described in Fig. 4. The titration vessel A,

taking 2 ml, is connected to a saturated calomel electrode B via a salt bridge C containing agar gel 1.5 *M* in ammonium nitrate and a solution of ammonium nitrate D. The other electrode E consists of a silver wire. Silver nitrate is added by the micro burette F of the type "Agla" constructed by the Wellcome Physiological Research Laboratories, England. The burette measures volumes down to 0.1 μl. The solution was stirred by blowing air through the capillary G with the rubber balloon H. The equivalence point was found to be located at 8.5 μl corresponding to 68 μg of bromide, *i. e.* 3.4 mg in the whole of the solution.

Retention: The retention of ^{82}Br in bromate was determined in the following way. 1 ml of the solution containing the irradiated material was added to a solution 0.1 *M* in potassium bromate and 0.01 *M* in sodium bromide. The bromide and bromate were separated using an apparatus as described in Fig. 5. The ion-exchange column contained a layer of Dowex 50 in hydrogen form in order to absorbe the radioactive potassium isotope ^{40}K present in the solution. The two fractions of the eluate indicated in Fig. 6 were collected, diluted to 100 ml, and 10 ml of each measured by a liquid counter.

Regardless of the mechanism, the decomposition is proportional to the time of irradiation, for constant neutron flux and γ-radiation. The rate of decomposition, D, expressed as moles of bromide per mole of bromate per hour, thus can be calculated:

$$D = 1{,}2 \cdot 10^{-4} \text{ hr}^{-1}$$

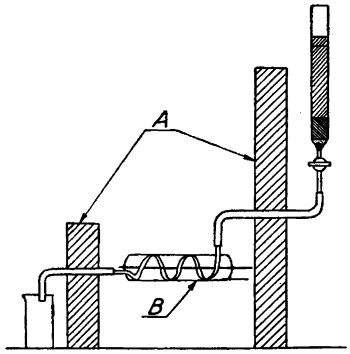
The decomposition is the sum of the effect due to neutrons and to γ-radiation:

$$D = D_\gamma + D_n \tag{5}$$

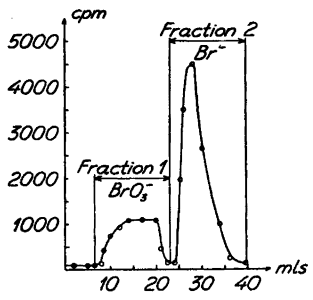
D_n can be roughly estimated. With a neutron flux of 10^{11} cm^{-2} sec^{-1} and a thermal activation cross section of about 2 barns $[\sigma(^{79}Br) + \sigma(^{80}Br)]$, there are $4.3 \cdot 10^{14}$ nuclear reactions per hour per mole in the target material. The magnitude of the decomposition following each nuclear reaction is rather uncertain and cannot be exactly calculated from the present knowledge of the hot-atom chemistry of bromates. However, if there is no chain reaction mechanism, the decomposition due to the very short range of the recoiling atom certainly can be assumed to be less than 10^{17} ions per mole per hour. This gives $D_n \leqq 1.7 \cdot 10^{-7}$. As D was found to be $1.2 \cdot 10^{-4}$, the decomposition is almost completely due to γ-radiation. The retention of the radioactive isotope is defined by

$$R = \frac{I(BrO_3^-)}{I(BrO_3^-) + I(Br^-)}, \tag{6}$$

where I = radioactivity. R was determined in two preparations irradiated under the same conditions, and was found to be 25 and 27 %.



5. Separation column connected to liquid flow counter (B). A = lead shields.



6. Radioactivity of eluate as a function of volume. Fractions used for the determination of R.

This value of the retention is not in agreement with that found by Libby [3], who determined R as (4 ± 4) % if solid sodium bromate is irradiated and dissolved in pure water, and $R = (10 \pm 8)$ % if a solution of sodium bromate 3 M in sodium hydroxide is irradiated. In Libby's work the reaction $^{79}Br(n,\gamma)^{80}Br$ was studied. The use of this reaction instead of $^{81}Br(n, \gamma)\ ^{82}Br$ should, however, be of no importance since it can be assumed that the excitation energy given to the recoiling bromine atom is about the same for ^{80}Br as for ^{82}Br; neither should it be of any importance that sodium bromate instead of potassium bromate was irradiated. The difference may be explained in the following way:

The brownish colour of the potassium bromate after the irradiation indicates that the decomposition product is, at least partly, free bromine. When dissolved in sodium hydroxide solution, this bromine reacts as follows:

$$3Br_2 + 6NaOH = 5NaBr + NaBrO_3 + 3H_2O \qquad (7)$$

The radioactivity is immediately evenly distributed between Br_2 and Br^- through the equilibrium

$$Br_2 + Br^- \rightleftharpoons Br_3^- \qquad (8)$$

Hence, if the amount of bromine is much larger than that of bromide, the retention according to (7) must be 16.7 % if the reaction is allowed to proceed to completeness. The lower value found by Libby can be explained by bromide impurities in the bromate irradiated. Libby's irradiation was carried out with a 200 mc Ra-Be source. In this case the decomposition is certainly very small

compared to the bromide impurities in usual analytical reagents. Furthermore, when dissolved in pure water of pH = 7, the reaction (7) at room temperature is a rather slow process.

The higher values of the retention found in this investigation must be due to the fact that R is a sum of two terms:

$$R = R_p + R_s \tag{9}$$

where R_p = primary retention, and R_s = secondary retention. Here the primary retention is that part of ^{82}Br which either recombines with oxygen atoms during the cooling of the hot recoiling atom, or is not liberated from the bromate ion as a consequence of the nuclear reaction. The secondary retention is that part of ^{82}Br which reacts to form bromate during the separation. As the isotopic exchange between bromate and bromine has a measurable rate only in acid solution the secondary retention is due only to reaction (7), and is determined by the ratio Br^-/Br_2 after the irradiation and by the completeness of reaction (7). From the experimental results available up to the present, nothing can be said with certainty about the chemical state of the decomposition products, *i. e.* the ratio Br^-/Br_2; hence it can only be stated that $R_s \leqq 16.7$ and $9 \leqq R_p \leqq 26$. Libby's results, in spite of the large errors stated, support the view that R_p is rather small. It is therefore probable that the decomposition product due to the γ-radiation is mainly free bromine and that the primary retention is 9 %. Nothing can be said about the chemical state which results as a consequence of the (n, γ)-reaction.

The question whether the primary retention is due to recombination during the cooling process or to the fact that the bromate ion in some cases is not decomposed is impossible to answer so long as the number and energy of the γ-quanta emitted from the intermediate nucleus are not known.

3. GENERAL CALCULATION OF THE SEPARATION PROCESS

The success of the preparation method outlined in part 1 depends on the concentration factor and the yield which can be obtained. The concentration factor F is defined as the ratio between the specific radioactivity obtained with the use of a Szilard-Chalmers reaction and that obtained without this process. F is determined by the following factors: 1) the initial bromide content of the irradiated bromate, 2) the decomposition during the irradiation, 3) the retention, and 4) the time necessary for the separation. The initial bromide content of the irradiated bromate can be made practically zero by purifying the bromate with the ion exchanger used for the separation. If the retention is assumed

to be independent of the length of the irradiation the specific radioactivity obtained is determined by the equation

$$I_s = \frac{100-R}{100} \frac{kx(1-e^{-\lambda t_i})}{Dxt_i} e^{-\lambda t_i} \tag{10}$$

where I_s = specific radioactivity of bromide, R = retention, k = saturation specific radioactivity of bromate, x = irradiated amount of bromate, D = rate of decomposition, λ = decay constant of ^{82}Br, t_i = time of irradiation, and t_s = time of separation. The time of separation is determined by the equation

$$t_s = \frac{x}{bvA} + t' \tag{11}$$

where b = solubility of potassium bromate, v = flow rate during the separation, and t' = time for elution of the bromide band and for purification in the cation-exchanger. For the ratios between bromide and bromate which usually appear, t' can be neglected in comparison with the other term.

The area of the column is determined by the equation

$$Al'(C + a[I^-]) = Dxt_i \tag{12a}$$

where C = capacity of ion-exchanger, l' = minimum length of the bromide band for the yield intended, a = volume fraction of liquid in the column, and $[I^-]$ = concentration of the iodide used for the elution. Usually $a[I^-]$ can be neglected in comparison with C. (For $[I^-] \leqq 0.01\ M$). Hence

$$Al'C = Dxt_i \tag{12}$$

From equations (11) and (12) t_s is found to be determined by

$$t_s = \frac{Cl'}{bDvt_i} \tag{13}$$

Hence

$$I_s = \frac{100-R}{100} \frac{k}{D} \frac{1-e^{-\lambda t_i}}{t_i} \cdot e^{-\frac{\lambda k_1}{\lambda t_i}} \tag{14}$$

where $k_1 = \frac{Cl'}{b\ v\ D}$

Hence

$$F = \frac{100-R}{100} \frac{1-e^{-\lambda t_i}}{t_i} \cdot e^{-\frac{\lambda k_1}{\lambda t}} \tag{15}$$

In order to find the time of irradiation which results in the best concentration factor, the derivative dF/dt_i is calculated and set equal to zero. This gives the equation for the optimum irradiation time t:

$$e^{-\lambda t} = \frac{t-\lambda k_1}{t-\lambda k_1 + \lambda t^2} \tag{16}$$

The following numerical values are used: $C = 4\times10^{-2}$ mmole/cm^3; $l' = 2$ cm; $b = 0.43$ mmole/cm^3; $v = 0.6$ ml/min cm^2; $\lambda = 0.01925$ hr^{-1}; and $R = 26$ %. Then k_1 is found to be 44.1 hr^2 and $\lambda k_1 = 0.85$ hr. Then from (16) t by approximative methods is found to be 9.5 hours. With this optimum time of irradiation the concentration factor according to (15) is 102. If the usual time of irradiation in the pile of A.E.R.E., *viz.* 5 days is used, F decreases to 46.

The diameter of the column is determined by the amount of potassium bromate irradiated. From equation (12) and the numerical values found for optimum time of irradiation,

$$A = 0.0143\ x \tag{17}$$

The yield of the separation process can now be calculated as a function of x from equations (4) and (17):

$$\eta = 0.8-\frac{[I^-]\ V_d}{0.11\ x} \tag{18}$$

DISCUSSION

The maximum concentration factor of about 100 considerably increases the specific radioactivity of the preparations, but for a Szilard-Chalmers reaction it is a rather low value. The concentration factor is limited by the rate of decomposition and by the time of separation. A gain in separation time is possible if sodium bromate, which is more soluble than potassium bromate, is irradiated. For the optimum time of irradiation, however, the decrease in F due to the time of separation is only 9 %. Hence there is no reason for using sodium bromate if the rate of decomposition cannot be decreased. Since the decomposition is due in the main to γ-irradiation, it should be possible to decrease the rate without any appreciable decrease in neutron flux. The absorption coefficients in lead for thermal neutrons and γ-radiation are $\mu_\gamma \leqq 0.45$ cm^{-1}, $\mu_n = 3.3\times10^{-5}$ cm^{-1}. Hence if the material is surrounded by about 5 cm of lead during the irradiation, the rate of decomposition should be reduced by a factor 10. The possibility of having such a lead container in the reactor was not able to be investigated by the author, but if it were possible, it would lead to a considerable improvement in the concentration factor.

SUMMARY

An ion-exchanger containing silver oxide and capable of exchanging halide ions for hydroxyl ions has been used for the preparation of ^{82}Br by Szilard-Chalmers reaction on pile-irradiated potassium bromate. The checking of the separation column by E.M.F. measurements in the column is described.

The radiation chemistry and hot-atom chemistry for pile-irradiated potassium bromate have been investigated, and the results applied to a calculation of the maximum concentration factor and yield obtainable if the irradiation is carried out in the reactor of the Atomic Energy Research Establishment, Harwell, England, and if use is made of the ion-exchange column described. It has been found that a specific radioactivity of about 5 c per gram can be produced. Possible improvements yielding about 50 c per gram are discussed.

My thanks are due to Dr. W. J. Arrol, A.E.R.E. for arranging the irradiation of potassium bromate in the reactor.

LITERATURE

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On the Analytical Determination of Radioactive Bromine

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The analytical determination of radioactive nuclides in tracer experiments involves the performance of accurate relative measurements of radioactivity. The measured radioactivity of a sample is, as is well known, determined by the equation

$$I = \omega \sum_i (k_a \; k_{sa} \; k_s \; k_r \Lambda)_i \qquad (1)$$

where I = measured radioactivity, ω = solid angle of the preparation, k_a = absorption factor, k_{sa} = self-absorption factor, k_s = scattering factor, k_r = realease factor, Λ = number of transmutations per unit time, and i = different types of radiation emitted by the sample.

In relative measurements, the factors determined solely by the counter tube and the arrangement of counter and sample can easily be held constant, and thus are of no importance for the measurements. These factors are ω, k_a, k_s, and k_r. The self-absorption factor, however, which depends mainly on the dimensions of the sample cannot always be kept constant during long series of measurements.

The purpose of this work has been to study the self-absorption of the radiation from ^{82}Br. The preparation of samples suitable for radioactivity measurements is the first problem. Further, the dependence of the self-absorption factor on the geometrical arrangement of the counter tube and the sample has to be considered together with the possibility of calculating the self-absorption corrections from the absorption in aluminium.

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* Operated under the auspices of the Swedish Atomic Energy Commission.

THE PREPARATION OF SAMPLES

The separation of bromide from a solution by precipitation with silver nitrate followed by preparation of samples for radioactivity measurements from silver bromide is a very unsuitable method. The light sensitivity of this compound would necessitate performing all the work in red light. This is very impractical and almost impossible during the different manipulations that have to be carried out with a preparation for which the radioactivity is to be measured. Among the other existing less soluble bromides the thallium compound was considered to be best suited for the preparation of samples.

A sample to be used for radioactivity measurements must possess a well defined and reproducible geometry. This fact raises additional problems regarding otherwise very simple chemical operations. Thallium bromide forms a crude precipitate which is not uniformly distributed when filtered through a horizontally mounted filter paper. This fact makes it necessary to separate the precipitate from the filter paper in order to put it in some geometrically defined form. As it is impossible to perform this operation quantitatively it must be possible to create samples with a reproducible composition. For the preparation of samples having well defined geometrical properties, the best method consists in pressing the preparation into pellets.

Experimental

Thallium bromide was prepared through precipitation of 5 ml of a solution 0.1 M in sodium bromide by an equivalent amount of 0.1 M thallium nitrate solution. The precipitate was filtered off with a "membrane filter", scraped off, dried at 80° C, and weighed. It was then dissolved in a large quantity of hot water (80° C) and bromide was determined by potentiometric titration with 0.1 M silver nitrate solution using a silver electrode and a saturated calomel electrode. From the determined bromide content, the weight of the thallium bromide was calculated. The results are shown in Table 1.

For the preparation of samples of good geometry, thallium bromide was pressed into pellets with a hydraulic press. In order to study the reproducibility, pellets containing

Table 1. Analysis of precipitated thallium bromide.

No.	Weight of TlBr (g)		Error %
	Found	Calculated	
1	0.1201	0.1199	+ 0.2
2	0.1195	0.1204	− 0.7
3	0.1192	0.1196	− 0.3

^{82}Br were prepared using a pressure of about 4 000 kg/cm^2. For every ten mg between 30 and 100 mg a pair of pellets of approximately the same weight was prepared. Each pellet was weighed and its radioactivity measured. The results are shown in Table 2.

From Table 1 it is found that the manner of preparing thallium bromide results in a compound of constancy satisfactory for the purpose concerned.

The difference in specific radioactivity of the different pellets listed in Table 2 is due to the difference in self-absorption. This difference, however, has been neglected for pellets belonging to the same pair. For each measurement of the radioactivity about 4 000 impulses were counted. Hence, the standard deviation of the radioactivity measurement is 1.6 %, and the probable difference can be calculated to be 2.3 %. All results except those for pellets 1 and 6 fall within this limit. Furthermore, the single occurrence

Table 2. Radioactivity of pellets from thallium bromide.

No.	Radioactivity (cpm)	Weight (mg)	Specific radioactivity	Difference (%)
1 a	1965	30.3	64.9	5.5
1 b	2029	29.7	68.3	
2 a	2411	40.8	59.09	1.4
2 b	2420	40.4	59.90	
3 a	2598	49.6	52.37	1.7
3 b	2657	49.9	53.24	
4 a	2957	59.8	49.44	0.4
4 b	2931	59.8	49.26	
5 a	3221	69.8	46.14	0.5
5 b	3233	69.7	46.38	
6 a	3437	80.4	42.74	3.5
6 b	3532	79.8	44.26	
7 a	3749	89.5	41.88	0.1
7 b	3748	89.5	41.87	
8 a	3979	99.6	39.94	1.1
8 b	4045	100.1	40.40	

of the error 3.5 % among 8 measurements constitutes no deviation from expected values since the probability of an error of 4.6 % is 0.18. Hence it has been shown that it is possible to prepare reproducible samples by the method outlined.

THE SELF-ABSORPTION CORRECTION

The factor k_{sa} is defined by the equation

$$I = I^{\circ} k_{sa} \tag{2}$$

where I = measured radioactivity, and I° = radioactivity measured if the same amount of radioactive nuclide had been a weightless sample on a support having $k_s = 1$.

The values of k_{sa} are so sensitive to the geometrical arrangement of sample and counter tube that it must be regarded impossible to determine all the correction factors necessary for the application of a self-absorption curve determined for one counting system to another arrangement. The purpose has, therefore, been to examine to what extent the calibration of different counting systems can be simplified. The preparation of samples for a determination of k_{sa} according to the usual methods [1] is rather time consuming and therefore it is desirable to calculate k_{sa} from an ordinary absorption curve measured with aluminium foils. If the difference in mass-absorption between thallium bromide and aluminium is neglected, the measured radioactivity of a thick sample on a support having the same back-scattering factor as thallium bromide is given by

$$I = k \int_{x=0}^{d} I_s A \, [\mathrm{f}_1(x) + (k_s - 1)\mathrm{f}_2(x)] \, \mathrm{d}x \tag{3}$$

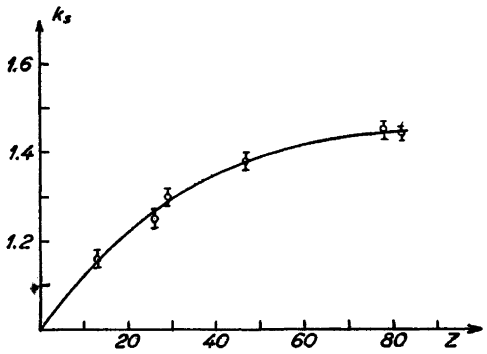
where I = measured radioactivity, I_s = specific radioactivity of the material for $k_s = k_{sa} = 1$, A = area of sample, $\mathrm{f}_1(x)$ = absorption function for primary radiation, $\mathrm{f}_2(x)$ = absorption function for back-scattered radiation, d = thickness of sample, k = a constant, and k_s = back-scattering coefficient for the material constituting the sample.

The measured radioactivity of the same amount of ^{82}Br when measured as a weightless sample on a support having back-scattering factor 1 is determined by

$$I^{\circ} = k I_s A d \tag{4}$$

Hence k_{sa} is determined by the equation

$$k_{sa} = \frac{1}{d} \int_{x=0}^{d} [\mathrm{f}_1(x) + (k_s - 1)\, \mathrm{f}_2(x)] \, \mathrm{d}x \tag{5}$$



1. Back-scattering coefficients for the radiation from ^{82}Br as a function of the atomic number of the back-scatterer.

For a determination of k_{sa} for a special arrangement, k_s, $f_1(x)$ and $f_2(x)$ should thus be known.

For practical purposes it is better to determine another function $f_3(x)$ defined by

$$f_3(x) = f_1(x) + (k_s - 1)\ f_2(x) \tag{6}$$

This is possible by measurement of an absorption curve for a weightless preparation on a support having the same back-scattering factor as the material constituting the sample. The self-absorption is then determined by

$$k_{sa} = \frac{1}{d} \int_0^d f_3(x)\ dx \tag{7}$$

The experimental determination of k_{sa} requires knowledge of the backscattering for different materials as the determination of $I°$ is usually not carried out using a support with $k_s = 1$.

Experimental

Back-scattering: To determine k_s as a function of the atomic number of the back-scatterer, a support which was regarded as weightless was prepared. A solution of a polyvinyl acetate in acetone was dropped on a water surface when a thin film of the polyvinyl compound was formed. This film was then allowed to cover an aluminium ring 50 mm in diameter and dried under an infrared lamp. The thickness of the film was determined by weighing to be about 100 $\mu g/cm^2$. On the center of this film a small drop of a solution containing ^{82}Br was evaporated. The weight of the substance could be calculated to be about 5 μg. This sample was regarded as essentially weightless. The radioactivity of the sample was measured at a distance of 15 cm to the nearest scattering material and 7 mm to the counter tube. The radioactivity so measured is denoted by $I°$. Then back-scatterers of materials with different atomic numbers and with a thickness exceeding the saturation value were placed immediately behind the sample and the radioactivity measured. The back-scattering factors so obtained are shown in Fig. 1.

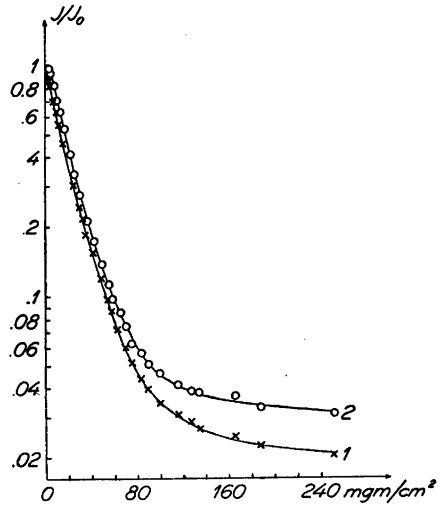


Fig. 2. Absorption curves for the radiation from ^{82}Br in aluminium. Curve 1 : 7 mm between sample and window, 0 mm between absorber and window. Curve 2 : 7 mm between sample and window, 7 mm between absorber and window.

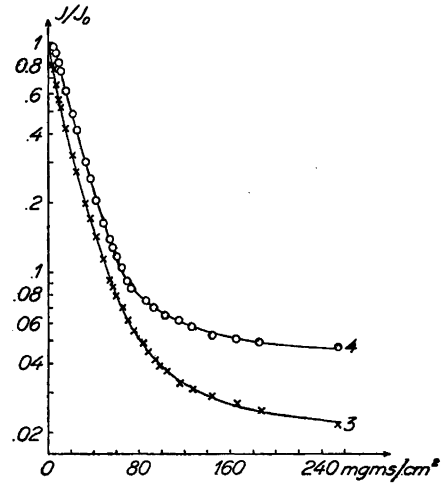


Fig. 3. Absorption curves for the radiation from ^{82}Br in aluminium. Curve 3 : 37 mm between sample and window, 0 mm between absorber and window. Curve 4 : 37 mm between sample and window, 37 mm between absorber and window.

Absorption in aluminium: Samples were prepared by evaporation of 0.01 ml of a radioactive solution on platinum discs 10 mm in diameter. Before the evaporation the discs were moistened with a wetting agent in order to produce a uniform layer of radioactive substance. This layer, having a thickness of 10—20 $\mu g/cm^2$, was regarded as weightless. The absorber foils of aluminium were prepared by stamping, which produces foils with an accurately determined diameter. The foils were calibrated by weighing and the thinnest of them were cemented on aluminium rings 0.2 mm thick and 1 mm wide. Foils between 2.9 and 535 mg/cm^2 were prepared. Absorption curves were measured using four different geometric arrangements. The resulting curves are shown in Figs. 2 and 3.

Self-absorption: The radioactivity of a solution containing ^{82}Br was determined by evaporation of 0.01 ml on platinum discs, as described above, followed by measurement of the radioactivity of these samples. The reproducibility of samples so prepared was found to be within 2 %. A measured volume of this solution was added to a solution containing a known amount of bromide, and thallium nitrate then added. The precipitated thallium bromide was filtered off, dried, and pressed into pellets weighing from 30 to 100 mg. The radioactivity of each pellet was measured using distances from 7—47 mm between the sample and the window of the counter tube. From the value of the radioactivity of the ^{82}Br-solution the specific radioactivity of the samples for different distances could be calculated. Empirical self-absorption curves determined from the measured radioactivities are shown in Figure 4.

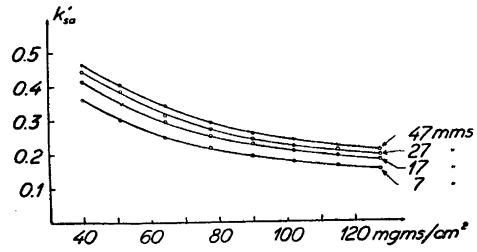


Fig. 4. Empirical self-absorption coefficients for ^{82}Br as a function of thickness of samples for different distances between sample and counter window.

In order to obtain values of the self-absorption for different window thickness, the radioactivity of the pellets was measured using absorber foils placed close to the window. The corresponding values for the specific radioactivity of the samples were calculated from the absorption curve 1 in Fig. 2. The results of these measurements are shown in Fig. 5.

DISCUSSION

The difference in the shape of the absorption curves measured under different geometrical conditions has earlier been reported by Johnston and Willard [2], and is due to scattering effects in the absorber foils. Consequently, the determination of $f_3(x)$ in (7) must take place under geometrical conditions similar to those operating during the measurements of thick samples, *i. e.* with the absorber foils close to the sample. The absorption curve must further depend on the backing material of the sample, since the back-scattered radiation must have a different energy spectrum. $f_3(x)$ thus ought to be determined using a support for the sample with the same back-scattering factor as thallium bromide. This factor can be calculated by

$$k_s(\mathrm{TlBr}) = \frac{k_s^{(Z=81)} + k_s^{(Z=35)}}{2} \tag{8}$$

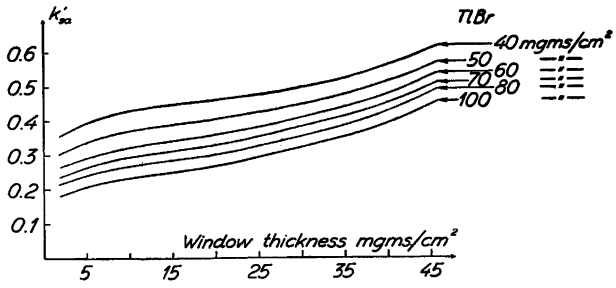


Fig. 5. Empirical self-absorption coefficients for ^{82}Br as a function of the thickness of the counter window for different sample thickness.

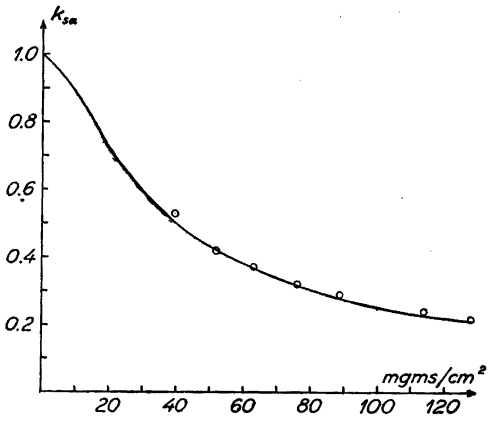


Fig. 6. Calculated self-absorption curve for ^{82}Br for a distance between sample and counter window of 7 mm and a window thickness of 1.78 mg/cm². The points marked in the figure are experimentally determined values.

Then from Fig. 1 we see that k_s (TlBr) = 1. 39, which corresponds to an atomic number of about 47, *e. g.* silver. In spite of this platinum has been used as backing material in the determination of $f_3(x)$ since it was considered probable that silver would react with bromide during the evaporation of solutions on this material and since no other suitable material with an atomic number of the right magnitude was available. This, of course, introduces an error in $f_3(x)$, which has, however, been neglected because of the small difference in back-scattering for silver and platinum and the relatively low contribution of $f_2(x)$ to $f_3(x)$.

It should be observed that the self-absorption factors shown in Figs. 4 and 5 do not represent k_{sa} as defined on page 1119, but another factor k'_{sa} determined by

$$k'_{sa} = \frac{I}{I'_s m} \tag{9}$$

where m = mass of sample, and I'_s = a relative value of the specific radioactivity which is valid in the event that the original solution is evaporated on platinum discs, and the specific radioactivity calculated directly from the measured radioactivity.

I'_s is determined by

$$I'_s = k_s \times I_s \tag{10}$$

where k_s, the back-scattering factor for the backing material used when determining the specific radioactivity is a function of the geometrical arrangements. The values of k'_{sa}, though not equal to the true self-absorption values, can be used to determine the ratio between k'_{sa} for different sample thickness. Hence they can be directly used as correction factors in relative measurements.

The self-absorption factor for a distance between sample and window of 7 or 37 mm has been calculated from curves 2 and 4 in Figs. 2 and 3. These curves, representing $f_3(x)$ in (7), were integrated by measuring the areas enclosed by the curves in a linear diagram. The calculation of the specific radioactivity of the sample for the distance 7 mm between sample and counter was performed using the value of $k_s = 1.45$ for platinum as found from Fig. 2. In figure 6 are shown the experimentally determined values of k_{sa} together with the calculated curve. For a distance between sample and window of 37 mm the ratio between the calculated values of k_{sa} and the empirical values of k'_{sa} was found to be 1.22. This means that the back-scattering factor for this distance should be 1.22.

From Fig. 6 it can be stated that the self-absorption factor for thallium bromide can be calculated with satisfactory accuracy from the absorption curves for aluminium as absorber, and from values of the back-scattering factors, provided the measurement of these values is carried out with the same geometrical arrangements used in the actual measurement of thallium bromide.

It has also been found that the values of k_{sa} and k_s are very sensitive to the distance between the sample and the window of the counter tube.

SUMMARY

The analytical determination of ^{82}Br has been investigated. The back-scattering, absorption, and self-absorption of the radiation has been studied. It has been found possible to calculate self-absorption factors from measured absorption in aluminium and values of the back-scattering factors.

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Short Communications

The Dissociation Constant of Silver Fluoride

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The emf of the following cell was measured at 25° C

$$\text{Ag, AgCl} \;\Big|\; \begin{matrix} c_{Ag}\ \text{C}\ AgClO_4 \\ c_F\ \text{C NaF} \\ (1-c_{Ag}-c_F)\ \text{C}\ NaClO_4 \end{matrix} \;\Big|\; 1\ \text{C}\ NaClO_4 \;\Big|\; 1\ \text{C}\ NaClO_4 \;\Big|\; \text{Hg, } Hg_2SO_4$$

The solutions to the left were made by adding aqueous sodium fluoride from a burette to a solution of silver and sodium perchlorate in the electrode vessel. Experimental details were the same as described in some previous papers on complex formation *Cf. e. g.* [1,2].

Some of the results are tabulated in Table 1, where E is the difference between the emf of the initial cell, where $c_F = 0$, and the emf of the same cell after the addition of fluoride. The concentration of free silver ions, $[Ag^+]$, has been calculated from Nernst's formula *Cf,* [2], and then β_1 was computed from equation 1.

Table 1. Titration of silver ions with fluoride.

$c_{Ag} \cdot 10^3$	$c_F \cdot 10^3$	E mV	β_1 C^{-1}	$c_{Ag} \cdot 10^3$	$c_F \cdot 10^3$	E mV	β_1 C^{-1}
10.51	0	0		105.1	0	0	
8.47	118	6.8	0.47	95.0	243	2.6	0.46
7.51	174	10.6	0.46	93.5	274	3.0	0.47
6.74	218	13.9	0.47	92.4	299	3.3	0.48
6.11	255	16.9	0.49	90.6	337	3.8	0.50
5.15	310	21.9	0.48	89.6	365	4.1	0.50
4.38	355	26.6	0.49				
3.50	405	33.1	0.51				
		Mean:	0.48			Mean:	0.48

This paper is a part of a study of the complex formation between silver and halogen ions, for which Statens Naturvetenskapliga Forskningsråd delivered a financial support to one of us (I. L.).

$$\beta_1 = \frac{[AgF]}{[Ag^+]\ [F^-]} = \frac{c_{Ag} - [Ag^+]}{[Ag^+] \cdot [c_F - (c_{Ag} - [Ag^+])]} \qquad (1)$$

The computation shows that AgF is the only complex formed in noteworthy concentrations. The slight increase of β_1, for increasing c_F could be interpreted as a formation of AgF_2^-, but liquid junction potentials and changes of activity coefficients make it impossible to draw any certain conclusion. Neither are polynuclear complexes formed, as the calculated β_1, remains constant, when c_{Ag} increases from 0.01 to 0.1 C.

Hence the dissociation constant of AgF, $1/\beta_1$, = 2.1 C at the ionic strength 1 C ($NaClO_4$).

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"Trans" 1,4-Chloro-Bromocyclohexane

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Crystallographic equivalency of chlorine and bromine atoms in organic compounds was first described by Hendricks [1] in the case of *p*-chloro-bromobenzene. Recently the same phenomenon was observed by Wang Lund and the present authors [2] who prepared and studied 1*x*, 2*x* dichloro-4*x*, 5*x*-dibromocyclohexane. This compound is dimorphous and for both modifications the crystallographic examination indicated the presence of a molecular center of symmetry. However, the cyclohexane derivative which would correspond most closely to the *p*-chloro-bromobenzene is the "trans" 1,4-chloro-bromocompound which has not, it appears, so far been prepared.

We first prepared a mixture of the two stereoisomeric 4-chlorocyclohexanols from pure chinitol (100 g) and 35% hydrochloric acid (100 ml) by slowly heating the reaction mixture to 100° C and keeping it at that temperature for 3—4 hours. After cooling, the oil formed during the reaction was separated from the acid solution and the latter, after dilution with water, neutralized with solid sodium bicarbonate. The solution was saturated with sodium chloride and extracted with ethyl ether. The ether solution was washed with bicarbonate solution, then with water and finally dried over sodium sulphate. After evaporation of the ether the chlorocyclohexanol mixture was distilled *in vacuo*. (Cl found 26.28, calc. 26.35).

100 g of phosphorous tribromide was added drop by drop to 45 g of the 4-chlorocyclohexanol mixture. When the greater part of the phosphorous tribromide had been added the cooling bath was removed and the temperature allowed to rise. Subsequently the reaction mixture was heated to 70° C for about an hour and then cooled. After addition of water the reaction product was extracted with ethyl ether. The ether solution was washed with bicarbonate solution, then with water and finally dried over calcium chloride. After evaporation of the ether the remaining liquid was fractionated *in vacuo*. A fraction boiling between 62 and 64° C (0.5 mm) and kept at about 5° C for some time gave a good yield of crystals. After recrystallization from ligroin or ethyl alcohol needle-shaped monoclinic crystals of m.p. 102° C were obtained ($a = 11.81$; $b = 5.52$; $c = 5.95$; $\beta = 103°$):

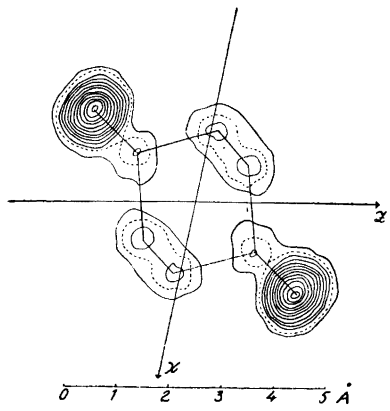


Fig. 1.

$C_6H_{10}ClBr$	Calc.	Br 40.48	Cl 17.93
	Found	» 40.37	» 18.12

X-ray analysis showed that the crystals are isomorphous with those of "trans" (*x*, *x*) 1,4-dibromocyclohexane m. p. 112° C and the corresponding diiodocyclohexane (m.p. 142° C)/. We may add that according to our observations the corresponding dichlorocyclohexane (m.p. 102° C) has a transition point at 12—13° C and that the modification stable *below* this temperature has a crystal structure corre-

sponding to that of the three trans 1,4-dihalogenocyclohexanes. A detailed X-ray study of the whole series of isomorphous compounds (including N,N′-dichloropiperazine [4]) is in progress. Part of a Fourier projection along the *b* axis of the bromine compound worked out by Bergskoug is reproduced in Fig. 1.[5]

In the case of the chloro-bromocompound the conclusion had to be drawn that the chlorine and bromine atoms are indeed crystallographically equivalent. No sign of a transition into a second modification could be observed in this case. It is therefore very probable that dichloro- and chloro-bromocyclohexane have different crystal structures at their melting points.

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5. Bergskoug, A.-R. Unpublished result.

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Preparation of Carboxylabelled Oleic Acid *

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The above-mentioned labelled acid, which was required for metabolic studies, was prepared from erythro-9,10-dihydroxystearic acid.

The acid (m.p. 129°) was acetylated and subjected to the silver salt bromine degradation. The diacetoxybromide was deacetylated to erythro-8,9-dihydroxy-1-bromo-heptadecane m.p. 102—103°. A nitrile synthesis using labelled potassium cyanide, followed by alkaline hydrolysis, yielded carboxylabelled erythro-9,10-dihydroxystearic acid.

This labeled acid was then transformed into the dibromo acid with hydrogen bromide in acetic acid-sulphuric acid according to Ames and Bowman [1]. By following their procedure of debrominating the esterified dibromo acid, sterically pure oleic acid was obtained.

If the low melting threo-9,10-dihydroxystearic acid is used as starting material elaidic acid should be obtained by the same method [2].

EXPERIMENTAL. Dihydroxystearic acid (m.p. 129°) was prepared from oleic acid by oxidation with permanganate [3].

Silver salt of erythro-9,10-diacetoxy octadecanoic acid. 4.5 g of 9,10-dihydroxystearic acid was dissolved in a mixture of acetic anhydride (5 ml) and pyridine (10 ml) and heated on the steam bath for one hour. After concentration *in vacuo* to a syrup, ice and ether were added. The ether solution was washed with *N* hydrochloric acid and water, dried over sodium sulfate and evaporated to dryness. The crude product was dissolved in 50 ml ethanol and neutralized with sodium hydroxide (phenolphthalein). A solution of 2 g silver nitrate in 30 ml 60 per cent ethanol was added with rapid stirring. The resulting sticky mass of the precipitated silver salt was filtered off, washed with a little water and dried overnight in a vacuum oven at 60°. The silver salt forms a brown gum which is soluble in hot carbon tetrachloride.

1-Bromo-8,9-dihydroxyheptadecane. 65.5 g of crude oven-dried silver salt was dissolved in a mixture of 1 l of carbon tetrachloride and 200 ml of methylene chloride and the solvents distilled until the b.p. was 76.5° and water no longer appeared in the condenser. The residual solution was mixed with dry silver acetate (10 g) according to a procedure described earlier [4] and treated with bromine (11 ml).

* Part 7 on the Metabolism of lipids.

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* Part 7 on the Metabolism of lipids.

The precipitated silver bromide was removed by filtration and the filtrate was washed successively with solutions of potassium iodide, sodium thiosulphate and sodium carbonate. After washing with water and drying, the solvent was removed yielding an amber-coloured oil (36.9 g). 5 g of this material was dissolved in 25 ml absolute methanol containing 4 milliequiv. HCl-gas and refluxed for 3 hours. After dilution with water and extraction with ether, the dihydroxybromide was crystallized from heptane or methanol. M.p. 102–103°.

$C_{17}H_{35}O_2Br$ (351.37)

Calc.	C 58.1	H 10.0	Br 22.8	
Found	» 59.1	» 10.1	» 22.6; 22.9	

Isotopic dihydroxystearic acid. 2.8 millimoles of bromide and 2.8 millimoles of $K^{13}CN$ were refluxed in a mixture of 10 ml of ethanol and one ml of water for 48 hours. 2 g of potassium hydroxide was added and the refluxing continued for a further 48 hours. Dihydroxystearic acid-1-^{13}C (m.p. 126–127°) was obtained in a yield of 85 per cent. Similar yields have been obtained with $K^{14}CN$ under the same conditions except that an excess of bromide was used.

Oleic acid. Treatment of 5 g of labelled dihydroxystearic acid with hydrogen bromide-acetic acid-sulphuric acid and subsequent debromination as described by Ames and Bowman [1] yielded 2.5 g oleic acid-1-^{14}C m.p. 12°.

Reported at the Second International Congress of Biochemistry in Paris July 21–27 1952.

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Crystalline Modifications of D-Mannitol

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The crystallographic data of two modifications of D-mannitol are quoted by Groth [1] *i.e.* α-mannitol and β-mannitol, both orthorhombic, the former with $a:b:c$ = 0.4718 : 1 : 0.5200, the latter $a:b:c$ =0.5121 :1 : 0,6577. According to Groth, β-mannitol crystallizes from aqueous or alcoholic solutions, but it is not stated when α-mannitol is formed, though it is said sometimes to occur in mixture with the β-form. Becker and Rose [2] determined the unit cell for mannitol (not stating from which solvent) as a = 10.36 A, b = 8.1 A, c = 4.55 A and $a:b:c$ = 1.275 : 1: 0.562. Marwick [3] reported for β-mannitol: a = 8.65 A, b = 16.90 A, c = 5.56 A and $a:b:c$ = 0.511: 1:0.329. She also suggested that β-mannitol belongs to the space group V^4, and that the molecule is parallel to [100]. Our determinations for β-mannitol are in agreement with the latter data, whereas those of B. & R. have not been verified. Calculated density from B. & R.'s values, with Z = 2, is 1.587, which differs from the actual sp. density of 1.49.

It has been found in the present work that two other forms of mannitol may be obtained by recrystallization from water-alcohol 1 : 1 or alcohol-aceton:water 5:5:2 (volume). In the first case, however, the result depends upon the rate of crystallization. Thus, rapid cooling produces the new form (γ), while slow cooling gives a mixture of α- and β-forms (see below). Powder photographs of the new γ-form can be indexed by assuming orthorhombic symmetry and a = 9.33 A, b = 17.36 A, c = 4.99 A and $a:b:c$ = 0.537:1:0.287, giving V = 808.2 A^3, Z = 4 and d_x = 1.496 g/cm^3. Reflexions $h00$ and $0k0$ are absent

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The crystallographic data of two modifications of D-mannitol are quoted by Groth [1] *i.e.* α-mannitol and β-mannitol, both orthorhombic, the former with $a:b:c$ = 0.4718 : 1 : 0.5200, the latter $a:b:c$ =0.5121 :1 : 0,6577. According to Groth, β-mannitol crystallizes from aqueous or alcoholic solutions, but it is not stated when α-mannitol is formed, though it is said sometimes to occur in mixture with the β-form. Becker and Rose [2] determined the unit cell for mannitol (not stating from which solvent) as a = 10.36 A, b = 8.1 A, c = 4.55 A and $a:b:c$ = 1.275 : 1: 0.562. Marwick [3] reported for β-mannitol: a = 8.65 A, b = 16.90 A, c = 5.56 A and $a:b:c$ = 0.511: 1:0.329. She also suggested that β-mannitol belongs to the space group V^4, and that the molecule is parallel to [100]. Our determinations for β-mannitol are in agreement with the latter data, whereas those of B. & R. have not been verified. Calculated density from B. & R.'s values, with Z = 2, is 1.587, which differs from the actual sp. density of 1.49.

It has been found in the present work that two other forms of mannitol may be obtained by recrystallization from water-alcohol 1 : 1 or alcohol-aceton:water 5:5:2 (volume). In the first case, however, the result depends upon the rate of crystallization. Thus, rapid cooling produces the new form (γ), while slow cooling gives a mixture of α- and β-forms (see below). Powder photographs of the new γ-form can be indexed by assuming orthorhombic symmetry and a = 9.33 A, b = 17.36 A, c = 4.99 A and $a:b:c$ = 0.537:1:0.287, giving V = 808.2 A^3, Z = 4 and d_x = 1.496 g/cm^3. Reflexions $h00$ and $0k0$ are absent

when h resp. k is odd, suggesting that the space group may be V^3—$P2_12_12$, but absences cannot be stated with certainty from powder patterns alone, and the crystals were too small for single crystal methods.

A powder photograph of mannitol from alcohol-aceton-water is identical with that of mannitol prepared from the triethylidene compound (see Meunier [4]). Purified by the latter method, followed by slow crystallization from 96 % alcohol, good crystals for X-ray investigations were obtained. Unit cell dimensions of the orthorhombic crystals were determined from oscillation and Weissenberg photographs: $a = 8.94$ A, $b = 18.41$ A, $c = 4.92$ A, $a:b:c = 0.486 : 1 : 0.267$, $V = 809.7$ A^3, $Z = 4$ and $d_x = 1.494$ g/cm^3. Many absences are recorded, thus, $h00$ when h odd, $0k0$ when k odd, $00l$ when l odd, $0kl$ when l odd, $h0l$ when $h+l$ odd and $hk0$ when h odd. No orthorhombic space group has all these absences, and some of them must, therefore, be due to pseudosymmetry. By re-exammination of the photographs a few exceedingly weak reflexions $0k0$ were found for which k is odd, thus [010] is no true screw axis. The remaining absences would suggest the space group V_h^4—$Pcna$, but no reasonable arrangement of the molecules could be found. On the assumption that absences of $0kl$ for l odd are also due to pseudosymmetry (these absences being less certain since reflexions $0kl$ for l even are also very weak), the others correspond to the space group C_{2v}^6—$Pmna$. A satisfactory arrangement of the molecules can be found such that the direction of their carbon chains is parallel to [010] and their approximate centers are located close to the positions $\frac{1}{4}\frac{1}{4}\frac{1}{4}$, $\frac{\bar{1}}{4}\frac{1}{4}\frac{1}{4}$, $\frac{\bar{1}}{4}\frac{\bar{1}}{4}\frac{\bar{1}}{4}$, $\frac{1}{4}\frac{\bar{1}}{4}\frac{\bar{1}}{4}$. This arrangement would also account for the systematic weaknesses.

The axial ratio of this form agrees roughly with that quoted by Groth for α-mannitol (with c-axis doubled), and this form may, therefore, possibly correspond to α-mannitol.

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3. Marwick, Th. C. *Proc. Roy. Soc.* **A 131** (1931) 621.
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The Presence of Djencolic Acid in Hydrolysates of Mammalian Tissues

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Djencolic acid was first isolated from urine of natives of Java who had eaten the djencolic bean and were suffering from djencolic poisoning. Later on Van Veen and Hyman isolated the compound from alkaline extracts of the bean [1,2]. Consden *et al.*[3] found the amino acid in hydrolysates of reduced wool treated with methylene bromide or formaldehyde. To our knowledge the amino acid has not been isolated from common protein hydrolysates of mammalian tissues.

In connection with microbiological and chemical analysis of a peptide fraction isolated from calf plasma [4] we found that the two-dimensional chromatograms of the acid-hydrolyzed material (3 N HCl, 16 hours at 120°) showed a spot close to the known positions of ethanolamine-phosphoric acid, djencolic acid and diaminopimelic acid [5–7].

The substance was eluted with HCl together with the leucine-isoleucine fraction from a Dowex 50 columns according to the method of Stein and Moore [8]. The dry mixture was packed on a small column and eluted with *tert.* — amyl alcohol where

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djencolic acid was insoluble. The amino acid was recrystallized three times from 6 *N* HCl in the form of needles. They were compared with djencolic acid synthesised according to du Vigneaud and Patterson [9]. They had the same R_F-values in paper chromatograms run with different solvents. The X-ray powder diagrams were the same. The molecular weight was 325 as calculated from X-ray diffraction data, suggesting the presence of four molecules per unit cell. The substance had the following composition.

3.855 mg substance:	0.306 ml N_2 at 28° and 763 mm.
3.813 » »	3.274 mg CO_2 and 1.539 mg H_2O.
6.464 » »	32.36 ml 0.01 *N* thiosulfate (Kirsten)
5.680 » »	3.584 ml 0.01 *N* $AgNO_3$

$C_7H_{14}O_4N_2S_2 \cdot 2$ HCl

Calc.	C 25.6	H 4.9	S 19.6	Cl 22.3	N 8.6
Found	» 23.4	» 4.5	» 20.2	» 23.0	» 9.0

Scarcity of material allowed only a single analysis. The results seem to indicate that djencolic acid is present in the plasma peptide fraction.

A spot corresponding to the position of djencolic acid has also been observed in chromatograms from acid-hydrolyzed plasma, liver and muscle tissues. With regard to the quantitative aspects the chromatograms showed the following pictures when amounts of material corresponding to 100 μg of material were used. The strongest spot was obtained from the peptide fraction. Liver and plasma contained smaller amounts of the amino acid and in muscle hydrolysates the spot was barely visible. Negative results were obtained with hydrolysates of proteins from the spleen and with a peptide fraction prepared from human urine. On the other hand, recent results in this laboratory with chromatograms of some mucoproteins seem to show the presence of a spot close to the position of djencolic acid [10].

Accordingly, our results are in good agreement with the opinion expressed already in 1936 by du Vigneaud [9]: "It is possible that the compound may be more widely distributed in nature and perhaps may be responsible for a portion of the non-cystine, non-methionine sulfur in certain protein." A full account of this work will appear elsewhere.

The investigation was supported by a grant from the Medical Research Council. The technical assistance of Mr. T. Persson is gratefully acknowledged.

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Separation of Growth Factors for *Lactobacillus lactis* Dorner *Lactobacillus leichmannii* and *Leuconostoc citrovorum*, by Means of Ionophoresis on Paper

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The usefulness of microorganisms for identifying and estimating biologically active substances is in some cases limited, because of the lack of specificity. In the

djencolic acid was insoluble. The amino acid was recrystallized three times from 6 *N* HCl in the form of needles. They were compared with djencolic acid synthesised according to du Vigneaud and Patterson [9]. They had the same R_F-values in paper chromatograms run with different solvents. The X-ray powder diagrams were the same. The molecular weight was 325 as calculated from X-ray diffraction data, suggesting the presence of four molecules per unit cell. The substance had the following composition.

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The usefulness of microorganisms for identifying and estimating biologically active substances is in some cases limited, because of the lack of specificity. In the

determination of vitamin B_{12} by lactobacilli, seven naturally-occurring entities are known to interfere, *viz.* five desoxyribosides and at least two different forms of the vitamin. Little is known about the factors causing growth of *Leuconostoc citrovorum* but, within the range of concentration used for the estimation of natural and synthetic citrovorum factor on agar plates, at least four factors stimulate the growth of this microorganism [1,2]. The introduction of partition chromatography on paper [3] greatly facilitated the separation of the small quantities of substances active in microbiological tests. The first report of a paper chromatographic separation of microbiological growth factors, and the determination of the position of the different factors by using a microbiological indicator was given by Winsten and Eigen [4] on the chromatography of vitamin B_{12} and related growth factors.

In 1948 Wieland and Fischer [5] and later Durrum [6] described a procedure for the separation of amino acids and proteins by electrophoresis or ionophoresis on paper. This method has proved very useful in the study of proteins as shown by Tiselius *et al.*[7] The present paper deals with an attempt to apply paper ionophoresis to the separation of some growth factors for *Lactobacillus lactis* Dorner (ATCC 8000), *Lactobacillus leichmannii* 313 (ATCC 7830) and *Leuconostoc citrovorum* (ATCC 8081). The apparatur used was essentially of the same type as that described by Kunkel and Tiselius [7]. Carbon rods or platinum sheets were used as electrodes. Voltages ranging from 200 to 1 000 volts were applied, the most suitable for our purpose seemed to be 600 volts. The paper strips — 2.5—3 cm broad and about 32 cm long — were cut from sheets of Whatman No. 1 paper. Phosphate buffers (0.03 *M*) which have the advantage of covering a very wide pH range were employed. The ionophoresis experiments were run at room temperature for 1—5 hours depending on the factors to be separated. The paper strips were then dried and placed on agar plates seeded with the microorganism in question.

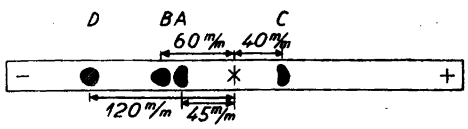


Fig. 1. Separation of vitamin B_{12}-factors by means of ionophoresis on paper. 600 volts, pH 5.0, 4 hours.
Cobemin protected from light: spot A.
Illuminated Cobemin: spots A, B, and C.
Normocytin, Reticulogen and fermentation liquor from Streptomyces griseus: spots A, B, C, and D.
Bacto Liver and Heptomin: A, B, and C.

Vitamin B_{12} or cyanocobalamin (Cobemin, Merck) protected from light migrates towards the cathode both at pH 5.0 and at pH 11.0 (Fig. 1 spot A). When the cyanocobalamin was illuminated two new spots appeared: one caused by a factor moving towards the anode (C) and the other (B) by a factor moving towards the cathod as does A, but faster than A. Normocytin (Lederle) that is supposed to contain only vitamin B_{12} and vitamin B_{12b} (hydroxy- or aquacobalamin) gave rise to four growth zones after ionophoretic separation. Three of these seemed to be identical with the spots A, B and C obtained with illuminated Cobemin, the fourth factor, causing the spot D in figure 1, migrated considerably faster towards the cathode than A and B. All four spots (A, B, C and D) could also be obtained with a fermentation liquor (*Streptomyces griseus*) and with Reticulogen (Lilly), whereas an aqueous extract of Bacto liver and an injectable liver concentrate Heptomin (Tika) only gave rise to three spots (A, B and C).

The spot C might be caused by a complex between the di- or trivalent ions of the buffer solution and the cobalamin, which could explain why it migrates in the opposite direction of B_{12} and B_{12b}. The

factor D, on the other hand, can hardly have been formed from B_{12} or B_{12b} during the ionophoresis as in that case it should appear when illuminated cyanocobalamin is employed. Normocytin, Reticulogen and the fermentation liquor of *Streptomyces griseus* thus contain a third B_{12} factor. It was observed that this factor was destroyed in alkali under conditions which destroy vitamin B_{12}. The factor D seemed to be equally active towards *Lb. lactis* Dorner and *Lb. leichmannii*. It will, however, need further research to demonstrate the nature of this new form of vitamin B_{12} and to decide if it is identical with any of the vitamin B_{12}-factors – different from B_{12} and B_{12b} – reported by other authors [8–10].

To obtain separation of the desoxyribosides (thymidine, desoxyguanosine and desoxyhypoxanthosine were employed) from the B_{12}-factors a pH of about 11 had to be employed. The separation was then complete within 3 hours and it could be shown that very little destruction of vitamin B_{12} had occurred in spite of the alkaline conditions. The desoxyribosides moved towards the anode at this pH, desoxyhypoxanthosine being the fastest moving of the three.

For separation experiments with *L. citrovorum*, only thymidine, synthetic citrovorum factor (Leucovorin, Lederle) and natural citrovorum factor (isolated from horse liver) [11] were available in pure form. Thymidine could easily be separated from the natural or the synthetic citrovorum factor at pH 8.0. At this pH thymidine migrates towards the cathode and both the natural and the synthetic citrovorum factor towards the anode. The separation was complete within 1 – 2 hours. The natural and the synthetic citrovorum factors seemed to migrate at the same speed towards the anode. The acidic property of the natural citrovorum factor was also observed by Lyman and Prescott [12] in their study on the electrolysis of growth factors for *L. citrovorum*. Thymidine and citrovorum factor occurring in liver extracts, fermentation liquors and in Reticulogen (Lilly) could also be separated by means of ionophoresis on paper.

The authors wish to express their thanks to Professor Harry Lundin, head of the division, for his cheerful interest in this work, to Dr, John C. Keresztesy, Bethesda, Maryland, U.S.A., for a gift of a calcium salt of natural citrovorum factor, and to Lederle Laboratories Division, American Cyanamid Company, New York, U.S.A., for Leucovorin and Normocytin.

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A Differential Dosimetry of Pile Radiations Using Fe^{2+} Oxidation

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Biological material irradiated in the pile is affected by different kinds of radiation [1]: (external) γ-radiation, fast, slow and thermal neutrons. The biological action of the latter is chiefly due to nuclear reactions with H, leading to (internal) γ-radiation through H(n)D, and with N, B and, in certain cases, Li, leading to the emission of heavy ionizing particles formed through the reactions $N^{14}(np)C^{14}$, $B^{10}(n\alpha)Li^7$, and $Li^6(n\alpha)T$ respectively. Since these radiations have very different biological effectivities, mainly due to differences in ionization densities [2], it is necessary to determine their relative importance in the total dose given. In the present communication the authors show the possibility of measuring the doses of γ-radiation (+ non-thermal neutrons — probably a small fraction) and thermal neutrons separately through the determination of the yield of the reaction $Fe^{2+} \rightarrow Fe^{3+}$, occurring in solutions of $FeSO_4$ in 0.1 *N* H_2SO_4. It is further demonstrated that the relative chemical efficiencies of the different kinds of radiation can easily be determined.

In a preliminary series of experiments pairs of silica tubes containing 3 ml of air-saturated $FeSO_4-H_2SO_4$ solution, one solution with, and the other without, an added amount of H_3BO_3 or $Li_2SO_4 \cdot H_2O$ have been irradiated simultaneously and at the same position in the Kjeller heavy water pile. The reaction yield in a solution without B or Li is due to γ-radiation (+ fast neutrons), whereas the yield increment in the solutions containing B or Li is due to the recoil nuclei produced in reactions with thermal neutrons. The yields of Fe^{3+} were determined spectro-photometrically at 520 mμ after the addition to the irradiated samples of 0.7 ml of 3 *N* KSCN. The Fe^{3+} concentrations corresponding to the extinctions were obtained from a colorimetric determination of $FeSO_4-Fe_2(SO_4)_3$ mixtures in the different milieus. The fact that Li_2SO_4 (concentrations up to 0.22 moles/l were used) lowered the extinction was allowed for. Admixtures of H_3BO_3 (concs. up to 0.08 moles/l) caused no change of the colour intensity.

The G values (number of ions oxidized per 100 eV absorbed) for X-rays were controlled for all solutions (roentgen apparatus run at 175 kV, filtration 0.5 mm Cu + 1 mm Al, dose rate, measured with a Victoreen type chamber, 50 r/min). The absorption of X-ray energy per ml was determined regarding absorption coefficients and densities [3]. Influence of eventual pH changes on the reaction yield was negligible. For X-rays a G value of 18.7 ions oxidized per 100 eV absorbed was found. When the pH dependence [6] of the reaction is considered, this is in close agreement with earlier values [4,5] of about 21 obtained in 0.8 *N* H_2SO_4. Simultaneously with the irradiation of the solutions the neutron flux was determined by activating sodium carbonate, followed by an absolute measurement of Na^{24} in a NaI(Tl) scintillating crystal [7]. In more accurate work a correction has to be made for the neutron absorption through H, B or Li in the solutions. Detailed experimental data will be published in a further communication.

From the neutron densities thus determined and from the known values of neutron cross sections of the nuclear reactions and reaction energies (see Table 1), the oxidizing efficiencies of the particle radiations relative to that of X-rays could be determined, *i.e.* the G values for these radiations (Table 1).

Conclusions: 1. The densely ionizing radiations give a lower reaction yield than do X- and γ-radiations. This agrees with the scant information from experiments with α-rays from Po and Rn [8,9]. The relatively high yield (Table 1) of the products from $Li^6(n\alpha)T$ is probably due to the less densely ionizing triton, the relative efficiency of which is calculated to be not far from 1, the efficiency of the α-particle being regarded equal to that of the recoil nuclei produced through $B^{10}(n\alpha)Li^7$. This finding

Table 1.

Nuclear reaction	Reaction		Ionizing particle			Oxidizing efficiency rel. to X-rays
	energy (MeV)	cross section (cm^{-24})	type	energy (MeV)	spec. ionization * ion pairs/μ H_2O	
$B^{10}(n\alpha)Li^7$	2.34 (+0.44 γ)	3 990	α Li^7	1.49 0.85	5 500 5 500 **	0.24 { 0.24 0.24
$Li^6(n\alpha)T$	4.78	910	α T	2.05 2.73	5 500 1 150	0.59 { 0.24 0.83
			e^- from * X-rays	~0.015	100	1.00

* Calcd. from range data given by Lea [10] and Siri [11] and from the assumption of an energy dissipation of 32.5 eV per ionization.

** Calcd. from the range of the Li ion (*i.e.* $Li^{3+} \rightarrow Li^{2+} \rightarrow Li^{+}$) given by Rossi and Staub (Ionization chambers and counters. New York, 1949, p. 187).

confirms the reliability of the fast neutron dosimetry [3] based on the oxidation of Fe^{2+}, the mean ionization density produced here being about 700.

2. The oxidation yield produced by the recoil nuclei is independent of the Fe^{2+} concentration within the region 0.3—3.0 mmoles/l.

3. The oxidation yields are independent of the thermal neutron intensity within the region examined, $0.27-4.54 \cdot 10^{11}$ n cm^{-2} sec^{-1}, and of the simultaneous γ intensities between 800 and 9 000 rep min^{-1}. After a standardization of the yield increment due to B or Li, the system described can thus be used for a determination of thermal neutron densities, irrespective of the contaminating γ-radiation.

4. A determination of the internal γ-radiation (and the form factor for absorption) from nγ captures in the irradiated object might be determined by a similar method after shielding off the external γ-radiation.

The experiments are continued with ionizing particles giving other ionization densities, *e.g.* protons from $N(np)C^{14}$ and fission fragments, and also with other chemical reactions, *e.g.* $Ce^{4+} \rightarrow Ce^{3+}$.

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Stepwise Degradation of Peptides

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In the stepwise degradation of peptides according to Ottesen's modification of Edman's method (Edman [1], Fraenkel-Conrat [2], Ottesen and Wollenberger [3], Christensen [4], the phenylthiocarbamido derivative formed in the reaction of a peptide with phenylisothiocyanate, is brought to react in aqueous solution to form thiohydantoin and a peptide with one amino acid less than the original peptide. In spite of the mild conditions maintained during the ring closure, 0.1 *N* HCl, 65° C during 2—24 hours, undesired side reactions sometimes occur *e.g.* hydrolysis of peptide bonds along the peptide chain. We have therefore considered it of interest to investigate the possibility of finding milder conditions for the ring closure. Among the preliminary results of these investigations we may mention that certain thiohydantoins form readily at 60—70° C in 0.05 *M* aqueous citrate buffer at pH 4.5—5 and may be extracted with an organic solvent. In order to reduce the possibility of a back-reaction between thiohydantoin and peptide and of a decomposition of the former, ring closure was effected at the given conditions but with simultaneous extraction with the organic solvent in a modified Steudel-Kutcher apparatus.

For lower peptides the following procedure has proven satisfactory. One ml of a 0.02 *M* solution of the peptides is reacted with phenylisothiocyanate at pH 8 according to Ottesen and Wollenberger. After removal of dioxane and excess of reagent by extraction with cyclohexane, 1 ml citrat buffer of the desired pH is added and continuous extraction with benzene at 70° is performed. After 10—24 hours extraction practically all thiohydantoin is found in the benzene. The quantity obtained is determined by U.V. absorption measurements after evaporation of the benzene and dissolution in ether. Since the uncharged phenylisothiocarbamido derivatives are slightly soluble in benzene pH should not be too low. These compounds have a maximum of absorption at $\lambda = 245$ mμ where the hydantoins have a minimum. A comparison of the extinction at the maxi-

Table 1.

Peptide	Time of extraction 70° (hours)	pH	Yield * %
Alanylglycine	5	5	98 alanine
Glycylaspargine	24	4.4	83 glycine, 80 aspargine **
Glycylglycine	18	5	94 glycine
Alanylglycylglycine	18, 17, 20	4.5	98, ala, 92, gly_1, 93 gly_2

* All hydantoins isolated were hydrolyzed in barium hydroxide and the amino acids involved investigated by filter paper chromatography. No other spots than the expected ones appeared.

** Determined by the ninhydrin method in the aqueous solution after extraction of the glycine hydantoin.

mum and minimum, 268 mμ and 245 mμ respectively may therefore give information about the purity of the extracted material. Above pH 4.3 only hydantoins are found in the benzene. Isopropylether (proposed by A. L. Levy) is probably a better solvent for thiohydantoins but it is not as selective as benzene and extracts appreciable amounts of the phenylisothiocarbamido derivatives of shorter peptides.

The role of the citrate buffer is as yet unclear, but definitely less satisfactory results were obtained in extraction experiments where no buffer was used and pH was continuously adjusted by addition of acid. Oxalate buffer may replace citrate but its buffer capacity is inferior to that of citrate in the important range pH 4—5.

The presence of buffer will cause the salt concentration of the reaction medium to rise from step to step in the degradation of peptides and will make the evaluation of the titration curves[3] difficult. Hence the yield in the reaction of phenylisothiocyanate can only be judged from the yield of thiohydantoin.

The table shows some results with simple peptides.

As regards the application of this method to proteins we may just point out that the phenylisothiocarbamido derivatives of these substances are insoluble in organic solvents and that lower pH-values and other extraction media (*e.g.* isopropylether) may be adopted. The stability of the protein chain naturally sets the ultimate limit for such modifications.

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The Reaction between Carbon Dioxide and Methoxy Substituted Phenylmagnesium and Tolylmagnesium Bromides

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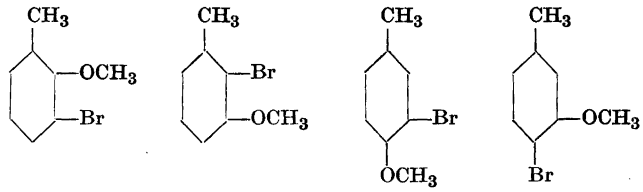
It has recently been shown [1] that besides *o*-methoxy-benzoic acid considerable amounts of 2,2′-dimethoxybenzophenone are formed when gaseous carbon dioxide is allowed to react with *o*-methoxyphenylmagnesium bromide. This ketone formation is not limited to the Grignard reagent mentioned, since Bodroux [2] isolated 4,4′-dibromobenzophenone and *p*-bromo-benzoic acid from the reaction product of *p*-bromophenylmagnesium bromide and carbon dioxide.

As it was of interest to determine whether ketone formation is a common reaction of methoxyarylmagnesium bromides, the reactions between carbon dioxide on one hand and methoxyphenylmagnesium bromides and methoxytolylmagnesium bromides on the other were investigated. Some of these reactions have been studied earlier. Bodroux [3] prepared *p*-anisic acid from *p*-methoxyphenylmagnesium bromide and carbon dioxide. Paty and Quelet [4] obtained *o*-methoxybenzoic acid in the corresponding reaction of *o*-methoxyphenylmagnesium bromide. Darzens and Levy [5] isolated 2-methyl-4-methoxybenzoic acid, Higginbottom, Hill, and Short [6] 2-methyl-5-methoxybenzoic acid, and Dubinin [7] 3-methyl-6-methoxybenzoic acid from the reaction products of the corresponding Grignard reagents and carbon dioxide. However, since in none of these investigations attention has been paid to the formation of ketones, it was considered necessary to repeat the experiments of Bodroux, Darzens and Levy, Higginbottom, Hill, and Short.

The Grignard reagents studied in the present investigation were, thus, prepared from the following bromides:

3-bromo-1-methoxybenzene
4-bromo-1-methoxybenzene
3-bromo-2-methoxytoluene
4-bromo-2-methoxytoluene
5-bromo-2-methoxytoluene
6-bromo-2-methoxytoluene
2-bromo-3-methoxytoluene
4-bromo-3-methoxytoluene
5-bromo-3-methoxytoluene
6-bromo-3-methoxytoluene
2-bromo-4-methoxytoluene
3-bromo-4-methoxytoluene

During the course of the work, it was found that Grignard reagents in which the methoxy group and the magnesium atom were in meta and para positions relative to each other give the corresponding acids exclusively. Two of the four compounds in which the methoxy group and the magnesium atom were in ortho position relative to each other, *viz.* those prepared from 3-bromo-2-methoxytoluene (I) and 2-bromo-3-methoxytoluene (II), reacted to give only the acids, whereas the Grignard reagents prepared from 3-bromo-4-methoxytoluene (III) and 4-bromo-3-methoxytoluene (IV) gave both ketone and acid.



I II III IV

If the ketone formation is to be considered a type of ortho effect, this effect is cancelled by a methyl group in the ortho position relative to either the methoxy group or the magnesium atom, *i.e.* the first ortho effect is cancelled by a second ortho effect.

In order to show that the ketone formation is a consequence of an abnormal reactivity of the *o*-methoxy substituted Grignard reagent, *o*-methoxyphenylmagnesium bromide was allowed to react with the reaction product of phenylmagnesium bromide and carbon dioxide. As 2-methoxybenzophenone has a low melting point (39°) and as it is readily soluble in most organic solvents, the ketone itself could not be isolated, but when the reaction product was treated with 2,4-dinitrophenylhydrazine, the corresponding hydrazone was formed. Phenylmagnesium bromide does not form a ketone under these conditions.

In connection with the preparation of the bromides some halogen substituted nitro compounds had to be reduced to the corresponding amines. When stannous chloride is used, a hydrogen atom may be substituted by chlorine or the halogen may be eliminated [8]. Weygand recommended the use of the method of West [9] in such cases. According to this method the nitro compound is dissolved in methyl alcohol, a small quantity hydrochloric acid is added after which iron powder is added in portions to the vigorously boiling mixture. It was, however, observed that the iron powder formed lumps during the addition and this could not be avoided by boiling nor by effective stirring. A consequence of this was that the reaction did not go to completion. The difficulty was eliminated by mixing the methyl alcohol solution of the nitro compound with iron powder and adding hydrochloric acid to the boiling mixture. In this way excellent yields were obtained.

EXPERIMENTAL

3-Bromo-1-methoxybenzene was prepared by bromination of nitrobenzene to 3-bromo-1-nitrobenzene [10], followed by reduction to 3-bromoaniline [10], diazotation to 3-bromophenol [10,11], and methylation with dimethyl sulphate [11].

4-Bromo-1-methoxybenzene was prepared by bromination of anisole [12].

3-Bromo-2-methoxytoluene. 3-Bromo-2-hydroxytoluene was prepared from *o*-cresol according to Brubaker and Adams [13]. The bromocresol (34.0 g) was dissolved in a solution of potassium hydroxide (14.0 g) in water (140 ml) and after addition of dimethyl sulphate (30.0 g) the mixture was shaken for one hour. 3-Bromo-2-methoxytoluene was distilled with steam and isolated by extraction with ether. The ether solution was shaken with dilute potassium hydroxide solution in order to remove any unchanged bromocresol. After drying of the ether phase, evaporation of the solvent left 3-bromo-2-methoxytoluene, which was distilled (b.p. 107—108°/22 mm).

C_8H_9OBr	Calc.	Br	39.74
	Found	»	39.87

The yield of 3-bromo-2-methoxytoluene was 60 per cent of the theoretical amount.

4-Bromo-2-methoxytoluene. *p*-Toluidine was nitrated using a large ratio of sulphuric acid to 2-nitro-4-aminotoluene [14]. This was converted into 4-bromo-2-nitrotoluene according to Gibson and Johnson [15]. When conducting the latter reaction, it was found advantageous to use a higher ratio of hydrobromic acid (3.5 equivalents) and to dissolve the amine in the acid before adding the sodium nitrite solution.

4-Bromo-2-nitrotoluene (34.0 g) was dissolved in methyl alcohol (110 ml) and iron powder (31.6 g) was added to the solution. The mixture was heated to boiling and concentrated hydrochloric acid (6 ml) was gradually added. After boiling for four hours, more concentrated hydrochloric acid (10 ml) was added and the mixture was filtered while still hot. The methyl alcohol was then distilled from the filtrate. After the residue had been made alkaline, a mixture of 4-bromo-2-aminotoluene and a small amount of unchanged nitro compound was isolated by steam distillation. The amine was converted into its hydrochloride and ether was added to dissolve the nitro compound. The solid hydrochloride was filtered, and the two layers of the filtrate were separated. The aqueous phase was made alkaline and the filtered hydrochloride was added to it. The amine was

dissolved in ether and this ether solution was dried with solid potassium hydroxide. After the ether had been evaporated, 4-bromo-2-aminotoluene, b.p. 138—140/20 mm, distilled. The yield (26.0 g) was 88.5 per cent of the theoretical amount.

A small quantity of the amine was acetylated with acetic anhydride. The acetyl derivative melted at 163—164°.

4-Bromo-2-aminotoluene (25.0 g) was dissolved in a mixture of concentrated hydrochloric acid (45 ml) and water (150 ml). After cooling to 5°, this solution was diazotized with an aqueous solution (30 ml) of sodium nitrite (9.5 g). The filtered solution was then gradually added to a boiling mixture of sulphuric acid (200 ml) and water (400 ml). During this addition, nitrogen was vigorously evolved and an oil distilled with water to the receiver. When the addition was completed, the reaction mixture was allowed to cool and water (600 ml) was added. Both the distillate and the contents of the reaction flask were extracted with ether. After the combined ether solutions had been dried with anhydrous sodium sulphate, the solvent was evaporated. The residue was dissolved in an aqueous solution (65 ml) of sodium hydroxide (6.4 g), and dimethyl sulphate (20 g) was added. After the mixture had been shaken for one hour, 4-bromo-2-methoxytoluene was isolated by steam distillation as in the case of 3-bromo-2-methoxytoluene. It distilled at 102—104°/13 mm.

C_8H_8OBr	Calc.	Br	39.74
	Found	»	39.95

The yield (16.3 g) was 60.4 per cent of the theoretical amount calculated on the quantity of 4-bromo-2-aminotoluene used.

5-Bromo-2-methoxytoluene. *o*-Cresol was methylated according to the method given for the synthesis of anisole in Organic Syntheses [16], the yield being 82 per cent of the theoretical amount. The methoxytoluene was then brominated in chloroform with iron filings as catalyst [17]. The yield was 82.5 per cent of the theoretical amount.

6-Bromo-2-methoxytoluene. Technical trinitrotoluene (TNT) was reduced with hydrogen sulphide in dioxane to 4-amino-2,6-dinitrotoluene according to Parkes and Farthing [18]. The amine was isolated from the reaction mixture by precipitation with dry hydrogen chloride. Owing to the poor quality of the starting material the yield was only 45—50 per cent of the theoretical amount. The amine was deaminated by diazotation followed by reduction with ethyl alcohol according to Qvist [19], which method gave a yield of 32—37 per cent of the theoretical amount. The 2,6-dinitrotoluene obtained in this way was converted into 2-nitro-6-aminotoluene by partial reduction with ammonium sulphide [20]. The synthesis was continued over 2-nitro-6-bromotoluene, 6-bromo-2-aminotoluene, and 6-bromo-2-hydroxytoluene to 6-bromo-2-methoxytoluene according to methods which have been previously described for the corresponding substances under the sub-heading "4-Bromo-2-methoxytoluene". The yields were the following: 2-nitro-6-bromotoluene 72 per cent, 6-bromo-2-aminotoluene 85 per cent, and 6-bromo-2-methoxytoluene 58 per cent of the theoretical amounts. The last mentioned substance, which has not been prepared previously, distilled at 115—116°/20 mm.

C_8H_9OBr	Calc.	Br	39.74
	Found	»	39.69

6-Bromo-2-acetaminotoluene, prepared for identification purposes from 6-bromo-2-aminotoluene, melted at 164—165°.

2-Bromo-3-methoxytoluene. 2-Bromo-3-methoxytoluene was prepared mainly according to Hodgson and Beard [21]. As these authors did not describe the experimental details, the procedure followed is given here.

m-Cresol (63 g) was dissolved in fuming sulphuric acid (250 ml; 18 per cent sulphur trioxide) and the solution was heated to 50° for half an hour. After cooling to 0—5°, the *m*-cresoldisulphonic acid formed was nitrated with a mixture of fuming sulphuric acid (65 ml; 18 per cent sulphur trioxide) and nitric acid (28 ml; *d* 1.52). Next morning the reaction mixture was diluted with water (300 ml). On steam distillation *m*-cresol passed over, but when the temperature of the steam was gradually raised to 180°, 2-nitro-3-hydroxytoluene (51 g; 58 per cent of the theoretical amount) distilled. After filtration the latter compound was dissolved in an aqueous solution (190 ml) of potassium hydroxide (19 g), and dimethyl sulphate (43 g) was added. After the mixture had been shaken for one hour and heated for two hours on the boiling water bath, 2-nitro-3-methoxytoluene (29 g; 52.5 per cent of the theoretical amount) was distilled with steam.

2-Nitro-3-methoxytoluene (35 g) was dissolved in ethyl alcohol (200 ml) and the solution was slowly added to a hot solution of stannous chloride (185 g) in concentrated hydrochloric acid (190 ml) and ethyl alcohol (50 ml). When the reduction was completed, the alcohol was distilled off. After cooling, a complex stannic compound (61 g) separated and was filtered. 2-Amino-3-methoxytoluene was liberated by alkali and removed by steam distillation, after which it was distilled under reduced pressure. The yield (23 g) was 80 per cent of the theoretical amount.

2-Amino-3-methoxytoluene (23 g) was dissolved in 48 per cent hydrobromic acid (50 ml) and diazotized with a solution of sodium nitrite (12 g) in water (20 ml) at a temperature just below 10°. When the addition was completed, the solution was immediately poured through a dropping funnel into a boiling mixture of cuprous bromide (14 g) and 48 per cent hydrobromic acid (15 ml) in a flask provided with steam inlet and a condenser for downward distillation. The 2-bromo-3-methoxytoluene formed was then distilled with steam and the organic layer in the distillate was dissolved in ether. After the ether solution had been freed from phenolic substances by extraction with alkali, it was dried with anhydrous sodium sulphate. After the ether had evaporated, 2-bromo-3-methoxytoluene (b.p. 142—143°/38 mm) distilled. The yield (17 g) was 50.5 per cent of the theoretical amount.

4-Bromo-3-methoxytoluene. *p*-Acetotoluide was converted into 3-nitro-4-aminotoluene according to Gattermann [22] and the synthesis was continued over 3-nitro-4-bromotoluene, 4-bromo-3-aminotoluene, and 4-bromo-3-hydroxytoluene to 4-bromo-3-methoxytoluene according to methods described for the corresponding substances under the sub-heading "4-Bromo-2-methoxytoluene". The yields were the following: 3-nitro-4-bromotoluene 82 per cent, 4-bromo-3-aminotoluene 82 per cent, and 4-bromo-3-methoxytoluene (b.p. 131—132°/30 mm) 68 per cent of the theoretical amounts.

4-Bromo-3-acetaminotoluene was prepared for identification purposes by acetylation of 4-bromo-aminotoluene with acetic anhydride. After crystallization from 40 per cent ethyl alcohol, its melting point was 121—122°.

$C_9H_{10}ONBr$	Calc.	N 6.14
	Found	» 6.24

The melting point of the compound has been reported to be 113.7—114.6° [23] and 164° [24].

5-Bromo-3-methoxytoluene. *p*-Acetotoluide was nitrated and the nitro compound hydrolyzed to 3-nitro-4-aminotoluene according to Gattermann [22]. By bromination the last-mentioned substance was converted into 3-nitro-5-bromo-4-aminotoluene [25], which was deaminated as follows:

Table 1.

Nr	Bromide, used	Carboxylic acid		Ketone	
		g	m.p.	g	m.p.
1	3-Bromo-1-methoxybenzene	1.30	106—107 [a]	—	
2	4-Bromo-1-methoxybenzene	0.76	183—184 [a]	—	
3	3-Bromo-2-methoxytoluene	0.94	82—83.5 [a]	—	
4	4-Bromo-2-methoxytoluene	0.54	144—145 [a]	—	
5	5-Bromo-2-methoxytoluene	1.30	193—194 [a]	—	
6	6-Bromo-2-methoxytoluene	1.77	150—151 [a]	—	
7	2-Bromo-3-methoxytoluene	1.84	137—138 [a]	—	
8	4-Bromo-3-methoxytoluene	0.56	104—105 [b]	1.05	116—117 [e]
9	5-Bromo-3-methoxytoluene	0.93	131—132 [c]	—	
10	6-Bromo-3-methoxytoluene	1.25	176—177 [a]	—	
11	2-Bromo-4-methoxytoluene	0.98	144—145.5 [a]	—	
12	3-Bromo-4-methoxytoluene	1.02	68—69 [d]	1.41	82—83 [e]

[a] After crystallization from water.
[b] After crystallization from a small quantity of ether.
[c] After crystallization from a mixture of acetic acid and water.
[d] After crystallization from a mixture of benzene and ligroin.
[e] After crystallization from ligroin.

3-Nitro-5-bromo-4-aminotoluene (46.2 g) was dissolved in a mixture of ethyl alcohol (500 ml) and concentrated sulphuric acid (80 ml). This solution was diazotized with an aqueous solution (90 ml) of sodium nitrite (15 g), the temperature being kept just below 0°. When the nitrite solution had been added, the reaction mixture was carefully warmed until the evolution of nitrogen ceased. The 3-nitro-5-bromotoluene was removed from the dark-coloured solution by steam distillation. After crystallization from alcohol, the substance melted at 82°. The yield (33.4 g) was 77 per cent of the theoretical amount.

The synthesis was continued over 5-bromo-3-aminotoluene and 5-bromo-3-hydroxytoluene to 5-bromo-3-methoxytoluene according to methods described above for the corresponding substances under the sub-heading "4-Bromo-2-methoxytoluene". The yields were the following: 5-bromo-3-aminotoluene 92 per cent and 5-bromo-3-methoxytoluene (b.p. 120—120.5°/22 mm) 55.4 per cent of the theoretical amounts.

5-Bromo-3-acetaminotoluene was prepared for identification purposes by acetylation of 5-bromo-3-aminotoluene with acetic anhydride. After crystallization it melted at 169°.

6-Bromo-3-methoxytoluene. *m*-Methoxytoluene (70 g), prepared from *m*-cresol and dimethyl sulphate according to the method given for anisole in Organic Syntheses [16], was reacted with bromine (92 g) in chloroform (70 ml), iron filings (1 g) being used as catalyst. During the addition of bromine, the temperature was kept at about 30°. When all the bromine had been added, the temperature was raised to 60° for two hours. After the solution had been shaken with an aqueous solution of sodium carbonate and dried with calcium chloride, the solvent was evaporated and the residue distilled under reduced

Table 2.

Nr	Benzoic acid	*p*-Nitrobenzyl ester		
		m.p.	N	
			Calc.	Found
—	2-Methoxy-	86—87	4.88	4.98
1	3-Methoxy-	79—80	4.88	4.95
2	4-Methoxy-	131—132		*
6	3-Methoxy-2-methyl-	96—97	4.65	4.73
10	4-Methoxy-2-methyl-	126.5—127.5	4.65	4.75
11	5-Methoxy-2-methyl-	110—111	4.65	4.74
7	6-Methoxy-2-methyl-	80—81	4.65	4.66
3	2-Methoxy-3-methyl-	75—76.5	4.65	4.71
5	4-Methoxy-3-methyl-	114—115	4.65	4.81
9	5-Methoxy-3-methyl-	113.5—114	4.65	4.65
12	6-Methoxy-3-methyl-	119—120	4.65	4.70
8	2-Methoxy-4-methyl-	112—113	4.65	4.74
4	3-Methoxy-4-methyl-	109—110	4.65	4.78

* Previously known.

pressure. The yield of 6-bromo-3-methoxytoluene (50 g), b.p. 115—116°/14 mm, was 43.5 per cent of the theoretical amount.

2-Bromo-4-methoxytoluene. *p*-Toluidine was converted over 2-nitro-4-aminotoluene and 2-nitro-4-hydroxytoluene into 2-nitro-4-methoxytoluene according to Ullmann and Dootson [26]. This nitromethoxy compound was reduced to 2-amino-4-methoxytoluene as follows:

2-Nitro-4-methoxytoluene (100 g) dissolved in ethyl alcohol (315 ml) was gradually added to a warm mixture of stannous chloride (405 g), concentrated hydrochloric acid (510 ml) and ethyl alcohol (175 ml). After this addition the mixture was heated for a quarter of an hour on the boiling water bath, after which it was made alkaline. On steam distillation, 2-amino-4-methoxytoluene, m.p. 44—45.5°, passed over and was collected by filtration. The yield (40.3 g) was 50.4 per cent of the theoretical amount.

2-Amino-4-methoxytoluene (40.3 g), dissolved in 48 per cent hydrobromic acid (110 ml), was diazotized with an aqueous solution (35 ml) of sodium nitrite (20.4 g), the temperature being kept between 0 and 5°. When the addition of the nitrite was completed, the solution was immediately poured through a dropping funnel into a boiling mixture of cuprous bromide (29 g) and 48 per cent hydrobromic acid (30 ml) contained in a flask provided with a steam inlet and a condenser for downward distillation. The 2-bromo-4-methoxytoluene formed was then distilled with steam and the organic layer in the distillate was dissolved in ether. After the solution had been freed from phenolic matter by extraction with alkali, it was dried with anhydrous sodium sulphate. After evaporation of the solvent, 2-bromo-4-methoxytoluene (b.p. 134—137°/38 mm) distilled. The yield (17.4 g) was 29.3 per cent of the theoretical amount.

3-Bromo-4-methoxytoluene. *p*-Cresol was methylated according to the method given for anisole in Organic Syntheses [16], the yield being 84 per cent of the theoretical amount. This methoxytoluene was then brominated in chloroform with iron filings as catalyst [17]. The yield was 54.5 per cent of the theoretical amount.

The reactions between Grignard reagents and carbon dioxide. The Grignard reagent was prepared from each bromide (5 g), an equivalent quantity of magnesium (0.70 g for bromomethoxybenzenes, 0.60 g for bromomethoxytoluenes), and dry ether (25 ml) by heating on the water bath for two hours. Carbon dioxide was then conducted into the solution for one hour. The reaction mixture was poured into dilute hydrochloric acid. After adding more ether, the mixture was shaken and the layers were allowed to separate. The organic phase was extracted successively with aqueous solutions of 1) sodium bicarbonate, 2) sodium carbonate, and 3) sodium hydroxide. The ether was evaporated from the organic phase and unchanged bromide and anisole or methoxytoluene were distilled off with steam. If ketone had formed during the reaction, it was isolated from the residue.

The sodium bicarbonate solution was acidified and the carboxylic acid was collected by filtration.

A very small amount of the carboxylic acid was isolated on acidifying the sodium carbonate solution.

In each case the sodium hydroxide solution contained only a very small amount of oily material which was not examined further.

The results are collected in Table 1.

One of the ketones, *viz.* 2,2′-dimethoxy-5,5′-dimethylbenzophenone, has previously been synthesized [27]. In order to characterize it, its 2,4-dinitrophenylhydrazone was prepared according to Wild [28]. The melting point of the latter substance was 164—165°.

$C_{23}H_{22}O_6N_4$	Calc.	N 12.44
	Found	» 12.45

2,2'-Dimethoxy-4,4'-dimethylbenzophenone, which was obtained by the reaction of the Grignard reagent of 4-bromo-3-methoxytoluene with carbon dioxide, melted at 117°.

$C_{17}H_{18}O_3$	Calc.	C	75.53	H	6.71
	Found	»	75.60	»	6.57

Its 2,4-dinitrophenylhydrazone melted at 194—195°.

$C_{23}H_{22}O_6N_4$	Calc.	N 12.44
	Found	» 12.32

All the carboxylic acids obtained in the present work have been described previously. Their *p*-nitrobenzyl esters were prepared for identification purposes [29]. The results are collected in Table 2.

The reaction between o-*methoxy phenylmagnesium bromide and the reaction product of phenylmagnesium bromide and carbon dioxide.* Carbon dioxide was conducted into a Grignard solution prepared from bromobenzene (4.00 g), magnesium (0.60 g), and dry ether (25 ml). When the reaction was completed, the mixture was gently warmed on the water bath in order to remove the unreacted gas. In the meantime, a Grignard reagent was prepared from *o*-bromoanisole (5 g), magnesium (0.60 g), and dry ether (25 ml). This solution was now rapidly added to the first Grignard solution. The oily layer formed by the reaction product between carbon dioxide and phenylmagnesium bromide grew thicker during the addition and its colour changed from black to brown. After the mixture had been stirred for a quarter of an hour, it was poured into a mixture of dilute hydrochloric acid and ether. When the solutions had cleared, they were separated and the

organic layer was freed from acid and phenolic matter by extraction with a dilute sodium hydroxide solution. The solvent was removed and volatile substances were distilled off by steam. The residue was a light-coloured oil (2.46 g). From this oil the 2,4-dinitrophenylhydrazone of *o*-methoxybenzophenone was isolated.

$C_{20}H_{16}O_5N_4$	Calc.	N	14.28
	Found	»	14.18

The compound melts at 146—147°.

SUMMARY

1. Methoxyphenylmagnesium bromides and methoxytolylmagnesium bromides in which the methoxy group and the magnesium atom are in meta or para position relative to each other react with carbon dioxide to give the corresponding carboxylic acids only.

2. A methoxy group in ortho position relative to the magnesium atom in a Grignard reagent prepared from bromo derivatives of benzene and toluene leads to the formation of ketone in addition to the acid. Ketone formation does not occur when a methyl group is in the ortho position relative to either the methoxy group or the magnesium atom.

3. *o*-Methoxybenzophenone is formed in the reaction between *o*-methoxyphenylmagnesium bromide and the reaction product of carbon dioxide and phenylmagnesium bromide.

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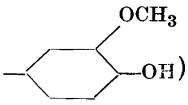
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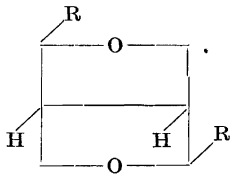
ACTA CHEMICA SCANDINAVICA 6 (1952) 1147–1151

Some New Derivatives of Pinoresinol *

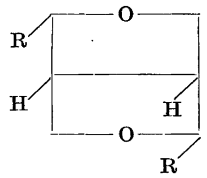
JARL GRIPENBERG and BJARNE LINDAHL

Department of Chemistry, Institute of Technology, Helsingfors, Finland

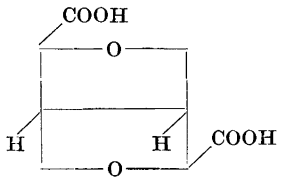
In previous papers of this series [1,2] it has been shown that pinoresinol has one of the two symmetrical configurations, I or II (R = ), but no decision concerning the correct structure has as yet been possible. If the aromatic rings could be converted into carboxylic groups (giving either III or IV) such a decision could possibly be made. Namely models of the two molecules III and IV show that in III the two carboxylic groups are in a position very close to another, so that cyclic anhydride formation should presumably be easy, whereas from IV no cyclic anhydride could be obtained.



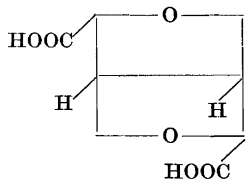
I



II



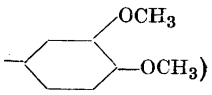
III



IV

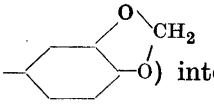
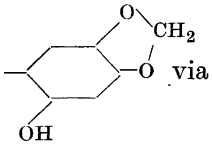
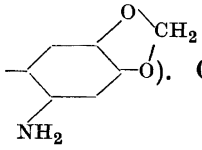
* Also XV contribution on the *Constitution of resin phenols and their biogenetic relations.* Part XIV *Acta Chem. Scand.* **4** (1950) 391.

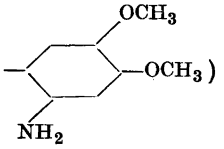
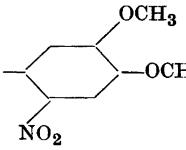
Although the preparation of the desired dicarboxylic acid could not be achieved, this investigation led to the preparation of some previously undescribed derivatives of pinoresinol, whose preparation together with a short account of the various methods tried for the oxidation of the aromatic rings in pinoresinol are described in the present paper.

Erdtman[3] oxidised pinoresinoldimethylether (I or II; R = ) with potassium permanganate and obtained veratric acid. It was however felt that if there were one free hydroxyl group, as in pinoresinol, the aromatic ring should be more susceptible to oxidation. In fact no vanillic acid was obtained on oxidation of pinoresinol in alkaline solution at room temperature, the only identifiable product being a small amount of oxalic acid.

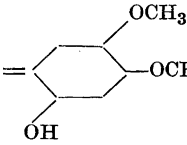
King and Grundon[4] have used hydrogen peroxide for the oxidation of the phenolic ring of tetrahydrochlorophorin to a carboxyl group. When this method was applied to pinoresinol, oxalic acid was again the only obtainable product. Oxidation with chromic acid in acetic solution gave no identifiable products.

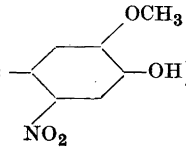
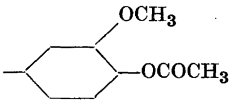
Thus as all attempts for the oxidation of the aromatic ring in either pinoresinol or its dimethylether had met with failure, the introduction of one more active group (either NH_2 or OH) in the aromatic ring was tried, in order to make this ring even more susceptible to oxidation and thus the oxidation possible under milder conditions.

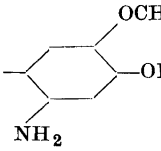
Dieterle and Schwengler[5] have described the conversion of the closely related asarinin (xanthoxylin-S) (I or II; R = ) into a dihydroxyasarinin (I or II; R = ) via diaminoasarinin (I or II; R = ). Oxidation of dihydroxyasarinin with hydrogenperoxide gave an acid, which according to Dieterle and Schwengler possibly could be the desired acid III or IV. The analytical data given by them are, however, in such a poor agreement with values calculated for $C_8H_{10}O_6$ that this claim appears highly questionable.

When this same sequence of reactions was applied to pinoresinoldimethylether, the diaminopinoresinoldimethylether (I or II; R = ) could relatively easily be obtained by catalytic hydrogenation of dinitropinoresinoldimethylether (I or II; R = ), but all attempts to obtain anything like a pure compound from the diazotisation of diaminopinoresinoldimethylether were unsuccessful.

Diaminopinoresinoldimethylether shows a remarkably high positive rotation ($[\alpha]_D^{20}$ + 255°) as compared to the rotations of dinitropinoresinoldimethylether ($[\alpha]_D^{20}$ −125°) and pinoresinoldimethylether ($[\alpha]_D^{20}$ + 64.5°).

Since the preparation of dihydroxypinoresinoldimethylether (I or II; R = ) was unsuccessful, attempts were made to oxidise diaminopinoresinoldimethylether with chromic acid to a diquinone, which on further oxidation should give the desired dicarboxylic acid, but no identifiable product could be obtained.

In order to get an even more reactive aromatic ring it was attempted to carry out the sequence of reactions mentioned above with pinoresinol. The dinitropinoresinol (I or II; R = ) has not hitherto been described. Attempted nitration of pinoresinoldiacetate (I or II; R = ) gave only unreacted starting material. Nitration of pinoresinol proved to be very dependent on the reaction conditions, but even in the best cases the yield of dinitropinoresinol was extremely poor (5—6%). In order to prove the structure of this compound its conversion into dinitropinoresinoldimethylether was attempted. However, methylation with dimethylsulphate in alkaline solution or with potassium carbonate and acetone, as well as methylation with diazomethane gave only unchanged starting material. The structure given above is thus based only on analogy with dinitropinoresinoldimethylether. The

dinitropinoresinol could be hydrogenated to diaminopinoresinol (I or II; R = ) but owing to the low yield in these reactions this approach was abandoned. Dinitropinoresinol and diaminopinoresinol do not show the very great difference in optical rotations observed with the corresponding methyl ethers.

EXPERIMENTAL

Diaminopinoresinoldimethylether. Dinitropinoresinoldimethylether (4.0 g) was dissolved in ethyl acetate, the solution warmed to about 70° and hydrogenated with a $Pt(O_2)$-catalyst at 2 atm. pressure. After 3 hours the consumption of hydrogen amounted to 540 ml (calc. 564 ml). The catalyst was filtered off from the still hot solution and the liquid concentrated. Upon cooling the diaminopinoresinoldimethylether crystallised out as colourless crystals. Yield 2.4 g (69 %). After recrystallisation from ethyl acetate it had m.p. 190—191°, $[\alpha]_D^{20}$ + 255° (c, 0.99 in chloroform). ($C_{22}H_{28}O_6N_2$ requires C, 63.4; H, 6.8; N, 6.7; found C, 63.2; H, 6.3; N, 6.4 %).

The diacetate was obtained when diaminopinoresinoldimethylether, dissolved in pyridine, was treated with acetic anhydride. It crystallised directly from the reaction mixture as microscopical needles and was purified by recrystallisation from alcohol. M.p. 289°, $[\alpha]_D^{20}$ + 117° (c, 1.02 in chloroform). ($C_{26}H_{32}O_8N_2$ requires C, 62.4; H, 6.5; N, 5.6; found C, 62.3; H, 6.4; N, 5.8 %).

The dibenzoate was obtained when diaminopinoresinoldimethylether, suspended in sodium hydroxide, was treated with benzoyl chloride. It was recrystallised from alcohol. M.p. 259°, $[\alpha]_D^{20}$ + 277° (c, 1.04 in chloroform). ($C_{36}H_{36}O_8N_2$ requires C, 69.2; H, 5.8; N, 4.5; found C, 68.7; H, 5.7; N, 5.0 %).

Dinitropinoresinol. Pinoresinol (5 g) was dissolved in propionic acid (40 ml). The solution was treated at −5° with a mixture of nitric acid (*d* 1.41, 2.6 ml) and propionic acid (10 ml). Water (400 ml) was then added and the amorphous precipitate obtained was extracted several times with chloroform. The chloroform solution was chromatographed on a column of alumina. Three different zones were obtained: a lower red, a middle light yellow and an upper brown. The lower zone gave, on further elution with chloroform and evaporation of the chloroform, dinitropinoresinol (0.34 g). Recrystallised from alcohol it formed golden yellow leaflets m.p. 184°, $[\alpha]_D^{20}$ + 20° (c, 1.01 in chloroform). ($C_{20}H_{20}O_{10}N_2$ requires C, 53.8; H, 4.5; N, 6.2; found: C, 54.0; H, 4.3; N, 6.3 %).

Diaminopinoresinol. Dinitropinoresinol (390 mg) dissolved in ethyl acetate (35 ml) was hydrogenated at 50° with a $Pt(O_2)$-catalyst at 2 atm. pressure. After 1 ½ hour 61 ml of hydrogen had been consumed (calc. 60 ml). The catalyst was filtered off and the solution evaporated to a 1/4 of its original volume. Upon cooling diaminopinoresinol (295 mg) crystallised as colourless crystals. After recrystallisation from ethyl acetate it had m.p. 216°, $[\alpha]_D^{20}$ + 59° (c, 0.196 in chloroform-metanol; 4 : 1). ($C_{20}H_{24}O_6N_2$ requires C, 62.2; H, 6.2; N, 7.2; found C, 62.0; H, 6.4; N, 6.8 %).

SUMMARY

Some new derivatives of pinoresinol have been prepared. Unsuccessful attempts have been made to oxidise the aromatic rings in pinoresinol to carboxylic groups.

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Flavanones from the Heart Wood of *Larix decidua* Mill. (*L. europea* D C.)

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The heart wood of conifers has proved to be a source of a wide variety of organic compounds [1]. Until recent times no investigation of the extractives of the heart wood of species belonging to the genus *Larix* appear to have been carried out. It was therefore deemed of interest to make such an investigation. The European larch, *Larix decidua*, has since long ago been cultivated in Finland, and was chosen as a representative of this genus.

By acidification of the sodium carbonate soluble part of the acetone extract of the heart wood, a crystalline precipitate was obtained which gave the colour reactions typical of 3-hydroxyflavanones [2]. It was readily soluble in hot water and crystallised therefrom upon cooling. It thus showed a striking resemblance to the dihydroquercetin (taxifolin) isolated by Pew [2] from the heart wood of *Pseudotsuga taxifolia*. By paper chromatography this precipitate was, however, shown to be a mixture of two compounds. Lindstedt [3] has recently shown the great value of paper chromatography for the identification of flavones and flavanones. In this case it was found more suitable to use another solvent mixture than those recommended by Lindstedt [3], *viz.* chloroform: alcohol: water (8 : 2 : 1, the bottom layer used). Use of this solvent gave after development of the chromatogram by dis-diazotised benzidine [4] two distinct spots. Different chromatograms gave somewhat varying R_F-values but there was always a definite difference between the two spots. The slower moving spot had R_F-values of about 0.2—0.4 and the faster moving 0.6—0.8. The slower moving spot was found to correspond exactly to the spot of taxifolin, when run simultaneously on the same paper strip. In the same way the faster moving spot was found to correspond to the dihydrokaempferol (3-hydroxynaringenin, coigue-flavanone) isolated by Pew [2] from the wood of *Nothofagus dombeyi*. (The author wishes to thank Dr. Pew and Prof. Erdtman for placing samples of taxifolin and coigue-flavanone to his

disposal.) For reasons given below the name aromadendrin will be given to this compound.

The conclusion that the two substances from *Larix decidua* should be identical with the two reference substances must however not be drawn from this evidence alone, because firstly both dihydroquercetin and dihydrokaempferol can exist in two diastereomeric forms which should not be expected to show any difference in R_F-values and secondly isomeric flavanones, with a different distribution of the hydroxyl groups should presumably not give very different R_F-values, and structural isomerism must not be ruled out.

In order to definitely prove or disprove the identity of the two pairs of compounds a preparative separation of the two substances in the mixture was carried out. This proved to be a rather difficult task. Fractional crystallisation failed to give any separation. Chromatography on alumina gave an eluate, which on a paper chromatogram showed only the presence of the faster moving compound, whereas the other was retained on the column and could not be eluted in a pure form. The use of silica-gel as absorbent gave no separation. Finally it was found that chromatography on a column of powdered cellulose, using chloroform: alcohol (9 : 1) as solvent gave a fairly good separation, giving only a small intermediate fraction containing both compounds.

The more easily eluted compound, as expected, proved to be the substance which on the paper chromatogram had the higher R_F-value. After purification it had m.p. 237—239° and corresponded to the formula $C_{15}H_{12}O_6$ and was for further characterisation converted into its tetra-acetate, m.p. 133—135°. This gave no depression of m.p. with the previously undescribed tetra-acetate of aromadendrin. Even the parent compounds gave no depression of m.p. on admixture, but this was found to be of rather little value for the purpose of identification, because even a mixture of aromadendrin and taxifolin produced only a very slight and difficultly observable depression.

The more difficultly eluted compound, which corresponded to the slower moving spot on the paper chromatogram had the composition $C_{15}H_{12}O_7$ and m.p. 238—240°. It was converted into its penta-acetate. This had m.p. 129—130°. The acetate of taxifolin prepared in the same way had m.p. 130—131° and no depression of m.p. was observed on admixture. On the other hand the acetates of aromadendrin and taxifolin whose m.p:s are rather close to one another show a depression of about 10° in the mixture test.

Graham and Kurth [5] have described the acetate of taxifolin as a non-crystallisable material of m.p. 82—85°. Obviously this was not pure.

It is thus evident that the two flavanones found in the heart wood of *Larix decidua* are identical with aromadendrin and taxifolin respectively. The two compounds were obtained from the chromatogram in the following yields:

aromadendrin 60 % and taxifolin 25 % of the original mixture. If the intermediate fraction containing both compounds is assumed to contain these in approximatively equal amounts, the proportion of aromadendrin to taxifolin is about 2 : 1. The total yield of the two flavanones together, based on air dried wood, is about 0.7 %.

When this work has almost completed there appeared a paper by Hasegawa and Shirato [6] describing the isolation from the heart wood of *Larix leptolepis* of a compound claimed to be identical with distylin. Distylin had earlier been isolated from the heart wood of *Distylium racemosum* [7] and shown to be a racemic dihydroquercetin [8].

Through the kindness of Dr. Hasegawa who sent a sample of his distylin, it was possible to submit it to a paper chromatographic analysis. This showed two spots corresponding exactly to the spots obtained from the mixture of flavanones from *Larix decidua*. Hasegawa's distylin is thus not a pure compound but presumably a mixture of aromadendrin and taxifolin. The amount available was, however, too small for a preparative separation and definite identification of these compounds.

In addition to the already mentioned occurrence of a dihydrokaempferol in *Nothofagus dombeyi* [2] there are a few other reports on the occurrence in nature of compounds with the same constitution. Uoda, Fukushima and Kondo [9] isolated from the wood of *Cercidiphyllum japonicum* a substance called katuranin (katsuranin), which was shown to be dihydrokaempferol. Later King and Acheson [10] isolated a substance with the same composition from the wood of *Afzelia* spp., which King [11] recently has shown to be identical with katuranin. Cohen and Hillis [12] have recently shown that aromadendrin, first isolated from the wood of *Eucalyptus* spp. by Maiden and Smith [13], is also a dihydrokaempferol. Dr. Cohen has been kind enough to provide the author with a generous sample of aromadendrin, from which a crystalline acetate easily could be obtained. This had m.p. 136—137° and gave no depression of m.p. with either the acetate of the dihydrokaempferol from *Nothofagus dombeyi* [2] or the acetate of the dihydrokaempferol from *Larix decidua*. The parent compounds can thus be considered identical and the name aromadendrin thus applies to all these three compounds.

Uoda, Fukushima and Kondo [9] report m.p. 128—129° for the acetate of katuranin. Acetylation of a sample of katuranin kindly provided by Dr. Hasegawa gave, however, an acetate with m.p. 134—135°. This gave no depression of m.p. when mixed with aromadendrin acetate. Even the optical rotation of katuranin is in agreement with the value reported by Cohen and Hillis [12] for aromadendrin. It is thus evident that even katuranin is identical with aromadendrin.

EXPERIMENTAL

Extraction of the heart wood. The finely powdered wood (5.5 kg) was extracted for 48 hours with acetone in a continuous extractor. Most of the acetone was distilled off from the dark coloured extract. The remaining viscous liquid was poured into ether. A small amount (4 g) of an amorphous precipitate was removed by filtration. The ether solution was then shaken with portions of sodium carbonate solution until no precipitate was obtained upon acidification. The combined sodium carbonate extracts were acidified with dilute sulphuric acid and the precipitate (94 g) removed by filtration. This contained still an appreciable amount of impurities, and was purified by dissolving in a small amount of ether and adding light petroleum in small portions. The addition of the first portions of light petroleum caused the precipitation of a resinous material. The later portions of light petroleum gave a crystalline, light yellow precipitate (37 g).

Separation of aromadendrin and taxifolin. The mixture (1 g) of the two flavanones, obtained as described above, was dissolved in chloroform: alcohol (9 : 1, 200 ml) and chromatographed on a column of powdered cellulose (37 × 1.8 cm). Elution was carried out with the same solvent mixture. The eluate was taken in fractions of about 200 ml each. Seven such fractions were obtained. Each fraction was then investigated by paper chromatography using chloroform: alcohol: water (8 : 2 : 1; bottom layer) as mobile phase. The chromatograms were developed by dipping the paper strip in a solution of bis-diazotised benzidine [4]. Because preliminary experiments with the two flavanones had shown that the R_F-values of the spots were rather variable, samples of authentic aromadendrin and taxifolin were always run simultaneously on the same paper strip. In this way the spots obtained from the different portions of the eluate could be definitely identified.

The fractions I—III showed the presence of only aromadendrin and fractions VI—VII of only taxifolin, whereas IV—V contained both compounds.

Aromadendrin. From the combined fractions I—III the solvent was removed *in vacuo* leaving a colourless crystalline residue (600 mg). This was purified by recrystallisation from chloroform-alcohol. M.p. 237—239°. $[\alpha]_D^{20}$ + 45° (c, 0.93 in equal volumes of acetone and water). Pew [2] records m.p. 237—241° and $[\alpha]_D^{20}$ + 45° in the same solvent mixture for aromadendrin from *Nothofagus dombeyi*. Aromadendrin from *Eucalyptus* spp. has m.p. 247—248° and $[\alpha]_D^{20}$ + 51.5° in the same solvent mixture [12]. Katuranin has $[\alpha]_D^{20}$ + 51° (c, 1.03 in the same solvent mixture), (measured on a sample provided by Dr. Hasegawa). A mixed m.p. determination showed no depression. ($C_{15}H_{12}O_6$ requires C, 62.5; H, 4.2; found C, 62.6; H, 4.4%).

The tetra-acetate was prepared by dissolving the flavanone in acetic anhydride and adding a drop of pyridine. After standing overnight the mixture was poured into water. The precipitate thus formed was recrystallised from methanol or ether. M.p. 133—135°. ($C_{23}H_{20}O_{10}$ requires C, 60.5; H, 4.4; found C, 60.3; H, 4.7%). When mixed with either the acetate of aromadendrin from *Nothofagus dombeyi* [2], m.p. 135—136°, or with the acetate of aromadendrin from *Eucalyptus* spp.[12], m.p. 136—137°, both acetates being prepared in the same way as described above, no depression of m.p. was observed.

Taxifolin. From the combined fractions VI and VII obtained in the chromatography the solvent was removed *in vacuo* yielding a slightly yellow crystalline residue (250 mg). This was recrystallised from chloroform containing a small amount of alcohol. M.p. 238—240°, $[\alpha]_D^{20}$ + 51° (c, 0.98 in equal volumes of acetone and water). Pew records m.p.

240—242° and $[\alpha]_D^{20}$ + 46 in the same solvent mixture for taxifolin from *Pseudotsuga taxifolia*. ($C_{15}H_{12}O_7$ requires C, 59.2; H, 4.0; found C, 58.4; H, 4.0%).

The penta-acetate was prepared in the same way as described above for the preparation of the acetate of aromadendrin. After recrystallisation from methanol it had m.p. 129—130°. ($C_{25}H_{22}O_{12}$ requires C, 58.3; H, 4.3; found C, 58.2; H, 4.3%). No depression of m.p. was observed when mixed with the acetate of taxifolin of m.p. 130—131°, prepared in the same way.

SUMMARY

Dihydrokaempferol and dihydroquercetin have been isolated from the heart wood of *Larix decidua*. Mill. The former has been shown to be identical with aromadendrin previously isolated from *Nothofagus dombeyi* and *Eucalyptus* spp. The latter has been shown to be identical with taxifolin previously isolated from *Pseudotsuga taxifolia*. The two flavanones were separated by chromatography on a column of powdered cellulose.

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The Monoketo- and Monohydroxyoctadecanoic Acids

Preparation and Characterization by Thermal and X-ray Methods

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In connexion with work on the autoxidation of unsaturated fatty acids [1–3], hydroxyoctadecanoic acids were isolated from the hydrogenated reaction products. In these cases the hydroxyl group should be located in the neighbourhood of the original double bond, and in order to identify the acids a number of DL-hydroxyoctadecanoic acids with the hydroxyl group near the middle of the chain were synthesized. In several other instances [*cf.* 4–7] monohydroxyoctadecanoic acids have remained unidentified owing to lack of reference compounds, and we have therefore prepared the complete series of the seventeen DL-monohydroxyoctadecanoic acids. Several of the isomers have been prepared earlier, although in some cases the methods used do not seem to ensure the structure or purity of the product.

In the course of the work to be described many cases of polymorphism have been observed among the hydroxy-acids and their methyl esters. The corresponding keto-compounds are less complicated in this respect, and for identification purposes it might therefore in certain cases be advantageous to convert the hydroxy-acid into the corresponding keto-acid. Optically active hydroxy-acids, for which synthetic material for comparison is lacking at present, will at any rate be best identified in this manner. We have therefore studied the crystal behaviour, by thermal and X-ray methods, of all the monoketo- and DL-monohydroxyoctadecanoic acids and their methyl esters. Acids that possess practically identical melting points may be distinguished

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by their X-ray diffraction patterns, and the data given in this paper should permit the identification of any keto- or hydroxyoctadecanoic acid, using only milligram quantities of material.

A further reason for the extension of the preparative work to include all positional isomers (66 compounds in all) was the fact that we wanted to study the influence of the position of the keto and hydroxyl group respectively on the monolayer properties and on the infra-red spectra. The results of these physico-chemical studies will be reported later.

Except the case of the 2- and 18-isomers the hydroxy-acids have been prepared via the methyl esters obtained by high pressure hydrogenation of the methyl esters of the corresponding keto-acids over Raney nickel. In some instances we have reduced the keto-ester over W 6-Raney nickel [8] at ordinary pressure and room temperature. The 2-hydroxyoctadecanoic acid was prepared by alkali treatment of the α-bromoacid whereas the 18-isomer was obtained by hydrogenolysis over Raney nickel of the monothiol ester of octadecane-1,18-dioic acid.

2-Ketooctadecanoic acid was prepared in 80 per cent yield by oxidation of methyl 2-hydroxyoctadecanoate with chromic trioxide in acetic acid at 37° [9], followed by hydrolysis of the ester.

Lower α-keto-esters have generally been prepared via the α-keto-nitrile, via the α-oximino-ester, or by oxalic ester condensation, *cf.* Adickes and Andresen [10] and Vogel and Schinz [11]. None of these methods appears suitable for the synthesis of long chain α-keto-acids, as the yields decrease with increasing chain-length. The highest member so far prepared (C_{15}) was obtained in a yield of 15 per cent by the oxalic ester condensation method [10].

3-Ketooctadecanoic acid was prepared [12] by acid hydrolysis of the methyl ester [13] according to the procedure of Mitz, Axelrod and Hofmann [14].

For the synthesis of keto-acids with the keto group in position 4 to 17 several methods are available, *cf. e.g.* [15, 16]. In the course of the present work, the 4-, 5-, 7-, 11-, 13-, and 17-ketooctadecanoic acids were prepared by alkylation of the appropriate β-keto-esters with esters of ω-iodocarboxylic acids, followed by hydrolysis and ketonic cleavage of the alkylation product. The procedure was that described by Ställberg-Stenhagen and Stenhagen [17]. The β-keto-esters required were synthesized as described by Hunsdiecker [18] and Ställberg-Stenhagen [13].

$$CH_3(CH_2)_nCOCH_2COOCH_3 + I(CH_2)_{14-n}COOCH_3 \xrightarrow[\text{pentanone-2}]{K_2CO_3}$$

$$CH_3(CH_2)_nCOCHCOOCH_3 \xrightarrow[CH_3OH]{KOH,H_2O} CH_3(CH_2)_nCO(CH_2)_{15-n}COOH$$

$$\qquad\qquad\quad |$$

$$\qquad\quad (CH_2)_{14-n}COOCH_3$$

This method gave directly practically pure keto-acids in 60—70 per cent yield.

The 4-, 5-, 6-, 8-, 9-, 10-, 12-, 14-, 15- and 16-ketooctadecanoic acids were prepared by the reaction of the halfester chloride of a dicarboxylic acid with organo-cadmium-compounds according to Gilman and Nelson [19]. For a summary see Cason [20].

$$(CH_3(CH_2)_n)_2Cd + 2\ CH_3OOC(CH_2)_{15-n}COOCl \longrightarrow$$

$$2CH_3(CH_2)_nCO(CH_2)_{15-n}COOCH_3 \xrightarrow[C_2H_5OH]{KOH,H_2O} CH_3(CH_2)_nCO(CH_2)_{15-n}COOH$$

The ketonic reaction products were isolated with Girard's reagent T [21]. The yield of keto-acids was only about 30 per cent of the theoretical. The low yield might at least partly be ascribed to a too short reflux time as in recent work considerably longer reaction times than those initially used have been found necessary [22]; *cf.* also Cason and Winans [23].

THERMAL AND X-RAY INVESTIGATIONS

Ketooctadecanoic acids, cf. Table 1 and Fig 1. This series shows a comparatively simple behaviour. With the exception of the 3-keto-isomer all keto-acids crystallize with vertical (V) chains (double molecules), and acids with the keto group in position 4 up to and including position 15 appear to form an isomorphous series as indicated by the identical side-spacings. The melting point curve of the acids given in Fig. 1 shows that acids with the keto group at even-numbered positions melt slightly higher than the odd-numbered neighbours. However, from the 12-keto-acids on, this behaviour is reversed. When the keto group is near either end, *viz.* in position 2, 16 or 17 respectively, the chains are vertical but the side spacings indicate that different lateral packing of the chains occurs in all these cases. The 3-keto-acid which shows the highest m.p. of the series *cf.* [12] has a structure with tilted double molecules. Polymorphism has only been observed for the 4-, 5- and 7-keto-acids. Specimens of the 7-keto-acid crystallized from light petroleum (b.p. 95°) showed the presence of a small amount of a crystalline modification with a long spacing of 46 Å. The diffraction pattern-temperature diagram (DPT diagram) [24] showed that this form disappeared about 10 degrees below the melting point.

The specimen of 5-keto-acid prepared by the organo-cadmium method melted at 87.1—87.3°, solidified at 86.8° in the form of well developed plates, and remelted at 87.1—87.3°. The specimen prepared by the β-keto-ester method and recrystallized from light petroleum (b.p. 95°) was found to be a mixture of the crystalline modification found for the form of m.p. 87.1—87.3° and a second

Table 1. Ketooctadecanoic acids.

Position of substituent	M.p.° C		Crystal spacings, Å.	
	Present investigation	Literature	Long spacing	Side spacings
2	74.3–74.6 (V)	— —	49.7 P, M	4.15, 3.58
3	99 decomp.[3c]	(64–65[3a]) 102–103 decomp.[3b]	42.0 P	4.45, 4.25, 3.87, 3.65
4	96.3–96.5 (V); 94.2–94.3	97[4a,b,c]	49.7 (V) P, M; 38.4 P, M	4.68, 4. 48, 4.01, 3.63
5	87.1–87.3 (V); 91.4–91.6	— —	49.7 P, M	
6	86.6–86.8 (V)	87[6a,b]; 86–87[6c]; 86[6d]		
7	82.8–83.0 (V)	75[7a]	49.7 P, M; 46 P	
8	83.6–83.8 (V)	— —		
9	81.7–81.9 (V)	83[9a,b]		
10	82.4–82.6 (V)	76[10a]; 74[10b]; 82(corr.)[10c]; 76[10d]; 82–82.8[10e]		
11	81.7–81.9 (V)	(65[11a])		4.10, 3.80, 2.99
12	81.5–81.9 (V)	82[12a]; 81[12b]; 81–81.5[12c]	49.7 P, M	
13	82.1–82.3 (V)	— —		
14	81.7–81.9 (V)	— —		
15	83.0–83.2 (V)	— —		
16	93.1–93.5 (V)	— —		4.50, 3. 76, 3.64
17	87.4–87.7 (V)	— —		4.30, 3.82

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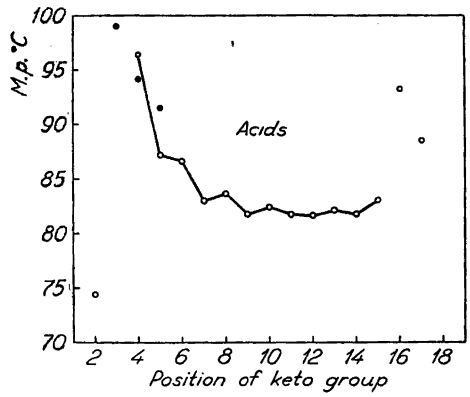


Fig. 1. Melting points of ketooctadecanoic acids. Open circles represent forms with vertically oriented chains. Points representing isomorphic forms are connected with a full drawn line.

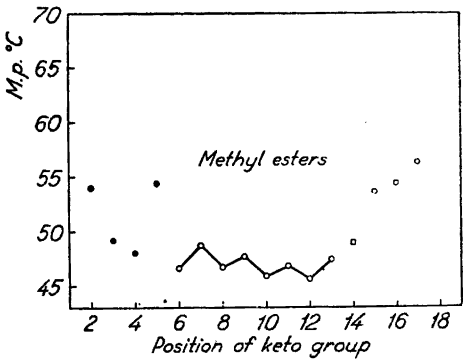


Fig. 2. Melting points of methyl ketooctadecanoates. Open squares represent forms with vertically oriented chains. Open circles represent β form. Tilted forms other than β are marked with filled circles.

form with tilted double molecules. In this case a complete transformation into the latter form took place on heating, followed by melting at 91.4—91.6°. After a second crystallization from the same solvent only the tilted form was present at room temperature. This modification melted at 91.4—91.6° and solidified at about 90° with the formation of long prisms in the melt.

The 4-keto-acid showed a similar behaviour. The specimen prepared by the organo-cadmium method was found to exist in a crystalline modification with

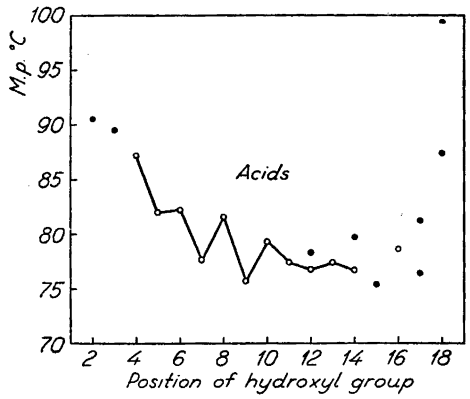


Fig. 3. Melting points of hydroxyoctadecanoic acids. Open circles represent γ form. Crystalline forms other than γ are represented by filled circles.

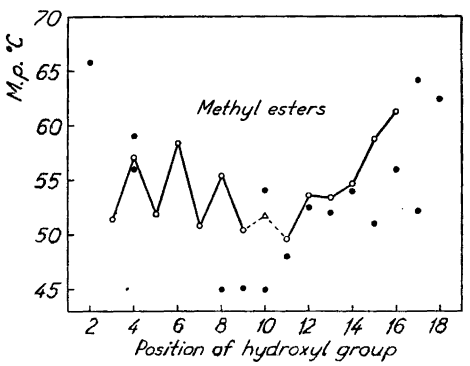


Fig. 4. Melting points of methyl esters of hydroxyoctadecanoic acids. Open circles represent β form. Other forms are represented by filled circles.

Table 2. Methyl esters of ketooctadecanoic acids.

Position of substituent	M.p.° C.		Crystal spacings, Å	
	Present investigation	Literature	Long spacing	Side spacings
2	54.0—54.1	— —	38.7 P, M	3.66, 4.05, 4.56, 4.98
3	49.2 *	49.2[3a]; 49[3b]	38.3 P, M	4.63, 4.40, 3.88, 3.69, 3.55, 3.39
4	47.9—48.0	49.5[4a]	45.6 P, M	4.27, 4.04, 3.90, 3.72, 3.56
5	54.3—54.5	— —	49.6 P, M	4.46, 4.28, 4.05, 3.78
6	46.3—46.8	— —		
7	48.7—48.8	— —		
8	46.4—46.9	42.7—44.3[8a]		
9	47.5—48.0	— —		
10	45.8—46.1	— —	47.6 (β) P, M	β: 3.73, 4.03, 4.27, 4.44
11	46.7—46.9	— —		
12	45.4—45.7	44.5[12a]		
13	47.3—47.7	— —		
14	48.9—49.0	— —	53.5 P, M	4.11, 3.76, 2.99
15	53.5—53.7	— —	47.6(β) P, M; 53 P, M	
16	54.3—54.5	— —	53.5 P, M	4.11, 3.76, 2.99
17	56.1—56.5	— —	47.6(β); 53 P	

* Sample synthesized by S. Ställberg-Stenhagen [13].

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tilted double molecules. The DPT diagram showed no transition, and melting occurred at 94.1—94.3°. Solidification took place at 93° with formation of beautiful long prisms extending through the whole mass, and the keto-acid remelted at 94.2—94.3°. The same acid prepared by the β-keto-ester method crystallized with vertical double molecules and melted and remelted at 96.3—96.5° without any transition being observed on heating.

Methyl ketooctadecanoates, cf. Table 2 and Fig. 2. In this series, the methyl esters with the keto group in position 6 up to and including 13 have similar crystal structures (β) with tilted double molecules, and the corresponding melting point diagram in Fig. 2 shows an alternation of the melting points, the esters with the keto group in an odd-numbered position melting higher than their even-numbered neighbours. The methyl esters of the 15- and 17-keto-acids also possess the same crystal structure but in these two cases minor amounts of a crystalline modification with vertical molecules have also been

Table 3. DL-*Monohydroxyoctadecanoic acids.*

Position of substituent	M.p.° C		Crystal spacings, Å.	
	Present investigation	Literature	Long spacing	Side spacings
2	90.9—91.1	91—92[2a]; 93[2b]; 84—86[2c]	41.9 P, M; 49 M	3.84, 4.33, 4.78 P
3	89.4—89.6 (γ) *)	89[3a]; 90[3b]; 89.4—89.6[3c]	46.5 (β), 44.6 (γ) P, 36.0 (ζ) M[3c]	4.11, 3.89 [3c]
4	87.0—87.4 (γ)	89[4a]; 87[4b]	44.1 P, M	
5	81.9—82.1 (γ)	(54—55[5a])	44.1 P, M	
6	82.0—82.4 (γ)	83[6a]; 84[6b]		
7	77.6—77.8 (γ)	82[7a,b]	44.4 P, M	
8	81.5—81.7 (γ)	— —		
9	75.4—75.9 (γ)	74—75[9a]	44.4 P, M; 47.5 (δ) P	γ: 4.36, 4.08, 3.90
10	79.2—79.5 (γ)	84—86[10a]; 81—81.5[10b]; 83—84[10c]; 84.5[10d]; 81—82[9a]; 85[10e]; 82.5[10f]	44.3 P, M	
11	77.2—77.5 (γ)	84—85[11c]; 76—77[9a]; 84[11a]; 82—85[11b]	44.3; 47.5 P, M	
12	76.6—76.9 (γ); 78.5—78.9	DL: 78—79[9a]; 80[12a]	44.3 P, M	
13	77.2—77.5 (δ)	77—77.5[9a]	47.5 (δ) P, M; 44.3 (γ) P, M	δ: 4.18, 3.85
14	76.6—76.8 (γ); 79.6—79.9 (ε)	— —	44.3 P, M; 42.2 (ε) M	
15	75.3—75.5 (δ)	— —	47.5 (δ) P, M; 44.3 M 42.2 (ε) M	δ: as 13—OH
16	78.4—78.6 (γ)	— —	44.3 P, M	γ: as 12—OH
17	76.4—76.6; 81.0—81.4	— —	40.2 P, M	4.36, 4.11, 3.93, 3.63
18	99.3—99.5; 87—88 (unst.)	96.6—97.2[18a]	46.8 P, M	4.61, 3.78, 3.46

* Sample synthesized by M. Skogh [3c].

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10 e. Radcliffe, L. G., and Gibson, W. *J. Soc. Dyers Colourists* **39** (1923) 4.
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11 a. Shukoff, A. A., and Schestakoff, P. J. *J. prakt. Chem.* **67** (1903) 416.
11 b. Saytzeff, M., C., and A. *J. prakt. Chem.* **37** (1888) 277.
11 c. Schreiner, O., and Shorey, E. C. *J. Am. Chem. Soc.* **32** (1910) 1674.
12 a. Reinger, E. *Ber. deut. pharm. Ges.* **32** (1922) 131.
18 a. Chuit, P., and Hausser, J. *Helv. Chim. Acta* **12** (1929) 488.

observed at room temperature. The 14- and 16-keto-esters have the same crystalline structure with vertical double molecules. The 2-, 3-, 4- and 5-keto-esters all crystallize in different tilted forms and no polymorphism has been observed. All keto-esters formed well developed crystals. In the case of the 2- and 4-isomers single crystals of very large size could be obtained.

Hydroxyoctadecanoic acids, cf. Table 3 and Fig. 3. In general, the hydroxy-acids show a more complicated behaviour than the corresponding keto-acids.

The 2-hydroxy-acid melted at 90.9—91.1° and solidified at about 86° with the formation of aggregates of needles. A sample of the original preparation of Le Sueur [2a] (Table 3), obtained through the courtesy of Dr. N. Sheppard, Cambridge, melted at 90.7—91.2° and began to solidify (without nucleus) at 87° in the same manner as our preparation. Remelting took place at 90.6—90.9°. After this specimen had been kept at 92° for 10 hours, the m.p. was about 80°, indicating the formation of lactide. Previously melted specimens of this acid showed the presence of a second crystalline form.

The crystal behaviour of 3-hydroxyoctadecanoic acid has been investigated by Skogh [25]. Three different crystalline modifications were found, which were called β, γ and ζ respectively.

A crystalline form corresponding to the γ form of the 3-hydroxy-acid has also been found for the 4- up to and including the 16-hydroxy-acid. It is the only form found for the 4-, 5-, 6-, 7-, 8-, 10- and 16-hydroxy-acids.

The 4- and 5-hydroxy-acids are on melting partly converted into the corresponding lactones, indicated by the successively lowered remelting points.

A form with a long spacing of 47.5 Å, which we have called δ, has been observed for the 9-, 11-, 13- and 15-hydroxy-acids. The DPT diagrams showed that in the case of the 9-hydroxy-isomer the δ form disappeared on heating,

and that the m.p. is that of the γ form. This was also usually the case with the 11-hydroxy-isomer but on one occasion a specimen melting at 76.6—76.9° was obtained in which the γ and δ forms coexisted up to the m.p. The 13- and 15-isomers both existed in the δ form close to the m.p.

The 12-hydroxy-acid exists in the γ form at room temperature. The acid melts at 76.6—76.9°, but on reheating immediately after solidification melting takes place at 78.5—78.9°. It is possible that the form with the higher m.p. corresponds to the ε form described below for the 14-hydroxy-acid.

The crystalline form with the long spacing 42.2 Å that we have called ε has been observed for previously melted specimens of 14- and 15-hydroxy-acids. In the case of the 14-hydroxy-acid previously melted specimens existed in the γ form at room temperature. On heating, transition to the ε modification began at 70° and this form melted at 79.6—79.9°.

The DPT diagram of the 15-hydroxy-acid showed that previously melted samples existing in the γ form at room temperature underwent a transition to a mixture of the δ and ε forms at about 60°.

The 17-hydroxy-acid melted at 76.4—76.6° but after solidification part of the specimen did not melt until 81.0—81.4°. The X-ray data refer to the low-melting form.

Two crystalline forms were also observed for the 18-isomer. The melt did not solidify until about 87° and on immediate reheating part of the sample melted at 87—88° but complete melting did not occur until 99.5°. The X-ray data refer to the higher-melting form.

The melting point diagram (Fig. 3) shows that for acids with the hydroxyl group in position from 5 to 11 the m.p.'s of the γ forms alternate, the even-numbered isomers having higher m.p.'s than their odd-numbered neighbours.

Methyl esters of monohydroxy octadecanoic acids, cf. Table 4 and Fig. 4. Only one crystalline form has been observed for the 2-hydroxy-ester. All hydroxy-esters from 3- up to the 16-isomer possess the same crystalline modification (β) with a long spacing of 47.5 Å. This is the only form observed for the 3-, 5-, 6- and 7-hydroxy-esters. All other esters show additional crystalline modifications.

Three different melting points have been observed for the 4-hydroxy-isomer. The β modification melts at 57.2—57.4°. The solidified melt on immediate reheating remelts at 58.6—59.1° but if the solidified melt is kept for 24 hours at room temperature the m.p. is again that of the β form. After rapid cooling of the melt the ester remelts at 56.2°.

The 8-, 9- and 10-isomers have a low-melting modification of m.p. 45°. For the 8- and 10-isomers this form is very unstable and has only been observed

Table 4. Methyl esters of DL*-monohydroxyoctadecanoic acids.*

Position of substituent	M.p.° C.		Crystal spacings, Å.	
	Present investigation	Literature	Long spacing	Side spacings
2	65.7—65.9	64—66[2a]	26.4 P	4.18, 3.64 P
3	51.1—51.4 (β)	— —	(β) 47.5 P, M	
4	56.1—56.4; 57.2—57.4; 58.6—59.3	— —		
5	51.7—52.1 (β)	— —		
6	58.2—58.5 (β)	— —		
7	50.7—51.0 (β)	— —		
8	55.3—55.6 (β); 45	— —		
9	50.3—50.6(β);45.0—45.3	45—46[9a]	~52 M	
10	45 (unst.); 51.5—52 (trans.); 54.1—54.2	46[10a]; 53—54[9a]	(β) 47.5 P	4.34, 4.11, 3.89 P, M
11	49.6—49.8 (β); 48 (unst.)	49—50[9a]		
12	53.4—53.6 (β); 52.3—52.7 (unst.)	50—51[9a]		
13	53.3—53.5(β);52.0—52.4	52—52.5[9a]		
14	54.5—54.8 (β); 54 (unst.)	— —		
15	58.7—58.9 (β); 51 (unst.)	— —		
16	61.2—61.5; 56 (unst.)	— —	40.6 (β) P, 40.6, 47.8 M	
17	64.1—64.3; 52—52.5 (unst.)	— —	47.5 P	4.64, 4.29, 4.07, 3.72
18	62.4—62.6 (38.5 Å-form)	61.5—62[18a]	38.5 P, 48 M	

2 a. Levene, P. A., and Young, P. S. *J. Biol. Chem.* **102** (1933) 557.
9 a. Tomecko, C. G., and Adams, R. *J. Am. Chem. Soc.* **49** (1927) 522.
10 a. Radcliffe, L. G., and Gibson, W. *J. Soc. Dyers Colourists* **39** (1923) 4.
18 a. Chuit, P., and Hausser, J. *Helv. Chim. Acta* **12** (1929) 488.

after very rapid solidification by cooling the m.p. tube in cold water. Immediately after melting the higher-melting β form crystallizes out.

On slow cooling the melt of the 9-isomer the low-melting modification of m.p. 45.0—45.3° crystallized in the form of long prisms.

In the case of the 10-hydroxy-isomer a transition point has been observed at 51.5—52°. The β form is transformed into another modification of m.p. 54.1—54.2°. The diffraction pattern is similar to that of the β form but additional lines appear.

A perusal of Tables 1—4 and Figs. 1—4 will show that a keto- or hydroxy-octadecanoic acid with unknown position of the substituent can be identified by

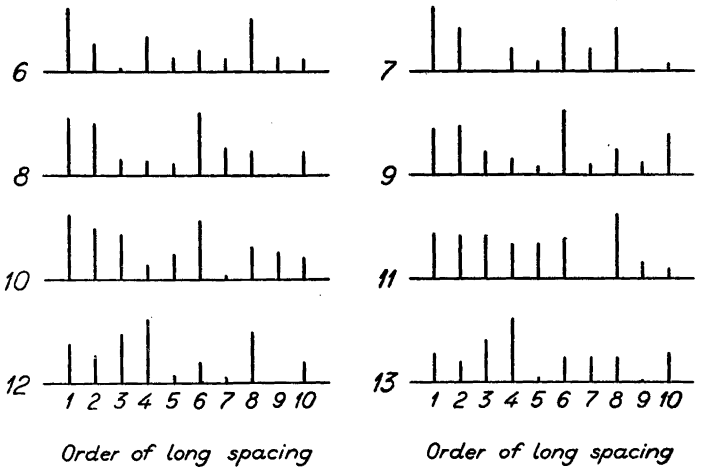


Fig. 5. Intensity distribution among the 00l reflexions of methyl esters of monoketooctadecanoic acids.

the thermal and X-ray spacing data, provided that the substituent is in a position near either end of the chain. However, in a hydroxy-acid or methyl ester with the hydroxyl group in position 5 to 14, the data in the tables will not be sufficient to distinguish clearly between the isomers. Conversion of the hydroxy-compound into the corresponding keto-compound decreases the uncertain region to position 6 to 13. In the case of an acid with the substituent in this region the identification may be achieved with the aid of the intensity distribution among the 00*l* reflexions in the X-ray diffraction patterns, which varies in a characteristic manner with the position of the substituent. The method was first employed by Shearer [26] for long chain keto-acids.

In the present case the identification is best performed on the methyl esters of the keto-acids, as the esters of acids with the ketogroup in position 6 to 13 do not exhibit polymorphism, which complicates the matter for the hydroxy-compounds and for the keto-acids. The visually estimated intensity distribution data for the 00*l* reflexions (up to *0010*) for the methyl ketooctadecanoates (β form) are given in Fig. 5.

EXPERIMENTAL

Due to the great difficulty in obtaining chemicals at the time when a large part of this work was done (1944—45), the availability of suitable starting material has in many cases determined the choice of the method used. The starting material was in every case purified with great care to ensure the absence of homologous impurities, generally by distillation through efficient columns or by crystallization. The final products have

been crystallized from different solvents until further crystallization brought about no further change in the properties of the compound. The acids were crystallized from methanol and acetone (in some cases with the addition of a small amount of water), or from light petroleum (b.p. 80—100°). The methyl esters were crystallized from methanol-water and from low-boiling light petroleum (b.p. below 40°).

As the procedures were identical in many cases, experimental details are given only for the 2-, 15-, 16- and 17-ketooctadecanoic acids, and the 4-, 5-, 17- and 18-hydroxyoctadecanoic acids. The 4-, 5-, 6-, 8-, 9-, 10-, 12- and 14-keto-acids were prepared by the general procedure described for the 15-keto-acid, and the 4-, 5-, 7-, 11- and 13-keto-acids by the procedure given for 17-ketooctadecanoic acid.

The melting points of the acids and their methyl esters are collected in Tables 1—4. The analyses given in Table 5 have been performed in the Microanalytical laboratory at the Institute of Medical Chemistry, Uppsala.

2-Hydroxyoctadecanoic acid. A pure specimen of stearic acid was converted into the α-bromo-acid, using the standard procedure. The α-bromo-acid was then treated with alkali, *cf.* Le Sueur [27].

2-Ketooctadecanoic acid. Methyl 2-hydroxyoctadecanoate (501 mg), obtained by esterification of the acid by means of diazomethane, was dissolved in 50 ml of glacial acetic acid at 37°. A solution of 169.6 mg of chromic trioxide in 50 ml 95 per cent acetic acid was added and the mixture kept at 37° for 60 minutes. The course of the reaction is shown in Fig. 6. One ml of methanol was then added and the solution taken to dryness *in vacuo*. The residue was dissolved in 80 ml of ether and a little water. The ether solution was washed twice with water, dilute sodium carbonate solution and water, dried over sodium sulphate and evaporated to dryness. The keto-ester was crystallized from methanol. The ester was saponified in methanolic potassium hydroxide at room temperature and the acid recrystallized from light petroleum (40—60° and 95°). Yield, 80 % of the theoretical.

3-Ketooctadecanoic acid and methyl ester. The specimens used were those previously described by Stenhagen [12] and Ställberg-Stenhagen [13] respectively.

3-Hydroxyoctadecanoic acid. A sample was kindly supplied by M. Skogh [25]. The methyl ester was prepared from this specimen by means of diazomethane.

4-Ketooctadecanoic acid. Specimens of this keto-acid were prepared both by the organo-cadmium and by the long chain β-keto-ester method. The initial materials in the former case were methyl hydrogen succinate and 1-iodotetradecane; in the latter case ethyl chloroacetate and methyl 3-ketoheptadecanoate [13] were employed.

5-Ketooctadecanoic acid. This acid was also prepared along both routes, starting in one case from methyl hydrogen glutarate and 1-iodotridecane, and in the other case from ethyl β-iodopropionate (obtained via the bromo-ester [28]) and methyl 3-ketohexadecanoate [13].

4- and 5-Hydroxyoctadecanoic acids. Methyl 4- and 5-ketooctadecanoate were hydrogenated at ordinary pressure and temperature over Raney Nickel W—6 in methanolic solution. The reductions were completed in one hour.

The crude 4- and 5-hydroxyoctadecanoic esters obtained after reduction of the corresponding keto-esters were hydrolyzed by excess alkali in refluxing ethanol. After cooling, diluting with water and acidification with the calculated amount of dilute acid, the hydroxy-acids crystallized. They were recrystallized from acetone at −25°. Both acids were obtained as well developed rhombic plates.

6-Ketooctadecanoic acid. Methyl hydrogen adipate and 1-iodododecane were used as in the organo-cadmium procedure.

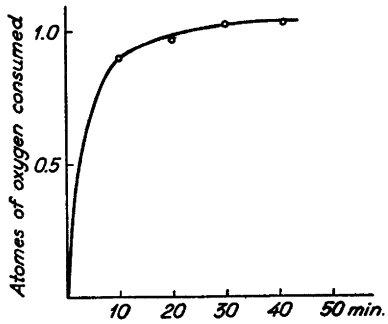


Fig. 6. *Oxidation of methyl 2-hydroxyoctadecanoate with chromic trioxide in 97.5 % acetic acid at 37° as followed by iodometric titration.*

7-Ketooctadecanoic acid. Ethyl 5-bromopentanoate was prepared from adipic acid by the method of Hunsdiecker and Hunsdiecker [29]. Ethyl 5-iodopentanoate, obtained from the bromo-ester by means of sodium iodide in acetone solution, was used for the alkylation of methyl 3-ketotetradecanoate [13].

8-Ketooctadecanoic acid was prepared from methyl hydrogen suberate and 1-bromodecane.

9-Ketooctadecanoic acid. This compound was prepared from methyl hydrogen azelate and 1-bromononane.

10-Ketooctadecanoic acid. Methyl hydrogen sebacate and 1-bromooctane were used.

11-Ketooctadecanoic acid was prepared by the β-keto-ester procedure, starting from ethyl 9-iodononanoate (obtained via the bromo-ester prepared from sebacic acid by the method of Hunsdiecker and Hunsdiecker [29]) and methyl 3-ketodecanoate [13].

12-Ketooctadecanoic acid. Methyl hydrogen dodecane-1,12-dioate and 1-bromohexane were used as initial materials.

13-Ketooctadecanoic acid. Ethyl 11-iodohendecanoate, obtained from undecylenic acid by the procedure of Ashton and Smith [30], was used in the alkylation of methyl 3-ketooctanoate [13].

14-Ketooctadecanoic acid was prepared from methyl hydrogen tetradecane-1,14-dioate and 1-bromobutane.

15-Ketooctadecanoic acid. 13.9 Grams of methyl hydrogen pentadecane-1,15-dioate were dissolved in dry benzene, an excess of thionyl chloride was added, and the mixture was left overnight at room temperature. The acid chloride was taken to dryness *in vacuo* two times after the addition of fresh benzene, and finally dissolved in 25 ml of dry benzene. A Grignard reagent was prepared from 9.26 g of 1-bromopropane and 1.8 g of magnesium turnings under dry nitrogen in a three-neck flask. 7.3 Grams of powdered dry cadmium chloride were added to the ice-cold, well stirred Grignard reagent. After five minutes the ether was distilled off by heating on a steam cone, and subsequently 45 ml of dry benzene were added and distilled off. The sticky reaction product was then stirred under reflux with 45 ml of fresh benzene and the solution of the acid chloride was added gradually during 10 minutes whereafter the mixture was refluxed for one hour. The mixture was then poured onto ice and sulphuric acid. Extraction with ether

yielded 13.5 g of crude reaction product. The ketonic part was isolated with Girard's reagent T according to Ruzicka and Prelog [31] in the following way:

The product was dried *in vacuo* over phosphorous pentoxide and dissolved in a mixture of 225 ml of dry methanol and 13.5 ml of glacial acetic acid. After addition of 12.5 g of "Girard T" the solution was refluxed for two hours. After cooling, about 250 g of ice and 225 ml of 5 per cent sodium carbonate were added and the mixture was extracted four times with ether. The aqueous phase was acidified and the ketones were isolated as usual. Yield 6.6 g of m.p. 48—50°. Saponification and separation of a small amount of neutral products yielded after recrystallization from ethanol or acetone 15-ketooctadecanoic acid. Eq. weight 298.5, Calc. 298.3. The methyl ester was prepared with diazomethane and recrystallized from aqueous methanol and from benzene-light petroleum (b.p. 60—80°).

15-Ketooctadecanoic acid. Ethyl 6-iodohexanoate was prepared from the ω-bromo-compound [32,33] by refluxing with sodium iodide in 2-butanone. *8-Ketopentadecane-1,15-dioic acid.* The methods described by v. Pechmann and Sidgwick [34] and English [35] for a lower homologue were followed. Equimolecular amounts of ethyl 3-ketopentanedioate and ethyl 6-iodohexanoate were heated together on the steam bath and 1 mole of sodium in ethanol was slowly added. When the solution had become neutral (10 hours) one mole iodo-ester was again added followed by sodium ethoxide as before. After the reaction mixture was neutral again the product was isolated in ether, saponified and decarboxylated with hydrochloric acid. Recrystallization from aqueous acetone yielded 8-ketopentadecane-1,15-dioic acid, m.p. 116—117° (m.p. 114° [36]). Yield 40 % of the theoretical.

$C_{15}H_{26}O_5$ (286.4)	Calc.	C 62.89	H 9.15
	Found	» 63.12	» 9.12

Pentadecane-1,15-dioic acid was prepared from the keto-acid by Clemmensen reduction using the conditions described by G. M. Robinson [37] and Stenhagen *et al.* [38]. 59.3 Grams of keto-acid were heated with 400 g of amalgamated zinc, 1 200 ml of conc. hydrochloric acid and 240 ml of acetic acid. The solvents were changed every day for 7 days. After saponification, 50 g of crude *n*-pentadecanedioic acid (m.p. 112—117°) were obtained. The dimethyl ester was purified by distillation, b.p. 199—203°, 11 mm, m.p. 40—43°. (Lit.: m.p. 42° [39], 43° [40], b.p. 203—205°, 11 mm [21]).

$C_{17}H_{32}O_4$ (300.4)	Calc.	C 67.94	H 10.74
	Found	» 68.18	» 10.77

Partial saponification yielded the mono-ester, m.p. 62—63°, after recrystallization from light petroleum (b.p. 60—80°). The acid chloride of this mono-ester and dipropylcadmium gave 15-ketooctadecanoic acid.

16-Ketooctadecanoic acid. Diethyl tetradecane-1,14-dioate was prepared from 1,10-diiododecane through a double malonic ester synthesis [41]. The crude ester had m.p. 24—29° and the yield was 60 per cent of the theoretical. The ester was reduced over copper chromite according to Adkins [42], yielding 1,14-dihydroxytetradecane. *1,14-Diiodotetradecane* was prepared in 94 per cent yield with iodine and read phosphorous [43] from the crude diol, and recrystallized from ethanol, m.p. 48—50°.

$C_{14}H_{28}I_2$ (450.2)	Calc.	C 37.35	H 6.27	I 56.38
	Found	» 37.42	» 6.08	» 55.90

To 58.4 g of diiodide in 250 ml ethanol were added 39 g of potassium cyanide in 65 ml of water and the mixture was left for 20 hours in the steam bath. A small sample of the dinitrile was distilled, b.p. 216—220°, 4 mm. M.p. 47—49°.

Table 5. Analyses.

Position of substituent	Ketooctadecanoic acids		Methyl ketooctadecanoates		DL-Hydroxyoctadecanoic acids		Methyl DL-hydroxyoctadecanoates	
	% C	% H	% C	% H	% C	% H	% C	% H
2	72.51	11.43	73.05	11.63	71.85	12.03	72.52	12.19
3	72.88	11.65[12]	72.50	11.52[13]	71.90	12.12[25]	72.47	12.15
4	72.74	11.45	72.97	11.60	72.10	12.06	72.71	12.14
5	72.48	11.47	72.96	11.50	71.86	12.05	72.77	12.20
6	72.32	11.53	73.07	11.60	71.85	12.11	72.63	12.16
7	72.37	11.46	73.10	11.68	71.86	12.04	72.39	12.12
8	72.56	11.45	72.90	11.64	71.64	12.03	72.82	12.26
9	72.71	11.48	72.76	11.56	71.92	12.08	72.58	12.25
10	72.73	11.49	73.05	11.61	72.09	12.04	72.67	12.18
11	72.29	11.41	73.16	11.67	71.84	12.17	72.90	12.27
12	72.48	11.41	72.75	11.57	71.90	12.06	72.52	12.09
13	72.47	11.43	73.04	11.58	71.94	12.04	72.60	12.22
14	72.17	11.43	72.58	11.47	72.05	12.14	72.77	12.16
15	72.75	11.49	73.07	11.63	72.10	12.07	72.57	12.19
16	72.37	11.46	73.07	11.57	71.65	12.00	72.58	12.17
17	72.25	11.42	72.77	11.60	71.36	11.97	72.57	12.19
18					71.99	12.18	72.28	12.14
Calc.	72.43	11.48	73.03	11.61	71.95	12.08	72.56	12.18

$C_{16}H_{28}N_2$ (248.4) Calc. C 77.36 H 11.36 N 11.28
Found » 77.09 » 11.44 » 11.40

The crude dinitrile was refluxed with ethanolic potassium hydroxide for 22 hours and the crude acid esterified with ethanol-sulphuric acid and distilled, b.p. 205—207°, 3 mm.

Methyl hydrogen hexadecane-1,16-dioate was prepared by partial saponification of the diester [29] and 16-ketooctadecanoic acid prepared from the acid chloride of this monoester and diethylcadmium.

17-Ketooctadecanoic acid. Methyl 15-bromopentadecanoate: 25 Grams of methyl hydrogen hexadecane-1,16-dioate (see above) were dissolved in 100 ml of hot methanol and one mole of potassium hydroxide in aqueous solution was added — the volume was increased to about 600 ml with water to give a clear solution of the potassium salt. This was poured into 500 ml of hot water that was vigorously stirred, simultaneously with the addition of 13.6 g of silver nitrate in 50 ml water. The precipitate was filtered, washed and dried over phosphorous pentoxide *in vacuo*. Yield 33.7 g. The dry silver salt (33.7 g) was suspended in 300 ml of dry carbon tetrachloride, about 100 ml of the solvent distilled off and 12.6 g of dry bromine slowly added [44,45]. When the reaction was complete, water was added and the carbon tetrachloride phase washed wi th alkali

and evaporated to dryness. Crystallization from methanol yielded 22.8 g of methy 15-bromopentadecanoate, m.p. 37.6—38.0°.

$C_{16}H_{31}O_2Br$ (335.3)	Calc.	C 57.30	H 9.32	Br 23.65
	Found	» 57.33	» 9.23	» 23.48

Methyl 15-iodopentadecanoate was prepared by refluxing 21.8 g of the bromo-ester with 40 g sodium iodide in 150 ml acetone for 4 hours. Crystallization from ethanol yielded 22 g of iodo-ester, m.p. 45.2—45.8°.

$C_{16}H_{31}O_2I$ (382.3)	Calc.	C 50.26	H 8.17	I 33.20
	Found	» 50.67	» 8.11	» 32.19

17-Ketooctadecanoic acid was prepared according to the general conditions used by Ställberg-Stenhagen and Stenhagen [17]. 13.87 Grams of the iodo-ester, 4.55 g ethyl acetoacetate and 15 g pulverized, dry potassium carbonate were refluxed in 100 ml of freshly distilled 2-pentanone for 24 hours; water and ether were added and the ether layer was washed with dilute acid, alkali and water. The ether solution was taken to dryness and the residue dissolved in 300 ml of methanol to which 30 g of potassium hydroxide in 30 ml of water were added. The solution was kept at 50° for 20 hours. The acid was isolated and crystallized from aqueous acetone yielding 7.7 g 17-ketooctadecanoic acid, m.p. 87.4—87.7°. The methyl ester was prepared with methanol-sulphuric acid and recrystallized from aqueous methanol, m.p. 56.1—56.5°.

17-Hydroxyoctadecanoic acid. The keto-ester was hydrogenated in methanol (150°, 150 atm., Raney nickel) and the hydroxy-ester was isolated with succinic anhydride as described by Dutcher and Wintersteiner [46]. The acid was recrystallized from aqueous acetone, m.p. 76.4—76.6° and 81.0—81.4°. Methyl 17-hydroxyoctadecanoate was obtained with diazomethane and recrystallized from aqueous methanol and from benzene-light petroleum (b.p. 60—80°).

18-Hydroxyoctadecanoic acid. The monomethyl ester of octadecane-1,18-dioic acid [47] was prepared according to Hunsdiecker *et al.*[29]. M.p. 76.5—77.5°. *Ethyl 17-carbomethoxythiolheptadecanoate.* $CH_3OOC(CH_2)_{16}COSC_2H_5$. 6 Grams of the monomethyl ester were dissolved in 25 ml of dry benzene with slight warming, 10 ml of thionyl chloride were added and the solution was left overnight at room temperature. It was then taken to dryness *in vacuo* and dissolved in fresh benzene to which 1.6 ml of pyridine and 5 ml of ethanethiol were added [48,49,50]. After 24 hours at room temperature, water and ether were added. The ether phase was washed with sodium hydroxide, hydrochloric acid and water, dried over sodium sulphate and evaporated to dryness. The yellow solid obtained (6.9 g) was dissolved in 100 ml of light petroleum (b.p. 40—60°) and filtered through a column of 20 g alumina that was then washed with 200 ml of the same solvent. All colour was retained on the alumina and 4.4 g of a crystalline solid of m.p. 44—47° were obtained. Two recrystallizations raised the m.p. to 48—49°.

$C_{21}H_{40}O_3S$	Calc.	C 67.69	H 10.82	S 8.60
	Found	» 67.89	» 10.95	» 8.33

Methyl 18-hydroxyoctadecanoate. One gram of the above thiol ester was refluxed 5 hours with approximately 10 g of Raney nickel [51] in 80 per cent ethanol. The reaction product was recrystallized 3 times from light petroleum (b.p. 40—60°) yielding very long needles of methyl 18-hydroxyoctadecanoate, m.p. 62.4—62.6°. Saponification yielded 18-hydroxyoctadecanoic acid, m.p. 99.3—99.5°. Chuit and Hausser [52] have isolated the hydroxy-acid, m.p. 96.6—97.2° (methyl ester m.p. 61.4—62°), as a by-product in the reduction of the dimethyl ester of octadecane-1,18-dioic acid with sodium and ethanol.

X-ray and thermal investigations

The X-ray work was carried out with powder methods, using copper Kα radiation (λ = 1.5418 Å, *cf.* ref.[53]). The technique was the same as that used previously in similar work [54]. The long crystal spacings were calculated from the 00*l* reflexions of diffraction patterns given by specimens deposited on glass plates by means of pressing or melting. Side spacings were calculated from diffraction patterns given by specimens contained in very thin-walled glass capillaries (Keesom-capillaries).

The crystal form found in a sample crystallized from solvents was in most cases independant of the solvent used (polar solvents such as methanol and acetone and non-polar solvents such as light petroleum of different boiling ranges were employed).

Apart from the general survey carried out at room temperature, the behaviour of all hydroxy-acids and several of the other compounds from room temperature up to the melting point has been studied by means of a continuously recording X-ray camera described by Stenhagen [24].

The melting points were taken in capillary tubes using the technique described previously [54].

We are indebted to Mrs B. Sjöbeck and Miss K. Pääbo for technical assistance and to Mrs S. Ställberg-Stenhagen for supplying several compounds used as intermediates.

This work was begun when three of the authors (S. B., G. A. E. and B. R.) worked at the Nobel Institute for Biochemistry in Stockholm and has been completed in the laboratories in Lund and Uppsala.

The work has been supported by grants from *Statens Tekniska Forskningsråd* and *Statens Naturvetenskapliga Forskningsråd.*

SUMMARY

The complete series of monoketo- and DL-monohydroxyoctadecanoic acids and their methyl esters has been synthesized. Great care has been taken to get homologue-free and pure compounds.

The polymorphism of the compounds has been studied by thermal and X-ray methods. The hydroxy-compounds are more complicated with regard to polymorphic behaviour than the keto-compounds.

The work described was originally undertaken in order to get synthetic material for the identification of hydroxy-acids derived from unsaturated fatty acids. The thermal and X-ray data given should permit the identification of any monohydroxyoctadecanoic acid, using a minimum amount of material.

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The Adsorption of S^{35} Labelled Sodium Tetradecyl Sulphate at the Solution-Air Interface of Solutions Containing Sodium Tetradecyl Sulphate and Sodium Dodecyl Sulphate

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Using surface tension-concentration measurements and the Gibbs adsorption equation the surface excess for systems containing one or two [1] surface-active substances has been calculated. In these calculations one usually approximates the activities of the solutes with their concentrations. This is perhaps accurate below the C.M.C. (critical micelle concentration) but not at higher concentrations [2]. At concentrations greater than the critical the surface excess calculated using this approximation will be smaller than the true one. This can be demonstrated by the following calculation using Gibbs' adsorption equation for one component in its exact (1) and approximate (2) forms where γ is the surface tension in dyn/cm and Γ the surface excess in mol/cm^2.

$$d\gamma = -\Gamma_a \; 2 \; RT \; \mathrm{d} \ln (f_{\pm} \cdot c) \tag{1}$$

$$\mathrm{d}\gamma = -\Gamma_c \; 2 \; RT \; \mathrm{d} \ln c \tag{2}$$

The two equations give

$$\Gamma_c/\Gamma_a = 1 + \frac{c}{f_{\pm}} \cdot \frac{\mathrm{d}f_{\pm}}{\mathrm{dc}} \tag{3}$$

where $\mathrm{d}f_{\pm}/\mathrm{dc} < 0$. This shows that the calculated surface excess (Γ_c) is smaller than the true one (Γ_a). The true surface excess can however be measured directly by application of a tracer technique using radioisotopes [3–9].

The tracer technique is as follows: Sodium tetradecyl sulphate was labelled with S^{35} and the activity from its solution was then measured with an end-window Geiger-Müller tube. Because of the relatively large range of beta particles from S^{35} one does not only measure the activity from the surface-adsorbed molecules but also from those which are

dissolved to a depth of about 0.3 mm (beta particle range in water) below the surface. To correct for this extra activity, solutions with different concentrations of the S^{35} labelled sulphuric acid used in the synthesis were prepared. Since this acid has exactly the same specific activity as the synthetized compound but is not surface-active in this concentration range [10], the activity from such a solution is the same as the activity from the interior of the surface-active solution at the same concentrations of the labelled substances when measured under the same conditions. From these two measurements the activity from the adsorbed molecules was determined.

The surface excess in mol/cm^2 was then calculated from a measurement of the specific activity, defined as the activity which one mol of the labelled substance would show under the same conditions of measurement as the adsorbed molecules.

Though the method is not yet very accurate its advantage lies in its application to systems with concentrations above the critical or to systems with many solutes. Gibbs' adsorption equation would in the first case be too inaccurate in its approximate form and in the second case it would be to complicated to handle.

EXPERIMENTAL

Pure *n*-dodecanol and *n*-tetradecanol, kindly supplied by Dr. Stenhagen of Upsala, were sulphated according to a method described elsewhere [8]. The *n*-tetradecanol was sulphated with S^{35} labelled sulphuric acid from Oak Ridge.

It is found [11] that if the alkyl sulphate contains 1 % of a normal alcohol with the same number of carbon atoms, then the alcohol will constitute 50—80% of the mixed surface film. When the C.M.C. for the soap is reached such a mixture will give a very sharp minimum in the surface tension-concentration curve [12]. Even as small amounts of alcohol as 0.05 % cause the minimum to appear [11]. The purifying of alcohol sulphates, where the most probable impurity is unsulphated alcohol, is therefore very important and the surface tension versus concentration measurements can be used as a very sensitive method for the indication of impurities.

The soap may be purified by ethyl ether extraction [12], foamextraction [13,14] or emulsion-extraction [13,14]. The two substances were extracted with diethyl ether in a micro extraction device and recrystallized several times from ethanol. As shown from the surface tension-concentration curves in Fig. 1, measured at 22 $\pm$ 2° C, the large depression in the sodium dodecyl sulphate curve has disappeared but the values are 1—3 dyn/cm lower than those of Miles and Shedlovsky [12]. Hence it is possible that traces of impurities ($\ll$ 0.05 %) remain in the soap. The sodium tetradecyl sulphate curve still shows a minimum of about 0.6 dyn/cm. It seemed to be impossible to purify this substance completely with this method. Some of the other methods may perhaps have been more effective, but because the loss of material is rather large with these methods they could not be used.

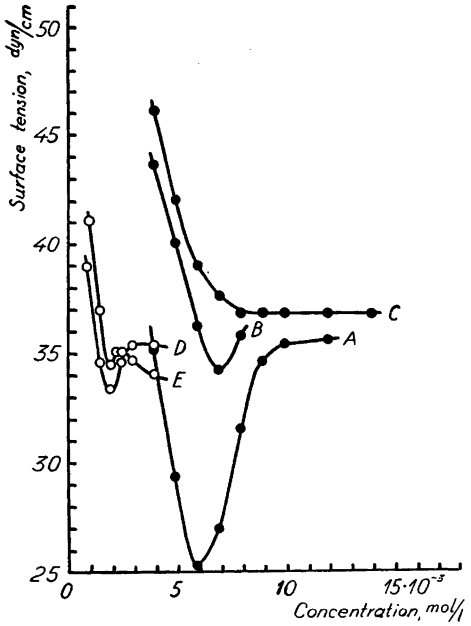


Fig. 1. Surface tension curves.
A, B and C = sodium dodecyl sulphate. (A before and C after purifying.)
D and E = sodium tetradecyl sulphate. (D before and E after purifying.)

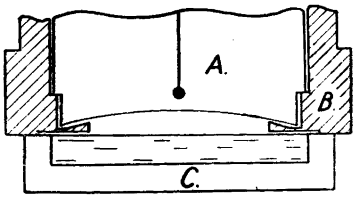


Fig. 2. The measuring device. A is the G.-M tube, B the aluminium tube and C the lucite cuve.

In the purifying process the minimum in the surface tension-concentration curve decreases and at the same time moves to higher concentrations (Fig. 1 the curves A and B). This may be explained by the fact that in the presence of an alcohol the C.M.C. is reduced [15] and that this reduction is a function of the alcohol concentration.

The measuring device is schematically shown in Fig. 2. The tube A is an Amperex 200-CB end-window Geiger-Müller tube with a window thickness of 1.4 mg/cm^2. A correction curve for the dead time of the tube was determined using J^{128} according to Kohman [16]. The tube rests on the flange of the aluminium tube B and the latter rests on the edges of the lucite cuve C. The bottom flange of the aluminium tube forms a screen for the beta particles coming from the edges of the cuve where the conditions are less defined because adsorption on the cuve may take place or the surface of the solution may be curved. The distance from the center of the curved G.-M window to the surface of the solution is 2.8 mm and the inner radius of the G.-M tube is 16 mm. With these figures and assuming that the sensitive volume of the G.-M tube starts 4 mm behind the window, the geometry is approximately 30 % (a point source in the centre of the surface of the solution according to Burtt [17]). The cuve is 2 mm deep and has a radius of 17.5 mm. It was filled to the brim with the solutions and the geometry was exactly reproduced except for small differences (about 0.2 mm) in the distance from the G.-M window to the surface of the solution. Experiments have however shown that these differences have no measurable influence upon the activity from the surface.

All measurements have been carried out in an air-thermostate, which at the same time protected the solutions from dust. With regard to the solubility of the sodium tetradecyl

sulphate, the temperature of the thermostate was held at 30 ± 1 °C, and to avoid evaporation from the solutions the air in it was kept at a relative humidity between 85 and 95 %.

In the first measurements it was found that water vapour condensed on the G.-M window and formed small droplets. To avoid this the G.-M tube was heated with a spiral of kanthal wire to 5° C above the temperature of the thermostate. This is very important because a water film of only 0.5 μ thickness reduces the activity by 1 %. Macaulay and Carson [18,19] have found that when, as in this case, the mica surface is not clean or the water vapour pressure is close to the saturation pressure it will be covered with a water film. The thickness of this film may however be regarded as constant under constant humidity and temperature, which is not the case for the film of water droplets. These droplets were never observed, when the tube was heated.

It was found that the activity from the solutions of sodium dodecyl sulphate and sodium tetradecyl sulphate decreased with time and after about 60 minutes reached a constant value, which was between 93 and 96 % of the initial one. The cuve was covered the whole time by the G.-M tube. If however the tube was taken away from the cuve the activity increased in five minutes to its initial value. If the cuve was covered with a glass plate, when the tube was taken away, the activity had not changed when measured again after five minutes. This indicates that the decrease in activity does not depend upon water vapour condensation on the G.-M window but has something to do with the evaporation from the surface of the solutions. This was supported by the fact that in all cases when water vapour condensed upon the glass plate the activity had increased when measured again after five minutes. These effects were not found in the measurements on S^{35} labelled sulphuric acid. Lottermoser and Stoll [20] have found that the surface tension of sodium tetradecyl sulphate at constant concentration decreases with decreasing temperature and from their curves it is seen that $d\gamma/dc$ at the same time increases. But an increase in $d\gamma/dc$ and a decrease in temperature gives, according to Gibbs' adsorption equation an increase in the surface excess and consequently an increase in the activity from the solution. This may explain the effect of increasing activity because evaporation from the solution decreases the temperature of the surface layer. This effect may also influence surface tension measurements and give lower absolute values than those obtained from a solution in equilibrium with its saturated vapour. The evaporation effect here discussed was obtained although the relative humidity in the air thermostate was between 85 and 95 %. Care was taken not to make measurements before equilibrium was obtained (*i.e.* constant value of the activity).

Before each measurement of the activity the surface of the solution was cleaned with the aid of a platinum wire mesh. The wire-mesh was put below the surface and then raised slowly. When the mesh broke the surface, the surface layer adhered to it and was

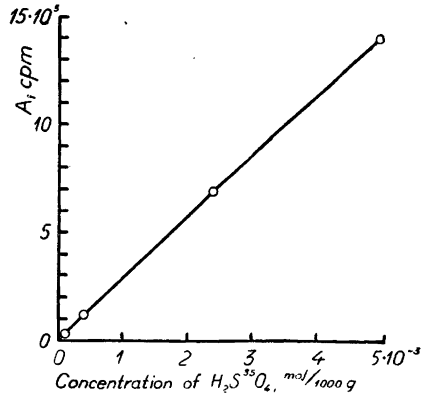


Fig. 3. The activity from the interior of the solution.

thus easily removed. A fresh prepared solution generally gave the lowest values of activity. When used several times the activity from the surface increased, sometimes as much as 20 %, perhaps due to the acumulation of impurities. Cleaning of the surface had in these cases only a little effect. For this reason a solution could be used for no more than one single measurement.

To determine the activity from the interior of the solutions different concentrations of the S^{35} labelled sulphuric acid used in sulphating the alcohol was carefully titrated and the activity measured in the same way as the activities from the surface active solutions. The activity versus concentration curve was almost a straight line (Fig. 3). The activity from the surface calculated as the total activity minus the activity from the interior of the solution has in these experiments only been between 4 and 19 % of the total activity.

To determine the specific activity as earlier defined 0.1 ml of a 0.110 *M* solution of the S^{35} labelled sulphuric acid was evaporated in the centre of a lucite plate and the activity measured. In this measurement the lucite plate replaced the cuve in the measuring device and had its surface at the same level as that of the solutions. The specific activity so measured was $2.39 \cdot 10^{11}$ cpm/mol.

The question now is if this measuring technique gives the specific activity according to the definition on page 2. For this to be true the measuring conditions for surface adsorbed molecules and the sulphuric acid molecules must be the same. The specific activity of the surface adsorbed and the sulphuric acid molecules is the same and they are measured in the same geometry in the same measuring device. The surface adsorbed molecules form an infinitely thin layer on a water layer as backing material whereas the layer of sulphuric acid is formed of droplets with an estimated thickness of about 1 μ on a backing layer of lucite. The thickness of the backing material is in both cases enough to give saturation backscattering. The average atomic number of water is 7.2 and of lucite 6.2. The backscattering factors are therefore almost the same. The monolayer may be considered to have no self-adsorption and no self-scattering. This is however not the case for the sulphuric acid layer.

If it were possible to make thin ($d < 1.5$ mg/cm^2) uniform layers of the S^{35} labelled sulphuric acid the value of the specific activity at $d = 0$ ("zero" thickness) could be found from an extrapolation of a specific activity versus d curve. At present only an estimation can be made.

When the surface density of the source is below the order of 5 % of the beta particle range (in this case 1.5 mg/cm^2) the specific activity in cpm/mol is found to increase instead of decrease with increasing surface density [16,21]. This means that the measured cpm/d versus d curve passes through a maximum. This phenomenon is termed self-scattering [16]. It depends upon the forescattering effect as defined by Zumwalt [22] and upon a difference in backscattering power between the source and the source supporting material. The forescattering effect is a focusing effect of the beta particles in the direction of the tube from an absorbing layer on the top of the active layer. The increase in counting rate due to this effect is at small surface densities greater than the decrease due to absorption in the focusing layer. The forescattering effect increases with increasing atomic number of the source. The backscattering effect also increases with the atomic number of the backing material. Because the average atomic number of sulphuric acid is 10.4 whilst that of the lucite plate only 6.2 a layer on the top of the source may, because of backscattering, show a greater activity than one on the bottom. So in this case we may expect that the forescattering effect and the increase in backscattering makes the measured specific activity slightly higher than that measured from an infinitely thin layer, but it is not possible to estimate the increase.

All activity values have been corrected for background, decay and dead time of the tube. The background was measured with the cuves filled with water.

The surface excess can then be calculated from the following equation also used by Aniansson [8]:

$$\Gamma = \frac{A_y}{s \cdot 2\pi \int_0^r r\ \eta\ (r)\ \mathrm{d}r} = A_y \cdot 0.01 \cdot 10^{-10}\ \text{mol/cm}^2 \qquad (4)$$

where A_y is the activity from the surface in cpm, s the specific activity in cpm/mol measured at $r = 0$, where r is the radius of the surface from which the activity is measured, and $\eta\ (r)$ is the counting efficiency of the tube as a function of r, measured with the aid of a point source (Fig. 4). The integral was calculated numerically.

The surface tension measurements were made by means of the ring method using a du Nouy tensiometer. The values were corrected according to Harkins and Jordan [23]. In all solutions (also in the radioactive measurements) water, purified using a mixed bed ion exchange column [24] and having a specific conductivity of about $8 \cdot 10^{-7}$ ohm^{-1}cm^{-1}, was used. Since only relative values are needed it was sufficient to ensure that the surface tension measurements were made in a reproducible manner with a definite time of waiting before each single measurement.

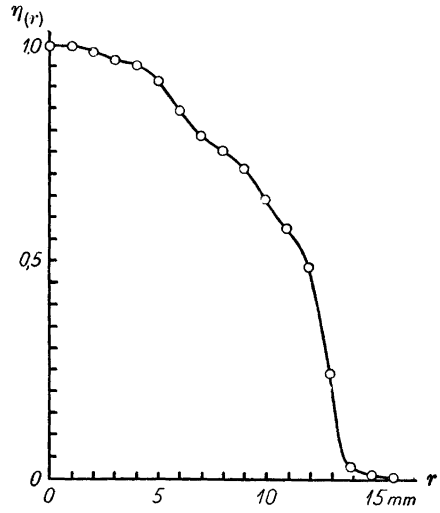


Fig. 4. The counting efficiency.

A few measurements of conductivity using a Philip's measuring bridge and a small conductivity cell have also been made.

RESULTS

In one series of measurements the total concentration of dodecyl and tetradecyl sulphate was constant ($5 \cdot 10^{-3}$ mol/1 000 g) throughout, but the proportions between the two substances varied. The activity values for different concentrations of surface active agent are given in Table 1, and in Table 2 for diff. conc. of S^{35} labelled sulphuric acid.

Table 1. Activity values from the first series of measurements.

C_{14} mol/1000 g	C_{12} mol/1000 g	$A_y + A_i$ cpm	A_y cpm	Rel. error in A_y %
$0.32 \cdot 10^{-3}$	$4.69 \cdot 10^{-3}$	1144 ± 2	154 ± 8	5.2
0.60	4.40	2068 ± 3	228 ± 10	4.4
0.90	4.10	3043 ± 3	328 ± 11	3.4
1.20	3.80	3982 ± 4	382 ± 12	3.1
1.60	3.40	5205 ± 4	455 ± 13	2.9
1.90	3.10	6121 ± 4	501 ± 15	3.0
2.04	2.96	6650 ± 5	640 ± 15	2.3
2.18	2.82	7075 ± 5	655 ± 16	2.4
2.51	2.50	8055 ± 5	705 ± 17	2.4
3.10	1.90	9817 ± 6	817 ± 19	2.3
3.11	1.89	9918 ± 6	878 ± 19	2.2
4.00	1.00	12521 ± 7	1041 ± 20	1.9
4.50	0.50	14037 ± 7	1187 ± 20	1.7

C_{14} = sodium tetradecyl sulphate. A_y = activity from the surface.
C_{12} = sodium dodecyl sulphate. A_i = activity from the interior of the solution.

Table 2. The activity from the interior of the solution.

$H_2S^{35}O_4$ mol/1000 g	A_i cpm
$0.110 \cdot 10^{-3}$	355 ± 2
0.396	1203 ± 7
2.380	6975 ± 16
4.948	14077 ± 23

These values are shown in Fig. 3. The curve so obtained was used in calculating the values of A_y in Table 1. The surface excess of sodium tetradecyl sulphate calculated from equation (4) is shown in Fig. 5.

In the second series of measurements the concentration of sodium tetradecyl sulphate was $0.5 \cdot 10^{-3}$ mol/1 000 g throughout and the concentration of sodium dodecyl sulphate was increased from 0 to $9 \cdot 10^{-3}$ mol/1 000 g. A_i is in this case constant. Its value (1617 ± 9) is taken from Fig. 3. Table 3 gives the activity values in this case.

Table 3. Activity values from the second series of measurements.

C_{12} mol/1000 g	$A + A_y$ cpm	A_Y cpm	Rel. error in A_y %
$0.00 \cdot 10^{-3}$	2010 ± 3	393 ± 10	2.5
2.01	1933 ± 3	316 ± 10	3.2
3.91	1906 ± 3	289 ± 10	3.5
5.05	1813 ± 3	196 ± 10	5.1
5.99	1785 ± 3	168 ± 10	6.0
6.95	1738 ± 3	121 ± 10	8.3
7.98	1716 ± 3	99 ± 10	10.1
8.97	1685 ± 3	68 ± 10	14.7

The surface excess of sodium tetradecyl sulphate calculated from equation (4) together with a surface tension and a specific conductivity curve are given in Fig. 6.

DISCUSSION

The C.M.C. for sodium dodecyl sulphate is about $6 \cdot 10^{-3}$ mol/1 and for sodium tetradecyl sulphate about $2 \cdot 10^{-3}$ mol/1 at 30° C. Fig. 5 shows that the surface excess of sodium tetradecyl sulphate increases greatly when its proportion in the mixture increases and that this is the case also when the C.M.C. for the mixture is passed. The largest surface excess measured gives an area of 12.7 Å^2 per molecule of tetradecyl sulphate. Because dodecyl-sulphate molecules also are present the true area per molecule will be smaller.

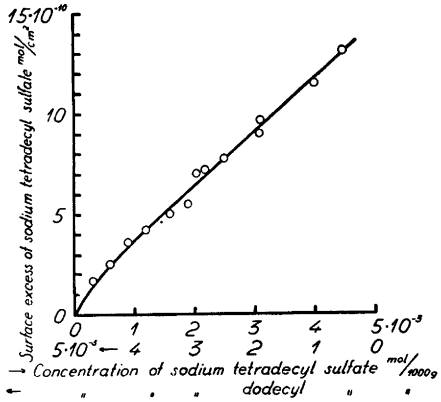


Fig. 5. The surface excess of sodium tetradecyl sulphate measured on a solution of this substance and sodium dodecyl sulphate. The total concentration was constant = 5 · 10⁻³ mol/1000 g.

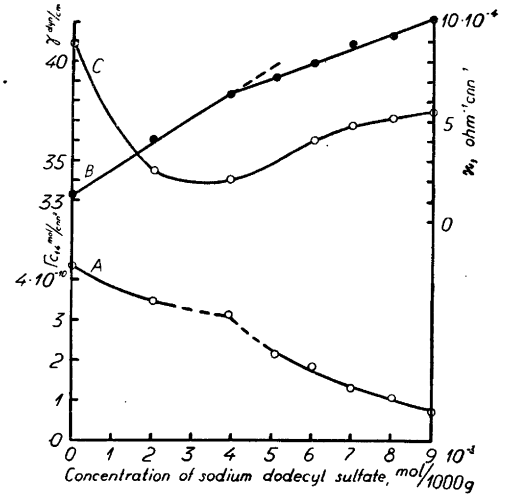


Fig. 6. The concentration of sodium tetradecyl sulphate is constant but the concentration of sodium dodecyl sulphate increases.
A = surface excess of sodium tetradecyl sulphate.
B = conductivity curve.
C = surface tension curve.

Since a molecule with a straight carbon chain in a monolayer occupies an area not smaller than 20.5 Å² the result will indicate that more molecules than the number needed to form a monolayer are present in the surface. The same was found by Dixon *et al.* concerning di-*n*-octyl sodium sulphosuccinate [3] and Aerosol SE[9].

The surface tension and the conductivity curves in fig. 6 indicate that the C.M.C. for sodium dodecyl sulphate in this case appears between 3 and 4 · 10^{-3} mol/1 000 g due to the presence of tetradecyl sulphate. The surface excess curve shows that as the concentration of dodecyl sulphate is increased the concentration of tetradecyl sulphate in the surface decreases and that this is more pronounced when the C.M.C. is passed. The latter statement is however only based on one measurement. What happens may be the following:

As the concentration of dodecyl sulphate increases it will replace tetradecyl sulphate in the surface. The total amount of surface active molecules in the surface increases however with the bulk concentration and so the surface tension decreases. When the C.M.C. for the mixture is reached micelles begin to form and tetradecyl sulphate is solubilized in these micelles. The result

of this is that the surface excess of tetradecyl sulphate decreases faster, while the conductivity increases slower than before, and the surface tension begins to increase up to values corresponding to a solution containing only dodecyl sulphate.

The statistical errors in the activity from the surface varied between 1.7 and 5.2 % in the first experiment and between 2.5 and 14.7 in the second one. These large errors are essentially due to the small activity from the surface compared with the total activity from the solution. It would have been possible to make these errors smaller by measuring the activities for longer times. Such an increase in accuracy was not possible however because the measuring conditions were not constant enough over long periods of time. In this work the times of measurement were not longer than 4 hours, the measuring device being checked with a reference source before every measurement.

The errors can be made smaller by increasing the activity from the surface relative to the total activity. This is attained by using a beta emitter of lower energy and a more surface active substance with greater specific activity. A calculation has shown that the use of tritium instead of S^{35} in this case would have increased the activity from the surface from on the average 10 % to 80 % of the total activity. The relative mean errors in the surface excess calculated from equation (4) are estimated to be 2—3 % greater than the relative errors in the activity from the surface.

SUMMARY

The surface excess of S^{35} labelled sodium tetradecyl sulphate has been measured at the solution-air interface of solutions containing this substance in the presence of sodium dodecyl sulphate.

The measurements show that the surface excess increases greatly when the proportion of sodium tetradecyl sulphate in the mixture increases and that this is the case also when the C.M.C. for the mixture is passed. They also show that at higher concentrations of sodium tetradecyl sulphate more molecules than the number corresponding to a monolayer are present in the surface, and that if the concentration of sodium tetradecyl sulphate is constant but the concentration of sodium dodecyl sulphate increases then the surface excess of the former substance decreases.

Also the experiments show that the radiotracer method can be used to determine quantitatively the surface excess of a selected substance in a mixture.

The authors have to express their thanks to Mr. G. Aniansson for valuable discussions, to Mr. I. Andersson for help in preparing and purifying the substances and to The Swedish Atomic Committee for financial support.

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Gradient Elution Analysis

II. Oligosaccharides

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During the last decade the study of chromatographic methods has made a most important contribution to analysis problems in sugar chemistry. A monograph [1] and several reviews have described the progress in this field [2, 3]. Different approaches have been made, using substituted or unsubstituted sugars, and both partition and adsorption methods have been applied. In adsorption systems both a polar adsorbent, as a clay [4], in a liquid less polar than water, and a nonpolar adsorbent (carbon) in water have been used. Especially for monosaccharides and the lower oligosaccharides both the qualitative separation and the quantitative analysis have been very successful.

For the higher oligosaccharides no good quantitative method has been published so far. Tiselius and Hahn [5] used displacement adsorption analysis for the resolution of a dextrinhydrolysate, but especially for the higher sugars the recovery was low due to irreversible adsorption. This was overcome by pretreating the carbon with a low concentration of the displacer. It was observed that by a stepwise increase in displacer concentration employed in the pretreatment, the sugars could be selectively washed from the column as elution peaks. Whistler and Durso have worked out a stepwise method, using etanol as an eluting agent [6], and Baily, Whelan and Peat have used this method for isolation of oligosaccharides with up to six glucose units [7]. A disadvantage of these methods is that the intermixing between adjacent components can be rather large and components present in small amounts can not easily be detected. Hough, Jones and Wadman mention [8] that they have applied paperchromatographic methods with success to the analysis of oligosaccharides

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without giving detailed results. Recently there has appeared a work by Jeanes, Wise and Dimler [9] describing a paper chromatographic method which is capable of resolving mixtures of oligosaccharides up to ten glucose units in a single molecule. No quantitative use of the method has yet been reported.

In the method for chromatographic analysis of oligosaccharides presented here a continuously increasing concentration of ethanol in water is applied to the saccharides which have been previously adsorbed on a carbon adsorbent. An earlier paper has discussed some of the general features of this technique [10], for which the name gradient elution analysis has been proposed.

On applying a concentration gradient of an eluting agent to a mixture of oligosaccharides adsorbed on carbon the higher members were only eluted with large losses of material, and as in displacement analysis this was overcome by pretreatment of the carbon. Such a pretreatment has been discussed earlier by other workers [11]. Thus Synge and Tiselius [12] used stearic acid in some model experiments with amino acids and peptides. The same compound has been tried with success in this work but employing a higher load. In addition to its advantageous effect on the adsorption properties of carbon a great convenience of the stearic acid pretreatment is that the compound is not dissolved from the carbon at moderate concentration of ethanol in water and so no contamination of the effluent occurs. The combination of pretreatment and a concentration gradient proved to be a most efficient method for the quantitative resolution of mixtures of oligosaccharides.

EXPERIMENTAL PART

Specimens of unhydrolysed and partially hydrolysed α-Schardingerdextrin were used as test substances. The author is grateful to Professor K. Myrbäck and Mrs. M. Jaarma Phil. Mag., Stockholms Högskola, for these samples. The dextrin is supposed to consist of a cyclic combination of six glucose units and the hydrolysate of it was considered to be a mixture of mono- to hexasaccharides and unchanged dextrin. (After some time it was observed that bacterial growth had developed in one of the samples.)

Three different carbons were tried as adsorbents. Carboraffin C, Norite FNX Special and Darco G-60. The carbons were usually mixed with an equal weight of Celite (analytical grade) and the pretreatment carried out by saturation in a column with the stearic acid solution in 96 % ethanol. The carbon was pressed out of the container and freed from adhering solution on a suction filter. It was stirred on the filter with 50 % ethanol, sucked dry and the operation repeated with 20 % ethanol. It was stored in glassbottles with 10 % ethanol. Negligible amounts of stearic acid were given off from the carbon by treatment with 50 % ethanol.

The adsorbent containers were made of Perspex. The carbon-Celite mixture was poured into the container in a thick slurry and allowed to settle under slight suction, afterwards it was pressed somewhat together with a stamper. After washing the column with water the surface was made as even as possible and sucked dry before applying

the solution to be analyzed, this was fed from an "Agla" micrometer syringe. The solution was sucked into the column under slight suction and followed by the same volume of distilled water, usually 0.3 ml. The empty space up to the top of the column, ca. 10 mm, was packed with moistened Celite which protected the adsorbent from being disturbed by the moving rod in the mixing chamber. The rate of flow through the column was kept at 8–10 ml/hour by applying an air pressure of ca. 0.2 atm.

The making of the gradient is described in detail in the previous paper [10] on gradient elution analysis. A "Shandon" automatic fraction collector was employed for collecting the fractions. It works on a weight basis and is equipped with weight-calibrated tubes. The smallest volume which could be used with some accuracy was 0.5 ml.

The orcinol-sulfuric acid reaction was used for the analysis of the fractions. The reaction is treated extensively by Sörensen and Haugaard [13], but the procedure suggested was somewhat modified in order to get a more rational method for the great number of fractions to be analyzed. The following reagents were required:

a) 15 g orcinol was dissolved in 500 ml water and added to a cooled mixture of 300 ml water and 200 ml conc. sulfuric acid.

b) 1800 ml conc. sulfuric acid was mixed with 1200 ml water and cooled.

In performing the analyses 60 ml of a) was mixed with 450 ml of b) and an aliquot of it was added to each tube with an automatic pipette, 8.0 ml of this mixture to each fraction of 0.5 ml. The volume in the tubes was too large to allow mixing by shaking and it was inconvenient to turn the tubes because the rim was apt to be contaminated with the strong sulfuric acid reagent. In order to overcome this difficulty a method of simultaneous mixing of the ingredients of all the tubes with glass rods was devised. Each series of measurements was accompanied through all operations by a set of control tubes containing known amounts of sugars (different dilutions of the solution applied to the column for analysis) which served as calibration points for the analytical procedure.

The tubes were heated for 15 min. in a boiling water bath, cooled down to room temperature in tap water and the extinction measured at 500 mμ in a Beckman spectrophotometer model B. This was equipped with an instalment for reading the tubes directly. The "Shandon" tubes were not fitted for immediate use in a spectrophotometer as they had heavy walls of somewhat uneven character. The simple devise of observing each tube through a complete rotation and then taking the smallest extinction as the real value was shown to be satisfactory. Tubes giving larger deviations from the mean spectrophotometer reading than ± 1.5 % by control with a solution of optical density 0.7 were not used.

The accuracy of the analytical procedure was limited by an absolute uncertainty in the spectrophotometer reading of about ± 0.01 corresponding to ca. 0.003 mg glucose and a relative error of about 5 %, the main sources of error being the uneven character of the tubes used and the variation in the fraction sizes which could amount to ± 10 %. This latter factor had an effect because the reaction was sensitive to the sulfuric acid concentration. The varying ethanol concentration in the fractions was shown to have only a very slight effect.

In the work of Sörensen and Haugaard [13] it is demonstrated that in lower oligosaccharides the monosaccharide unit gives the same analytical value as the monosaccharide itself, when correction is applied for the water taken up by hydrolysis. This was not given any further trial for the sugars analysed here and the analytical figures for the different peaks in the diagrams refer to amount of sugar calculated as glucose. The control solutions were made up from the solutions applied to the column.

The total work-time required for the chromatographic analysis of a sugar mixture using 100 fractions was about 5 hours.

RESULTS AND DISCUSSION

The general mode of operation of "gradient elution analysis" is treated in a previous paper [10] and will not be discussed here.

Five different preparations of α-Schardingerdextrin have been employed in the experiments reported below. They are designated here: 1) *Hydrolysate I*, which was contaminated by bacterial growth. 2) *Hydrolysate I, f 6*, a fraction obtained from Hydrolysate I by displacement adsorption analysis [5] and containing tetra- and penta-saccharides. 3) *Hydrolysate I, M*, a mixture of fractions from displacement analysis of Hydrolysate I containing reduced amounts of the lower sugars, 4) *Hydrolysate II*, obtained by hydrolysis of a highly purified dextrin. 5) *Unhydrolysed α-Schardingerdextrin.*

The hydrolysate is supposed to contain a mixture of mono- to hexa-saccharides and unchanged dextrine *i.e.* seven components and the chromatographic analysis shows the presence of these seven components. The α-Schardingerdextrin is the most strongly adsorbed as is seen by comparison of the experiments recorded in Fig. 2 H and I. So far no identification of the other peaks has been done but it seems clear that they leave the column in the order of increasing molecular weight [7].

Different degrees of saturation with stearic acid were tried in the pretreatment. The degree of saturation finally adopted was that obtained by equilibrating the adsorbent with 1 % stearic acid in 96 % ethanol. In Fig. 2 A and B is shown the effect of pretreating with a 0.2 % stearic acid solution (80 mg of stearic acid being adsorbed pr. g adsorbent). However, equilibrating with a 1 % solution (150 mg stearic acid adsorbed pr. g adsorbent) was better for the recovery of the highest sugars. With this pretreatment the carbon still had a high adsorption activity and separation power. Thus even the disaccharide had a curved isotherm in water as judged from Fig. 2 G, which shows that the elution peak of this saccharide is of an unsymmetrical form, corresponding to a curved isotherm under the condition of the elution, *i.e.* a very slight butanol gradient.

In the calculation of the recovery the large number of fractions should diminish the analytical error described above. On the other hand an exact determination of the baseline is difficult and small uncertainties in its position can cause slight errors in the recovery figures. Although the recovery cannot be rigidly controlled because of this uncertainty it is believed that there is substantially no loss of material during the passage of the sugars through the column.

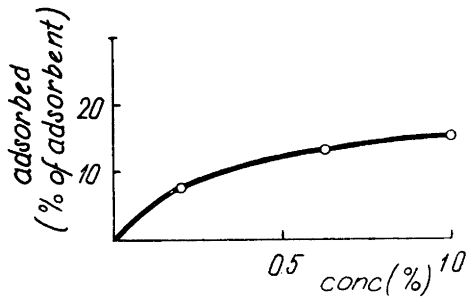


Fig. 1. Adsorption isotherm of stearic acid in 96 % ethanol on Darco G-60.

From the adsorption isotherm of stearic acid in 96 % ethanol of Darco G-60, shown in Fig. 2, it is seen that a concentration of 1 % of stearic acid gives a high degree of saturation of the carbon surface. One might expect that the stearic acid in addition to neutralizing the most active spots, modifies the properties of the whole surface. On the basis of the present experiments it is not possible to say what influence the pretreatment may have on the specificity although such influences might well be expected. The pretreatment of carbon should be compared with the deactivation of polar adsorbents by water as discussed for example by Stewart [14].

Later work in this laboratory has shown that the general effect of different saturators on carbon is much the same [15].

The stearic acid treatment caused depressed wetting properties of the carbon and flocculation of the carbon particles. This was to some degree counteracted by mixing the carbon with Celite and by adding ethanol to the adsorbent slurry before packing of the columns but clusters of flocculated particles must still be expected. This will have a bad effect on column efficiency and the spreading of zones. Pretreatment with a compound which produces, in addition to the deactivation characteristic of stearic acid, improved suspension properties of the carbons, should be expected to improve the column efficiency.

The pretreated carbon can be used repeatedly in chromatographic runs without noticeable changes in properties. The experiments recorded in Fig. 2 G and H are the 4th and 5th respectively carried out on the same column. Between the runs the column was washed with 33 % ethanol and water.

Other eluting agents than ethanol were tried. Propanol and metyletylketone gave very similar results. Their eluting power relative to ethanol was ca. 2 and 5 respectively. Normal butanol was too little soluble in water to be usable as is seen from the experiment recorded in Fig. 2 G, where a nearly saturated n-butanol solution in water is fed to the mixing chamber. The approximately straight lines drawn in Fig. 2 G and I denote the concentration in effluent of butanol and ethanol respectively calculated as described [10]. The

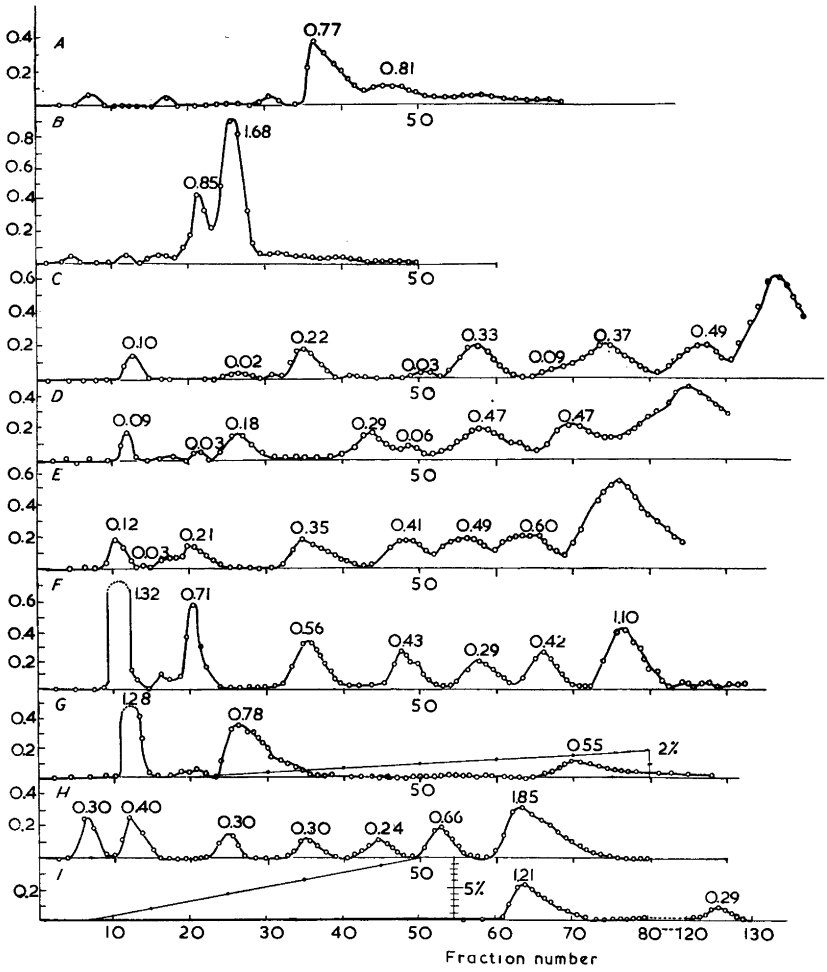


Fig. 2. Adsorbent: A, B, E, F, G, H, I, Darco G-60 + Celite 1/1: C, Carboraffin C + Celite 1/1; D, Norite FNX Special + Celite 1/1.

Column size: A, B, 8 × 60 mm; C, D, E, F, G, 8 × 130 mm; H, I, 10 × 200 mm.

Pretreatment: A, none; B, equilibrated with 0.2 % stearic acid in 96 % ethanol; C, D, E, F, G, H, I, equilibrated with 1 % stearic acid in 96 % ethanol.

Substance analysed: A, 3.0 mg, B 3.2 mg. Hydrolysate I f 6; C, D, E, 5.0 mg. Hydrolysate I M; F, G, 5.0 mg Hydrolysate I; H, 4.20 mg. Hydrolysate II; I, 1.50 mg. Unhydrolysed-α-Schardingerdextrin.

Volume of mixing chamber: A, B, C, D, E, F, G, 62 ml.; H, I, 256 ml.

Solution fed to mixing chamber: A, B, 50 % ethanol; C, D, E, F, H, I, 30 % ethanol; G 5.6 % (by weight) n-butanol. In I changed to 96 % from the 98th fraction. Fraction size: A, B, C, D, E, F, G, 0.50 ml.; H, I, 2.1 ml.

Recovery: A 57 %; B 94 %; F 98 %; H 97 %; I 100 %.

The dead volume is equivalent to the retention volume of the front of the monosaccharide peak. The amount of sugar is designated by a number (mgs.) above each peak.

butanol values are corrected for the observed retention volume in addition to the dead volume of the column. The circles denote analytical values found by examining the effluent with an Abbe refractometer. It is seen that ethanol has a negligible retention volume on the pretreated carbon, while butanol, though it has a noticeable retention volume, must have a linear adsorption isotherm in concentrations up to 2 %.

A component travelling in an eluting agent of increasing concentration will move through the column with an increasing speed [10], and the volume of solvent in which it leaves the column is dependent upon the mutual relationship, betwen the gradient slope and column size. The conditions are usually so chosen that the sugars will leave the column when travelling with low adsorption and consequently in a small volume. The importance of this point for the quantitative estimation of the component is seen by a comparison of the trisaccharide peak in Fig. 2 F with that in Fig. 2 G.

The three different carbons employed showed a marked difference in specificity with regard to the α-Schardingerdextrin as is seen from the relative positions of the dextrin peak in Fig. 2, C,D,E. When Darco G-60 is used, the dextrin appears after and separated from the hexasaccharide while on Carboraffin C it appears close to and partially mixed with the pentasaccharide. Norite FNX Special has an intermediate effect. *Hydrolysate I*, which was contaminated by bacterial growth contains some additional compounds in small amounts. The full resolution of these trace components has not been attempted, and they are probably to some degree the cause of the "tailing" exhibited by some of the peaks in Fig. 2 F being partially mixed with the main components.

In Fig. 2 H and I are shown the results of analyses of *Hydrolysate II* and *Unhydrolysed α-Schardingerdextrin*. The second peak in Fig. 2 I is not identified, but is probably the β-Schardingerdextrin. It should be noted that the solution fed to the mixing chamber is changed to 96 % ethanol after the 97 th fraction. The fractions 81—119 which are not plotted in the diagram, contained no sugars. (Because of the large fraction sixe in these runs aliquotes were pipetted out for the quantitative analysis.)

It seems that the application of "gradient elution analysis" in combination with pretreatment of the carbon adsorbents offers great possibilities for the quantitative resolution of sugar mixtures including those containing much larger molecules than present in the α-Schardingerdextrinhydrolysate. The work described here obviously can be subject to several refinements in technique such as an improvement in column efficiency and a more accurate quantitative analysis of the fractions.

SUMMARY

A new method for the quantitative chromatographic analysis of mixtures of oligosaccharides is described.

The method is characterized by the application of a continuously increasing concentration of ethanol in water, as an eluting agent, applied to a mixture of sugars adsorbed on a carbon adsorbent which has been pretreated with stearic acid.

The analysis of a hydrolysate of α-Schardingerdextrin containing a mixture of mono- to hexa-saccharides and unchanged dextrin is described.

The author wishes to thank Prof. A. Tiselius for the privilege of carrying out this work in his laboratories and for the kind interest he has shown in it. The investigation is financially supported by the Swedish Natural Science Research Council.

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Sheet Ion Exchangers

I. General Aspects of Synthetic Ion Exchangers in Sheet Form

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During the development of experimental methods for beta radiography [1] there arose a definite need for a beta radiation source, which possessed a very uniform emissive power over an area of a few sq.cms. At that time this problem of producing an extended beta radiation source seemed to have no general solution. As most radioactive materials are supplied as inorganic ions, it was tried to obtain *ion exchanging* substances in a suitable form *e.g.* as thin *plane parallel sheets* (instead of usual grain form).

Experiments in this direction were successful in May 1949 [2], when it was observed, that after the immersion of plates, rods or sheets of commercial *polystyrene* in chlorosulphonic acid [3] at room temperature the surface layer had been converted into polystyrene sulphonic acid, which has ion exchange properties (due to the sulphonic acid group). This layer was able to sorb cations in much the same way as other ion exchangers in the usual grain form and the original need for a uniform extended beta source was met by the sorption of radioactive ions, but soon other applications were also found [2]. (Beischer [4], faced with similar problems, has used Langmuir-Blodgett multilayers for their solution *e.g.* radioactive Ca^{45} as calcium stearate).

It is proposed to discuss briefly aspects of nomenclature, preparation methods, properties and applications of this new type of ion exchanger. It is hoped later to publish a series of papers on special topics of this field.

NOMENCLATURE

The above-mentioned layer of ion exchanger (mostly 0.1—200 μ thick, corresponding to 0.05—100 μeq/cm^2) is lying directly on the untreated section of the original material, which in general has large dimensions. In many

cases it is easy to tear off the exchanging layer or to dissolve the untreated high polymer, getting the pure ion exchanger free for various purposes. During our later developments this technique has appeared more and more important and the term "*sheet ion exchanger*" (swedish: skiktjonbytare) seems suitable and will be used in the following discussion even when the layer is not separated from the untreated body. (Originally the term "surface ion exchanger", swedish "ytjonbytare" [2] was used, but is now abandoned to avoid confusion with true surface ion exchange phenomena). An ion exchanger of this sheet type is most probably identical to the usual grainy substances with regard to chemical composition and ion exchange capacity per gram of material but differ chiefly in physical form (and also in special properties *e.g.* diffusion rate for ions as well as water, mobility of sorbed ions and swelling properties). Since the new form is, however, of great experimental importance, making possible new ways of studying ion exchange phenomena and new applications (see below), it therefore seems justified to introduce a special nomenclature. The special criteria of the sheet ion exchangers (in addition to insolubility) should be:

1. Two dimensions (*e.g.* length and width for plane sheets) many times greater than the third *i.e.* the thickness (the sheets may of course be curved in one or two dimensions e.g. cylindrical and spherical surfaces).

2. The substance should be homogeneous in all its properties especially in composition and ion capacity calculated per area as well as per mass unit.

3. Complete coherency with no macro- or microstructure down to 5 μ or preferably to the limit of optical resolution.

More special points of view relating to experimental requirements are: (a) High ion exchange capacity per mass unit (1—11 meq/gram of dry material), (b) only functional group (*e.g.* $-SO_3H$, $-COOH$, $-OH$, $-NH_2$ and amino derivatives). Further the substances should preferably withstand temperatures up to 100° C, chemical attacks and be colorless and transparent to light. Most of the visualized applications require a thickness of about 0.1—1 000 μ.

There exist some substances both natural and artificial, which partially fulfil our criteria *e.g.* layers of zeolites, clays, artificial mineral membranes [5], feldspar surfaces, micas, glasses, organic materials such as cellulose derivatives and gelatin. Many of these are lacking either in homogeneity or coherency or they have so low a capacity per unit area (at reasonable thickness) that their use for our purposes is in most cases doubtful.

During the development of the sheet ion exchangers (since 1949) some papers have been published which contain similar trends to our works: Wyllie[6], Juda [7] and Kressman [8] have taken the logical step from Marshalls artificial mineral membranes [5] made for membrane potential studies, to make membranes

containing synthetic organic ion exchangers for the same purposes. From Wyllie's paper it is clear that plastic binders (30—80 %) are used and that a mosaic structure is present and thus these membranes are supposed not to be homogeneous in structure and properties. Juda's paper [7] states that the membranes are "coherent" and of "high conductivity" but does not give enough details to permit a discussion of them from our point of view. Kressman's membranes seem to have very interesting properties, but before the preparation procedures have been published their relations to the present sheets cannot be discussed. — It is felt that all membranes made for potential studies, including both inorganic and organic ones, are of a thickness (mostly 1—5 mm) which is too great for our visualized applications, but it is possible that they will in future prove to be for great interest even outside the potential studies.

PREPARATIONS

Many high-polymers can be treated to yield sheet ion exchangers. Of special importance are those, which yield colorless, transparent products *e.g.* polystyrene and polyacrylates and their derivatives.

On sulphonation, polystyrene plates, rods or sheets from commercial sources give satisfactory sheet cation exchangers. Sheets of polystyrene gave, for instance, after 1 minute's immersion in chlorosulphonic acid at 20°, 15 μ thick layers on each side of an unsulphonated section, the three layers being easily separable after immersion first in benzene for some minutes and then in water. The thickness is varied by altering the time of contact and the temperature. The sulphonation is also easily made with fuming sulphuric acid at room temperature.

The mechanical stability of the sheets affords the greatest problem. Due to swelling (especially in distilled water) the sulphonated layer flakes off, when it is too thick probably due to the very small cross-linkage of the high polymer used (commercial thermoplastic material). Still it is possible with some care, to get large sheets of up to 30—40 μ thickness (10 × 10 cms are easily made). After preliminary experiments, copolymerized products of styrene-divinylbenzene *i.e.* with a small but definite degree of cross-linkage yield much better results.

After partial sulphonation the boundaries can be easily detected, because the treated part is highly hydrophilic and thus wetted, whereas the untreated part is hydrophobic and not wetted. A valuable test method is to measure the electrical resistance between two points, say 5 mm apart. The untreated surface has a resistance much higher than 10^{10} ohm whereas the sulphonated surface only 10^{5}—10^{6} ohm. Another method is to sorb nickel ions, rinse with distilled

water and develop with dimethylglyoxime when the red colour immediately indicates where the surface has developed ion exchange properties. The separated product showed a capacity (within the experimental error) similar to that of polystyrene monosulphonic acid (5.4 meq/gram).

"Surface hydrolysis" of polyacrylate plates (Lucite, Perspex *etc.*) with alcoholic alkalies yield a hydrophilic layer of polyacrylic acid (or derivatives). For example, boiling a Lucite plate in a 30 % solution of KOH in 96 % alcohol yielded after 5 hours a layer of ion exchanger of 1.5 mg/cm^2 in dry state. Because of the weak acidity of the carboxyl group it is very sensitive to acid solutions where the surface becomes hydrophobic at once (and shows a very much higher surface resistance). The layer is rather slimy, due probably to a very low degree of cross-linkage of the high polymer.

Other polymers *e.g.* bakelite and other plastics of aromatic type can be given on exchange properties after immersing in chlorosulphonic acid.

Only one way of preparing anion exchanger of the sheet type has been studied: Polystyrene plates were dipped into fuming nitric acid for about 10 seconds (with the probable formation of a polynitrostyrene) and subsequently reduced by $TiCl_3$ or $SnCl_2$ solutions in HCl. An anion capacity of the order 0.1 μeq/cm^2 was observed (probably due to a polyaminostyrene). The determination of the capacity was made by means of a radioactive tracer method using P^{32} as H_3PO_4.

APPLICATIONS

It has already been mentioned that a very uniform sorption of ions on the sheet ion exchanger takes place. This is of interest for the preparation of many kinds of radioactive sources. The capacities of the cation exchangers made are of the order 0.1—100 μeq/cm^2 and thus the method is of certain value when a high specific activity is present, which is the case for the uranium fission products, nuclides made by the Szilard-Chalmers reaction, many daughter products in the radioactive series, products from (n,p) and (n,α) reactions *etc.* For example the method has hitherto been used for the nuclides Sr^{90} + Y^{90}, RaD + E, ThB + C, Ca^{45}, UX_1 *etc.* Because the exchange reaction is not complete, quantitative preparations can not be made using the usual single stage technique. However the method should be of value for those cases where losses can be tolerated *e.g.* the preparation of radiation sources for beta radiography, beta thickness gauges, the calibration of photographic emulsions and also as standard preparations all of which have been preliminarily studied. Also other similar fields are suggested: for use in medical applicators, sources for beta spectroscopy (where the uniform and low self-absorption is the most important feature), preparation of static eliminators, phosphors *etc.* Because

of the hygroscopic nature of the layers they should be carefully sealed when high precision is required in radiation studies *e.g.* when working with standards.

It is also possible to carry out separations of different ion species on treated plates or strips, both as one- and two-dimensional chromatography. Only a few trial experiments have been made. The supposition that the submicroscopical "grain size" would give very sharp fronts was supported by experimental observations. On the other hand, the relatively closedpacked structure gives a rather low transport velocity and thus a long experimental time. The plates or strips are easily developed by spraying with colour-developing solutions using techniques usual in paper chromatography. Working with radioactive substances the chromatogram is easily studied by means of a radiation detector provided with a narrow slit. The detection of soft beta rays and alpha rays, difficult to realise in the usual column apparatus, is rather easy in this case due to the low self absorption. Autoradiography gives a complete picture of the result. More work has to be done before practical methods for sheet ion exchange chromatography can be recommended. Such works are in progress.

The ions in the sheet ion exchangers possess a certain mobility in electric fields, which, when the layer is wet, is of the order of 1—10 % of the known mobility for aqueous solutions. These properties have been used to study the migration of inorganic ions and experiments are now being directed towards the separation of ions and radiocolloids (in collaboration with Mr L. W. Holm).

SIE loaded with different ions are useful as ionic absorbers *e.g.* for beta rays and neutrons. This method is now being applied in an extended study of the effect of chemical state on the absorption of beta rays.

Sheet ion exchangers can also be used as filters rendering other applications possible *e.g.* "pile chromatography", test methods for radiocolloids, indicator "papers", semipermeable membranes, ultrafilters.

One modification of the technique is to make solid precipitates in the layer itself after having loaded it with certain ions. Silver bromide photographic emulsions can be made using the steps:

$$H^+ X^- + Ag^+ \rightarrow Ag^+X^- + H^+$$

$$Ag^+ X^- + K^+Br^- \rightarrow AgBr_s + K^+ X^-$$

rinsing with distilled water after each reaction (X^- represents the negative group of the ion exchanger). From the above equations it can be seen that such a process yields a AgBr content of 50 %, but the process can be repeated again giving theoretically 75 %. Thus it is possible to obtain light sensitive layers with similar AgBr content to those of ordinary photography as well as to the

socalled nuclear emulsions. The ion exchanging high-polymer gel takes the role of gelatin as protection colloid for the silver bromide.

The same technique has been used to produce layers containing $BaSO_4$, $Cu_2Fe(CN)_6$, ZnS, PbS, Ag_2S *etc.*

The sheet ion exchangers possess promising features for the study of the ion exchangers themselves and the ion exchange process *e.g.* rendering possible electronmicroscopic preparations, studies of diffusion and ion mobility, water sorption properties and swelling, optical and mechanical properties *etc.*

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The Equilibria between Nickel and Acetate Ions

An Ion Exchange and Potentiometric Investigation

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In a previous paper [1] the present author has developed and tested a method to investigate the complex equilibria between a bivalent cation M^{2+} as the central group and a univalent anion A^- as the ligand quantitatively by means of a cation exchanger. Within systems of this kind the free central group M^{2+} and the first complex MA^+ can be sorbed by the cation exchanger. For the quotient φ between the total concentrations of the central group in the ion exchanger (C_{MR}) and in the solution (C_M) at equilibrium the following relation was deduced [1, p. 861]

$$\varphi = \frac{l_0 + l_1\beta_1\,[A^-]}{X} \qquad (1)$$

The notations used are explained in the previous paper. Eq. (1) is *general* with regard to the composition of the complex system, provided C_{MR} and C_M are kept sufficiently small [1, p. 862].

Generally, the mass law products k_0 and k_1 of the two exchange equilibria*:

$$M^{2+} + 2\,Na_R^+ \rightleftharpoons M_R^{2+} + 2\,Na^+$$

$$MA^+ + Na_R^+ \rightleftharpoons MA_R^+ + Na^+$$

of the ion exchanger in the sodium form are no constants, even if C_{MR} and C_M are kept small in comparison with the ionic strengths in the two phases. Thus, under such conditions the distribution coefficients $l_0 = [M^{2+}]_R \cdot [M^{2+}]^{-1}$ and $l_1 = [MA^+]_R \cdot [MA^+]^{-1}$ are no constants either but depend upon the

* In this paper the ions in the ion exchanger phase are denoted M_R^{2+}, MA_R^+, Na_R^+ and their concentrations $[M^{2+}]_R$, $[MA^+]_R$, $[Na^+]_R$.

composition of the ion exchanger phase. This fact complicates the calculation of the complexity constants of the system.

If the free central group M^{2+} could solely enter the resin phase, then at a fixed value of C_{MR} the coefficient l_0 would have the same value at all measurements, and this would be valid at every appearance of the sorption isotherm of M^{2+}.

For systems of the type $M^{2+}-A^-$ we have as a rule $l_1 > 0$. However, from the result of the preceding investigation we arrive at the conclusion that also when the complex MA^+ is taken up by the ion exchanger, l_0 and l_1 are, at small values of C_{MR}, dependent practically *solely* upon C_{MR} and not upon the ligand number $\bar{n}_R = [MA^+]_R \cdot C_{MR}^{-1}$ in the ion exchanger phase. The theoretical background of this behaviour will be developed in a subsequent paper. Thus, if the measurements are carried out at a fixed value of C_{MR}, the coefficients l_0 and l_1 can be treated as constants at the calculations.

In the present investigation this ion exchange method was applied on the nickel acetate system. Furthermore, to get a check on the results obtained a potentiometric investigation was also performed. Both investigations were carried out at a constant ionic strength, $I = 1$ C, with sodium perchlorate as a supplementary salt in the solutions.

The complex formation between nickel and acetate ions has very seldom been studied quantitatively. The investigations that are reported in the literature are essentially of a qualitative nature and scarcely give any information about the composition of the complex system or the strength of the complexes formed. Cryoscopic measurements by Calame [2] and an extinction investigation by Major [3] are of this kind.

THE ION EXCHANGE INVESTIGATION

On the calculation of the complexity constants

The calculation of the complexity constants of the system is not performed in exactly the same way as in the previous paper [1], but has been somewhat modified. From corresponding values of φ and $[A^-]$, determined at a fixed value of C_{MR} (see p. 1204), the constants are calculated as follows. The function φ is extrapolated graphically to $[A^-] = 0$, and this can be performed with great accuracy owing to the weak complexity of the present system. At the extrapolation we obtain:

$$l_0 = \lim_{[A^-] \to 0} \varphi$$

When l_0 has been determined, we can form the function φ_1*:

$$\varphi_1 = (l_0\varphi^{-1} - 1) \cdot [A^-]^{-1} \qquad (2)$$

* The same as $l_0\varphi_1$ in the preceding paper.

If we put $l_1\beta_1 l_0^{-1} = l$ in eq. (1) we find that an extrapolation of φ_1 to $[A^-] = 0$ gives:

$$\beta_1 - l = \lim_{[A^-] \to 0} \varphi_1$$

Now, for the sake of conveniency we introduce a new function f containing the two quantities l_0 and $\beta_1 - l$:

$$f = \frac{l_0\varphi^{-1}\{(\beta_1 - l)[A^-] - 1\} + 1}{[A^-]^2} \tag{3}$$

It is easy to verify that eq. (1) can be written:

$$f = \beta_1\varphi_1 - \sum_{j=2}^{N} \beta_j [A^-]^{j-2} \tag{4}$$

If the complexity is slight and at not very great $[A^-]$-values we can with sufficient accuracy put $N = 3$. Then, if f and φ_1 are determined at different $[A^-]$, we get the following relation between the differences:

$$\Delta f = \beta_1 \cdot \Delta\varphi_1 - \beta_3 \cdot \Delta[A^-] \tag{5}$$

It must be possible to represent graphically the connection between $\Delta f/\Delta[A^-]$ and $\Delta\varphi_1/\Delta[A^-]$ by a straight line, from the slope of which β_1 is obtained. After β_1 has been determined, the constants of the other mononuclear complexes can easily be computed according to eq. (4) from corresponding values of $[A^-]$ and $f - \beta_1\varphi_1$.

Measurements

Chemicals used. Nickel perchlorate was prepared from nickel carbonate, p.a., and perchloric acid, p.a., and was recrystallized from water to be released from the excess of perchloric acid. A stock solution was used and its concentration of nickel was checked by electroanalysis in ammoniacal solution according to Okáč [4].

Potassium dithio-oxalate was prepared from diphenyl oxalate and potassium hydrosulphide according to Auger and Billy [5]. As water solutions of the salt are not stable, they were made up just prior to use.

The *cation exchanger* was Amberlite IR-105 transferred into the sodium form and air-dried at room temperature. It was stored in a closed vessel, so that the content of water was constant during all the measurements. The exchange capacity was 2.30 meq. per gram of the dried exchanger in the sodium form.

The *other chemicals* used were prepared in the same way as in a previous treatise [6]. All stock solutions had the ionic strength 1 C.

The measurements were performed in the same manner as before [1, p. 864]. v liters of the solution:

$$\begin{cases} C'_M \text{ mC } Ni(ClO_4)_2 \\ C'_A \text{ mC NaAc} \\ 0.5\ C'_A \text{ mC HAc} \\ (1000 - 3\ C'_M - C'_A) \text{ mC } NaClO_4 \end{cases}$$

were shaken in a thermostat at 20.0° C with m grams of the dried ion exchanger, until an equilibrium was attained. Then the solution was separated and the equilibrium concentrations C_M and C_A of nickel and acetate were ascertained.

The nickel concentration was extinctiometrically determined. Potassium dithio-oxalate solution was added in excess, and the extinction was measured with a Beckman Quartz Spectrophotometer (Model DU) at the wave length 5 000 Å, where the extinction curve has a relative maximum. As the nickel dithio-oxalate complex formed is very strong, the molar extinction, obtained from solutions with known nickel concentration, is independent of the acetate ion concentration. The molar extinction at the wave length used is great, so even very low nickel concentrations can be ascertained with great accuracy.

The acetate concentration C_A at equilibrium was determined by extinctiometric titration in the way described before [1, p. 865]. Cupric perchlorate was added and the extinction measurements were carried out at the wave length 3 000 Å.

The nickel concentration C_{MR} in the resin phase was calculated from the relation:

$$C_{MR} = \frac{v}{m}(C'_M - C_M \cdot \delta) \quad , \tag{6}$$

As the value of m/v was kept constant (40.0 g · l^{-1}) at all measurements, the relative volume decrease of the solutions, caused by the swelling of the ion exchanger, was also the same for all solutions measured. Thus δ had a constant value and was obtained from the relation $\delta = C'_A/C_A$ at $C_M = 0$. The value of δ was 0.993.

It should be emphasized here that C_{MR} cannot be determined by analysis of the resin phase, for if the exchanger is washed with water to be released from adherent outer solution, then all of the acetate disappears from the resin phase because of the dissociation of the complex $NiAc^+$, taken up by the exchanger. As at the same time an equivalent amount of cations must leave the resin phase, part of the nickel concentration C_{MR} could be lost.

The concentration values obtained at the measurements and the calculated values of C_{MR} and φ are collected in Table 1. In Fig. 1 the function $\varphi(C_A)$ is

Table 1. Ion exchange measurements on the nickel acetate system.

$M^{2+} = Ni^{2+}$, $A^- = Ac^-$

C'_M mC	C_A mC	C_M mC	$C_{MR} \cdot 10^3$ mmole · g^{-1}	$\varphi \cdot 10^3$ l · g^{-1}
1.000	21.1	0.492	12.80	26.0
1.000	41.7	0.508	12.40	24.4
1.000	68.7	0.525	11.95	22.8
1.000	101.1	0.545	11.45	21.0
1.000	153.9	0.589	10.40	17.6
1.000	201	0.624	9.50	15.2
1.000	251	0.657	8.70	13.2
1.000	304	0.688	7.93	11.5
1.000	404	0.733	6.80	9.30
1.000	504	0.770	5.88	7.65
2.50	23.5	1.38	28.3	20.5
2.50	41.7	1.41	27.5	19.5
2.50	68.1	1.46	26.3	18.0
2.50	99.9	1.52	24.8	16.3
2.50	150.7	1.61	22.5	14.0
2.50	201	1.69	20.5	12.1
2.50	252	1.77	18.5	10.5
2.50	302	1.82	17.3	9.50
2.50	403	1.90	15.3	8.05
2.50	503	1.98	13.3	6.70
5.00	23.0	2.99	50.7	16.9
5.00	40.7	3.06	49.0	16.0
5.00	66.8	3.16	46.5	14.7
5.00	101.1	3.29	43.3	13.2
5.00	154.4	3.48	38.5	11.1
5.00	201	3.61	35.5	9.85
5.00	252	3.72	32.8	8.80
5.00	302	3.85	29.5	7.65
5.00	403	3.99	26.0	6.50
5.00	504	4.18	21.3	5.10

represented and in Fig. 2 the function C_{MR} (C_A), in both cases at the three given values of C'_M. From these curves three pairs of values (C_{MR}, φ) are obtained at every given C_A. If the function $\varphi(C_{MR})$ is graphically represented at different C_A, it is very easy to determine by interpolation or extrapolation the function $\varphi(C_A)$ at a constant value of C_{MR} (Table 2, last column). The value $C_{MR} = 10^{-2}$

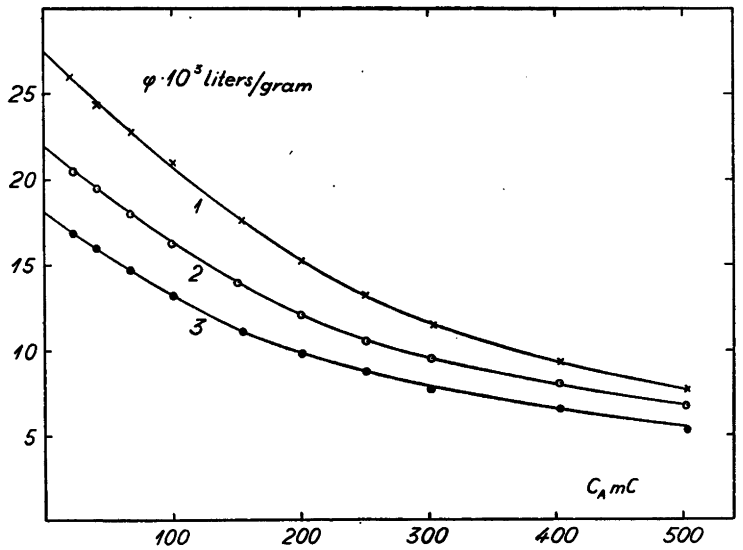


Fig. 1. The function $\varphi(C_A)$ at given values of C'_M. 1. $C'_M = 1.000$ mC; 2. $C'_M = 2.50$ mC; 3. $C'_M = 5.00$ mC.

mmoles.g^{-1} was selected. As this corresponds to very low equilibrium concentrations C_M, we can put $[A^-] = C_A$. Then $[H^+]$ and $[H^+]_R$ are constant, and this is important, as the affinity of Ni^{2+} for the ion exchanger used is dependent on $[H^+]_R$ and thus on $[H^+]$. In the outer solution $p[H^+]$ was potentiometrically determined and had the value 4.90.

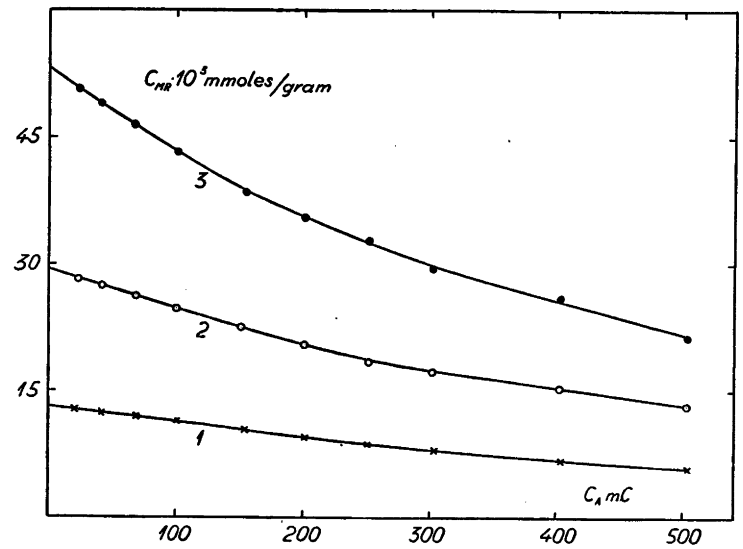


Fig. 2. The function $C_{MR}(C_A)$ at given values of C'_M. 1. $C'_M = 1.000$ mC; 2. $C'_M = 2.50$ mC; 3. $C'_M = 5.00$ mC.

Table 2. Determination of corresponding values of C_A, C_M, and φ at a constant value of C_{MR}.

C_A mC	C'_M = 1.000 mC		C'_M = 2.50 mC		C'_M = 5.00 mC		$C_{MR} = 10^{-2}$ mmole·g^{-1}
	$C_{MR} \cdot 10^3$ mmole·g^{-1}	$\varphi \cdot 10^3$ l · g^{-1}	$C_{MR} \cdot 10^3$ mmole·g^{-1}	$\varphi \cdot 10^3$ l · g^{-1}	$C_{MR} \cdot 10^3$ mmole·g^{-1}	$\varphi \cdot 10^3$ l · g^{-1}	$\varphi \cdot 10^3$ l · g^{-1}
0	13.20	27.6	29.4	22.0	53.1	18.1	29.0
20.0	12.80	26.1	28.5	20.7	51.1	17.0	27.3
40.0	12.50	24.7	27.6	19.6	49.1	16.0	25.7
70.0	11.95	22.6	26.2	17.9	46.2	14.6	23.4
100.0	11.40	20.7	24.8	16.3	43.4	13.2	21.2
150.0	10.45	17.8	22.6	14.1	39.0	11.3	18.0
200	9.50	15.2	20.5	12.1	35.6	9.85	15.0
250	8.75	13.3	18.8	10.6	32.6	8.70	12.9
300	8.01	11.6	17.5	9.50	29.9	7.70	11.1
400	6.90	9.40	15.4	8.05	25.7	6.45	8.85
500	5.91	7.65	13.4	6.70	21.8	5.15	7.15

When the function $\varphi([A^-])$ at $C_{MR} = 10^{-2}$ mmoles.g^{-1} is extrapolated to $[A^-] = 0$ we get $l_0 = 29.0 \cdot 10^{-3}$ l · g^{-1}. In Table 3, column 2 the function φ_1 has been calculated according to eq. (2) and by graphic extrapolation to $[A^-] = 0$ we obtain $\beta_1 - l = 2.80$ C^{-1}. With the two limit values determined the function f has been calculated from eq. (3). When calculating only such $[A^-]$-values have been used, at which the experimental random errors do not affect the f-values too much. In Table 3 the differences $\Delta[A^-]$, $\Delta\varphi_1$, and Δf have the following meaning:

$$\Delta[A^-] = [A^-] - 0.200 \text{ C}, \quad \Delta\varphi_1 = \varphi_1 - 4.67 \text{ C}^{-1}, \quad \Delta f = f - 3.7 \text{ C}^{-2}$$

The relation between $\Delta\varphi_1/\Delta[A^-]$ and $\Delta f/\Delta[A^-]$ can be represented by a straight line, the slope of which gives us the first complexity constant

$$\beta_1 = 4.7 \pm 0.6 \text{ C}^{-1}$$

Then we have $l = 1.9 \pm 0.6$ C^{-1}. In the last column of Table 3 the polynomial $X_2 = \sum_{j=2}^{N} \beta_j [A^-]^{j-2}$ has been calculated at the higher $[A^-]$-values. According to eq. (4) we have the following expression: $X_2 = \beta_1\varphi_1 - f$. The values of X_2 in Table 3 are constant within the limits of experimental errors and we get

$$\beta_2 = 18 \pm 3 \text{ C}^{-2}$$

Table 3. Determination of corresponding values of φ_1, f, and the polynomial X_2 at different values of $[A^-]$ and at $C_{MR} = 10^{-2}$ mmoles · g^{-1}.

$[A^-]$ mC	φ_1 C^{-1}	f C^{-2}	$\frac{\Delta\varphi_1}{\Delta[A^-]}$ C^{-2}	$\frac{\Delta f}{\Delta[A^-]}$ C^{-3}	X_2 C^{-2}
0	*2.80*				
40.0	3.20				
70.0	3.41				
100.0	3.68				
150.0	4.07				
200	4.67	3.7			18.2
250	4.99	5.2	6.4	30	18.3
300	5.30	6.5	6.3	28	18.4
400	5.70	8.7	5.2	25	18.1
500	6.11	10.5	4.8	22.5	18.2

Owing to the weak complexity of the system no formation of $NiAc_3^-$ or higher complexes can be detected at the acetate ion concentrations used.

With the constants obtained at the ion exchange measurements the ligand numbers in the outer solution and in the resin phase and the composition of the complex system have been calculated. In Table 4 α_j means the fraction $[MA_j^{2-j}] \cdot C_M^{-1}$.

On the mass law products k_0 and k_1

From the intercepts on the φ — and C_{MR} — axes in Figs. 1 and 2 corresponding values of l_0 and C_{MR} are obtained. These values are collected in Table 5. For

Table 4. The ligand numbers $\bar{n}$ and $\bar{n}_R$ and the composition of the nickel acetate system at different $[A^-]$.

$[A^-]$ mC	$\bar{n}$	$\bar{n}_R$	$100 \cdot \alpha_0$	$100 \cdot \alpha_1$	$100 \cdot \alpha_2$
10	0.050	0.020	95.3	4.5	0.2
50	0.255	0.085	78.1	18.4	3.5
100	0.505	0.160	60.6	28.5	10.9
200	0.895	0.28	37.6	35.3	27.1
300	1.155	0.36	24.8	35.0	40.2
500	1.445	0.49	12.7	29.9	57.4

Table 5. The mass law product k_0 as a function of C_{MR} at $p[H^+]=4.90$.

$C_{MR} \cdot 10^3$ mmole $\cdot g^{-1}$	$l_0 \cdot 10^3$ $l \cdot g^{-1}$	C_M mC	$k_0 \cdot 10^{-3}$ $l^{-1} \cdot g$
13.2	27.6	0.478	5.34
29.4	22.0	1.34	4.34
53.1	18.1	2.93	3.69

the calculation of k_0 we have the relation: $k_0 = l_0 \cdot [Na^+]^2 \cdot [Na^+]_R^{-2}$. Because of the low nickel concentrations $[Na^+]$ and $[Na^+]_R$ are nearly constant and are given by the following connections, valid at $C_A = 0$:

$$[Na^+] = 1\,000 - 3\ C'_M + 2\,(C'_M - C_M)\ mC$$
$$[Na^+]_R = 2.30 - 2\ C_{MR}\ mmoles \cdot g^{-1}$$

From Table 5 it is obvious that k_0 at p $[H^+] = 4.90$ is dependent on the value of C_{MR}, though this quantity is kept very small in comparison with the exchange capacity. This fact will be discussed in a following paper, and here mention will only be made that the variation of k_0 indicates that the nickel ions are not free in the same way as the sodium ions in the resin phase, but are partly coordinated to groups, fixed at the exchanger and in minute concentration.

For l_1 we have only the value that corresponds to $C_{MR} = 10^{-2}$ mmoles $\cdot g^{-1}$. It is calculated from the relation $l_1 = l_0 l \beta_1^{-1}$ and then k_1 from $k_1 = l_1 \cdot [Na^+] \cdot [Na^+]_R^{-1}$. For $[Na^+]$ and $[Na^+]_R$ the approximate values 1 000 mC and 2.30 mmoles $\cdot g^{-1}$ can be used, and we obtain $k_1 = 5.0$. The value of k_1 shows that the sorption affinity of the ion $NiAc^+$ for the exchanger is much greater than that of Na^+. This fact again indicates that at the p$[H^+]$ used all the ions $NiAc^+$ are not free in the resin phase.

THE POTENTIOMETRIC INVESTIGATION

In order to get some check on the results obtained a potentiometric investigation of the nickel acetate system was also performed. The measurements for the determination of $[Ac^-]$ were carried out with the quinhydrone electrode, and the cells were of the same type as in a previous paper [7, p. 78]. The solution of the one half-cell had the composition:

$$\begin{cases} C_M\ mC\ Ni(ClO_4)_2 \\ C_A\ mC\ NaAc \\ 0.5\ C_A\ mC\ HAc \\ (1000 - 3\ C_M - C_A)\ mC\ NaClO_4 \end{cases}$$

Table 6. Determination of $\bar{n}/[A^-]$ as a function of $[A^-]$ from emf measurements.

C_M mC	C_A mC	E_A mV	$-\vartheta$ mC	$[A^-]$ mC	$\bar{n}/[A^-]$ C^{-1}
101.0	12.99	13.6	0.32	7.95	5.89
101.0	19.23	12.7	0.32	12.02	5.68
101.0	31.3	12.1	0.32	19.8	5.60
101.0	45.5	11.7	0.3	29.0	5.53
101.0	58.8	11.3	0.3	38.0	5.34
101.0	83.3	10.9	0.3	54.5	5.09
101.0	125.0	10.2	0.3	83.8	4.83
101.0	159.1	9.9	0.3	108	4.67
101.0	200	9.5		137	4.57
101.0	250	9.0		175	4.24
101.0	313	8.4		224	3.94
101.0	400	7.7		295	3.52
50.0	12.99	7.2	0.16	10.01	5.64
50.0	19.23	6.8	0.16	14.94	5.53
50.0	31.3	6.4	0.16	24.5	5.39
50.0	45.5	6.1	0.2	36.0	5.17
50.0	58.8	5.9	0.2	46.8	5.04
50.0	83.3	5.7	0.2	66.8	4.88
50.0	125.0	5.4	0.2	101.5	4.65
50.0	159.1	5.1	0.2	130	4.46
50.0	200	4.8		165	4.24
50.0	250	4.6		209	3.92
50.0	313	4.3		264	3.71
50.0	400	3.8		344	3.26

Measurements were performed with $C_M = 0$, 50, and 101 mC and with varying values of C_A. At $C_M = 0$ the emf of the cell is independent of C_A and denoted E_0. The difference between E_0 and the emf at the other C_M-values is called E_A. The values obtained of E_A are collected in Table 6. The quantities $[A^-]$ and $\bar{n}/[A^-]$ have been calculated from the eq. (19—21) of the previous paper [7, p. 79]. In these equations we have only to replace [B], C_B, and E_B by $[A^-]$, C_A, and E_A and put $\vartheta_0 = 0$. In the present case the correction term ϑ is equal to the difference between $[H^+]$ in the complex solution and $[H^+]$ in the solution with $C_A = 0$ but with the same C_M-value.

From Fig. 3 it is obvious that the function $\bar{n}/[A^-]$ at small values of the variable $[A^-]$ is not entirely independent of C_M, but the dependence is very slight, proving that practically only mononuclear complexes are formed,

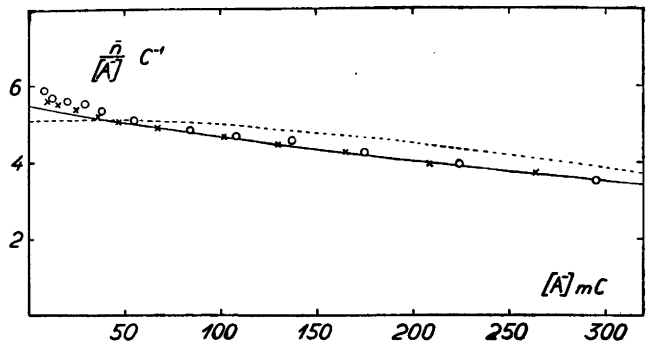


Fig. 3. $\bar{n}/[A^-]$ *as a function of* $[A^-]$. — *Circles and crosses relate to the emf measurements at* $C_M = 101$ *and 50 mC respectively, and the full drawn curve represents the limiting function at* $C_M = 0$. — *The dashed curve is the same limiting function calculated from the ion exchange measurements.*

and the limiting function valid at $C_M = 0$ can be obtained at once. From its value at $[A^-] = 0$ we get $\beta_1 = 5.5 \pm 0.3$ C^{-1}.

For the sake of comparison in Fig. 3 the same limiting function has been calculated from the values of $\bar{n}$ and $[A^-]$ in Table 4. The agreement between the two curves of the same function is quite satisfactory, if we take into consideration that the potentiometric measurement of $[A^-]$, which is the only potentiometric method available in this case, is not very suitable for an accurate determination of β_2 and higher complexity constants, if the complexity is as slight as that of the nickel acetate system.

Thus, as the polynomial X and the complexity constants are uniquely determined [7, eq. (12)] by the limiting function mentioned above, the results from the ion exchange investigation are confirmed.

SUMMARY

The complex equilibria of the nickel acetate system are investigated by the use of a cation exchanger and potentiometrically.

The results from the two investigations are consistent and prove the existence of two mononuclear complexes, *viz.* $NiAc^+$ and $NiAc_2$, at acetate ion concentrations $\leqq 500$ mC. From the emf measurements it can be concluded that practically no polynuclear complexes are formed. At the temperature 20° C and the ionic strength $I = 1$ C the complexity constants of the complexes formed are:

$$\beta_1 = 4.7 \pm 0.6 \text{ C}^{-1}, \ \beta_2 = 18 \pm 3 \text{ C}^{-2}$$

The sorption of $NiAc^+$ by the ion exchanger is studied. The fraction of the total nickel concentration in the resin phase that consists of the complex mentioned, is obtained as a known function of $[Ac^-]$ in the outer solution.

My thanks are due to Professor S. Bodforss for kindly putting an assistant at my disposal and for his interest in this work.

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Constituents of Pine Heartwood

XXIX.* A Synthesis of Strobochrysin Dimethyl Ether (5,7-Dimethoxy-6-methylflavone)

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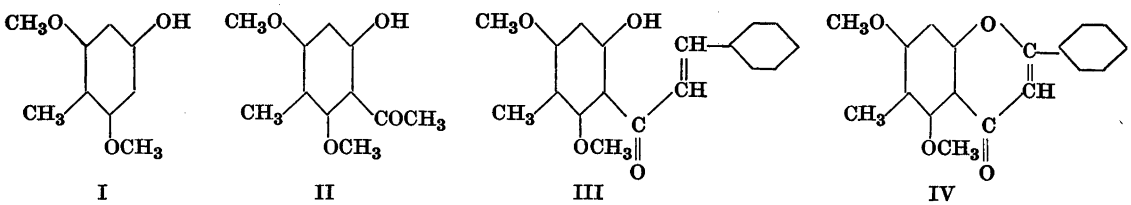
In Part XXIV of this series [1], the isolation from the heartwood of *Pinus strobus* L. of a new flavone, named strobochrysin, was recorded. It was found to be a C-methylchrysin with the methyl group in either the 6- or the 8-position. The dimethyl ether, m.p. 170—171° **, was also prepared, and as this compound was not identical with 5,7-dimethoxy-8-methylflavone (m.p. 230—231°), we concluded that the C-methyl group in strobochrysin occupies the 6-position. Lack of material prevented us from proving this structure in a more direct way.

An unambigous synthesis of 5,7-dimethoxy-6-methylflavone has now been carried out by a method analogous to that used for the synthesis of the 8-methyl isomer [1]. The main difficulty lies in the relative inaccessibility of the key intermediate, 2,6-dimethoxy-4-hydroxytoluene (I). Of the three published methods of synthesising this substance, that of Gruber [2] appears to be the simplest, consisting as it does of a Gattermann aldehyde synthesis on phloroglucinol dimethyl ether and reduction of the *p*-hydroxyaldehyde (formed as a by-product) by the Clemmensen method. In our hands, however, the Gattermann synthesis (using zinc cyanide and hydrogen chloride) yielded the *o*-hydroxyaldehyde exclusively. Of the other two methods available, we preferred that described by Asahina and co-workers [3,4] to the older synthesis due to Robertson and co-workers [5]. We have modified one of the steps of the synthesis slightly.

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* XXVIII. *Acta Chem. Scand.* **6** (1952) 744. XXX. *Svensk Papperstidn.* **55** (1952) 602.

** All melting points uncorrected.



I II III IV

Robertson and co-workers [6] prepared 2-hydroxy-5-methyl-4,6-dimethoxyacetophenone (II) from the phenol (I) by Fries rearrangement of the acetate, and obtained the ketone as an oil. We, however, succeeded in getting the substance crystalline, m.p. 38—39°, after purification by vacuum distillation. Condensation of this ketone with benzaldehyde gave 2′-hydroxy-5′-methyl-4′,6′-dimethoxychalkone (III), m.p. 94—95°, oxidised in good yield to the desired 5,7-dimethoxy-6-methylflavone (IV) by selenium dioxide. This flavone, m.p. 170—171°, was found to be identical with strobochrysin dimethyl ether, which we had previously prepared in an impure state [1], and thus, strobochrysin is 6-methylchrysin.

The chalkone (III) was rearranged to the corresponding 5,7-dimethoxy-6-methylflavanone, m.p. 130—131°, either by boiling with hydrochloric acid in alcohol or by allowing it to stand in dilute sodium hydroxide at room temperature (method of Löwenbein [7]). The flavanone was obtained in good yield, in contrast to the 8-methyl isomer, which is very difficult to prepare from the corresponding chalkone [1]. The difficulty of rearranging the latter chalkone is probably due to steric hindrance.

The following table lists the melting points and the reduction colours of the fully methylated 6- and 8-methylflavones and -flavanones we have prepared.

Substance	M.p.	Mg-HCl colour
5,7-dimethoxy-**6**-methylflavone	170—171°	Lemon yellow
—»— flavanone	130—131°	Pale yellow
5,7-dimethoxy-**8**-methylflavone [1]	230—231°	Pink
—»— flavanone [1]	141—142°	Pale pink
3,5,7-trimethoxy-**8**-methylflavone [8]	154—155°	Pink

It is noteworthy that the 8-methyl derivatives have higher melting points and give deeper colours on reduction than the corresponding 6-methyl derivatives.

The information now available sheds some light on the structure of strobobanksin, a C-methylflavanone isolated from the heartwood of *P. strobus* [1]. Strobobanksin is a

3,5,7-trihydroxyflavanone with a C-methyl group either in the 6- or in the 8-position. When its partial methylation product was dehydrogenated to a flavonol with palladium-cinnamic acid, a yellow substance was obtained, which on exhaustive methylation yielded a product which was not purified owing to lack of material. However, it gave a strong yellow colour with magnesium-hydrochloric acid and, therefore, it can not be 3,5,7-trimethoxy-8-methylflavone, but is probably the 6-methyl isomer. Consequently, strobobanksin should belong to the 6-methyl series.

EXPERIMENTAL

2,6-Dimethoxy-4-hydroxytoluene (I). The methylation of 3,5-dihydroxy-4-methylbenzoic acid [3] proved to be the most difficult step in the synthesis. Methylation with dimethyl sulphate and alkali, as described by the Japanese workers [3], led to mixtures, which were difficult to separate. Better results were obtained using the following method:

3,5-dihydroxy-4-methylbenzoic acid (26.5 g) in acetone (1 l) was refluxed for three hours with dimethyl sulphate (62 ml) and freshly ignited potassium carbonate (180 g). The precipitate was filtered off and washed with hot acetone, and the filtrate was concentrated to a syrup, which soon deposited crystals. Recrystallisation of the solid material from methanol gave colourless crystals of 3,5-dimethoxy-4-methylbenzoic acid methyl ester, m.p. 104—105°. The syrupy fraction, on distillation at low pressure, yielded four fractions, the highest-boiling of which (b.p. 148—150°/4 mm) consisted of an additional amount of the substance desired. The total yield of the pure ester was 13.0 g.

The preparation of the phenol (I) from this ester by Curtius rearrangement and diazotisation of the amine formed was carried out as described by Asahina and Yanagita [4], and with about the same yields as found by those authors.

2-Hydroxy-5-methyl-4,6-dimethoxyacetophenone (II). — The phenol (I) was acetylated (97 % yield), and the acetate was rearranged with aluminium chloride in nitrobenzene as described by Robertson and co-workers [6]. The product, after distillation at 1 mm pressure (b.p. 131—135°), was a yellow oil which solidified after being set aside in the refrigerator. M.p. 35—36°; 2.3 g from 3.0 g of the acetate.

The solid product was dissolved in light petroleum and the solution was cooled to 0°. A small amount (0.2 g) of the phenol (I) which separated was removed and the solution was evaporated and the residue again distilled at 1 mm pressure. B.p. 128—131°, m.p. 38—39°. The melting point remained unchanged after a third distillation. The substance gives a violet colour with ferric chloride.

$C_{11}H_{14}O_4$ (210.2) Calc. OCH_3 29.5 Found OCH_3 29.5

2'-Hydroxy-5'-methyl-4',6'-dimethoxychalkone (III). — The ketone (II) (1.5 g) was dissolved in ethanol (15 ml), and benzaldehyde (1.1 ml) and potassium hydroxide (6 g) in water (6 ml) were added. The red homogeneous solution was left overnight at room temperature; then acidified and extracted with ether. The ether solution was dried over anhydrous sodium sulphate and filtered through aluminium oxide, which adsorbed part of the red colour. The filtrate was concentrated to a reddish syrup, which crystallised on standing. The crude product (2.0 g) was recrystallised once from methanol and thrice from ethanol, and then formed yellow needles, m.p. 94—95°. The chalkone gives a brownish colour with alcoholic ferric chloride.

$C_{18}H_{18}O_4$ (298.3)	Calc.	C	72.5	H	6.08	OCH_3	20.8
	Found	»	72.6	»	6.05	»	20.0

5,7-Dimethoxy-6-methylflavone (IV). — The chalkone (III) (0.5 g) was refluxed with selenium dioxide (0.7 g) in *iso*-amyl alcohol (8 ml) for 42 hours. The precipitated selenium was filtered off, and the alcohol was removed by steam distillation. The residue was dissolved in chloroform, and the solution washed with dilute sodium hydroxide, dried over anhydrous sodium sulphate, filtered through aluminium oxide and evaporated. The brownish crystalline residue was washed with a little ether and recrystallised twice from ethanol, yielding colourless needles (0.4 g), m.p. 170—171°, undepressed in admixture with the crude strobochrysin dimethyl ether (m.p. 170—171°) previously prepared [1]. The compound gives a lemon yellow colour with magnesium-hydrochloric acid.

$C_{18}H_{16}O_4$ (296.3)	Calc.	C 73.0	H 5.44	OCH_3 20.9
	Found	» 72.8	» 5.40	» 20.0

5,7-dimethoxy-6-methylflavanone. — The chalkone (III) (0.2 g) was dissolved in ethanol (5 ml) and refluxed with 2 *N* hydrochloric acid (4 ml) for 30 hours. After evaporation of the ethanol, the solution was extracted with ether. The ether solution was dried over anhydrous sodium sulphate, filtered through aluminium oxide and concentrated to a reddish brown oil, which soon crystallised. The coloured impurities were removed by washing with a little ether, and the residue, after two recrystallisations from methanol, yielded colourless crystals, m.p. 130—131° (0.15 g). The substance gives a faint yellow colour on reduction with magnesium-hydrochloric acid.

$C_{18}H_{18}O_4$ (298.3)	Calc.	C 72.5	H 6.08	OCH_3 20.8
	Found	» 71.7	» 6.09	» 20.7

The rearrangement could also be carried out by dilute sodium hydroxide at room temperature. The chalkone (0.1 g) in ethanol (1 ml) was heated with 1.5% sodium hydroxide (1.5 ml) until the solution was homogeneous, then set aside for two days, during which time a yellow precipitate formed. The solid was collected, decolourised by washing with ethanol and ether, and then recrystallised from methanol. The m.p. either alone or in admixture with the flavanone prepared by the first method was 130—131°.

SUMMARY

5,7-Dimethoxy-6-methylflavone and -flavanone have been synthesised by unambigous methods. The flavone has been shown to be identical with the dimethyl ether of strobochrysin, which thus must be 5,7-dihydroxy-6-methylflavone.

The evidence now available indicates that strobobanksin belongs to the 6-methyl series (3,5,7-trihydroxy-6-methylflavanone).

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The Polarographic Determination of Small Amounts of Tin in Zinc Die Casting Alloys

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Zinc alloys for die-castings should, on account of the danger of intergranular corrosion, contain only minute amounts of Cd, Pb, Tl, Bi and Sn.

The alloys designated Zamak 2, 3 and 5, for example, may contain a maximum of 0.005 % Cd, Pd and Sn. For some other zinc alloys (Commercial rolled zinc) and Zilloy 15 and 10 the maximum content of Sn is 0.001 %. These small percentages may be determined polarographically, as it is very difficult to apply ordinary methods of chemical analysis.

When the sample contains traces of Cu, a reliable analysis of Pb, Cd and Sn is easily obtained; the polarogram gives the copper content as well. With a larger copper content, such a high voltage has to be applied that a direct determination of Sn cannot be carried out. In such cases Sn has to be separated from Cu. Methods have been described according to which Cu may be electrodeposited from solutions containing hydrochloric [1] or sulphuric acid [2]. As the ratio of the copper and tin percentages is often 10 000 : 1, there is great risk, however, that Sn is codeposited with Cu. Direct determination of Sn in amounts less than 0.0015 % is not to be recommended [1]. The tin content, therefore, has to be enriched and this may be brought about by precipitation as sulphide together with Cu [3], as sulphide together with Mo (in steel) [4], as metastannic acid together with manganese dioxide, as metastannic acid together with aluminium hydroxide and finally with kupferron [2]. In these methods the Sn content is determined volumetrically with potassium iodate [3,5,6] or with colorimetric [6] or polarographic methods [2,4,7]. For routine analysis all these procedures are far too time-consuming. Moreover, they are not very suitable for the determination of traces of Sn in Zn alloys with high Cu content.

The present investigation was undertaken with a view to obtaining a direct and sufficiently accurate method for routine analysis.

The procedure developed is the following: The Zn alloy is dissolved in hydrochloric acid. After this operation Cu and Sn are obtained as a metallic residue, which is dissolved in nitric acid. Sn is precipitated as metastannic acid, which is fused with sodium hydroxide and the Sn is determined polarographically as Sn^{4+}.

PRECIPITATION OF TIN AS METASTANNIC ACID

Precipitation of small amounts of tin as metastannic acid has been studied by, among others, Hagen, Hoffman-Bang and Gjertsen [5]. These authors point out that metastannic acid is thereby precipitated in a very finely divided, partly colloidal form. Quantitative filtration through filter paper is therefore impossible. Furthermore, a considerable loss of tin is caused by the fact that metastannic acid adheres very firmly to the beaker. In an experiment, carried out by these authors, 6.61 mg of Sn was precipitated as metastannic acid after boiling with nitric acid. 57 % of the tin content stuck to the beaker and could not be removed. Heating was done with a small flame.

It is also known that the presence of iron and chromium ions fosters the formation of metastannic acid in colloidal form. The precipitation of metastannic acid is also disturbed by the presence of phosphoric acid, hydrochloric acid and sulphuric acid. As to the adsorption of the metastannic acid on the bottom of the beaker in the investigation referred to above, this was undoubtedly due to too high a temperature, caused by the flame. This difficulty was to a large extent avoided in the present investigation by heating the beaker on a heating plate with a temperature of 280—300° C.

The precipitates have been filtered through close-texture filter paper with filterpulp (filter paper macerated with nitric acid to make it more retentive). By this means it has also been possible to avoid the use of any collecting substance (as manganese dioxide), which adsorbs copper, which in its turn interferes with the polarographic determination of tin.

In order to investigate how completely metastannic acid can be precipitated from a nitric acid solution containing a large excess of copper ions, the following series of experiments was carried out: 2.50 g of cupric oxide (Baker's C.P.) was added to a known quantity of hydrochloric acid tin standard solution (1 ml = 1 mg of Sn), dissolved in 15 ml of conc. nitric acid and evaporated to 3 ml. In order to volatilize completely the hydrochloric acid two further portions of 5 ml of nitric acid were added and after each addition the solution was evaporated to 3 ml. Finally 5 ml of conc. nitric acid was added, and then the solution was diluted with hot water to 50 ml and was kept in a warm place over-night. The precipitated metastannic acid was filtered

off on a close-texture filter paper, which was coated with filter pulp. The beaker was thoroughly wiped with filter paper. The precipitate was washed with a warm solution containing 5 % ammonium nitrate and 2 % nitric acid until Cu had been washed out. The filter paper was placed in a platinum crucible, dried and ignited in an electric furnace at 500—600° C. The residue was transferred to a silver crucible and fused with 0.2—0.3 g of sodium hydroxide over a gas flame. Before heating, the silver crucible was placed in a porcelain crucible. After the fusion, which proceeds very rapidly and is completed in 2—3 min. after the sodium hydroxide has melted, the contents of the crucible were dissolved in 3—4 ml of water, which was added in 3 portions and heated

Table 1. Precipitation of tin as metastannic acid.

The tin was determined polarographically as Sn^{4+}.

Experiment No.	Addition of Sn mg	Diffusion current in relative units (mm) Sample	Reference solution ***	Tin mg	Corrected tin content mg	Error %
1	0.00	5	0.0	0.05 *	—	—
2	0.30	31.5	27.6	0.34	0.29	— 3.3
3	0.30	30.8		0.33	0.28	— 6.7
4	0.30	29.7		0.32	0.27	—10.0
5	0.50	37.4	35.1	0.53	0.48	— 4.0
6	0.50	36.2		0.52	0.47	— 6.0
7	0.50	35.0		0.50	0.45	—10.0
8	1.00	44.3	48.2	0.92	0.87	—13.0
9	1.00	45.4		0.94	0.89	—11.0
10	2.00	54.7	57.7	1.90	1.85	— 7.5
11	2.00	53.0		1.83	1.78	—11.0
12	3.00	59.8	64.8	2.75	2.70	—10.0
13	3.00	59.5		2.75	2.70	—10.0
14	3.00	33 **	35.1 **	2.85	2.80	— 6.7
15	3.00	32.6		2.79	2.74	— 8.7
16	3.00	34.9		2.98	2.93	— 2.3
17	3.00	35.5		2.86	2.81	— 6.3

* Corresponds to the tin content of the cupric oxide.

** The sample was diluted to 25 ml.

*** Contained exactly as much Sn as was added to the test example.

to boiling each time. Finally the crucible was washed with 3 ml of conc. hydrochloric acid, which was mixed with the alkaline solution previously obtained. 1 ml of perchloric acid was added and the solution heated to boiling. After cooling the sample was diluted to 10 ml with polarographic supporting electrolyte (330 ml of conc. hydrochloric acid, 100 ml of a 2 % gelatin solution and 570 ml of water) and was run into the polarographic cell. At the same time reference solutions containing a known amount of tin standard solution and sodium hydroxide, hydrochloric acid and perchloric acid in amounts mentioned above and tempered under the same conditions as the samples were run into the cell.

In the polarographic tin determination the tin was determined as Sn^{4+}, this being the simpler procedure compared to the determination of Sn^{2+} which involves reduction of Sn^{4+} and the use of an inert atmosphere. The advantages gained by the determination of Sn^{4+} have been pointed out by Stackelberg [8]. The conditions in this determination have been more closely investigated by Lingane [9]. For the investigation the present author has used the Radiometer-København Type PO 3a polarograph.

The results of the experiments with the isolation of tin as metastannic acid are shown in Table 1.

SEPARATION OF TIN FROM ZINC ALLOYS BY INTERNAL ELECTROLYSIS

After the favourable results reported above a series of experiments was made on the separation of tin (together with copper) from zinc alloys. The alloy designated Zamak 5 and containing 2.84 % of copper and a very low percentage of tin was used for this purpose. It was dissolved in hydrochloric acid and to this solution was added a known volume of tin standard solution. The same result was obtained, if the tin standard solution was directly added to the sample.

Separation of tin is assumed to proceed in the following way. In the dissolution of the alloy, copper which has been dissolved primarily is reduced to the metallic state by the zinc. An element Zn/HCl/Cu is then formed. Here, Zn is the anode and goes into solution and tin is deposited on the cathode. This may be regarded as an example of internal electrolysis, in which both electrodes are supplied by the specimen that is to be dissolved.

Lead, cadmium and bismuth can be deposited on the copper in the same way as tin but lead does not interfere with the determination of tin, since it goes into solution in the subsequent treatment of the sample with nitric acid. It has been verified experimentally, however, that no appreciable amounts of lead or cadmium accompany the copper, and the filtrate is well suited for

the polarographic determination of lead and cadmium. This has also been verified by L. C. Copeland and F. S. Griffith[10]. The copper is so sparingly soluble in the filtrate that it does not interfere with the polarographic determination of lead and cadmium.

Elementary silicon ("black" silicon), that is often present in Zamak alloys, is carried along with the tin but in this case it plays a useful role since it aids the separation and ignition of the metastannic acid.

PROCEDURE

100 g of zinc alloy turnings was dissolved in 330 ml of conc. hydrochloric acid in a 3 l beaker, cooled in running water during the dissolution. When only one or two turnings were left, the precipitated copper and tin was filtered off on a large glass filter (Jena GL) and washed 5 times with water (altogether 500 ml).

The precipitate was transferred to a 100 ml beaker and after most of the water had been decanted the precipitate was dissolved in 15 ml of conc. nitric acid. After the dis-

Table 2. Separation of tin from a zinc alloy (containing 2.84% of copper) by internal electrolysis. The tin was determined polarographically as Sn^{+4}.

Exp. No	Addition of tin mg	Diffusion Current in relative units (mm) Sample	Reference solution ***	Tin content mg	Corrected tin content mg	Error %
18	0.00	3	—	0.03 *	—	—
19	0.30	31.0	28.8	0.32	0.29	— 3.3
20	0.30	31.6		0.33	0.30	0.0
21	0.30	30.5		0.32	0.29	— 3.3
22	0.50	37.6	35.5	0.53	0.50	0.0
23	0.50	37.7		0.53	0.50	0.0
24	0.50	37.5		0.53	0.50	0.0
25	0.50	29.0		0.48	0.45	—10.0
26	1.00	44.7	47.9	0.93	0.90	—10.0
27	1.00	46.3		0.97	0.94	— 6.0
28	2.00	55.0	59.0	1.87	1.84	— 8.0
29	2.00	36.4 **	38.3 **	1.90	1.87	— 6.5
30	3.00	31.3 **	33.3 **	2.82	2.79	— 7.0

* Determined with reference solution 0.3 mg Sn.

** Solution diluted to 25 ml.

*** Contained exactly as much Sn as was added to the test example.

solution was completed the solution was evaporated to 3 ml. In order to expel the hydrochloric acid completely two further portions of 5 ml of conc. nitric acid were added and after each addition the solution was evaporated to 3 ml. Finally 5 ml of conc. nitric acid was added, and then the solution was diluted with hot water to about 50 ml and was allowed to stand hot over-night. The metastannic acid was treated further as described above.

The results are shown in Table 2.

As seen the error range is between 0 % and − 10 %. If the result is increased by 5 % (or multiplied with 1.05) a value is obtained having an error of ± 5 %.

SUMMARY

A procedure has been developed for the determination of tin in amounts from 0.0003 % to 0.003 % Sn in zinc die-casting alloys with high copper contents. The sample is treated with hydrochloric acid, and copper and tin are obtained as a residue, which is dissolved in nitric acid. Tin is precipitated as metastannic acid, then fused with sodium hydroxide and finally tin is determined polarographically as Sn^{4+} as the diffusion current between −0.4 and −0.6 V. By this method, tin can be determined with an accuracy ± 5 % when present in amounts from 0.0003 % to 0.003 %. The procedure is useful for the routine determination of trace amounts of tin in zinc die-casting alloys with a high copper content.

The author would like to thank Husqvarna Vapenfabriks AB for permission to carry out the investigation and to publish the results. Thanks are due to Mr. Evert Andersson for his advice and Mr. Carl Otto Östman for his aid with the analytical work. Thanks are also due to Mr. Gösta Wranglén and Mr. Cecil Black for their help with the English text.

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The Influence of pH on the Enzymic Hydrolysis of Acetylcholine

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Since Sörensen [1] in 1909 pointed out the influence that pH has on the activity of enzymes a great many systems have been investigated in this respect. As far as acetylcholinesterase is concerned, the estimation of the activity of the enzyme is usually based upon a determination of the acetic acid liberated by the hydrolysis of acetylcholine. The acid can be determined either by titration or by measuring the amount of CO_2 which is evolved from a bicarbonate buffer. The Warburg manometric method is most frequently used, but is not suitable for studies on the influence of pH because the pH of the reaction mixture can only be varied within narrow limits. Recently two new procedures for the estimation of the activity have been reported, one colourimetric (Hestrin [2]) and one electrometric (Michel [3]). In the present investigation, the colourimetric method was employed.

One of the main purposes of this investigation was to determine the influence of pH on the activity-pS curve of the system acetylcholine-acetylcholinesterase, *i.e.*, the plot of enzyme activity versus pS (= −log molar concentration of the substrate). In addition the stability of enzyme solutions at various values of pH was studied. The stability of the enzyme in various buffers at 4° and 25° C was also measured, and the rate of enzymic hydrolysis of some esters other than acetylcholine has been determined at different values of pH and pS.

METHODS

The enzymic hydrolysis was studied by determining the amount of unchanged substrate, by conversion of the ester to a hydroxamic acid by treatment with alkaline hydroxylamine. Treatment of the hydroxamic acid with ferric chloride in acid solution gives a red complex, and the extinction coefficient of the solution at 5400 Å was measured

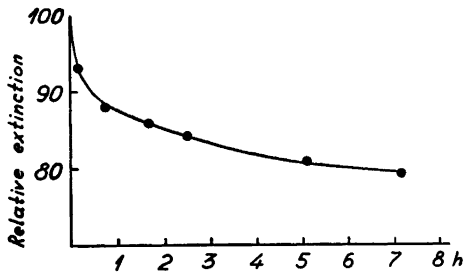


Fig. 1. Relative extinction of a coloured solution as function of the time of standing.

promptly with an EEL* colorimeter. As pointed out by Hestrin and clearly demonstrated by Fig. 1 the colour fades rather rapidly.

Crystallised cobra venom was used as the enzyme preparation as it contains no other esterases [4]. The substrates used were acetylcholine iodide (ACh), acetylsalicylcholine chloride (ASaCh), acetylsalicylic acid (ASa) and salicylcholine chloride (SaCh) [5]. Substrates and enzyme were dissolved in buffers, and in order to get optimal activity, all the solutions contained magnesium ions in the form of $MgCl_2$, $6H_2O$ at a concentration of 1.73×10^{-3} molar. All reactions were carried out in a thermostate at 25° C.

RESULTS

The reaction of hydroxylamine with esters. Various esters were converted to the corresponding hydroxamic acids and treated with ferric chloride, and the extinctions of the coloured solutions were determined. A 2.00×10^{-3} molar solution of ACh was taken as standard, and the extinction of the solution of the derived complex was assigned the value 100. The extinctions of other compounds investigated, at the same molarity, are collected in Table 1. Substances insoluble in water were dissolved in 95 % ethanol (0.1 ml) which does not affect the colour reaction.

The relative extinction values for substances with only one ester linkage are very scattered, varying from 210 (ASa) to 0 (prostigmine). On the other hand, compounds containing two or more ester linkages seems to have extinctions predominantly around 100.

One pair of isomers, 9- and 3-acetoxyretenes, have extinctions of 85 and 87, but the difference can hardly be regarded as significant.

Hestrin has determined the extinction of about 40 compounds, and some of his values are collected in Table 1. With the exception of the values for ethyl acetate and methyl butyrate the agreement is good.

Influence of pH on enzyme stability. In order to determine the influence of pH on the stability of enzyme solutions, the activities of solutions of various pH, all with ACh as substrate, were determined every month for six months.

* Evans Electroselenium LTD, Harlow, Essex.

Table 1. Relative extinction of the ferric complex of hydroxamic acids of some esters.

Compound	Extinction	Extinction per ester-linkage	Values obtained by Hestrin
Acetylcholine iodide	100	100	100
Acetyl-β-methylcholine chloride (mecholyl)	106	106	107
Salicylcholine chloride	160	160	—
Benzoylcholine chloride	139	139	—
N-acetyl-*p*-aminobenzoylcholine chloride	146	146	—
Ethyl acetate	50	50	97
Iso-propyl acetate	33	33	—
Methyl butyrate	50	50	87
Ethyl butyrate	33	33	—
Methyl benzoate	142	142	—
Ethyl benzoate	117	117	—
Acetylsalicylic acid	210	210	—
Prostigmine	0	0	0
3-Acetoxyphenyltrimethyl-ammonium iodide	103	103	—
9-Acetoxyretene	87	87	—
3-Acetoxyretene	83	83	—
Succinylcholine iodide	150	75	—
o-Acetylsalicylcholine chloride	250	125	—
Diacetylmorphine chloride	206	103	—
Triacetin	294	98	98
Tripropionin	314	105	—
Tributyrin	285	95	95
Glucose pentacetate	540	108	110
Maltose octacetate	840	105	—

The molar concentration of the substrate was 7.32×10^{-3}, and the enzyme solutions were kept in the refrigerator at 4° C. The results are shown in Fig. 2.

The activity seems to decrease during the first three months, but then appears to reach a constant value. The final activity depends on the pH but is 53—64 per cent of the original value. A measure of the stability may be obtained by determining the time required for the activity to be reduced to a certain degree, *e.g.* 75 per cent. On this basis the stability is greater at pH 6.7 than at pH 5.8 or 8.1.

Enzyme stability in various buffer solutions, and the influence of these solutions on the colour reaction and enzyme activity. It is well known that the buffer

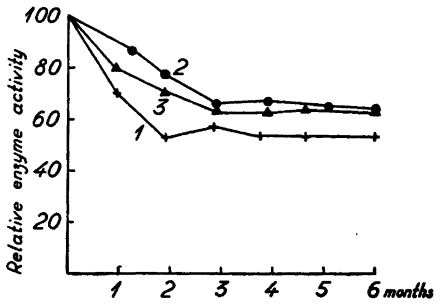


Fig. 2. Enzyme activity in buffers of various pH as function of time.
1. pH 5.8 2. pH 6.7 3. pH 8.1

solutions may influence the activity of enzymes. The activity of acetylcholinesterase has been determined in three different buffer solutions (bicarbonate, veronal and phosphate), all having a pH of 7.2. None of these buffers was found to disturb the colour reaction. 1.46×10^{-3} molar ACh was used as substrate.

In connection with these determinations the stability of the enzyme in different buffers was studied. The stability was measured both at 4° and 25° C. The activity was determined every week for three weeks with the results shown in Table 2.

It is clear from this table, that the rate of hydrolysis of ACh is the same in all three buffer solutions. At 4° C the solutions buffered with phosphate or bicarbonate have the greatest stability, the stability of the enzyme in a veronal buffer is much lower. Naturally the stability at 25° C is lower, but when a phosphate buffer is employed the change is not great. The enzyme dissolved in bicarbonate buffer shows a remarkably low stability at 25° C.

The hydrolysis of acetylsalicylcholine. Augustinsson [4] has shown that only one of the two ester linkages of ASaCh, the acetate link, is split with cobra venom when the concentration of the substrate is between 6.62×10^{-3} and

Table 2. Relative enzyme activity in various buffers as function of time and temperature.

Buffer		$t = 0$	$t = 1$ week	$t = 2$ weeks	$t = 3$ weeks
Phosphate	4° C	100	96	91	87
	25° C	100	83	74	70
Veronal	4° C	100	70	57	48
	25° C	100	52	39	32
Bicarbonate	4° C	100	100	87	83
	25° C	100	56	17	0

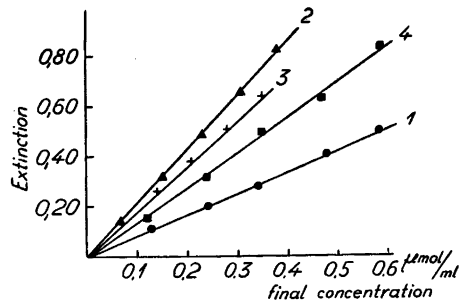


Fig. 3. Calibration curves of ACh(1), ASaCh(2), ASa(3) and SaCh(4).

1.99×10^{-4} molar, although both ester linkages are split when the molarity of the substrate was 6.62×10^{-2}—1.99×10^{-2}. These measurements were made at pH 7.4.

To investigate the influence of pH on these reactions ASaCh was enzymically hydrolysed at pH values in the range 5.0 to 8.1, and the extinction was determined when the hydrolysis had ceased. The molar concentration of the substrate used were 8.00×10^{-3} and 1.46×10^{-3}. From the calibration curves of ACh, ASaCh, ASa and SaCh (Fig. 3) the extinctions of the possible hydrolysis products have been calculated (Table 3).

Table 3. Calculated extinction when the hydrolysis of ASaCh has ceased as function of pS and the number and type of broken ester linkage.

	pS	
	2.1	2.8
Two ester linkages split	0	0
One » linkage » : SaCh left	0.22 *	0.40
» » » » : ASa »	0.30 *	0.52

* dilution 1:10

Because the three curves are quite distinct the difference in the final extinction is significant in the two cases where only one ester linkage is split. The results (Table 4) show that with ASaCh only one ester linkage is split irrespective of the pH of the reaction mixture, and that the ester linkage split is that of the acetyl group. The same results were obtained at both substrate concentrations.

Table 4. The extinction when the hydrolysis of ASaCh has ceased as function of pS and pH.

pH		5.0	5.8	6.7	8.1
pS	2.1	0.22 *	0.22 *	0.23 *	0.22 *
	2.8	0.41	0.42	0.40	0.38

* dilution 1:10

The quantitative determination of ASaCh is complicated by the fact that when the acetyl group is split off, the compound formed, SaCh, also gives a red colour with the hydroxylamine reagent. A theoretical calibration curve may be calculated as follows. The extinction of ASaCh can be written $E_{\mathrm{ASaCh}} = k_{\mathrm{ASaCh}} \cdot c_{\mathrm{ASaCh}}$ where k_{ASaCh} is a constant and c_{ASaCh} is the molar concentration of ASaCh. Likewise we obtain $E_{\mathrm{SaCh}} = k_{\mathrm{SaCh}} \cdot c_{\mathrm{SaCh}}$. If the concentration of ASaCh is s initially and x after a certain time then the concentration of SaCh at the same time will be $s-x$, and the extinction is given by:

$$E = E_{\mathrm{ASaCh}} + E_{\mathrm{SaCh}} = k_{\mathrm{ASaCh}} \cdot x + k_{\mathrm{SaCh}}(s-x) \quad \text{or}$$

$$E = E_0 + k \cdot x$$

where $E_0 = s \cdot k_{\mathrm{SaCh}}$ and $k = k_{\mathrm{ASaCh}} - k_{\mathrm{SACh}} \cdot k_{\mathrm{ASaCh}}$ and k_{SaCh} may be found from the calibration curves for ASaCh and SaCh, and thus x may be calculated from E. This method has been used in the following.

Influence of pH on the activity-pS curve of ACh. ACh solutions of various concentrations were enzymically hydrolysed at pH values varying from 5.8 to 9.8 (Fig. 4). All values have been corrected for non-enzymic hydrolysis which was noticeable only at pH values greater than 8.1.

Fig. 4 shows that with increasing pH pS_{opt} and the activity at pS_{opt} increase to a maximum. After that pS_{opt} and the activity at pS_{opt} decrease. These results are in agreement with the general tendency predicted by Wilson and Bergmann [6], and will be discussed below.

It is known that acetylcholinesterase of cobra venom gives a bell-shaped activity-pS curve both with ACh and ASaCh [4,7]. Two cases may be visualized. Either the two curves intersect, or one of them will lie inside the other. In the first case the enzyme will split one substrate at a higher rate than the other at a certain concentration and at a lower rate at another concentration, in the second case one substrate will be split at a higher rate at all substrate concentrations.

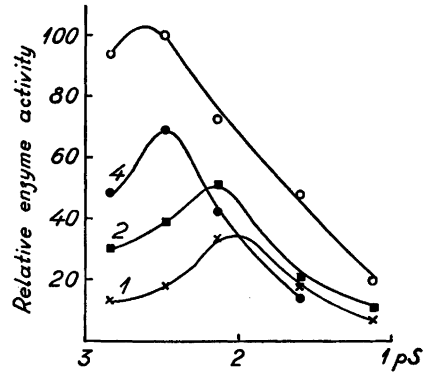


Fig. 4. Influence of pH on the activity-pS curve of ACh.

1. pH 5.8 2. pH 6.7 4. pH 9.8. The uppermost curve pH 8.1.

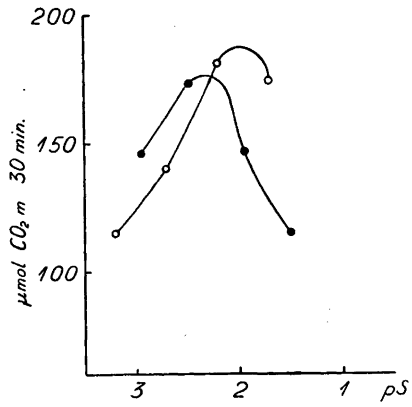


Fig. 5. Activity-pS curves of ACh(1) and ASaCh(2) at pH 7.4.

A preliminary experiment using the Warburg manometric method at pH 7.4 gave the result shown in Fig. 5, and clearly demonstrated that at this pH the system ACh—ASaCh is of the former type.

Enzymic hydrolysis were carried out with ACh and ASaCh at acidities varying between 5.8 and 8.1, and the substrate concentration given in Table 4. For comparison SaCh and ASa were also hydrolysed enzymically, but only at the higher concentration (Table 5). All the values are corrected for non-enzymic hydrolysis which, however, was significant only at pH 8.1.

When the molarity of the substrates is 8.00×10^{-3} (pS = 2.1) ASaCh is split at a higher rate than ACh, SaCh or ASa, the last two substances being almost unaffected. The difference between the rates of hydrolysis of ASaCh

Table 5. Relative enzyme activity towards ACh, ASaCh, ASa and SaCh as function of pH and pS.

Substrate \ pH	5.8		6.7		8.1	
	pS 2.1	pS 2.8	pS 2.1	pS 2.8	pS 2.1	pS 2.8
ACh	25	11	42	25	61	77
ASaCh	31	12	56	20	100	52
ASa	1		1		1	
SaCh	—		—		—	

and ACh decreases with decreasing pH. When the substrate concentration is 1.46×10^{-3} ($pS = 2.8$) and the pH 6.7 or 8.1 ACh on the other hand is split more rapidly than is ASaCh. The difference at pH 5.8 is insignificant.

DISCUSSION

Deductions on the influence of pH on the enzymic hydrolysis with acetylcholinesterase have been made by Wilson and Bergmann [6]. Between pH 7 and 9, $[S]_{opt}$ is expected to have a minimum, 3.2×10^{-3} molar, when the activity at $[S]_{opt}$ is a maximum. Outside this pH range $[S]_{opt}$ is higher and the activity at $[S]_{opt}$ lower.

As shown in Table 5, it is necessary to use the same substrate concentrations when comparing the activity of the enzyme towards various esters. If ACh is taken as the standard ester, it is convenient to make the comparison at the optimum concentration of ACh.

At pH 6.7 where pS_{opt} is 2.1 (Fig. 4) the rate of enzymic hydrolysis of ASaCh is higher than that of ACh (Table 5). On the other hand at pH 7.4 where pS_{opt} is 2.5 the rate of enzymic hydrolysis of ACh is higher than that of ASaCh (Fig. 5).

The results clearly show the importance of pH in studies on specificity.

SUMMARY

The determination of acetylcholinesterase (cobra venom) was carried out using the colourimetric method recently described by Hestrin [2]. The relative extinctions of the coloured complexis from various esters were determined.

The stability of enzyme solutions of different pH was examined and the stability of the enzyme in various buffers was studied at 4° and 25° C.

The influence of pH on the activity-pS curve of ACh was investigated.

The rate of enzymic hydrolysis of ACh, ASaCh, ASa and SaCh has been investigated at different pH and substrate concentrations.

The importance of pH in specificity studies has been discussed.

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The Amino Acid Composition of Bence-Jones Protein

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Dent and Rose [1] recently published a chemical study of Bence-Jones protein and reported that the sample isolated by them contained no methionine. In a preliminary note from this laboratory [2] a comparison was made (paper chromatography) of the amino acid composition of two samples of Bence-Jones protein, one prepared from urine, the other from a tumor extirpated for diagnostic purposes, both from the same person. The two samples of protein contained most of the common amino acids with exception of methionine and hydroxyproline. However, it was pointed out that the paper chromatographic method did not allow the identifying of methionine in a hydrolysate from a protein with the very low methionine content reported by previous workers. A microbiological amino acid analysis was therefore carried out on both of our samples of Bence-Jones protein.

MATERIAL AND METHODS

Preparation of material *. Both samples were prepared from the same patient with multiple myelomatosis. Most of a fresh small tumor extirpated for diagnostic purposes was ground and extracted with 0.9 per cent NaCl. The solution, adjusted to a pH of about 5 with acetic acid, was rapidly heated to 100°. From the hot, filtered solution the Bence-Jones protein precipitated at about 60°. The precipitate was washed with distilled water and dried with ethanol and ethyl ether. The urine was also made slightly acid with an acetate buffer, heated to 100°, filtered and allowed to cool to about 60°. The precipitate was washed and dried as above. Hydrolysates of tryptophan were prepared with barium hydroxide in nickel containers according to Miller and Ruttinger [3]. Acid hydrolysates, used for the determinations of the other amino acids, were prepared by hydrolyzing samples of protein for 6 hours at 15 pounds pressure with 3 *N* HCl in sealed tubes.

* The preparations were carried out by professor Blix in this department and kindly supplied to me.

Microbiological procedures. Only a few milligrams of protein from the tumor were available. A microadaptation of the microbiological procedure as described from this laboratory [4] was accordingly used throughout this investigation. Microorganisms, basal medium, ranges of standard curves, and incubation times are given in Table 1. The procedure for culture and inoculum has been described in previous papers [5,6]. A casein

Table 1. Experimental condition for the microbiological analyses.

Amino acid	Medium	Microorganism	Standard curve mμg per 50 μl	Incubation time, hours
Alanine	Sauberlich and Baumann [7]	*L. citrovorum* (8091)	125—1000	24
Arginine	Henderson and Snell [8*]	*L. casei*	62—500	48
Aspartic acid	Steele *et al.*[9]	*L. mesenteroides* P—60	125—1000	48
Cystine	Sauberlich and Baumann [7]	*L. citrovorum* (8081)	31—250	24
Glutamic acid	Steele *et al.*[9]	*L. mesenteroides* P-60	62—500	48
Glycine	Dunn *et al.*[10]	*R. citrovorum* (8081)	62—500	48
Histidine	Henderson and Snell [8]	*L. mesenteroides* P—60	62—500	48
Isoleucine	Sauberlich and Baumann [7]	*L. citrovorum* (8081)	62—500	24
Leucine	Henderson and Snell [8]	*L. arabinosus* 17—5	31—250	48
Lysine	—»—	*L. mesenteroides* P—60	62—500	72
Methionine	—»—	*L. arabinosus* 17—5	31—250	29
Phenylalanine	—»—	—»—	62—500	48
Proline	—»—**	*L. mesenteroides* P—60	62—500	48
Serine	—»—*	*S. faecalis* R (8043)	62—500	48
Threonine	Steele *et al.*[9]	*L. mesenteroides* P—60	62—500	48
Tryptophan	—»—	—»—	62—500	48
Tyrosine	Henderson and Snell [8]	—»—	62—500	48
Valine	—»—	*L. arabinosus* 17—5	62—500	48

* Single-strength medium diluted with an equal volume of water [3,5].

** pH of basal medium 6.0.

hydrolysate was always included as an extra control in the determinations of the amino acids. For each amino acid several separate assays were carried out with an average deviation of approximately ± 10 per cent for all amino acids. In each series five assay levels were used. The amino acids used as standards were dried *in vacuo*, at room temperature, and kept *in vacuo* in a desiccator containing silica gel. DL-forms of threonine and valine were employed. The natural isomers of the other were used.

Other analyses. The water content was measured by drying to constant weight at 100°. The ash was estimated by ignition to constant weight in a muffle furnace. The nitrogen content was determined by a micro-Kjeldahl procedure.

RESULTS AND DISCUSSION

In Table 2 the microbiological results are presented according to the usual procedure [11]. In the case of the urine protein, it is possible to account for 100 per cent of the weight of the protein and 96 per cent of the nitrogen. The

Table 2. Amino acid composition of Bence-Jones protein from a tumor and the urine of the same patient.

The total nitrogen corrected for ash and moisture was 16.1 per cent for the urine protein (UP). The ash content was 0.47 per cent and moisture 9.2 per cent. All values for the urine protein in Table 2 are expressed on an ash- and moisture-free basis. The figures for the tumor protein (TP) are calculated on the basis of the nitrogen content in the hydrolysate and on the assumption of 16.1 per cent of total nitrogen in the TP.

	G per 100 g protein		G residue per 100 g protein		G nitrogen per 100 g protein	
	UP	TP	UP	TP	UP	TP
Alanine	6.0	3.5	4.8	2.8	0.935	0.550
Arginine	5.7	5.4	5.1	4.9	1.830	1.740
Aspartic acid	6.1	7.8	5.3	6.7	0.640	0.820
Half-Cystine	3.8	0.67	3.2	0.57	0.445	0.079
Glutamic acid	16.6	13.8	14.6	12.1	1.580	1.310
Glycine	2.3	1.8	1.7	1.4	0.430	0.336
Histidine	1.0	1.2	0.88	1.1	0.271	0.325
Isoleucine	4.2	2.6	3.6	2.2	0.450	0.260
Leucine	10.6	8.8	9.2	7.6	1.135	0.943
Lysine	4.4	4.2	3.9	3.7	0.845	0.807
Methionine	0.40	0.1	0.35	0.01	0.038	0.009
Phenylalanine	2.7	2.0	2.4	1.8	0.230	0.170
Proline	6.4	5.6	5.4	4.7	0.780	0.685
Serine	16.7	8.1	13.8	7.2	2.220	1.078
Threonine	9.5	6.9	8.1	5.9	1.120	0.815
Tryptophan	3.9	2.5	3.5	2.3	0.535	0.343
Tyrosine	7.3	4.0	6.6	3.6	0.562	0.306
Valine	11.0	6.9	9.3	5.9	1.315	0.825
Total	118.6	85.9	101.7	74.5	15.4	11.4

amide nitrogen is not included in these figures. The corresponding figures for the protein from the tumor are 75 per cent and 71 per cent. These figures are calculated from the nitrogen content of the tumor protein hydrolysate and on the assumption that the total nitrogen content of the tumor protein also is 16.1 per cent. The very limited amount of tumor protein available did not permit any nitrogen and ash analyses. To a certain degree this complicates a comparison of the amino acid composition of the two sources of protein. Even so it is quite obvious that dissimilarities exist. This observation indicates the need for a certain degree of precaution in considering the connection between function and amino acid composition of Bence-Jones proteins isolated

from the urine. The amount of methionine found in our two samples of Bence-Jones protein was below the limit of detection of the chromatographic method for the quantities of hydrolysate previously examined [2].

The total nitrogen and ash contents of our urine protein is in good agreement with those found by Dent and Rose [12]. Contrary to the finding of these authors our two preparations contained methionine. The generally accepted molecular weight for Bence-Jones protein is 35 000 [13]. The calculated value for one molecule of methionine per molecule of protein is 0.43 per cent, in good agreement with the 0.40 found in our analysis. The most recent complete figures for the amino acid composition of Bence-Jones protein isolated from the urine are given by Roberts *et al.*[14] These authors also found methionine in their preparation. In general, there is comparatively good agreement with the values found in this investigation. Of special interest is the high content of serine and threonine found by Roberts *et al.* and in this investigation. The American authors stress the similarity in amino acid composition between their sample of Bence-Jones protein and the proteins from different strains of tobacco mosaic virus, with regard to the very high amount of aliphatic hydroxy amino acids. The high content of these acids in Bence-Jones protein is another structural analogy, in addition to the absence or very low content of methionine in both types of material [12]. Dent and Rose who first suggested that multiple myelomatosis might be due to the invasion of the body by a virus, also thought that the Bence-Jones protein, when combined with nucleic acid in the cells, was the virus itself. This brings us back to the fact that while we could account for 100 per cent of the urine material in form of amino acids, the corresponding figure for the tumor protein was only 75 per cent. Accordingly, it would seem to be of considerable value to further test the virus hypothesis by determining whether the unaccountable part of the tumor material is nucleic acid.

SUMMARY

Quantitative estimations of 18 amino acids have been carried out on two samples of Bence-Jones protein, one isolated from urine, the other from an extirpated tumor belonging to the same patient. Both proteins were characterized by a low content of methionine and by very high amounts of serine and threonine. The analysis accounted for 100 per cent of the weight of the urine protein and 75 per cent of the protein from the tumor. Possible reasons for this discrepancy are discussed.

The technical assistance of Mr. S. Eklund, Mr. E. Kristenson and Mrs. I. Kristenson is greatfully acknowledged.

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Purification of Bacitracin and Some Properties of the Purified Bacitracin

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Bacitracin is a polypeptide which in its chemical and physical properties is especially suitable for studies of possible separation methods applicable to peptides. The aim of this investigation has been to purify the crude bacitracin as far as possible, to determine its approximate molecular weight and, finally, to estimate its amino acid composition.

The purification was performed by means of carrier displacement analysis [1]; the molecular weight determination by means of ultracentrifugation (according to the calculation method suggested by Archibald [2]) and the estimation of the amino acid composition mainly by means of the usual paper chromatographic methods.

Williams, Hagdahl and Tiselius have recently shown that certain difficulties are involved in the use of carrier displacement analysis on carbon (in press). These difficulties also appear in connection with the separation of polypeptides. It is to be expected, however, that better separation results could be obtained, if there were a large excess of one component the intention being to purify this main component. By means of the method described below a far-reaching purification is possible. A specimen of bacitracin partly purified by counter current distribution according to Craig [3] proved to be an excellent starting material.

THE CARRIER DISPLACEMENT METHOD

For the displacement analysis of compounds *e.g.* A_1, A_2 and A_3 (A_3 "the displacer") with increasing adsorption affinity a diagram such as that shown in Fig. 1 is obtained.

If small amounts of substances with intermediary adsorption affinity are introduced, these ought to appear at the boundaries v_1, v_2, and v_3. Suppose

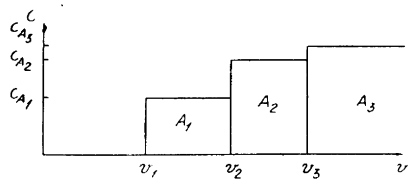


Fig. 1. Displacement analysis diagram for three components, concentration in effluent against effluent volume.

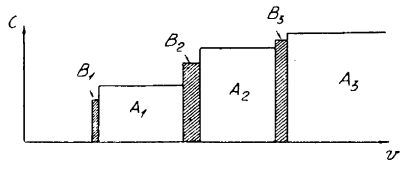


Fig. 2. Ideal carrier displacement analysis diagram. The components of a mixture (B_1, B_2 and B_3), separated by three carriers (A_1, A_2 and A_3).

B_1, B_2 and B_3 which are substances having such adsorption properties are introduced simultaneously with A_1 and A_2 into a column. If the isotherms of the system A_1, A_2, A_3 and the system B_1, B_2, B_3 are of the same shape, a diagram shown in Fig. 2 is obtained. The advantage of this method, the carrier displacement method, lies in the fact that the components of the mixture can be separated in the column by large volumes of interposed substances of a different nature [1]. These latter substances should be chosen in such a way as to make it possible to remove them easily from the required compounds.

If the isotherms of the carriers and those of the components in the mixture cross each other, some complications arise. If, alcohols for example are used as carriers for peptides and the column is loaded with large amounts of peptides, high elution peaks will be seen in the diagram (Fig. 3). There is more or less tailing in the regions behind the zone boundaries as indicated in the diagram. In this case it is even more important to separate the zone boundaries by large volumes of the carriers.

If the carriers are pressed into the column as in an ordinary displacement analysis (Fig. 1) the volumes of v_1, (v_2-v_1) and (v_3-v_2) will be determined by the retention volume v_3 of the displacer. The retention volume v_3 can be increased either by lowering the concentration C_1 of the displacer or by increasing the filter volume.

A much simpler way to vary at will the distances between the zone boundaries is to introduce the carrier solutions at equilibrium concentration. This will give a diagram shown in Fig. 4. This diagram differs from Fig. 1 in one respect.

The volumes $(v_2'-v_1')$ and$(v_3'-v_2')$ can be chosen arbitrarily at the fixed concentrations C_{A_1}, C_{A_2} and C_{A_3}.

This method offers several advantages. A small filter can be utilized for comparatively large amounts of substance and the distances between the zone boundaries can be conveniently chosen at will. A great number of carriers

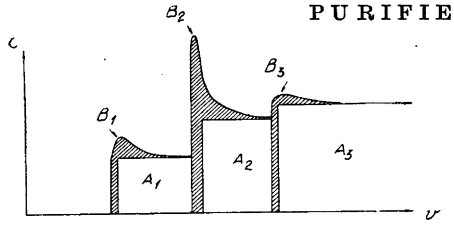


Fig. 3. Carrier displacement analysis diagram obtained for systems with crossing isotherms.

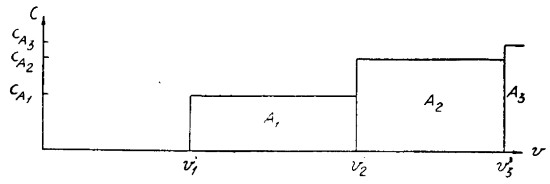


Fig. 4. Displacement analysis diagram with elongated steps.

can thus be used in a single experiment. It is also possible to get long steps over a greater range of concentrations of the carriers, a fact which seems to be of certain importance.

EXPERIMENTAL

Starting material: The preparation used had been purified by means of counter current distribution at the Research laboratories of Merck and Co. Inc., Rahway, N. J., U.S.A. ("Bacitracin 50 R 4996"). Its original activity was about 50 units/mg, but at the time of this investigation the activity had declined to 36—37 units/mg. The lyophilized product was faintly yellowish but the concentrated water solution was of a very deep red-brown colour. Paper chromatograms developed with acetic acid : butanol : water (1 : 4 : 5) indicated that the substance was a mixture consisting of at least five components. These components could be detected after treatment with ninhydrin by examination in visible and ultraviolet light.

Apparatus: A sectioned column of plastic filters each surrounded by a steel support of the type described by Hagdahl and Porath [5] was used. The progress of the analysis was followed by means of the Tiselius-Claesson interferometer [6].

Procedure: 50 % ethanolic solution in 0.1 *N* HCl, was used throughout as a solvent for the carriers. Quite as good a separation was obtained in neutral solution but complete inactivation occurred. Using this solvent, solutions of 0.5, 1.0 and 2.5 % *n*-heptanol-1 of *n*-octanol-1 and *n*-nonanol-1 respectively were prepared (volume percentages.) An ordinary displacement analysis was performed. From this experiment the equilibrium concentrations corresponding to 2.5 % nonanol were obtained: 0.43 % heptanol and 1.70 % octanol.

The filters (2, 4, 8 and 14 ml) were packed with a suspension of carboraffin supra charcoal and celite (1 : 3 parts by weight) in water. After washing with 0.1 *N* HCl/50 % ethanol solution, 50 mg bacitracin, dissolved in 3 ml of this solvent, was pressed into the column. This solution was followed successively by 20 ml 0.43 % heptanol solution, 25 ml 1.70 % octanol solution and finally by 2.5 % nonanol solution. The eluate was collected in graduated test tubes. Higher alcohols were removed from the fractions by extraction twice with an equal volume of hexane each time. The solvent was evaporated *in vacuo* over NaOH-pellets.

Fig. 5 shows a diagram of a typical experiment. Several experiments have given curves of the same shape. The nitrogen recovery varied from about 50 to 72 %. Most of the substance (about 60 % of the amount obtained in the fractions) was found in fraction 10 at the boundary between the heptanol and the octanol. The large amount of material in this fraction is indicated by the high elution peak shown in the diagram. There is also a free elution peak in fraction 3—5. As will be seen below, these fractions contain lower peptides.

The distribution of the nitrogen in the fractions of one experiment is shown in the table below.

Table 1. Nitrogen distribution.

Fraction	Mg N	Percent of the total amount of N
1— 4	0.144	2.0
5— 8	0.216	3.0
9	0.312	4.4
10	2.918	40.8
11	0.810	11.3
12	0.250	3.5
13—17	0.464	6.5
	5.11	71.5

INVESTIGATION OF THE FRACTIONS

1. Qualitative bacteriological tests. Corynebacterium xerosis* was used in order to determine qualitatively the distribution of the bacteriostatic activity over the fractions [4].

* A quantitative determination of the material in fraction 10 made at the Research Laboratories of Merck and Co. Inc., Rahway, N.J., U.S.A., indicated a decrease in the activity to about 27 units/mg (calculated on the free base). The inactivation may have occurred in the strongly acid solution or during shipment.

It might be considered that the comparatively low activity yield indicates that some still more active compound has not been displaced from the carbon. This is, however, probably not the case. 50 % propanol, a very powerful eluting agent, does not elute any higher peptide [7].

A completely inactivated material cannot be distinguished from the active substance neither by means of adsorption analysis nor by any other method, here used, except biological assay. Therefore it seems reasonable to assume that the inactivation must be due to some intramolecular rearrangement or at any rate a change which does not alter considerably the molecular weight. In order to study this it is necessary to follow more carefully the inactivation at the different steps during purification. Such controls have not been possible at these Institutes.

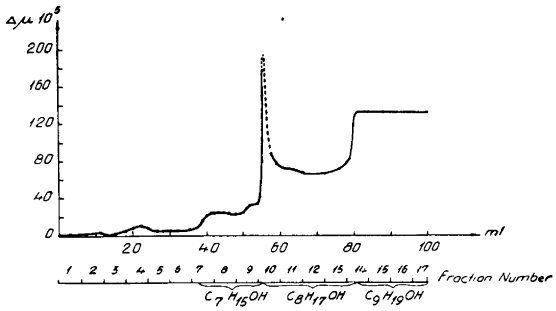


Fig. 5. Carrier displacement analysis diagram for a crude bacitracin preparation. Carriers: n-heptanol-1, n-octanol-1 and n-nonanol-1. Refractive index increment of effluent plotted against effluent volume.

The dried fractions were dissolved in small volumes of water. Each solution was sucked up into a circular filter paper of 10 millimeters' radius. Only about 0.5 mg of fractions 9 and 10 was taken, while about half the material of the other fractions was used. The papers were placed on blood agar plates containing 24 hours' old beef broth for 10 minutes at 37° C. The papers were removed and the blood agar on each plate were inoculated with one loopful of culture. After incubating 24 hours at 37° C the inhibition zones were measured.

The results are compiled in Table 2.

Table 2. Activity distribution.

Fraction no.	Activity *	Fraction no.	Activity *
1	0	8	0
2	0	9	++
3	0	10	+++
4	0	11	+++
5	0	12	++
6	0	13	+
7	0	14	+
		15	(+)

2. Paper chromatography. Small amounts of the material of the evaporated fractions were transferred to paper (Whatman no. 4). One-dimensional chromatograms were developed in the usual manner with acetic acid : *n*-butanol : water (1: 4 : 5). After treatment with 0.1 % ninhydrin dissolved in *n*-butanol the components were localized by examination in visible and ultraviolet light.

Fig. 6 is a map of the chromatograms showing the distribution of the peptides in the fractions obtained by the carrier displacement experiment.

* +++ indicates very strong act.
++ » » »
+ indicates very low act.
(+) » » » »
0 » no »

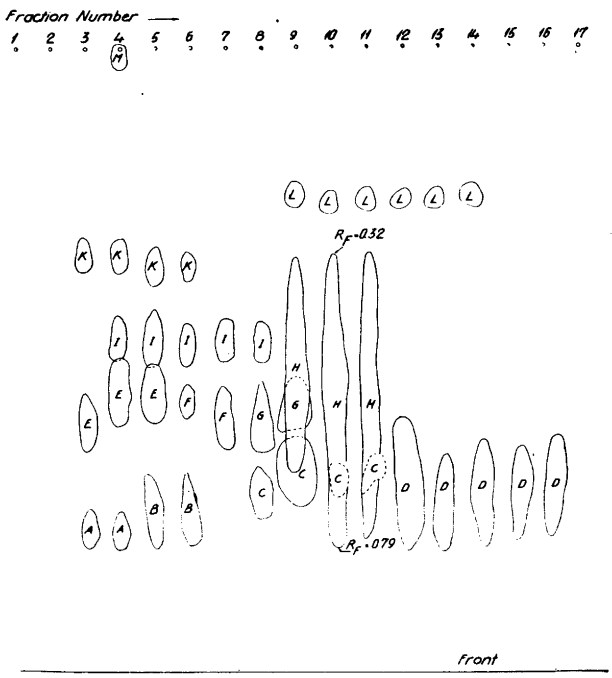


Fig. 6. Map of an one-dimensional paper chromatogram of the fractions obtained from the carrier displacement analysis of crude bacitracin (see Fig. 5).

The colours of the spots are given in Table 3. Fig. 7 shows a photography of chromatogram of the combined fractions 1—9 and fraction 10.

The most active fractions (10 and 11) contain at least three components corresponding to the spots H, C and L in the diagram. From the first nine fractions only small amounts of substance were recovered. Therefore, as the colours of the spots E and I were very intense, it seems reasonable to assume that the material of these fractions consists of lower peptides and, possibly, of amino acids. Since these compounds are inactive no further examination was made.

To establish that the lower peptides did not originate from the active peptide (as a results of a breakdown of the peptide in the acid medium influenced by the active carbon) a column was loaded with fraction 10 and another run was performed in exactly the same manner as before. Investigation of the new fractions shows that cleavage of the peptide chains does not occur. There was no detectable material in the new fractions 1—8 and only a very small amount in fraction 9. The chief material came in fraction 10 as was expected and a slight amount was also found in fractions 11 and 12. The paper chromatograms of fractions 10—12 show exactly the same spots as in the original experiment, but C and L were extremely weakly coloured. From elution

Fig. 7. Photography of chromatograms of the combined fractions 1—9 and fraction 10.

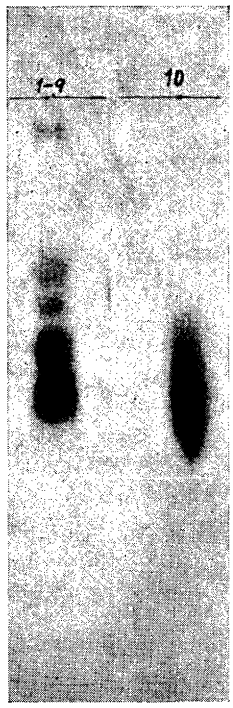


Table 3.

Spot	Colour in visible light	ultraviolet light
A	pink	dark blue
B	violet	yellow
C	— — —	light green
D	green	dark green
E	dark violet	blue green
F	pink	— — —
G	faint violet	light blue
H	greenish violet	dark green
I	faint violet	— — —
K	pink	blue *
L	pink	yellow
M	pink	blue *

experiments it has become clear that the original preparation only contains traces of the component L.

From the very faint colour of spot L in fraction 10 it was clear that it must contain an extremely small amount of material. In order to estimate the amount of material which causes the strong ultraviolet fluorescence of spot C, a series of chromatograms containing 50 mg total of crude bacitracin was developed. The system used was acetic acid: diethyl carbinol: water (1 : 4 : 5). All the material except the fluorescent matter remained in the original spot. A yellow spot (coloured before the ninhydrin treatment) at R_F 0.22 exhibited a very strong ultraviolet fluorescence, thus proving the identity with the material of spot C in fraction 10. Since the ninhydrin test was negative the material in spot C was probably not a peptide. This substance was eluted from the paper and the eluate was lyophilized. A very small amount (about 0.5 mg) of a bright yellow powder was obtained. The bacteriological test indicated that this pigment was completely inactive.

* Sometimes these spots appeared yellow.

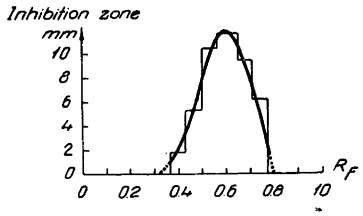


Fig. 8. Activity curve showing the distribution of activity for the fraction 10 from the carrier displacement analysis in a one-dimensional paper chromatogram (see Fig. 6).

In order to establish which component (or components) is responsible for the activity a test of fraction 10 was made as described below.

About 1 mg of dried substance from fraction 10 was transferred to a 2.5 cm broad paper strip (Whatman no. 4). A chromatogram was developed by use of acetic acid : *n*-butanol : water (1 : 4 : 5). The front was marked on the moist paper and after that the paper was washed with ether and dried. The paper was cut into 1 cm broad strips perpendicular to the direction of the development. The paper strips were placed on plates containing blood agar and were inoculated as described above. After 24 hours the inhibition zones were measured, inhibition zone defined as

$$\frac{\text{(width of the culture-free region)} - \text{(width of the paperstrip)}}{2}$$

Fig. 8 shows the activity curve obtained by plotting the width of the inhibition zones *versus* the R_F-values of the paper strips. The position of the activity coincides (as far as can be read from the diagrams) with that of spot H in Fig. 6, as also the maxima of intensity of both activity and colour.

SOME PROPERTIES OF THE PURIFIED BACITRACIN (FRACTION 10)

1. Check of the purity by means of electrophoresis on paper. Electrophoresis on paper was performed according to Kunkel and Tiselius [8], using the apparatus constructed by them.

About 1 mg of the substance dissolved in buffer solution was put on to a paper (Munktell no. 20) previously soaked in the same buffer. The paper was placed between two glass plates coated with silicone grease and the run was made by using 8 volt/cm for 5 hours. Acetate buffer, pH 4.8, μ 0.1, and veronal buffer, pH 8.6, μ 0.1, were used. The position of the peptide was determined by treatment of the paper with ninhydrin solution. On heating the paper from the electrophoresis runs only one spot could be detected in both cases (see Fig. 9). One paper strip from a run at pH 4.8 was cut into pieces and tested on biological activity in the manner described before.

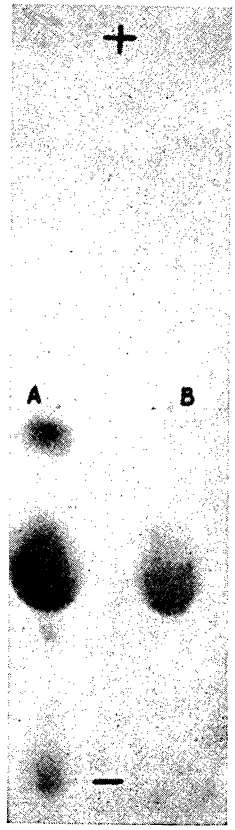


Fig. 9. Paper electrophoresis diagrams of crude bacitracin (A) and purified bacitracin (B). The run was made at pH 4.8 $\mu = 0.1$ using a field strength of 8 volt/cm during 5 hours.

The activity curve showed a maximum at a distance from the origin corresponding to that of the ninhydrin spot. A low activity was however observed throughout the whole region between the maximum and the origin. This indicates that there is some adsorption of the active peptide on the paper. In any event the experiments show that the bacitracin preparation is electrophoretically homogeneous as far as the sensibility of the ninhydrin reaction is concerned.

2. *Determination of the molecular weight.* Archibald has suggested a method of calculation which can also be used for determination of the molecular weight of comparatively small molecules by means of the ultracentrifuge [2]. The principle of this method is briefly described.

Consider that we are dealing with a monodisperse solution. The material flux Φ through the surface at a distance x form the axis of revolution will then be determined by the equation,

$$\Phi = x\,\Theta\,h\left(\omega^2 x\,n\,s - \mathrm{D}\frac{\delta n}{\delta x}\right) \tag{1}$$

where n is the concentration, ω the angular velocity of the rotor, s the sedimentation constant, D the diffusion constant, (x_a, x_b, Θ and h determine, as is shown in Figure 10, the shape and size of the cell).

If equation 1 is written,

$$\Phi = D\omega^2 x^2 n\,\Theta\,h\left(\frac{s}{D} - \frac{1}{\omega^2 x\,n}\frac{\delta n}{\delta x}\right) \tag{2}$$

it is easily seen that Φ will vanish for $\frac{1}{\omega^2 x\,n}\frac{\delta n}{\delta x} = \frac{s}{D}$. This is apparently the case

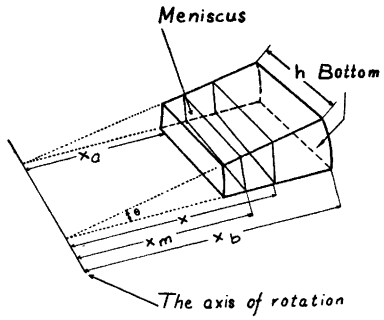


Fig. 10. Schematical drawing of the sedimentation cell of the ultracentrifuge.

when equilibrium is reached. At equilibrium this is valid for all points between x_a and x_b. It is possible, however, to determine $\frac{s}{D}$ at earlier stages of centrifugation. At the meniscus $x = x_m$ and at the bottom $x = x_b$ the material flow must evidently be zero and independent of the time from the start of the centrifugation. As $\frac{1}{\omega^2 x\, n} \cdot \frac{\delta n}{\delta x}$ can be calculated from the variation of refractive index (this is made in the same manner as described by Svedberg and Pedersen [9]) $\frac{s}{D}$ can be found by extrapolating the values of $\frac{1}{\omega^2 x\, n} \frac{\delta n}{\delta x}$ to the meniscus or the bottom. This determination is of importance, as the diffusion constant for low molecular compounds cannot easily be determined. The molecular weight can then be calculated from Svedberg's formula,

$$M = \frac{s}{D} \frac{RT}{1 - V\varrho} \tag{3}$$

Bacitracin hydrochloride (13.3 mg of preparation 10) was dissolved in 0.8889 ml 0.1 *M* potassium dihydrogen phosphate solution. The run was performed by means of an oil-driven ultracentrifuge at the speed of 400 r.p.s. At certain intervals exposures were taken. The calculated values of $\frac{1}{\omega^2 x\, n} \cdot \frac{\delta n}{\delta x}$ plotted against x gave a set of curves, some of which are shown in Fig. 11. From each curve two values of $\frac{s}{D}$ were obtained. The results are compiled in Table 4.

As sufficient amounts of the pure substance were not available, the partial specific volume of the unpurified preparation was used for the calculation of

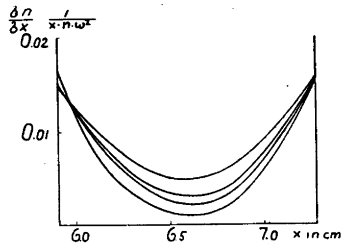


Fig. 11. Four curves showing the variation in the function $\frac{\delta n}{\delta x} \cdot \frac{1}{x\, n\, \omega^2}$ *with the distance from the axis of rotation (x) at four different times. The upper curve is obtained 840 minutes after the start and the lowest curve at 420 minutes after the start (see formula 2).*

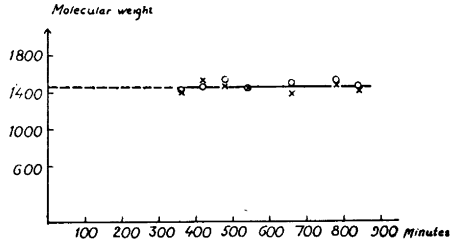


Fig. 12. Molecular weight of bacitracin as calculated from the values obtained for $\frac{s}{D}$ *at the meniscus (×) and at the bottom of the cell (○), for different times after the start of the run.*

the molecular weight. $V_{20} = 0.734$, the value used, is the mean value of two determinations according to Drucker [10] (0.732 and 0.736).

Fig. 12 shows the calculated molecular weights as a function of time. It is clearly seen that the molecular weight of the material at the bottom as well as at the meniscus is independent of time. This indicates that the preparation only contains molecules of the same or nearly the same weight. The average value of the molecular weight of these 14 determinations was found to be 1 460.

Table 4. s/D and M at different times after the start of centrifugation.

Time in minutes	s/D		M	
	Meniscus	Bottom	Meniscus	Bottom
360	0.0150	0.0152	1 410	1 430
420	0.0163	0.0157	1 540	1 480
480	0.0156	0.0164	1 470	1 550
540	0.0154	0.0155	1 450	1 460
660	0.0147	0.0160	1 390	1 510
780	0.0157	0.0162	1 480	1 530
840	0.0149	0.0157	1 400	1 480
			Average: 1 460	

In this actual experimental series the maximum deviation from the average is somewhat more than $\pm$ 6 % *.

3. *Some preliminary analytical data.* Purified bacitracin hydrochloride was hydrolyzed in a sealed tube with 6 *N* hydrochloric acid at 110° for 30 hours.

* A more detailed description will appear elsewhere.

From paper electrophoresis and paper chromatograms the following number of residues in the bacitracin molecule seem to be the most probable: aspartic acid 2, glutamic acid 1, leucine 1, isoleucine 2 or 3, phenylalanine 1, cysteine 1, histidine 1, lysine 1 and ornithine 1. A peptide so composed should have a molecular weight in excellent agreement with the value found by the ultracentrifugation. The amino acid analysis is however only to be considered as an estimation.

The bacitracin hydrochloride was found to contain 9.83 % Cl, 13.53 % N and 1.95 % S. The sulphur content, which was determined by a method recently developed by Paulson [11] corresponds to an equivalent weight of 1496, calculated on the free peptide.

SUMMARY

The investigation described above has shown how it is possible to reveal analytically the complexity of a preparation, previously highly purified by means of counter current distribution, and to further purify the main component, to give a monodisperse, electrophoretically homogeneous product.

By ultracentrifugation the molecular weight of bacitracin has been found to be *about 1460*.

The author wishes to thank Professor A. Tiselius and Dr. K. Pedersen for valuable discussions, Dr. Karl Folkers and Dr. B. Zetterberg for help with the bacteriological assays, Fil.mag. S. Paulson for the analysis of the sulphur content and Mr. E. Hellman for help in connection with the molecular weight determination. He is also indebted to Mr. S. Jerstedt for technical assistance. The investigation has been supported by a grant from the *Nobel Foundation* (kemiska prisgruppens särskilda fond).

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The Influence of the Solvent on Reaction Velocity

III. The Hydrolysis of Valerolactone

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In the previous investigations [1,2] of this series the influence of the solvent on the alkaline hydrolysis of carboxylic esters was studied. It was found that a small addition of an alcohol, acetone, or dioxan into the water used as solvent often slightly enhances the velocity of the reaction, especially if the temperature is low, but that larger amounts of these organic substances always powerfully decrease the reaction rate. Much more interesting features revealed the determination of the parameters of the Arrhenius equation. In binary mixtures of water with aliphatic alcohols, acetone, or dioxan the activation energy and frequency factor have a minimum at the mole fraction x_{water} 0.94 —0.90, methanol-water and glycol-water being the only exceptions found to this rule. Besides, several other peculiarities appear, *e.g.* a steep fall of the frequency factor in aqueous alcohol at high alcohol percentages.

To see in what extent these observations have a wider bearing we have investigated the hydrolysis of valerolactone. The present communication contains the results of these experiments. The reaction has previously been studied by Hegan and Wolfenden [3] in ethanol-water, but their experiments did not cover the whole range of mixtures. However, on the ground of their results a minimum in E and A could be expected also in this case.

EXPERIMENTAL

Preparation of valerolactone. For the reduction of laevulinic acid to γ-hydroxyvaleric acid we used hydrogenation under pressure in presence of a catalyst, which is much more profitable than the older methods, *e.g.* that given by Taylor and Close [4]. 58 g (ca. 0.5 moles) of laevulinic acid was neutralized with 20 g of sodium hydroxide dissolved in 100 ml of water, this solution diluted up to 200 ml with water and introduced in a 500 ml hydrogenation bomb of stainless steel. Raney-nickel W6, prepared according to Adkins and Billica [5], was used as catalyst. The bomb was placed in an electric heater

mounted on a shaking apparatus. Hydrogen of a pressure of 95 atmospheres was admitted to the bomb, while it was shaken, through a supply tube. The reaction set in at about 50° C, at which temperature the reduction was then performed. After two hours no more hydrogen was absorbed. The bomb was left to cool and opened; 65 ml of concentrated hydrochloric acid was added to the solution and the solution saturated with ammonium sulphate. The liberated γ-hydroxyvaleric acid was extracted with ether in an extraction apparatus. The extract was dried over anhydrous sodium sulphate, the ether distilled off, and the residue repeatedly distilled until a constant boiling point testified to the purity of the product. Usually two or three distillations are enough. The valerolactone obtained, a colourless liquid boiling at 207°, was practically pure. The yield depends greatly on the thoroughness of the extraction; a yield of 75 per cent is fairly easily reached.

The methanol, ethanol, acetone, and water used as solvent in the kinetic experiments were of the following qualities.

Methanol: A commercial synthetic product was distilled over anthranilic acid (10 g per 1 liter) and then treated by the method of Lund and Bjerrum [6], with the only exception that in the final distillation anthranilic acid was used instead of tribromo-benzoic acid. The purity of the product was checked by comparing its refractive index with that of methanol obtained from pure methyl oxalate.

Ethanol: Usual 95 per cent alcohol was dried by boiling with calcium oxide and finally purified by the method of Lund and Bjerrum.

Acetone: A commercial product of the quality "pure" was dried over ignited potassium carbonate and distilled.

Water: Only freshly distilled water was used.

Method. Wolfenden and Hegan used the conductivity method. Owing to the rapidity of the reaction in water, we, however, chose the titration method employed in this laboratory. The making use of the reaction vessels previously described [7] renders it possible to follow the course of fast reactions, too. The experiments were carried out by using both lactone and sodium hydroxide in equal concentrations. Except in experiments for the investigation of the dependence of the specific rate on the initial concentration of the reactants, 5 ml of a 0.05 *M* solution of the lactone was run into the one compartment of the reaction vessel, and 5 ml of a 0.05 *M* NaOH into the other, so that, after mixing, the initial concentration of each reactant was 0.025 moles/liter. The reaction vessel was kept in the thermostat until the reactants had acquired its temperature, and the reaction was then started by mixing of the two solutions. The reaction was stopped by adding a slight excess of 0.02 *N* hydrochloric acid and the acid titrated with 0.02 *N* baryta solution, cresol red being used as indicator.

The temperature of the thermostats could be held constant within $\pm 0.02°$. The thermometers used in the thermostats were checked against a thermometer calibrated by the National Bureau of Standards (U.S.A.).

The velocity constants were calculated by the ordinary bimolecular formula. To save space, merely the means of the runs (usually eight measurements) are given below. As an illustration the following two runs chosen at random may be sufficient.

1. Solvent water, $a = 0.025$ mol/l, temperature 40.00° C.

Time sec.	10	15	20	40	50	60	70	80
Percentage change	19.6	28.0	32.8	49.2	55.3	60.0	63.2	66.0
k l mol^{-1} sec.$^{-1}$	0.970	1.038	0.978	0.969	0.990	1.000	0.982	0.972

2. Solvent 60 per cent methanol, $a = 0.025$ mol/l, 0° C.

Time mins.	60	100	180	325	365	525	675	750
Percentage change	16.0	23.6	34.4	48.8	52.7	61.2	67.2	69.2
10^5 k l mol^{-1} sec.$^{-1}$	211	206	194	195	201	200	202	200

RESULTS AND DISCUSSION

The rate constants. The rate constant k is practically independent of the initial concentration a of the reactants and of an added salt, as is seen from the following results in water at 25° C:

a mol/l	0.05	0.025	0.01	0.025	0.025
KCl mol/l	—	—	—	0.1	0.2
k l mol^{-1} sec.$^{-1}$	0.411	0.400	0.412	0.411	0.409

This being stated, all the measurements were, as mentioned above, carried out using the initial concentrations 0.025 mol/l.

The experimental rate constants, corrected for change of concentration due to thermal expansion of the solvent, are given in Table 1. It is interesting to note that methanol always retards the reaction, whereas ethanol, when present in small amounts accelerates, but when present in large amounts retards it. The lower the temperature, the greater is the accelerating effect, and obviously it does not occur above a certain temperature. A corresponding acceleration is observed also in the alkaline hydrolysis of ethyl acetate [1,8]. A possible explanation is that in this composition range of the solvent the solvation of the transition state is strongly increased [1], which causes a great decrease in the energy of activation.

The retarding influence of methanol is much greater than that of ethanol, as is the case also in the alkaline hydrolysis of ethyl acetate [1]. Acetone has in greater concentrations a weaker effect than ethanol. No exact mathematical relationship between the rate constant and the composition of the solvent can be given, but it may be mentioned that in methanol-water in the range from pure water to the mole fraction $x_{\mathrm{MeOH}} = 0.85$ (0—90 % by weight of methanol) log k is practically a linear function of the methanol content: at 25° $\log k = -0.400 - 2.625\ x_{\mathrm{MeOH}}$. Log k then falls with rapidly increasing slope (Fig. 1). For the other mixtures only very rough relations can be given.

The Arrhenius equation. Table 1 also contains the values of the activation energy E and the frequency factor A, calculated by the Arrhenius equation $k = Ae^{-E/RT}$ from the experimental values of k at different temperatures using the method of least squares. In most cases the Arrhenius equation was

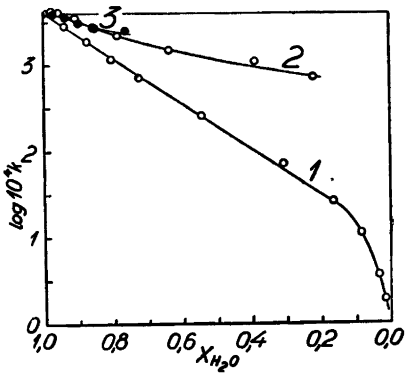


Fig. 1. Plot of log k vs. $x_{\text{water, 25° C}}$.
1. Methanol-water.
2. Ethanol-water.
3. Acetone-water.

obeyed within the limits of the experimental error, but in many methanol-water mixtures there appeared deviations from a linear relationship between log k and $1/T$. These will be treated later on separately. The data of Table 1 are mean values for the whole temperature range used, obtained by the method of least squares. They are fit enough for the examination of the general solvent effect.

It may be mentioned that our results are in good agreement with the data of Hegan and Wolfenden: | p % EtOH, E, log A | 0, 11 200, 7.89 | 21.5, 10 800, 7.46 | 40, 12 600, 8.57 | 60, 14 500, 9.78 |.

From the table or more cleary from Figs. 2 and 3 we see that E and A continuously rise with increasing methanol content of the solvent, the influence of methanol being very powerful. At the mole fraction $x_{\text{MeOH}} = 0.75$ A has its highest value, after which it rapidly falls. In ethanol-water and in acetone-

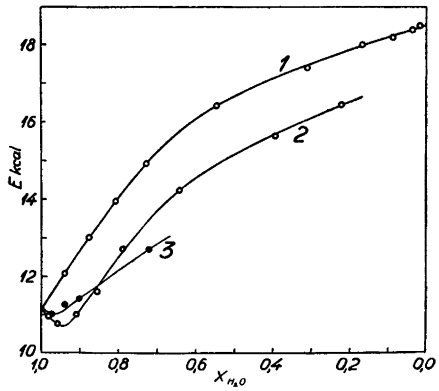


Fig. 2. Graph to show the correlation between E and x_{water}.

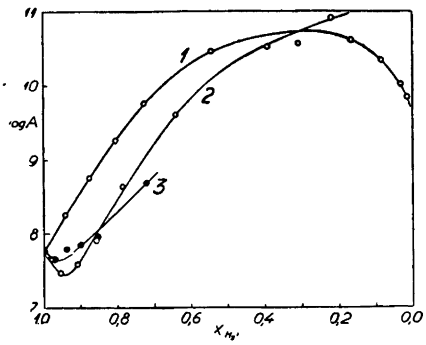


Fig. 3. Graph to show the correlation between log A and x_{water}.

Table 1. The values of k, E, log A, and ΔS.*

Solvent	x_{H_2O}	k 1 mol^{-1} sec.$^{-1}$					E cal	log A	ΔS^* E.U.
		0.00°	15.00°	25.00°	40.00°	50.00°			
Water	1	0.0714	0.208	0.400	0.987		11 150	7.776	−25.0
10 wt. % Methanol	0.941	0.0434	0.137	0.283	0.732		12 050	8.286	−22.6
20 » »	0.877	0.0244	0.0873	0.187	0.516		13 000	8.772	−20.4
30 » »	0.806	0.0128	0.0510	0.115	0.341		13 950	9.282	−18.0
40 » »	0.727	0.00666	0.0291	0.0697	0.223		14 930	9.780	−15.8
60 » »	0.543	0.00198	0.00978	0.0255	0.0943		16 420	10.468	−12.6
80 » »	0.308	0.0_3454	0.00242	0.00663	0.0271		17 400	10.565	−12.2
90 » »	0.165	0.0_3160	0.0_3872	0.00260	0.0109		18 000	10.602	−12.0
95 » »	0.085		0.0_3380	0.00110	0.00465	0.0120	18 200	10.365	−13.1
98 » »	0.035			0.0_3359	0.00158		18 400	10.020	−14.7
99 » »	0.018			0.0_3190	0.0_3894	0.00519 (60°)	18 500	9.839	−15.5
5 wt. % Ethanol	0.980	0.0758	0.220	0.411	1.004		10 960	7.651	−25.5
10 » »	0.958	0.0775	0.217	0.405	0.969		10 730	7.471	−26.4
20 » »	0.910	0.0638	0.184	0.349	0.847		11 000	7.596	−25.7
30 » »	0.857	0.0432	0.136	0.269	0.656		11 600	7.912	−24.2
40 » »	0.788	0.0291	0.101	0.216	0.575		12 700	8.641	−21.0
60 » »	0.640	0.0158	0.0642	0.147	0.452		14 250	9.609	−16.6
80 » »	0.390	0.00878	0.0404	0.104	0.347		15 650	10.517	−12.4
90 » »	0.221	0.00526	0.0262	0.0708	0.251		16 450	10.906	−10.6
10 wt. % Acetone	0.972	0.0715	0.203	0.389	0.950		11 000	7.651	−25.5
20 » »	0.939	0.0636	0.183	0.355	0.898		11 250	7.800	−24.8
30 » »	0.900	0.0535	0.165	0.309	0.789		11 400	7.847	−24.6
40 » »	0.854	0.0461	0.141	0.274	0.712		11 600	7.956	−24.2
60 » »	0.721	0.0342	0.116	0.247	0.674		12 700	8.688	−20.7

water E and A have a minimum at $x_{water} = 0.95$. In these media E is always and A generally much lower than in methanol-water of the same molar composition. The curves in Figs. 2 and 3 have about the same form as those connected with the alkaline hydrolysis of carboxylic esters.

The correlation between E and log A is seen from Fig. 4. For ethanol-water and acetone-water the relationship is linear before and after the minimum. For methanol-water a slight deviation from linearity is observed, and besides, at high methanol contents the line is strongly curved. The linear relationship

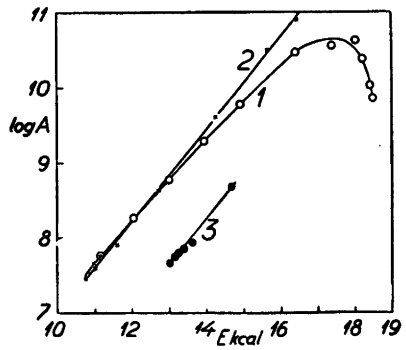


Fig. 4. Plot of log A against E.

1. *Methanol-water.*
2. *Ethanol-water.*
3. *Acetone-water, E + 2 kcal.*

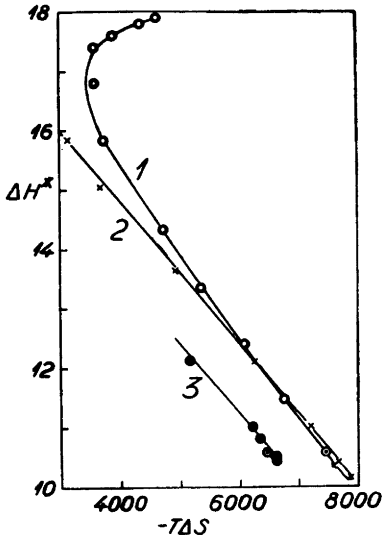


Fig. 5. Plot of ΔH^* vs. $T\Delta S^*$.

1. *Methanol-water.*
2. *Ethanol-water.*
3. *Acetone-water, $-T\Delta S^* + 1\,000$.*

between E and log A is equivalent to a linear relationship between the heat of activation and the entropy of activation (Fig. 5), as expressed by the equation [1].

$$T\Delta S^* = \alpha \Delta H^* + \beta$$

From Fig. 5 we obtain for ethanol-water and acetone-water mixtures in which the mole fraction of water is less than 0.9, $\alpha = 0.84$, which corresponds to the ratio $\Delta \log A/\Delta E = 0.000616$ in Fig. 4. For the narrow region between pure water and the minimum $\alpha \approx 1$. For methanol-water $\alpha \approx 0.7$ or $\Delta \log A/\Delta E \approx 0.0005$. These values are thus of the same order of magnitude as for the alkaline hydrolysis of carboxylic esters.

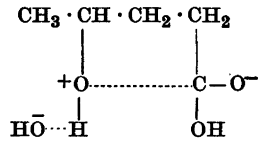
The entropies of activation are calculated by the formula

$$\log A = e \frac{kT}{h} e^{\Delta S^*/R}$$

and the heats of activation from the equation $E = \Delta H^* + RT$.

The theory put forward in Part I of this series applies also to the case of the hydrolysis of valerolactone. According to this theory, if the transition state has a structure containing strongly hydrophilic and hydrophobic parts,

in some region of a continuous series of mixtures of water with an organic solvent a situation may arise, where the solvation of the transition state is greatly increased, which leads to a minimum in the curves representing E and A as functions of the composition of the solvent. Here the transition state may be written



Thus it is of the type just mentioned. The reactants, *i.e.* the lactone and OH^-, are also strongly solvated by water, since valerolactone is in all proportions mixible with water. Probably owing to this the minima are not so pronounced as in the hydrolysis of carboxylic esters. The rise of E and A after the minimum indicates that here the total solvation of the transition state rapidly decreases or that of the lactone increases or, perhaps, that both of these processes occur. The increase of E in high alcohol percentages is probably due also to a shift of the alcoholate equilibrium [1].

An addition of an organic solvent which contains a very small hydrocarbon part, *e.g.* methanol, to the water, does not markedly increase the solvation of the transition state; on the contrary, since methanol molecules are only weakly attracted to the hydrocarbon part of the transition state, it is possible that with the decreasing concentration of the water molecules the total solvation of the activated complex continuously decreases and, accordingly, no minima appear in the curves of E and A.

The energy of activation and temperature. In aqueous methanol containing 20—60 per cent by weight of methanol the deviation of the ratio between $\log k$ and $1/T$ from linearity is very pronounced and far beyond the experimental errors connected with the reproducibility of the measurements. In these media the energy of activation is clearly the higher the lower the temperature. This seems to be the case also in aqueous ethanol between 30 and 60 per cent of ethanol (Table 2). In acetone-water no definite dependence of E on T could be ascertained.

A dependence of activation energy on temperature was found also in the alkaline hydrolysis of ethyl acetate in *tert.*-butanol-water mixtures *i.e.* in mixtures of water and an alcohol with a very great hydrophobic part [1]. Here, in the region of the minimum, E increases with the temperature. The explanation given was that with the rise of the temperature the solvation of the activated complex decreases, which causes an increase in E. In the hydro-

Table 2. Values of the activation energies in various temperature ranges.

Solvent	E_{0-15}	E_{15-25}	E_{25-40}
Water	11 120	11 120	11 230
10 wt. % Methanol	12 000	12 380	11 860
20 » »	13 330	13 000	12 560
30 » »	14 410	13 880	13 450
40 » »	15 370	14 900	14 370
60 » »	16 650	16 360	16 180
80 » »	17 450	17 200	17 420
90 » »	17 680	18 650	17 730
10 wt. % Ethanol	10 740	10 650	10 790
20 » »	11 040	10 930	10 970
30 » »	11 960	11 650	11 200
40 » »	12 970	12 980	12 110
60 » »	14 620	14 140	13 900

lysis of valerolactone a shift of E is observed in aqueous ethanol and more powerfully in aqueous methanol, *i.e.* it is the more pronounced the more water-like the organic component of the solvent, and E decreases as the temperature rises. Besides, the phenomenon is here observed in the middle region of the mixtures, in aqueous ethanol after the minimum of E. Thus it is likely that here the situation is governed by a change in the solvation of the reactants with the temperature, for a decrease of the solvation of the reactants with rising temperature would tend to decrease the energy of activation. Such an assumption is well justified; the hydrocarbon heads of the alcohol molecules can attach to the hydrocarbon portion of the lactone, but the attraction is weak and easily disturbed by the thermal motion of the molecules. In the case of methanol this disturbance is greater than in the case of ethanol, where, owing to the greater hydrocarbon head, the attachement to the lactone must be firmer. If the alcohol concentration is high, this temperature-dependence is small. It is insignificant also in small alcohol concentrations in which the solvation by the alcohol molecules is very weak. Naturally also the transition state is less solvated at high than at low temperatures, which has a temperature effect opposite to the former, but this effect seems to be weaker than that caused by the change in the solvation of the reactants.

SUMMARY

The hydrolysis of valerolactone in methanol-water, ethanol-water, and acetone-water mixtures shows many common features with the alkaline hydrolysis of carboxylic esters. The details are discussed.

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Chemical Factors Affecting Associations of Lactic Acid Bacteria

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It is known that various micro-organisms are able to form associations in which one organism may stimulate or inhibit the growth of another. In particular, lactic acid bacteria have the ability to enter into associative inter-relationships in mixed cultures [Cf. e.g. 1–4]. However, although such phenomena were early recognised, the associative, and especially symbiotic, relationships of lactic acid bacteria in mixed populations are very little explored, and the chemical mechanism of associative action is mostly not definitely known.

In the present investigation the associations of lactic acid bacteria in a medium of known chemical composition have been studied. Attention has been focussed upon chemical factors affecting these associations.

EXPERIMENTAL

Cultures and inocula

The organisms used in this study were *Lactobacillus arabinosus* 17—5, *Streptococcus faecalis* R, and *Leuconostoc mesenteroides* P-60. The cultures were handled in the manner usually employed in microbiological techniques[5]. These were carried by bi-weekly transfer as stab cultures in a medium containing 1% glucose, 1% sodium citrate, 0.5% Bacto-tryptone, 1.5 % agar and 20 per cent by volume yeast extract. (The yeast extract was prepared as follows. 1 kg fresh yeast was suspended in 1 litre water, kept for approximately 24 hours at 42° C. It was then centrifuged and the cell-free solution used.) The inoculum used for the experiments was prepared from a 12—18 hour culture grown at 37° C in a medium above mentioned, without agar. The cells were washed twice by centrifugation and resuspension in sterile 0.9 % saline, and one drop of the barely visible suspension (96—98 % transmission, Klett-Summerson photoelectric colorimeter with filter no. 66) was used to inoculate each experimental tube.

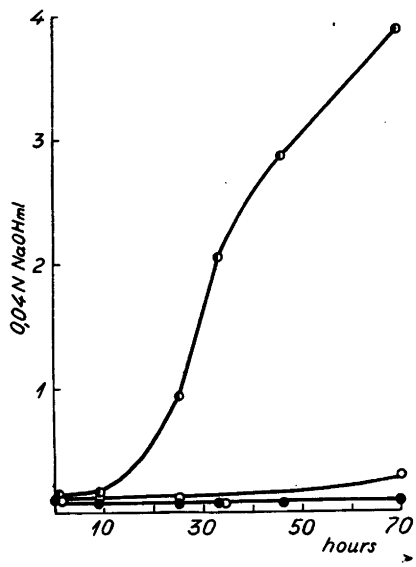


Fig. 1. The growth of Lactobacillus arabinosus 17—5 and Streptococcus faecalis R in symbiosis. Basal medium without phenylalanine and folic acid.

- ● *L. arabinosus 17—5 (phenylalanine-requiring strain).*
- ○ *Str. faecalis R (folic acid-requiring strain).*
- ◐ *L. arabinosus 17—5 and Str. faecalis R together.*

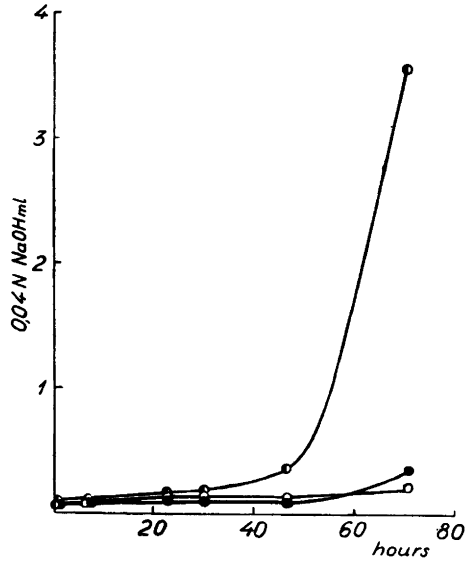


Fig. 2. The growth of Lactobacillus arabinosus 17—5 and Streptococcus faecalis R in symbiosis. Basal medium without threonine, phenylalanine and folic acid.

- ● *L. arabinosus 17—5 (phenylalanine-requiring strain).*
- ○ *Str. faecalis R (threonine and folic acid-requiring strain).*
- ◐ *L. arabinosus 17—5 and Str. faecalis R together.*

Basal medium and methods

The microbiological techniques used in these experiments are essentially the same as those described by Henderson and Snell [5]. The uniform basal medium used contained 0.5 times the concentration of all the ingredients in the medium used by these authors, with the exception of glucose, which was kept at the original concentration. All media were prepared at twice their final concentrations, adjusted to pH 6.8, added to test tubes in 1 ml quantities, diluted with water or supplements (vitamin or amino acid) to 2 ml, plugged with cotton wool, and autoclaved at 112° C for 5 minutes. After cooling, the tubes were inoculated, then incubated at 37° C in a covered water bath. Growth response was followed titrimetrically, and turbidimetrically. The lactic acid produced was titrated electrometrically directly in the test tubes with 0.04 *N* NaOH. Turbidity was measured with the Klett-Summerson photoelectric colorimeter with filter no. 66.

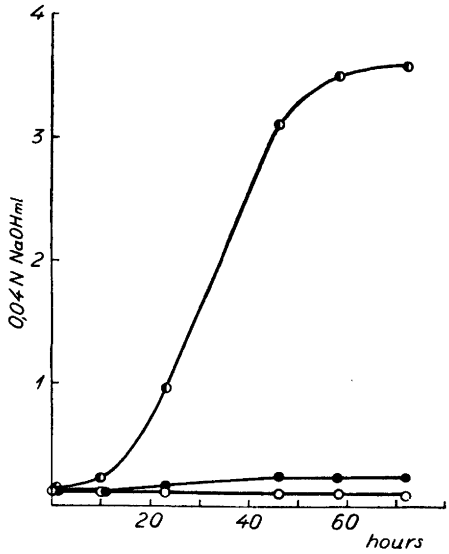


Fig. 3. The growth of Leuconostoc mesenteroides P-60 and Streptococcus faecalis R in symbiosis. Basal medium without proline and folic acid.

- ○ *Leuconostoc mesenteroides P-60 (proline-requiring strain).*
- ● *Str. faecalis R (folic acid-requiring strain).*
- ◐ *Leuconostoc mesenteroides P-60 and Str. faecalis R together.*

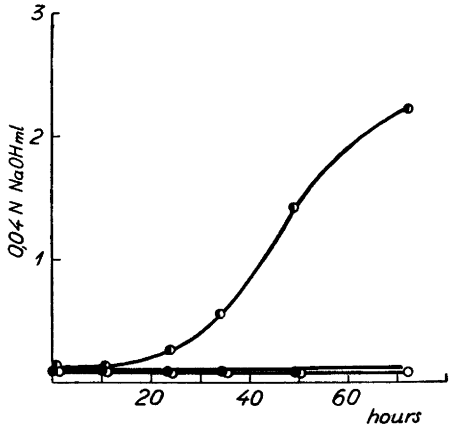


Fig. 4. The growth of Leuconostoc mesenteroides P-60 and Streptococcus faecalis R in symbiosis. Basal medium without proline, phenylalanine, glycine and folic acid.

- ○ *Leuconostoc mesenteroides P-60 (proline, phenylalanine, and glycine-requiring strain).*
- ● *Str. faecalis R (folic acid-requiring strain).*
- ◐ *Leuconostoc mesenteroides P-60 and Str. faecalis R together.*

RESULTS

In all experiments either one vitamin and one or more amino acids, or amino acids only, which were essential for the growth of bacteria, were omitted from the basal medium. Amino acid and vitamin requirements of organisms employed, had previously been investigated separately for each experiment.

It can be seen from Fig. 1 that *Str. faecalis* R and *L. arabinosus* 17—5 do not grow alone, but are able to grow together in a medium lacking folic acid and phenylalanine, although folic acid is required by *Str. faecalis* R and phenylalanine by *L. arabinosus* 17—5. If threonine, which has been found to be an essential amino acid for the growth of *Str. faecalis* R, was also omitted

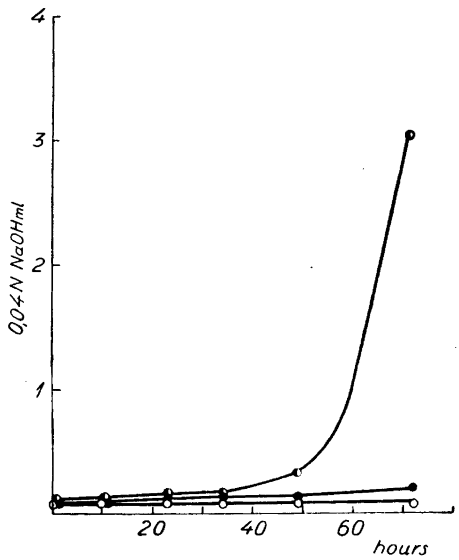


Fig. 5. The growth of Lactobacillus arabinosus 17—5 and Streptococcus faecalis R in symbiosis. Basal medium without phenylalanine and threonine.

- ● *L. arabinosus 17—5 (phenylalanine-requiring strain).*
- ○ *Str. faecalis R (threonine-requiring strain).*
- ◐ *L. arabinosus 17—5 and Str. faecalis R together.*

from this medium (already free of folic acid and phenylalanine), the above mentioned bacteria can still be grown together, as shown in Fig. 2. In this case, however, the rate of growth in the association was slower. When serine was absent from this medium instead of threonine, the bacteria still grew in association, but it was shown microscopically that *L. arabinosus* 17—5 was grown more rapidly than *Str. faecalis* R.

As shown in Fig. 3, the proline-requiring strain *Leuconostoc mesenteroides* P-60 and the folic acid-requiring strain *Str. faecalis* R could be grown together, but not alone, in a medium from which proline and folic acid had been omitted. If, in addition to these compounds, phenylalanine and glycine, which are essential amino acids for *Leuconostoc mesenteroides* P-60 (but not for *Str. faecalis* R) were absent from this medium, both bacteria still grew together, as can be seen from Fig. 4.

From Fig. 5 it appears that *Str. faecalis* R and *L. arabinosus* 17—5 grew together, but not alone, in a medium lacking the two amino acids phenylalanine (required by *L. arabinosus* 17-5 alone) and threonine (required by *Str. faecalis* R alone). The lag phase in this association was relatively long. —However, when one of the missing amino acids was essential for both strains together (in this case methionine), they could not be grown in association, as can be seen from Fig. 6.

In the experiment illustrated by Fig. 7. *Str. faecalis* R was cultured first with growth-limiting amounts (0.005 γ per test tube) of folic acid in a medium

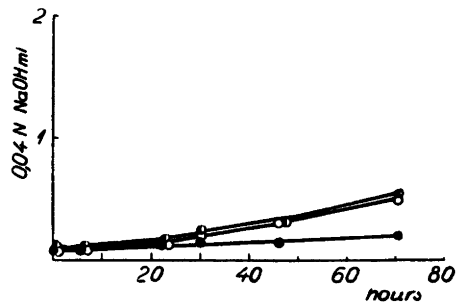


Fig. 6. Lactobacillus arabinosus 17—5 and Streptococcus faecalis R in a basal medium lacking methionine and phenylalanine.

- ● *L. arabinosus 17—5 (methionine and phenylalanine-requiring strain).*
- ○ *Str. faecalis R (methionine-requiring strain).*
- ◐ *L. arabinosus 17—5 and Str. faecalis R together.*

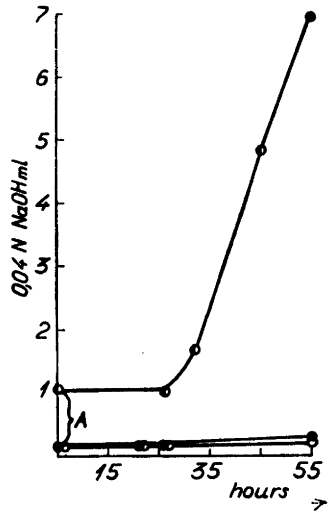


Fig. 7. The growth of Lactobacillus arabinosus 17—5 after Streptococcus faecalis R. Basal medium without phenylalanine and folic acid. Str. faecalis R was first cultured with growth-limiting amounts of folic acid. A indicates the amount of acid produced by Str. faecalis R, corresponding to a weak growth.

- ● *L. arabinosus 17—5 (phenylalanine-requiring strain).*
- ○ *Str. faecalis R (folic acid-requiring strain).*
- ◐ *L. arabinosus 17—5 grown after Str. faecalis R.*

lacking phenylalanine. After 25 hours the cultures were centrifuged, and the clear solutions were each added to one ml of a basal medium lacking folic acid and phenylalanine. The test tubes were then kept for 3 minutes at 100° C, after cooling, inoculated with *L. arabinosus* 17-5. This was able to grow in this medium after *Str. faecalis* R. As mentioned above, phenylalanine is required for growth of *L. arabinosus* 17-5.

Fig. 8 shows that *Leuconostoc mesenteroides* P-60 was not grown after *L. arabinosus* 17-5 in a medium deficient in phenylalanine, because this amino acid was a requirement common to both bacteria. (*L. arabinosus* 17-5 was cultured first with growth-limiting amounts (2 γ per tube) of L-phenylalanine, and was then handled in the manner described in the preceding experiment.)

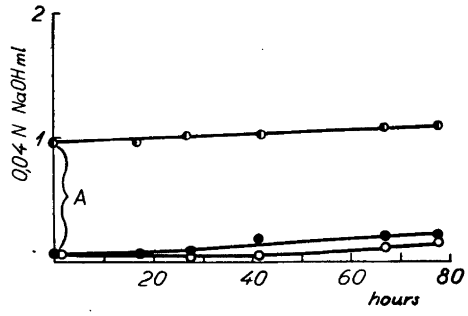


Fig. 8. Leuconostoc mesenteroides P-60 in a phenylalanine-deficient medium, in which Lactobacillus arabinosus 17—5 was first cultured with growth-limiting amounts of phenylalanine. A indicates the amount of acid produced by L. arabinosus 17—5, corresponding to a weak growth.

- ● *Leuconostoc mesenteroides P-60 (phenylalanine-requiring strain).*
- ○ *L. arabinosus 17—5 (phenylalanine-requiring strain).*
- ◐ *Leuconostoc mesenteroides P-60 inoculated after L. arabinosus 17—5.*

Finally, it has been found that a dialysing membrane (Cellophane) could not prevent the growth of *L. arabinosus* 17-5 and *Str. faecalis* R in association, in a medium from which phenylalanine and folic acid was omitted. (See also the experiment illustrated by Fig. 1.)

DISCUSSION

The results obtained in this preliminary investigation show that the two different strains of lactic acid bacteria can be grown together in symbiosis in a synthetic medium, each producing growth factors needed by the other. As a rule, this seems to be possible only in such cases where none of the vitamins or amino acids omitted is a requirement common to both organisms (Figs. 1—5 and 7). In cases in which a common amino acid requirement is omitted, bacteria cannot be grown in symbiosis (Figs. 6 and 8).

Although it seemed probable that the growth factors affecting the associations of lactic acid bacteria are these vitamins and amino acids which are required for the growth of organisms in a deficient synthetic medium, direct evidence has not been obtained in the present study about the type of these compounds. In view of the observation obtained using a dialysing membrane, the molecules in question are comparatively small and may be rapidly dialysed. In any case, the results obtained suggest that the chemical factors produced

by bacteria in symbiosis under the conditions used, have the activities of those amino acids and vitamins (in some experiments these factors seem to have activities which are greater than those of the corresponding amino acid or vitamin), which are absent from the synthetic basal medium, but which have been found to be essential metabolites for the growth of lactic acid bacteria which form these associations.

In view of these findings, it appears probable that the above mentioned growth factors also play an important part in the associations of lactic acid bacteria in milk and dairy products. For this reason, and especially because of the special theoretical interest of the symbiotic relationships among lactic acid bacteria, further work on their associations is in progress.

SUMMARY

Symbiotic inter-relationships have been shown to exist between different strains of lactic acid bacteria in a medium of known chemical composition. When certain vitamin and amino acid(s), which are essential substances for the growth of two lactic acid bacteria, were omitted from the synthetic medium, both organisms were able to grow in symbiosis together, but not alone, each producing the chemical factors needed by the other.

The results are discussed briefly.

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Metal Ammine Formation in Aqueous Solution

VIII. Acid-base, cis-trans, and complex equilibria in the cobalt(III)-ethylenediamine system

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In this paper the following system of acid-base and cis-trans equilibria in solutions of diaquobis(ethylenediamine)cobalt(III) salt

$$\begin{array}{ccccccc} \text{cis-Co en}_2(H_2O)_2^{+++} & \rightleftharpoons & \text{cis-Co en}_2(H_2O)(OH)^{++} + H^+ & \rightleftharpoons & \text{cis-Co en}_2(OH)_2^{+} + H^+ \\ \downarrow\uparrow & & \downarrow\uparrow & & \downarrow\uparrow \\ \text{tr.-Co en}_2(H_2O)_2^{+++} & \rightleftharpoons & \text{tr.-Co en}_2(H_2O)(OH)^{++} + H^+ & \rightleftharpoons & \text{tr.-Co en}_2(OH)_2^{+} + H^+ \end{array}$$

has been examined by means of spectrophotometric and glass electrode measurements. Further the hydrolysis constant

$$k_{h,1} = \frac{[\text{Co en}_2(OH)_2^+]\ [\text{en}]}{[\text{Co en}_3^{+++}]\ [OH^-]^2} \tag{1}$$

for the exchange of one ethylenediamine molecule in the luteo complex with two hydroxyl ions has been determined by optical analysis of alkaline complex solutions in which the equilibrium was established by using charcoal as a catalyst [1, 2]. The hydrolysis constant found ($k_{h,1} = 1.76$ liter/mol) combined with the acid-base and cis-trans constants obtained for the bis(ethylenediamine) system, gives the following values for the two 3rd formation constants in the cobalt(III)-ethylenediamine system:

$$k_3(\text{cis}) = \frac{[\text{Co en}_3^{+++}]}{[\text{cis-Co en}_2(H_2O)_2^{+++}]\ [\text{en}]} = 10^{13.28} \tag{2}$$

$$k_3(\text{tr.}) = 10^{15.24}$$

The absorption spectra of the two isomeric diaquobis(ethylenediamine) cobalt(III) ions have previously been measured by several investigators [3–5]. Uspensky and Tschibisoff [3] made measurements at 0° C. to diminish the velocity of the establishment of the cis-trans equilibrium, which adjusts itself spontaneously at a measurable rate in aqueous solution. Uèmura and Hirasawa [4] and Basolo [5] also gave some data for the influence of changes in pH. The authors mentioned examined the chlorides and bromides. To avoid halogeno complex formation, nitrates have been used here, and in order to exclude salt effects a constant salt medium of 1 N $NaNO_3$ was used. The equilibria were examined at 25° C.

The two acidic dissociation constants for the cis-diaquobis(ethylenediamine) ions are given by

$$k_{s,1}(\text{cis}) = \frac{[H^+]\,[\text{cis-Co en}_2(H_2O)(OH)^{++}]}{[\text{cis-Co en}_2(H_2O)_2{}^{+++}]}, \qquad k_{s,2}(\text{cis}) = \frac{[H^+]\,[\text{cis-Co en}_2(OH)_2^+]}{[\text{cis-Co en}_2(H_2O)(OH)^{++}]} \tag{3}$$

and analogously $k_{s,1}$(tr.) and $k_{s,2}$(tr.) denote the acidic dissociation constants for the trans-diaquo ion. The constants for the cis-trans equilibrium of the diaquo, the aquohydroxo and the dihydroxo ions will be denoted by

$$q_0 = \frac{[\text{cis-Co en}_2(H_2O)_2^{+++}]}{[\text{tr.-Co en}_2(H_2O)_2^{+++}]}, \quad q_1 = \frac{[\text{cis-Co en}_2(H_2O)(OH)^{++}]}{[\text{tr.-Co en}_2(H_2O)(OH)^{++}]}, \quad q_2 = \frac{[\text{cis-Co en}_2(OH)_2^+]}{[\text{tr.-Co en}_2(OH)_2^+]} \tag{4}$$

and finally for the gross acidic dissociation constants for solutions in cis-trans equilibrium, we will introduce

$$k_{s,1}(\text{gr.}) = \frac{[H^+]\,([\text{cis-Co en}_2(H_2O)(OH^{++})] + [\text{tr.-Co en}_2(H_2O)(OH)^{++}])}{[\text{cis-Co en}_2(H_2O)_2^{+++}] + [\text{tr.-Co en}_2(H_2O)_2^{+++}]}$$

$$k_{s,2}(\text{gr.}) = \frac{[H^+]([\text{cis-Co en}_2(OH)_2^+] + [\text{tr.-Co en}_2(OH)_2^+])}{[\text{cis-Co en}_2(H_2O)(OH)^{++}] + [\text{tr.-Co en}_2(H_2O)(OH)^{++}]} \tag{5}$$

Among the 6 acidic dissociation constants and the 3 cis-trans equilibrium constants, q_0, q_1 and q_2, we have four independent relations

$$q_0 = \frac{k_{s,1}(\text{tr.}) - k_{s,1}(\text{gr.})}{k_{s,1}(\text{gr.}) - k_{s,1}(\text{cis})} = \frac{k_{s,1}(\text{tr.}) \cdot k_{s,2}(\text{tr.})}{k_{s,1}(\text{cis}) \cdot k_{s,2}(\text{cis})} \cdot q_2$$
$$q_1 = \frac{k_{s,2}(\text{tr.}) - k_{s,2}(\text{gr.})}{k_{s,2}(\text{gr.}) - k_{s,2}(\text{cis})} = \frac{k_{s,2}(\text{tr.})}{k_{s,2}(\text{cis})} \cdot q_2 \qquad (6)$$

As a consequence of these relations, knowledge of five of the constants is enough to calculate the remaining four. In this investigation all 6 acidic dissociation constants were estimated by means of glass electrode measurements, and the constant q_2 was determined by means of spectrophotometric measurements. The two other cis-trans constants, q_0 and q_1, therefore, could be estimated in different ways. The most reliable values were calculated by combining the direct determined value for q_2 with the expressions containing the products of the acidic dissociation constants, and all experimental data showed satisfactory agreement within the uncertainty of the experiments. For the 9 mass action constants, which govern the system, the following values were found in 1 N $NaNO_3$ at 25° C:

$$k_{s,1}(\text{cis}) = 10^{-6.06}, \qquad k_{s,2}(\text{cis}) = 10^{-8.19}$$
$$k_{s,1}(\text{tr.}) = 10^{-4.45}, \qquad k_{s,2}(\text{tr.}) = 10^{-7.94}$$
$$k_{s,1}(\text{gr.}) = 10^{-5.80}, \qquad k_{s,2}(\text{gr.}) = 10^{-8.10}$$
$$q_0 = 58, \quad q_1 = 1.42, \quad q_2 = 0.80$$

From the dissociation constants it is seen that the trans-diaquo ion is an acid about 40 times stronger than the cis-diaquo ion with regard to the dissociation of the first hydrogen ion, but not essentially stronger with regard to the dissociation of the 2nd hydrogen ion. Further the q-values show that the equilibrium in acid solution is strongly displaced in favour of the cis ion, *i.e.* in acid solutions of diaquo salt less than 2 per cent is to be found as trans ion, while neutral and basic solutions contain about equal amounts of cis and trans complex in equilibrium.

The rates of isomerization of the diaquo ions in acid solution and of the dihydroxo ions in basic solution are of the same order of magnitude, but strongly dependent on the purity of salts; with ordinary purified preparations equilibrium is usually reached in about a week. The rates of isomerization of the aquohydroxo ions are much higher [5] and equilibrium, even with the most thoroughly purified salts, is reached within one hour. It was, therefore, necessary, when determining the cis and trans acidic dissociation constants, to work quickly and to extrapolate the potentials measured to the time zero.

Some preliminary kinetic experiments on the isomerization of trans diaquo salt in acid solution (see Table 3) showed that the reaction was of 1st order,

and almost independent of pH and salt concentration. Addition of small amounts of charcoal increased the reaction velocity many times, and it is, therefore, beyond any doubt that the cis-trans isomerization is partly an heterogenously catalyzed reaction. Charcoal also has a strongly catalyzing effect on the cis-trans isomerization in basic solution, but here, though more slowly, it also brings about an exchange of ligands [1, p. 220]. In this way using charcoal as a catalyst, it was possible to determine the 1st hydrolysis constant of the luteo ion ($k_{h,1}$), and our thanks are due to Mr. Flemming Woldbye, who has performed all the experimental work in connection with the estimation of this constant.

Comparison with other systems

Lewis *et al.*[6] have found that the exchange of radioactive ^{60}Co between cobalt(II)-ammonia solutions and the hexamine-cobalt(III) complex is much slower than the exchange between cobalt(II)-ethylenediamine solutions and the tris(ethylenediamine)cobalt(III) complex. This is a simple question of electron transfer, and it is not at variance with the fact that the cobalt(III) complexes with ethylenediamine have by far the greatest robustness.

ter Horst [7] has shown qualitatively that the trans-diaquotetrammine-cobalt(III) ion is a stronger acid than the corresponding cis compound. The adjustment of the cis-trans equilibrium is here more rapid than for the analogous ethylenediamine complexes, and the acidic dissociation constants for the diaquotetrammine-cobalt(III) ion determined by Lamb and Damon [8], $k_{s,1} = 10^{-5.32}$ and $k_{s,2} = 10^{-7.30}$ ($\mu = 0.02$, 25° C), are, therefore, undoubtedly gross dissociation constants. J. Bjerrum's calculation of k_5 in the cobalt(III)-ammonia system [1, p. 284] must consequently be correct.

That the trans-diaquo ion is a stronger acid than the cis-diaquo ion is also true for the diaquodiammine-platinum(II) ions [9].

The cause of such a "trans" effect was first discussed by Grünberg [10], who tried to explain it on a purely electrostatic basis. Some calculations made in Table 1 seem to show that the effect only appears in complexes with typical covalent bonds. The data compiled are computed on the following basis:

In an octahedral system where β_1 denotes the Ostwald factor by which the ethylenediamine molecule first bound to the metal ion lowers the tendency to take up a 2nd molecule in the "planar position", and analogously β_2 denotes the corresponding factor for the uptake in the "angle position", the ratio of the consecutive formation constants is given by

$$\frac{k_1}{k_2} = \frac{24}{5} \cdot \frac{5\beta_1}{4\beta_1 + \beta_2} \cdot \beta_2, \qquad \frac{k_2}{k_3} = \frac{75}{8} \cdot \frac{(4\beta_1 + \beta_2)^2}{25\beta_1^2} \cdot \beta_2, \qquad q_0 = \frac{4\beta_1}{\beta_2} \tag{7}$$

Table 1. Equilibrium constants and Ostwald factors in some ethylenediamine systems. $\mu = 1$, 30° C.

	$\log k_1$	$\log k_2$	$\log k_3$	$\log K_3$	q_0	β_1	β_2
Mn^{++} + 3 en	2.73	2.06	0.88	5.66	(2.1)	0.60	1.15
Fe^{++} + 3 en	4.28	3.25	1.99	9.52	(5.2)	2.76	2.14
Co^{++} + 3 en	5.89	4.83	3.10	13.82	(1.5)	1.15	3.16
Ni^{++} + 3 en	7.66	6.40	4.55	18.61	(1.7)	2.08	4.78
Co^{+++} + 3 en	(18.7)	(16.2)	13.81	48.69	58	632	43.5

The formulae (7) are obtained by J. Bjerrum [1, p. 95] using the assumption that the factor by which the ethylenediamine molecules in an angle position to each other resist the uptake of the 3rd ethylenediamine molecule, is equal to the product of the action of the individual ligands — or, in this particular instance, β_2^2. It follows directly from the formulae that the ratio between the consecutive constants may be regarded as a product of the statistical factor (24/5 and 75/8 respectively), the β_2-factor and an asymmetry factor which vanishes when $\beta_1 = \beta_2$. In this special case we have for the cis-trans constant of the diaquo ions $q_0 = 4$.

In the systems of divalent iron group metal ions the values for q_0 given in parenthesis and the Ostwald factors β_1 and β_2 are estimated from the previously [1, p. 217] determined consecutive formation constants in 1 N KCl at 30° by means of formulae (7). In the cobalt(III)-ethylenediamine system the gross complexity constant $K_3 = k_1k_2k_3$ has previously been determined in 1 N salt solution at 30° [1, p. 233] by combination of the normal potentials of the aquo ions and the tris(ethylenediamine) ions with the corresponding value for K_3 in the cobalt(II) system. Of the values found in this paper the cis-trans constant ($q_0 = 58$) has probably the same value at 25° and 30° (*cf.* p. 1278), and the 3rd formation constant (k_3(gr.) $= 10^{13.99}$ in 1 N $NaNO_3$ at 25°) has been converted to 30° using some thermochemical data of Yatsimirskiĭ [11]. The estimated values for the 1st and 2nd formation constants given in parenthesis in Table 1, as well as the β-factors have been computed on this basis, and it will be seen that it is especially the β_1-factor for planar uptake of the 2nd ethylenediamine that has an extraordinarily high value.

EXPERIMENTAL

Preparation of salts. With minor modifications the complex salts used were prepared according to methods described in the literature. Tris(ethylenediamine)cobalt(III) nitrate was made in excellent yield from cobalt(II) nitrate and aqueous ethylenediamine using decolorizing charcoal as a catalyst [1, 2]. *Anal.* Calc. for Co $en_3(NO_3)_3$: Co 13.86,

N 29.65. Found: Co 13.89, N 29.89. It was shown that further recrystallization from water had no demonstrable influence on the absorption spectrum of the salt in aqueous solution.

Cis-diaquobis(ethylenediamine)cobalt(III) nitrate was prepared from the corresponding bromide [12], which again was prepared from Werner's brown nitrate [13]. The oxidation of cobalt(II) nitrate to brown salt was made in photographic trays over which a stream of air was blown from a ventilator. In this way the time of preparation was reduced from days to hours. The crude product was recrystallized once and dried at 60°. *Anal.* Calc. for $[Co(Co(OH)_2en_2)_3](NO_3)_6$: N 23.34. Found: N 23.15.

During the further treatment of brown nitrate with 10 N HBr a stream of air was blown over the reaction mixture and not more than 5 g were treated in every run as it is difficult with greater portions to remove the bromine formed during the reaction quickly enough to get a good yield. In order to convert to the nitrate, the freshly prepared bromide was dissolved in dilute nitric acid at − 5°, aerated to remove bromine, and precipitated with the double volume of alcohol. The salt was reprecipitated using the same procedure and dehydrated over $CaCl_2$. *Anal.* Calc. for $[Co\ en_2(H_2O)_2](NO_3)_3$: Co 14.69. Found: Co 14.61. Further reprecipitation using the same procedure had no influence on the absorption spectrum, and the salt could be kept unchanged for months in a bottle with a tight fitting stopper.

Trans-diaquobis(ethylenediamine)cobalt(III) nitrate was prepared directly from the trans-dihalogeno salts $[Co\ en_2\ Br_2]\ Br$ or $[Co\ en_2\ Cl_2]Cl$. The halogeno salts were treated in portions of 2 g at 0° C with the equivalent amounts of Ag_2O and about twice the equivalent amounts of water. In the case of the chloride it was necessary to start the reaction before cooling the mixture. The solutions were quickly filtered from the silver halogenide and at − 10° C twice the calculated amount of fuming nitric acid was added and the solution slowly precipitated with icecold alcohol. The trans-salt was easily freed from the more soluble cis-salt by reprecipitation with alcohol in presence of nitric acid. It was purified until the spectrum was unchanged by further reprecipitation. *Anal.* Calc. for $[Co\ en_2(H_2O)_2]\ (NO_3)_3$: Co 14.69. Found: Co 14.70.

Analytical methods. Cobalt was determined after complete decomposition of the complexes with concentrated sulphuric acid. In the later experiments the decomposition was made and excess of H_2SO_4 conveniently removed by heating on an infrabath. In analysis of pure complex salts and of solutions also containing other metal salts, different methods were used. In the first case cobalt was determined by weighing as $CoSO_4$ after heating in an electric furnace at 550° C, and in the second case according to Yardley [14], by potentiometric titration of the cobalt(II)-citrate complex in ammoniacal solution with ferricyanide in an atmosphere of oxygenfree nitrogen [Cf. 1, p. 266 ff.].

In the sulphuric acid destruction of the cobalt(III) complexes ethylenediamine is partly oxidized to N_2. For this reason nitrogen in the solid complex salts was determined by the Dumas method, and a potentiometric method worked out for the determination of free ethylenediamine in alkaline solutions of the cobalt(III) complexes (see p. 1281).

Solutions. The different solutions were prepared in calibrated measuring flasks by weighing the pure complex salts and by weighing or pipetting from stock solutions of sodium nitrate, nitric acid, and ethylenediamine as previously described [15, p. 12]. Carbon dioxide-free sodium hydroxide solutions were prepared according to the Sørensen method.

ACID-BASE CONSTANTS OF THE SYSTEM OF DIAQUO IONS

The acid-base constants of the system of diaquobis(ethylenediamine) ions were determined by means of glass electrode measurements in solutions of the complex salts to which suitable amounts of sodium hydroxide had been added. The data are given in Table 2. The composition of the solutions is given in terms of the total concentrations of cobalt(C_{Co}) and of sodium hydroxide (C_{NaOH}). The cobalt concentrations are denoted by cis and trans, respectively, as well as by gr. for solutions in equilibrium with respect to cis and trans. The true hydrogen ion concentration [H^+] was determined in all cases by standardizing against a standard acid solution of the composition: $C_{HNO_3} = 0.00499$, $C_{NaNO_3} = 1.000$. The measurements were performed in an electrically heated air thermostat with a "Radiometer" valve potentiometer, Model PHM 3.

Due to the great velocity with which the isomerization of the diaquo salts occurs in the buffer range, it was necessary to work very quickly in order to obtain the acidic dissociation constants for the pure isomers. An experiment was started by the addition from a Krogh syringe pipette of the proper amount of titrated sodium hydroxide to a freshly made and suitably diluted solution of the cobalt salt. In this way it was possible to take the first reading about 30 seconds after the addition of the base.

A typical example is the following: 0.997 ml 0.1072 N NaOH was added to 19 ml of a solution containing 4.00 ml 5 N $NaNO_3$ and 0.08734 g trans-nitrate, total volume corrected 20.03 ml (sol. no. 4 in Table 2), and the following potentials were obtained at the times mentioned:

	extrapol.						
t (sec.)	0	40	70	100	130	160	∞
E (volt)	−0.1410	−0.1420	−0.1428	−0.1438	−0.1445	−0.1455	−0.2201

In the extrapolated potential E ($t = 0$) is a little uncertainty due to the time it takes for the glass electrode to adjust itself, but this uncertainty scarcely amounts to more than 0.5 mV. In the example given $E_{st.}$, the potential of the standard acid solution with [H^+] = 0.00499, had the value −0.0138 at the time $t = 0$, and two hours later the value −0.0140, at the time when the equilibrium potential E ($t = \infty$) was measured.

The average number of protons produced by the diaquo ion is given by

$$\bar{\nu} = \frac{C_{NaOH} + [H^+] - [OH^-]}{C_{Co}}$$

Table 2. The acidic dissociation constants of the diaquo-bis(ethylenediamine)cobalt(III) ions in 1 N sodium nitrate solution at 25°.

$C_{NaNO_3} = 1.000$, $-\log[H^+]_{st.} = 2.312$

a. The first dissociation constant. $\Delta = \frac{(2-\bar{\nu})k_{s,2}}{(1-\bar{\nu})[H^+]}$

No.	C_{Co}(cis)	C_{NaOH}	$\bar{\nu}$	$E_{st.}-E$	$-\log[H^+]$	$\log\frac{1-\bar{\nu}}{\bar{\nu}}$	$\log(1+\Delta)$	$-\log k_{s,1}$
1	0.01084	0.005337	0.4923	0.2210	6.051	0.013	0.009	6.074
2	0.01066	0.005337	0.5006	0.2199	6.033	−0.001	0.009	6.041
3	0.01059	0.005337	0.5040	0.2221	6.071	−0.007	0.009	6.073
	C_{Co}(tr.)						$-\log k_{s,1}$(cis):	6.06
4	0.01086	0.005337	0.4945	0.1272	4.464	0.010	0	4.474
5	0.01046	0.005337	0.5136	0.1273	4.461	−0.023	0	4.438
6	0.01040	0.005337	0.5164	0.1278	4.470	−0.028	0	4.442
	C_{Co}(gr.)*						$-\log k_{s,1}$(tr.):	4.45
7	0.009968	0.003969	0.3982	0.1956	5.622	0.179	0.004	5.805
4	0.01086	0.005337	0.4913	0.2061	5.799	0.015	0.006	5.820
5	0.01046	0.005337	0.5103	0.2063	5.803	−0.018	0.006	5.791
8	0.02050	0.01024	0.5110	0.2076	5.825	−0.020	0.007	5.812
9	0.01040	0.006217	0.5978	0.2148	5.948	−0.172	0.011	5.787
10	0.01017	0.006947	0.6831	0.2251	6.114	−0.334	0.014	5.800
							$-\log k_{s,1}$(gr.):	5.80

b. The second dissociation constant. $\Delta = \frac{\bar{\nu}[H^+]}{(\bar{\nu}-1)k_{s,1}}$

No.	C_{Co}(cis)	C_{NaOH}	$\bar{\nu}$	$E_{st.}-E$	$-\log[H^+]$	$\log\frac{2-\bar{\nu}}{\bar{\nu}-1}$	$-\log(1+\Delta)$	$-\log k_{s,2}$
11	0.01493	0.02150	1.440	0.3429	8.113	0.095	−0.013	8.194
12	0.01453	0.02150	1.479	0.3461	8.168	0.036	−0.010	8.195
13	0.01419	0.02150	1.515	0.3490	8.217	−0.026	−0.008	8.183
	C'_{Co}(tr.)						$-\log k_{s,2}$(cis):	8.19
14	0.01516	0.02150	1.418	0.3237	7.788	0.144	−0	7.932
15	0.01424	0.02150	1.510	0.3340	7.963	−0.017	−0	7.946
16	0.01404	0.02150	1.531	0.3360	7.997	−0.054	−0	7.943
	C_{Co}(gr.) *						$-\log k_{s,2}$(tr.):	7.94
17	0.009816	0.01343	1.368	0.3280	7.862	0.246	−0.014	8.094
14	0.01516	0.02150	1.418	0.3340	7.963	0.144	−0.010	8.097
18	0.01013	0.01488	1.473	0.3397	8.060	0.047	−0.007	8.100
19	0.01682	0.02482	1.475	0.3403	8.071	0.043	−0.007	8.107
20	0.01418	0.02150	1.527	0.3462	8.170	−0.047	−0.005	8.118
21	0.01347	0.02150	1.596	0.3510	8.251	−0.169	−0.004	8.078
22	0.01026	0.02150	1.623	0.3555	8.326	−0.218	−0	8.108
							$-\log k_{s,2}$(gr.):	8.10

* Nos. 7, 8, 9, 10, 17, 18, 19, 21 are prepared from cis-salt, nos. 4, 5, 14, 20, 22 from trans-salt.

and connected with the acidic dissociation constants through the expression:

$$\bar{\nu} = \frac{k_{s,1}[H^+] + 2\,k_{s,1} \cdot k_{s,2}}{[H^+]^2 + k_{s,1}[H^+] + k_{s,1} \cdot k_{s,2}}$$

When this equation is solved with respect to $k_{s,1}$ and $k_{s,2}$ and arranged in the proper way [Cf. 16, p. 13], we get the following expressions for the two constants for $\bar{\nu} < 1$ and $\bar{\nu} > 1$, respectively:

$$-\log k_{s,1} = -\log[H^+] + \log\frac{1-\bar{\nu}}{\bar{\nu}} + \log\left(1 + \frac{(2-\bar{\nu})\,k_{s,2}}{(1-\bar{\nu})\,[H^+]}\right.$$

$$-\log k_{s,2} = -\log[H^+] + \log\frac{2-\nu}{\bar{\nu}-1} - \log\left(1 + \frac{\bar{\nu}\,[H^+]}{(\bar{\nu}-1)\,k_{s,1}}\right.$$

The reciprocal influence of $k_{s,1}$ and $k_{s,2}$ finds its expression in the last terms, which are negligibly small in the trans system and in the other cases only give a minor correction. $\bar{\nu}$ is varied to some extent in the equilibrium solutions, but is kept close to 0.5 and 1.5, respectively, in the solutions of the unstable isomers in order to obtain the smallest possible uncertainty in the estimated constants. Table 2 gives, besides the constants found and their mean values, the values of the individual terms in the calculation.

SPECTROPHOTOMETRIC INVESTIGATION OF THE SYSTEM OF DIAQUO IONS

In Fig. 1 are given extinction curves for tris- and bis(ethylenediamine) cobalt(III) complexes in 1 *N* $NaNO_3$. The molar extinction coefficient

$$\varepsilon = \frac{\log_{10} I_0/I}{C_{Co} \cdot d}$$

(I_0 and I denote the intensity of the incident and the transmitted light, respectively, and d the thickness of the absorbing layer in cm) is plotted as ordinate, and the wave number in cm^{-1} as abscissa; for orientation purposes the wavelength in mμ is also plotted. Cobalt(III) complexes have practically no light absorption above 700 mμ, and below 350 mμ the nitrate ion begins to absorb ($\varepsilon_{350} = 0.018$), and for this reason the extinction coefficients are examined in the range 350—700 mμ only. For the diaquo and dihydroxo ions the extinction coefficient is denoted by ε_0 and ε_2, respectively, in accordance with the designations of the q-constants in formulae (4). The effect of the sodium nitrate medium on the spectra is negligibly small, and as a whole the extinction coefficients of the complexes are only 1—2 % higher in 1 *N*

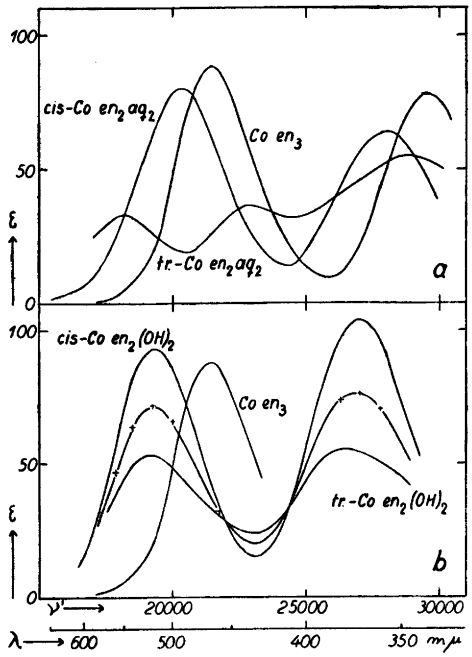


Fig. 1. Extinction curves for tris- and bis(ethylenediamine)cobalt(III) complexes in 1 N $NaNO_3$. — ε = the molar extinction coefficient, ν' the wave number in cm^{-1}, and λ the wavelength in mμ. a: Curves for luteo, cis and trans diaquo ions. b: Curves for luteo, cis and trans, and the equilibrium of isomeric dihydroxo ions. The equilibrium curve of cis and trans dihydroxo ions is calculated from the spectra of the pure isomers by means of the average value found for the cis-trans constant: $q_2 = 0.80$. The +-points mark the experimental mean values for $\varepsilon_2(\infty)$ in Table 4a.

$NaNO_3$ than in water. The extinctions were measured at 23 $\pm$ 1° C. A Beckman DU spectrophotometer with ordinary equipment was used for the measurements.

Cis-trans isomerizations. The velocity with which trans-diaquo salt changes into cis salt was examined at 492 mμ, a wavelength which is especially suited for an investigation of this kind (see Fig. 1a). The acidic solutions of the diaquo salt were placed (or in the experiments with added charcoal shaken) in a water thermostat at 25° C, and at proper intervals of time samples were taken for optical analysis. The data are given in Table 3. The constancy of $\frac{-\log(1-\alpha)}{\text{time}}$ (α = degree of reaction) shows that the isomerization is approximately a 1st order reaction in the experiments both with and without added charcoal. $t_{1/2}$ gives the times of half-completion. The end values for the extinction as determined in experiment 3 and 4 are seen to correspond very nearly to complete conversion into cis salt: $\varepsilon_0 = 80.9$ ($\frac{0.786}{C_{Co}} = 81.0$ in exp. 3 and $\frac{0.655}{C_{Co}} = 81.4$ in exp. 4, respectively). The experiments mentioned were made with our purest preparation of trans-diaquo salt and were reproducible as long as the same preparation was used.

Table 3. Isomerization velocity of trans-diaquobis(ethylenediamine)cobalt(III) nitrate in acidic solution at 25°.

Wavelength 492 mμ: Mean extinction coefficients used

ε_0(tr.) = 19.2, ε_0(cis) = 80.9 (C_{NaNO_3} = 1), 80.0 (dilute)

Exp.	C_{NaNO_3}	C_{HNO_3}	C_{Co}	g Norite / 100 ml	t (min.)	$\log \frac{I_0}{I} / d$	$-\log(1-\alpha)/t$	$t_{1/2}$ (min.)
1	0	0.05	0.00989	0	2	0.191		
					56	0.216	0.000322	
					118	0.239	0.000305	
					177	0.264	0.000316	
					239	0.291	0.000330	
					298	0.316	0.000339	
					1383	0.587	0.000338	
						Mean	0.000325	926
2	0	0.10	0.01002	0	3	0.192		
					119	0.239	0.000302	
					183	0.260	0.000284	
					243	0.286	0.000300	
					299	0.303	0.000294	
					344	0.318	0.000294	
						Mean	0.000295	1020
3	1.00	0.05	0.00970	0	3	0.187		
					193	0.296	0.000456	
					243	0.325	0.000470	
					297	0.354	0.000480	
					356	0.386	0.000494	
					3236	0.786		
						Mean	0.000473	637
4	1.00	0.05	0.008046	0.040	75	0.428	0.00467	
					132	0.550	0.00525	
					205	0.610	0.00533	
					1565	0.655		
						Mean	0.0051	59

In alkaline solution it is possible to follow the transformation of cis- as well as of trans-dihydroxo ion to a state of equilibrium. A selection of the measurements made is given in Table 4. In part *a* of the table the isomerization is followed directly by measuring the extinction coefficients of the alkaline solutions at various times, and in part *b* by transforming dihydroxo ions to diaquo ions before measuring the extinction coefficients. In connection with

the neutralization and acidification, the volume was doubled and enough sodium nitrate added to obtain a 1 N solution of the salt in question.

The tables give mean values for the extinction coefficients of the pure isomers as well as for the equilibrium mixtures at various wavelengths. Complete isomerization is reached after about 7 days at 25° C and after further standing for 589 days the equilibrium spectrum is practically unchanged. The equilibrium spectra at 25° C as well as the spectra of solutions on the way towards equilibrium are within the uncertainty of the measurements composed of only 2 components. The common points of intersection (see also Fig. 1) as well as the independence of wavelength of the computed values for the degrees of reaction of cis salt

$$\alpha_{cis} = \frac{\varepsilon_2(\text{cis}) - \varepsilon_2(\text{time})}{\varepsilon_2(\text{cis}) - \varepsilon_2(\text{tr.})} \text{ , or } \frac{\varepsilon_0(\text{cis}) - \varepsilon_0(\text{time})}{\varepsilon_0(\text{cis}) - \varepsilon_0(\text{tr.})}$$

and of trans salt ($\alpha_{tr.}$), respectively, give clear evidence of this. The cis-trans constant q_2 is calculated from the extinction coefficients corresponding to equilibrium $\varepsilon_2(\infty)$, or $\varepsilon_0(\infty\varepsilon_2)$, and the extinction coefficients of the pure isomers, by the expressions

$$q_2 = \frac{[\text{cis}]}{[\text{tr.}]} = \frac{\varepsilon_2(\infty) - \varepsilon_2(\text{tr.})}{\varepsilon_2(\text{cis}) - \varepsilon_2(\infty)} \text{ , or } \frac{\varepsilon_0(\infty\varepsilon_2) - \varepsilon_0(\text{tr.})}{\varepsilon_0(\text{cis}) - \varepsilon_0(\infty\varepsilon_2)}$$

The uncertainty of the estimated values of q_2 is approximately proportional to the differences of extinction coefficients $\varepsilon_2(\text{cis}) - \varepsilon_2(\text{tr.})$, or $\varepsilon_0(\text{cis}) - \varepsilon_0(\text{tr.})$, of the pure isomers at the wavelength in question. For this reason rounded off values of the extinction coefficient differences mentioned were used as weights in the calculation of the mean values for q_2 as well as for the degrees of reaction given in the last column of Tables 4. The average value, $q_2 = 0.81$, found at 25° C in weaker alkaline solution (Table 4a: $C_{NaOH} = 0.027 - 0.24$) is seen to be in satisfactory agreement with the average value, $q_2 = 0.79$, at 25° C in Table 4b ($C_{NaOH} = 0.50$), and the average of these 2 values, $q_2 = 0.80$, has been taken as the final mean value for the cis-trans constant in alkaline solution.

The estimated values for the degrees of reaction given in Tables 4 give some information about the kinetics of the isomerization. The 1st order rate constant for the transformation of cis into trans is given by the expression

$$k_{rate} = \frac{-\log(1 - \alpha_{cis}(1 + q_2))}{\text{time} \cdot (1 + q_2)}$$

and the time of half-completion by

$$t_{1/2} = \frac{\text{time} \cdot \log 2}{-\log(1 - \alpha_{cis}(1 + q_2))}$$

Table 4. Cis-trans equilibria of the dihydroxobis(ethylenediamine)cobalt(III) ions.

a. Extinction coefficients measured directly in the alkaline solution.

$C_{Co} \sim 0.01$, $C_{NaNO_3} = 1.00$

	No.	time at t °C	C_{NaOH}	360	370	380	460	500	520	540	560 mμ	Av.
ε_2(cis), av.	1—4	0	0.03—0.10	95.5	103.6	97.5	33.3	85.5	93.0	82.1	55.4	
ε_2(tr.), av.	5	0	0.0268	50.5	53.9	54.9	30.5	48.1	53.0	49.3	38.6	
ε_2(cis)—ε_2(tr.)				45.0	49.7	42.6		37.4	40.0	32.8	16.8	
Cis salt	3	2 days at 25°	0.0268	77.0	83.3	79.4	32.3	69.8	75.8	66.6	47.5	
a_{cis} (2 days)				0.412	0.408	0.425		0.420	0.430	0.472	0.470	0.427
Cis salt	»	4 » » »	»	73.2	78.5	76.1	32.3	66.7	72.7	63.9	46.7	
» »	»	7 » » »	»	71.1	76.4	74.2	31.8	65.7	71.4	63.7	46.9	
» »	»	19 » » »	»	71.4	76.5	74.2	31.9	65.8	72.5	64.7	47.3	
» »	4	47 » » »	0.0970	69.0	74.9	73.1	—	65.3	71.2	63.6	—	
» »	»	589 » » »	»	71.0	77.0	75.0	31.8	65.0	70.5	63.2	46.5	
» »	6	13 » » »	0.2422	70.1	—	74.2	—	65.1	71.0	63.0	46.6	
Trans salt	5	2 » » »	0.0268	64.1	69.5	68.1	32.2	60.0	65.0	58.9	43.3	
$a_{tr.}$(2 days)				0.302	0.314	0.310		0.318	0.300	0.293	0.280	0.305
Trans salt	»	4 » » »	»	67.7	73.1	71.5	32.4	63.5	68.9	61.2	45.0	
» »	»	7 » » »	»	69.5	75.0	72.9	32.4	65.7	71.0	63.4	47.0	
$\varepsilon_2(\infty)$, av.	3—6			70.2	75.7	73.7	32.0	65.5	71.4	63.7	46.8	
q_2 (25°)				0.78	0.78	0.79		0.87	0.85	0.78	0.95	0.81
Cis salt	7	5 min. at 80°	0.0254	76.0	79.7	76.6	—	63.7	69.2	62.5	46.3	
» »	»	15 » » »	»	75.9	79.7	76.9	—	—	69.3	62.4	46.2	
» »	»	105 » » »	»	74.9	79.4	76.9	33.4	64.0	69.1	62.9	46.6	
q_2 (80°)				(1.18)	(1.05)	(1.07)		0.74	0.67	0.71	0.91	0.72

b. Extinction coefficients measured shortly after neutralization with nitric acid.

Concentrations in the alkaline solution: $C_{Co} \sim 0.02$, $C_{NaNO_3} = 1 - C_{NaOH}$,

and after acidifying etc.: $C_{HNO_3} \sim 0.05$, $C_{Co} \sim 0.01$, $C_{NaNO_3} = 1.00$.

	No.	time at t °C	C_{HNO_3}	370	380	410	444	480	492	520	550 mμ	Av.
ε_0(cis), av.	8—11	0	0.01	57.0	44.2	14.3	33.2	76.2	80.9	62.7	32.8	
ε_0(tr.), av.	12—15	0	0.05	46.0	40.9	32.0	35.1	20.0	19.2	26.2	32.8	
ε_0(cis)—ε_0(tr.)				11.0		—17.7		56.2	61.7	36.5		
			C_{NaOH}									
Cis salt	16	368 min. at 25°	0.503	55.5	43.5	15.0	—	71.4	75.8	59.6	—	
a_{cis}(368 min.)				0.136		0.040		0.085	0.083	0.085		0.085
Cis salt	»	26½ hours » »	»	53.1	42.8	18.8	—	60.1	63.1	—	—	
a_{cis}(26½ hours)				0.355		0.254		0.287	0.288			0.290
Cis salt	»	50 » » »	»	52.0	42.7	21.3	—	53.1	55.3	—	—	
a_{cis}(50 hours)				0.454		0.395		0.411	0.415			0.415
Cis salt	»	42 days » »	»	—	42.9	24.1	—	46.5	47.9	42.9	—	
» »	17	7 » » »	0.504	51.5	44.0	24.5	34.4	44.1	46.4	42.1	—	
» »	»	17 » » »	»	52.2	43.6	25.1	34.8	45.0	46.2	42.3	32.6	
Trans salt	18	7 » » »	»	50.6	42.3	24.4	34.2	44.2	45.6	41.2	31.8	
» »	»	17 » » »	»	50.6	42.3	24.2	34.1	44.8	46.1	42.1	32.5	
$\varepsilon_0(\infty\varepsilon_2)$, av.	16—18			51.2	43.0	24.5	34.4	44.9	46.4	42.1	32.3	
q_2 (25°)				0.90		0.74		0.80	0.79	0.77		0.79
Cis salt	19	c. 1 min. at 100°	0.050	—	45.1	26.4	35.0	44.5	46.0	41.0	32.3	
q_2 (100°)						(0.46)		0.77	0.77	0.68		0.75

By inserting the data for sol. 16, Table 4b, in these formulae we get

C_{NaOH}	t (min.)	α_{cis}(av.)	$\frac{-\log(1-1.8\,\alpha_{cis})}{1.8 \cdot t}$	$t_{1/2}$(min.)
0.503	368	0.085	0.000109	1539
»	1590	0.290	0.000112	1496
»	3000	0.415	0.000111	1512

The time of half-completion is seen to be longer than for the isomerization in acidic solution, but it must be mentioned that one of the authors (J. Bjerrum) has once investigated a cis salt with a several times longer time of half-completion than the salt examined here. The average values for α_{cis} and $\alpha_{tr.}$ after two days (sols. 3 and 5, Table 4a) satisfy the equation $q_2 = \frac{\alpha_{tr.}}{\alpha_{cis}}$, fairly well, but this should be considered as a simple coincidence, which shows that the two salts in question are of the same purity and therefore also heterogenously catalyzed to about the same extent.

At temperatures above 80° C the cis-trans equilibrium is established in a few minutes as shown by sols. 7 and 19 in Tables 4. The extinction coefficients for the cis-trans equilibrium are too high in the near ultraviolet (probably due to small amounts of ultraviolet absorbing by-products) — and after prolonged heating the solutions became visibly opaque. The values for q_2 estimated from the ultraviolet extinction coefficients in basic solution are too high for this reason, and conversely the value estimated at 410 mμ from the extinction coefficients in acidic solution is too low (see Fig. 1). But the average values for visible light show clearly that the influence of temperature on q_2 is very small.

q_2 can also be calculated alone from the acidic dissociation constants of the systems of diaquo ions. From the formulae (6) this can be done in 2 ways giving $q_2 = 0.66$ and 1.34, respectively. These values are much more uncertain than the direct spectrophotometrically determined value, $q_2 = 0.80$, and the best values for q_0 and q_1 are obtained by combining the last mentioned value for q_2 with the expressions which contain only products of the mass action constants. Thus we get $q_0 = 58$, and $q_1 = 1.42$ (*cf.* p. 1267).

THE HYDROLYSIS CONSTANT ($k_{h,1}$) OF THE TRIS(ETHYLENEDIAMINE)-COBALT(III) COMPLEX (IN COLLABORATION WITH FLEMMING WOLDBYE)

In alkaline solution charcoal catalyzes the hydrolysis equilibrium of the tris(ethylenediamine)cobalt(III) ion:

$$Co\ en_3^{+++} + 2\ OH^- \rightleftharpoons Co\ en_2(OH)^+ + en$$

but the introductory experiments showed that the equilibrium adjustment takes place with much more difficulty than in the corresponding cobalt(III) ammonia system. The charcoal acts as a catalyst only for a short time after its addition though at the same time it decomposes the complexes to some extent, whereupon its catalytic effect comes almost to a stop. As a consequence of this, equilibrium could be obtained only by adding the charcoal in small portions until further addition caused no change in the calculated hydrolysis constant.

The same kind of "Norite" charcoal was used in all experiments. In every experiment 200 ml of the initial solution, the composition of which is given in Table 5a, were shaken with the charcoal in 250 ml flasks (kept tightly closed by a rubber cover over the glass stopper) in a water thermostat at 25.0° C. Table 5b gives the amounts of charcoal added during the equilibrium adjustment, and the times (in hours) the solutions have been shaken since the first addition of charcoal. Usually when portions of charcoal were added, samples of the solutions were analyzed spectrophotometrically after being carefully filtered. The extinction curves of tris(ethylenediamine) salt and of the cis-trans equilibrium mixture of dihydroxo ions are given in Fig. 1b. The common point of intersection of the curves $\varepsilon_{494} = 61.0$ was used to compute the cobalt concentration

$$C_{Co}(\text{opt.}) = \frac{\log_{10} I_0/I}{61.0 \cdot d}$$

and the degrees of conversion of luteo salt into dihydroxo salt

$$\alpha_\lambda = \frac{\varepsilon_\lambda - \varepsilon_{Co\,en_3}{}^{+++}}{\varepsilon_2(\infty) - \varepsilon_{Co\,en_3}{}^{+++}}$$

were calculated at 560 and 580 mμ by inserting the following values for the extinction coefficients:

	560	580 mμ
$\varepsilon_{Co\,en_3}{}^{+++}$	2.12	1.24
$\varepsilon_2(\infty)$	46.8	28.7

The data collected in Table 5b show that α_{560} and α_{580} follow each other very closely, and further that the cobalt concentration C_{Co} (titr.) determined by potentiometric titration (see p. 1270) in the equilibrium solutions agrees well with the optically determined cobalt concentration. This can be taken as a proof that in all the solutions examined we have a mixture of luteo and dihydroxo salt only.

Table 5. Estimation of the hydrolysis constant $k_{h,1}$ in ~ 1 N $NaNO_3$ at 25°.

a. Composition of initial solutions.

No.	$C^0_{NaNO_3}$	C^0_{NaOH}	C^0_{en}	C^0_{Co}	Co-salt
1	1.000	0.06655	0.0943	0.01512	Co en_3 $(NO_3)_3$
2	1.000	0.1023	0.0979	0.01558	»
3	1.000	0.1023	0.0616	0.01581	»
4	1.000	0.1137	0.0965	0.01644	Co en_2 $(H_2O)_2$ $(NO_3)_3$
5	1.000	0.1485	0.0647	0.01658	Co en_3 $(NO_3)_3$
6	0.800	0.1930	0.0656	0.02247	Co_4 $en_6(OH)_6$ $(NO_3)_6$
7	0.500	0.5080	0.0636	0.01658	Co en_3 $(NO_3)_3$
8	0.250	0.7620	0.0216	0.01525	»

b. Equilibrium adjustment and optical analysis.

No.	Hours shaken	g Norite / 200 ml	C_{Co} (opt.)	C_{Co} (titr.)	ε_{560}	ε_{580}	a_{560}	a_{580}
1	0; 16; 24; 41; 48	1; 0.5; 0.5; 0.5; 0.3	0.01034		4.06	2.45	0.0434	0.0430
	65; 85	0.5; 0.3	0.00872		4.22	2.59	0.0470	0.0479
	105		0.00792	0.00793	4.21	2.57	0.0468	0.0472
2	0; 14; 24	0.8; 0; 0	0.01380		4.30	2.64	0.0488	0.0497
	38	0	0.01378		4.80	2.91	0.0600	0.0594
	111	0.6	0.01378		6.12	3.76	0.0895	0.0895
	135	0.6	0.01228		7.00	4.38	0.1092	0.1116
	156		0.01048	0.01053	7.19	4.44	0.1135	0.1138
3	0; 14	1; 0	0.01363		5.16	3.14	0.0680	0.0675
	24	0	0.01358		6.40	4.06	0.0958	0.1001
	38	0	0.01361		6.78	4.16	0.1043	0.1039
	62	0.2	0.01358		7.68	4.75	0.1244	0.1249
	110	0.2	0.01274		8.60	5.36	0.1450	0.1465
	135	0.6	0.01181		9.34	5.91	0.1616	0.1660
	156		0.01017	0.01014	9.27	5.82	0.1600	0.1629
4	0; 14	1; 0.5	0.01486		26.68	16.29	0.550	0.535
	25; 39	0.5; 0.5	0.01225		14.23	9.02	0.2710	0.2761
	63	0.5	0.01122		10.83	6.84	0.1949	0.1989
	80	0.5	0.01042		9.57	5.95	0.1667	0.1673
	116		0.00952	0.00942	9.59	5.98	0.1672	0.1683
5	0; 16; 24; 41; 48	1; 0.5; 0.5; 0.5; 0.3	0.01135		13.91	8.64	0.2639	0.2628
	65; 88; 105	0.5; 0.3; 0.3	0.00892		15.52	9.77	0.3000	0.3029
	108	0.3	0.00818		16.03	10.11	0.3113	0.3153
	128		0.00744	0.00739	15.39	9.59	0.2970	0.2967
6	0; 18; 43; 72	1; 1; 0.5; 0.5	0.01541		30.25	19.09	0.630	0.635
	92		0.01469	0.01496	30.64	19.39	0.638	0.645
7	0; 15; 24; 39	1; 0.5; 0.5; 0.5	0.01282		39.63	25.25	0.840	0.854
	63; 65	0.5; 0	0.01114	0.01122	39.43	24.95	0.835	0.843
8	0; 18; 43; 72; 92	1; 1; 0.5; 0.5	0.01092	0.01100	45.53	28.65	0.972	0.974

Table 5. continued. c. Analysis of equilibrium solutions.

No.	$2C_{en} + C_{OH^-} + 1.89aC_{Co}$	$C_{en} + 1.79aC_{Co}$	C_{en} (exp.)	C'_{en} (calc.)	C_{OH^-} (exp.)	C'_{OH^-} (calc.)	$k_{h,1}$ (gr.)
1	0.2240	0.0896	0.0889	0.1163	0.0455	0.0442	2.12
2	0.2740	0.0972	0.0951	0.1142	0.0816	0.0847	1.83
3	0.1946	0.0592	0.0563	0.0802	0.0789	0.0820	1.75
4	0.2468	0.0772	0.0744	0.1027	0.0950	0.0895	1.66
5	0.2005	0.0527	0.0488	0.0945	0.0988	0.1165	2.11
6	0.2553	0.0448	0.0277	0.0640	0.1818	0.1850	1.51
7	0.5686	0.0722	0.0554	0.0891	0.4401	0.4731	1.49
8	0.8024	0.0436	0.0245	0.0451	0.7332	0.7278	1.64
						Mean	1.76

The concentrations of hydroxyl ion and free ethylenediamine in the equilibrium solutions were determined by glass electrode titration with a Radiometer pH meter. Fig. 2 gives graphs of such a titration. At the endpoint of the titration for $E \sim 0.165$ volt, pH ~ 4.89, ethylenediamine is titrated as a diacidic base, and at the inflexion point for $E \sim 0.405$ volt, pH ~ 9.04, as a monoacidic base. In the figure, besides E, $\left|\frac{\Delta V}{\Delta E}\right|$ is plotted against the volume V of added nitric acid. According to Gran's calculations [16] this plot

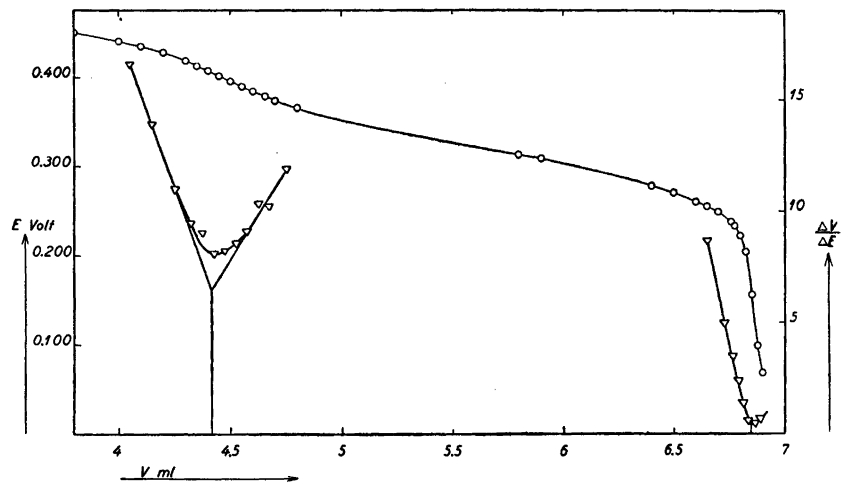


Fig. 2. Potentiometric titration of hydroxyl ion and free ethylenediamine in an equilibrium solution. In the example given 25.00 ml of sol. 2, Table 5, are titrated with 0.999 N HNO_3, and the o-points give directly the potential E (volt) plotted against the volume V, ml of nitric acid added. The Δ-points give the same titration, but, according to Gran [16], $\left|\frac{\Delta V}{\Delta E}\right|$ is plotted against V.

should transform the curve in the vicinity of the equivalent points into straight lines which intersect each other and the V-axis at the equivalent point. It will be seen that the inflexion points come out much more explicitly in Gran's plot. The reason why the first inflexion point is transformed into a U-shaped curve only, is that the bindings of the 1st and 2nd hydrogen ion to ethylenediamine overlap considerably ($pk_{enH_2}{}^{++} \sim 7.6$ and $pk_{enH}{}^{+} \sim 10.3$ at room temperature).

The average number of protons taken up by the dihydroxo cobalt(III) ion is at pH: 9.04, $2 - \bar{\nu} = 0.10$ and at pH: 4.89, $2 - \bar{\nu} = 1.89$. For this reason the total amount of acid consumed at the endpoint of the titration corresponds to $2\,C_{en} + C_{OH^-} + 1.89\,\alpha \cdot C_{Co}$, while the amount of acid consumed between the inflexion point and the endpoint is given by $C_{en} + 1.79\,\alpha \cdot C_{Co}$. The data are given in Table 5c.

The concentrations of hydroxyl ion and free ethylene diamine were also calculated from the initial concentrations using the assumption that the cobalt lost by every addition of charcoal is absorbed as cobalt(III) hydroxide exclusively [cf. 1, p. 262]. This gives the following relations:

$$C'_{en} = C_{en} + \Delta C_{en} = C^{\circ}_{en} + \alpha \cdot C_{Co} + 3(C^{\circ}_{Co} - C_{Co})$$

$$C'_{OH^-} = C_{OH^-} + \Delta C_{OH^-} = C^{\circ}_{NaOH} - 2\,\alpha \cdot C_{Co} - 3(C^{\circ}_{Co} - C_{Co})$$

when luteo salt is added originally, and similar expressions are easily derived when diaquo salt and brown nitrate are added originally. ΔC_{en} and ΔC_{OH^-} in the formulae indicate the hypothetical concentrations which were removed from the solutions by adsorption of ethylenediamine and hydroxyl ions, respectively. Comparison of the C'-concentrations (see Table 5c) with the directly titrated concentrations seems to show that ethylenediamine is adsorbed to a considerable extent, while the adsorption of hydroxyl ions is smaller and lies within the uncertainty of the computation. This explanation was supported by a few separate adsorption experiments under conditions corresponding to the equilibrium experiments.* In these it was found that the concentration of a 0.1 molar aqueous ethylenediamine was diminished by 13 %, and of a 0.01 molar solution by about 20 %, when shaken with 2 g Norite per 100 ml. In similar experiments with aqueous sodium hydroxide the concentration of a 1 N solution decreased by 1.4 % and of a 0.1 N solution by 4 %, when shaken with 2 g Norite per 100 ml.

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* For the making of these measurements the authors are indebted to Miss Lene Rasmussen.

The last column of Table 5c gives the hydrolysis constant $k_{h,1}$ (gr.) calculated from the expression:

$$k_{h,1}(\text{gr.}) = \frac{([\text{cis-Co en}_2(\text{OH})_2^+] + [\text{tr.-Co en}_2(\text{OH})_2^+])\,[\text{en}]}{[\text{Co en}_3^{+++}]\,[\text{OH}^-]^2} = \frac{\alpha \cdot C_{\text{en}}}{(1-\alpha)\,C_{\text{OH}^-}^2}$$

where α is taken as the average of α_{560} and α_{580} in the equilibrium solutions. It will be seen that $k_{h,1}$(gr.) is satisfactorily constant in the whole range examined, where α is varying from 0.04 to 0.97.

The gross hydrolysis constant $k_{h,1}$(gr.) is connected with the partial cis and trans constants through the equation:

$$k_{h,1}(\text{gr.}) = k_{h,1}(\text{cis}) + k_{h,1}(\text{tr.})$$

Using the mean value found, $k_{h,1}$(eq.) = 1.76 ,and combining with the cis-trans constants for the dihydroxo ions, $q_2 = 0.80$, we get:

$$k_{h,1}(\text{cis}) = 0.78,\ k_{h,1}(\text{tr.}) = 0.98$$

The 3rd formation constants in the cobalt(III)-ethylenediamine system are connected with the hydrolysis constants determined here, the acidic dissociation constants of the diaquo ions, and the ionic concentration product of water $k_{\text{H}_2\text{O}}$ through the expression:

$$k_3 = \frac{k_{h,1} \cdot k_{s,1} \cdot k_{s,2}}{k_{\text{H}_2\text{O}}^2}$$

Inserting in this expression the gross, cis and trans constants of $k_{h,1}$, $k_{s,1}$ and $k_{s,2}$, respectively, and $k_{\text{H}_2\text{O}} = 10^{-13.82}$, *i.e.* the value given by N. Bjerrum and Unmack [17] in 1 *N* KCl at 25°, we get in 1 *N* salt solution:

$$k_3(\text{gr.}) = 10^{13.99},\ k_3(\text{cis}) = 10^{13.28},\ k_3(\text{tr.}) = 10^{15.24}.$$

SUMMARY

In the extremely inert cobalt(III)-ethylenediamine system it was possible to establish equilibrium in alkaline solutions by a vigorous treatment with charcoal, and the 1st hydrolysis constant of the tris(ethylenediamine) ion could be estimated by analytical methods. In the system of diaquobis(ethylenediamine)cobalt(III) ions the cis-trans equilibria establish themselves spontaneously without exchange of amine molecules, and all acidic dissociation and cis-trans equilibrium constants were determined by glass electrode and spectro-

photometric measurements. In this way not only the 3rd consecutive formation constant, but also the partial formation constants, k_3 (cis) and k_3(tr.), could be estimated. The data support the idea that Grünberg's "trans" effect appears only in complexes with typical covalent bonds (*vide* Table 1).

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10-Aminoacylphenothiazines

II. Aminobutyryl Derivatives

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The interesting pharmacological properties of 10-aminoacetyl- and 10-aminopropionyl-phenothiazines, described in a preceding paper [1] prompted us to extend our investigations to some aminobutyryl derivatives. α-Bromobutyrylphenothiazine and γ-bromobutyrylphenothiazine were prepared from phenothiazine and the haloacyl halides. The β-bromobutyryl and β-bromo-*iso*butyryl compounds were prepared from phenothiazine and the corresponding halogenated acid by the convenient method for obtaining acid amides described by Human and Mills [2]. α-Bromo*iso*butyryl bromide did not react with phenothiazine under the usual conditions. By treating the halogenated compounds with a number of secondary amines, the corresponding 10-aminobutyryl-phenothiazines were readily obtained.

The hydrochlorides of the compounds have been tested (S. Wiedling) for local anesthetic, antispasmodic and antihistaminic activities in the same way as the acetyl and propionyl compounds in the previous communication [1]. The results of the tests are summarised in Table 1.

Local anesthetic effect. All the compounds possessed a marked activity. They were, however, rather irritating to the rabbit eye.

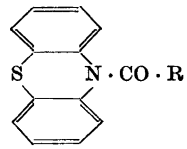
Antihistaminic effect. The most effective compound was slightly inferior to diphenhydramine but the majority had a substantially weaker effect. The most active compounds belonged to the γ-butyryl series.

Antispasmodic effect. Some of the compounds had outstanding spasmolytic properties. The β-butyryl and γ-butyryl derivatives had about the same activity and of these the diethylamino compounds were about as potent as atropine against spasms produced by acetylcholine. The marked difference in effect between the β-butyryl and the β-*iso*-butyryl derivatives is noteworthy. All the piperidino compounds were almost inactive.

Nicotinolytic effect. Some of the compounds have been tested against nicotine-induced tremors in rabbit. Their nicotinolytic activity was weaker

than that of the aminoacetyl and aminopropionyl derivatives, some of which have a very strong effect [3]. This shows that the nicotinolytic action has no relation to the antimuscarinic activity.

Table 1. Pharmacological properties of 10-aminobutyrylphenothiazines.



	R	Local anesthetic activity	Effect in reducing the spasm produced by		
			Acetyl-choline	$BaCl_2$	Histamine
V	$-CH(C_2H_5) \cdot N(CH_3)_2$	2	1.2		0.1
VI	$-CH(C_2H_5) \cdot N(C_2H_5)_2$	1	3	0.9	0.01
VII	$-CH(C_2H_5) \cdot NC_5H_{10}$	1	0.65		0.002
VIII	$-CH(C_2H_5) \cdot NC_4H_8$	5	1.9	2	0.01
IX	$-CH_2 \cdot CH(CH_3) \cdot N(CH_3)_2$		24	5	0.07
X	$-CH_2 \cdot CH(CH_3) \cdot N(C_2H_5)_2$	5	30	12	0.2
XI	$-CH_2 \cdot CH(CH_3) \cdot NC_4H_8$		10	1.1	0.1
XII	$-CH(CH_3) \cdot CH_2 \cdot N(CH_3)_2$		2.2		0.18
XIII	$-CH(CH_3) \cdot CH_2 \cdot NC_5H_{10}$		0.14		0.01
XIV	$-CH(CH_3) \cdot CH_2 \cdot NC_4H_8$		2		0.04
XV	$-(CH_2)_3 \cdot N(CH_3)_2$	2	13	7.5	0.8
XVI	$-(CH_2)_3 \cdot N(C_2H_5)_2$ *		30	2.9	0.6
XVII	$-(CH_2)_3 \cdot NC_5H_{10}$		0.1		0.01
XVIII	$-(CH_2)_3 \cdot NC_4H_8$	3.5	10	6	0.5
	Atropine sulphate		30		
	Papaverine-HCl			0.33	
	Xylocaine-HCl	1.0			
	Diphenhydramine-HCl		1.0	1.0	1.0

* The oxalate was used in the tests.

EXPERIMENTAL

Halogenobutyrylphenothiazines

10-(α-Bromobutyryl)-phenothiazine (I). α-Bromobutyryl bromide (35 g, 0.15 mole) was added to a solution of phenothiazine (19.9 g, 0.1 mole) in boiling toluene (100 ml). The mixture was refluxed for four hours, the solvent was evaporated and the residue (25 g, 72%) was recrystallised from ethanol; m.p. 120—121°. (Found: C 55.1; H 4.11; Br 22.7. $C_{16}H_{14}BrNOS$ (348.3) requires C 55.2; H 4.05; Br 22.9 %.)

10-(β-Bromobutyryl)-phenothiazine (II). β-Bromobutyric acid [4] (8.4 g, 0.05 mole) and pyridine (3.95 g, 0.05 mole) were dissolved in anhydrous ether (125 ml), thionyl chloride (5.95 g, 0.05 mole) in ether (10 ml) was cautiously added, and the mixture was set aside for three days at room temperature. Any excess of pyridine was precipitated as the hydrochloride by adding a small volume of ethereal hydrogen chloride, the reaction mixture was filtered and a solution of phenothiazine (6.6 g, 0.033 mole) in toluene (50 ml) was added. The ether was evaporated and the toluene solution kept at 85° for four hours. After cooling the resulting precipitate (9.4 g, 81%) was collected and recrystallised from benzene; m.p. 167—168°. (Found: C 54.6; H 3.89; Br 23.5. $C_{16}H_{14}BrNOS$ (348.3) requires C 55.2; H 4.05; Br 22.9%.)

10-(β-Bromoisobutyryl)-phenothiazine (III). This compound was prepared in the same way as II. β-Bromo*iso*butyric acid [5] (8.4 g) and phenothiazine (6.6 g) gave 9.6 g (83 %) of crude product, which after recrystallisation from ethanol melted at 110—111°. (Found: C 55.1; H 4.12; Br 22.7. $C_{16}H_{14}BrNOS$ (348.3) requires C 55.2; H 4.05; Br 22.9%).

10-(γ-Bromobutyryl)-phenothiazine (IV). Phenothiazine (5.3 g) and γ-bromobutyryl chloride [6] (7.5 g) were refluxed in toluene (60 ml) for three hours and the crude product (8.5 g, 91%) was recrystallised from ethanol; m.p. 88—90°. (Found: C 55.8; H 4.00; Br 23.2. $C_{16}H_{14}BrNOS$ (348.3) requires C 55.2; H 4.05; Br 22.9%).

Aminoacylphenothiazines

These compounds were prepared and isolated in essentially the same way as described in the previous communication in this series [1].

10-(α-Dimethylaminobutyryl)-phenothiazine (V). Dimethylamine (17 g) and 10-(α-bromobutyryl)-phenothiazine (I) (34.8 g) in benzene (100 ml) were heated in a sealed bottle at 85° overnight. The product (29.0 g, 93%) was recrystallised from methanol; m.p. 98—99°. (Found: C 69.5; H 6.32; N 9.17. $C_{18}H_{20}N_2OS$ (312.4) requires C 69.2; H 6.45; N 8.97%.)

10-(α-Diethylaminobutyryl)-phenothiazine (VI). Prepared similarly from diethylamine and I. Yield 77%. M.p. 64—66° (from ethanol). (Found: C 70.3; H 7.17; N 8.25. $C_{20}H_{24}N_2OS$ (340.5) requires C 70.5; H 7.10; N 8.23%.)

Hydrochloride. M.p. 202—203° (dec.), (from ethanol-ether 1 : 1). (Found: C 63.6; H 6.61; Cl 9.44. $C_{20}H_{24}N_2OS$. HCl (377.0) requires C 63.7; H 6.69; Cl 9.41%).

10-(α-Piperidinobutyryl)-phenothiazine (VII). Prepared from piperidine and I. Reflux five hours in toluene. Yield 83%. M.p. 86—88° (from methanol). (Found: C 71.1; H 7.15; N 7.84. $C_{21}H_{24}N_2OS$ (352.5) requires C 71.5; H 6.86; N 7.95%).

Hydrochloride. M.p. 214—216° (dec.), (from ethanol-ether 1 : 1). (Found: C 64.6; H 6.48; Cl 9.13. $C_{21}H_{24}N_2OS$. HCl (389.0) requires C 64.8; H 6.48; Cl 9.12%.)

10-(α-Pyrrolidinobutyryl)-phenothiazine hydrochloride (VIII). Prepared similarly from pyrrolidine and I. The oily base was directly converted to the hydrochloride. Yield 57%. M.p. 206—208° (dec.), (from acetone-ethanol 10 : 1). (Found: C 64.4; H 6.43; N 7.26. $C_{20}H_{22}N_2OS$. HCl (375.0) requires C 64.1; H 6.18; N 7.47%.)

10-(β-Dimethylaminobutyryl)-phenothiazine (IX). Prepared similarly from dimethylamine and II. Yield 87%. M.p. 94—95° (from ethanol). (Found: C 69.4; H 6.58; N 8.98. $C_{18}H_{20}N_2OS$ (312.4) requires C 69.2; H 6.45; N 8.97%.)

10-(β-Diethylaminobutyryl)-phenothiazine (X). Prepared similarly from diethylamine and II. Yield 21%. M.p. 62—63° (light petroleum). (Found: C 70.8; H 6.80. $C_{20}H_{24}N_2OS$ (340.5) requires C 70.5; H 7.10%.)

10-(β-Pyrrolidinobutyryl)-phenothiazine (XI). Prepared similarly from pyrrolidine and II in 74% yield. M.p. 119—120° (light petroleum). (Found: C 70.7; H 6.89; N 8.20. $C_{20}H_{22}N_2OS$ (338.5) requires C 71.0; H 6.57; N 8.28%).

10-(β-Dimethylaminoisobutyryl)-phenothiazine hydrochloride (XII). Prepared from dimethylamine and III. Yield 68%. The slightly soluble hydrochloride which separated when the reaction mixture was treated with hydrochloric acid melted at 251—252° (dec.) (from ethanol). (Found: C 62.0; H 6.22; N 7.79. $C_{18}H_{20}N_2OS$. HCl (348.9) requires C 62.0; H 6.07; N 8.03%).

10-(β-Piperidinoisobutyryl)-phenothiazine (XIII). Prepared in 71% yield from piperidine and III; m.p. 79—81° (light petroleum). (Found: C 71.2; H 6.94; N 8.02. $C_{21}H_{24}N_2OS$ (352.5) requires C 71.5; H 6.86; N 7.95%.)

10-(β-Pyrrolidinoisobutyryl)-phenothiazine hydrochloride (XIV). Prepared similarly from pyrrolidine and III. Yield 45%. The hydrochloride which separated when the reaction mixture was treated with hydrochloric acid melted at 231—233° (dec.). (From ethanol). (Found: C 63.8; H 6.46; N 7.24. $C_{20}H_{22}N_2OS$. HCl (375.0) requires C 64.1; H 6.18; N 7.47%.)

10-(γ-Dimethylaminobutyryl)-phenothiazine (XV). Prepared from dimethylamine and IV in 85% yield. M.p. 93—95° (from ethanol). (Found: C 68.9; H 6.29; N 8.99. $C_{18}H_{20}N_2OS$ (312.4) requires C 69.2; H 6.45; N 8.97%.)

10-(γ-Diethylaminobutyryl)-phenothiazine oxalate (XVI). Prepared similarly from diethylamine and IV; yield 58%. The oily base was converted to the oxalate; m.p. 151—153° (dec.), (from acetone). (Found: C 60.8; H 6.09; N 6.70. $C_{20}H_{24}N_2OS$. $(COOH)_2$ (430.5) requires C 61.4; H 6.09; N 6.51%.)

10-(γ-Piperidinobutyryl)-phenothiazine (XVII). Prepared similarly from piperidine and IV. Yield 22%. M.p. 78—80° (from light petroleum). (Found: C 71.8; H 7.03; N 7.93. $C_{21}H_{24}N_2OS$ (352.5) requires C 71.5; H 6.86; N 7.95%.)

10-(γ-Pyrrolidinobutyryl)-phenothiazine (XVIII). Prepared similarly from pyrrolidine and IV in 83% yield. M.p. 106—107° (light petroleum-ethanol 1 : 1). (Found: C 70.8; H 6.72; N 8.17. $C_{20}H_{22}N_2OS$ (338.5) requires C 71.0; H 6.57; N 8.28%.)

SUMMARY

The synthesis of fourteen 10-aminobutyryl derivatives of phenothiazine is described. Most of the compounds are powerful local anesthetics and some of them show outstanding spasmolytic properties.

The authors wish to thank Mr. L.-E. Österberg for skilful experimental assistance.

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The Structure of Aqueous Solutions of Perchloric Acid

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Perchloric acid is a member of the series of mineral acids which has not been previously studied by X-ray diffraction methods. Three different aqueous solutions have been examined. They contain 72, 65 and 58 g perchloric acid per 100 g solution, corresponding respectively to about 2, 3 and 4 molecules of water per molecule perchloric acid.

The theory for our investigations was republished recently [1]. The average intensity functions, transformed to theoretical backgrounds, are illustrated by Fig. 1, and the corresponding $\sigma(r)$-curves are given in Fig. 2. We have also verified the $\sigma(r)$-curves by different modifications, which will be published later.

The oxygen atoms of a perchloric acid molecule are tetrahedrally arranged around the chlorine atom. Our $\sigma(r)$-curves give values varying between 1.48 and 1.51 Å for the Cl—O distance, and the $\frac{\sigma(r)}{r}$-curves [2] give 1.47 to 1.50 Å. 1.49 Å is in fair agreement with the available data [3–5], and corresponds to a distance 2.43 Å between the oxygen atoms of the ClO_4^--tetrahedron. Our $\sigma(r)$-curves have maxima at about 2.4 Å. The maxima come out much smaller than one should expect according to the weight of this distance. This is probably due to the deep minimum found in the general form of the $\sigma(r)$-curves around 2.4 Å.

Close packing of molecules and bonds between atoms of different molecules may both cause preferred distances that influence the distribution curves. The presence of hydrogen bonds is indicated by the shape of the curves at about 2.85 Å. An accurate determination of this distance from the $\sigma(r)$-functions is impossible. Interference with other distances at both sides may take place, and the bond length may vary a little with the character of the oxygen atoms linked together. However, 2.85 Å is in good agreement with earlier results [6–8].

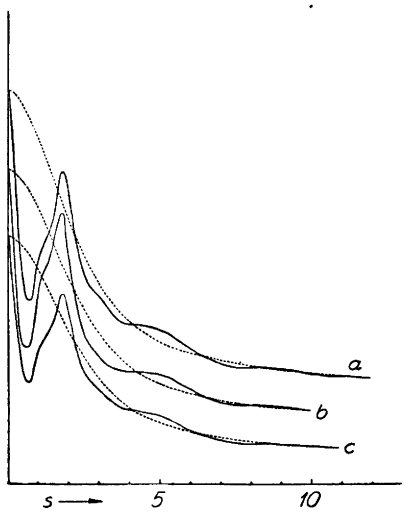


Fig. 1. Transformed intensity-curves (unbroken lines) and corresponding theoretical backgrounds (broken lines). a) 72 % acid. b) 65% acid. c) 58% acid.

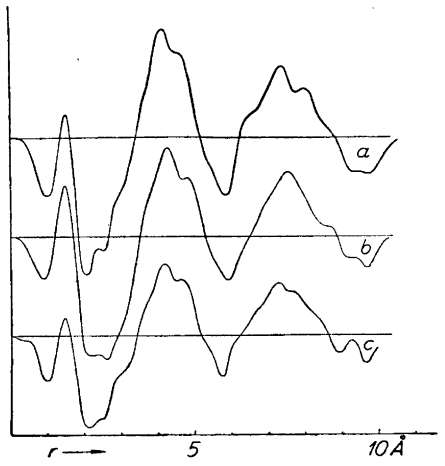


Fig. 2. σ (r)-functions corresponding to Fig. 1.

The hydrogen bonds may connect two water molecules, two molecules perchloric acid or one of each sort. The first case leads to no new distances. For the other two we have tried different ways of placing the molecules relative to each other, and have found the structures of Figs. 3 and 4 to be the average arrangements agreeing best with the σ (r)-curves. As one will see, the atoms

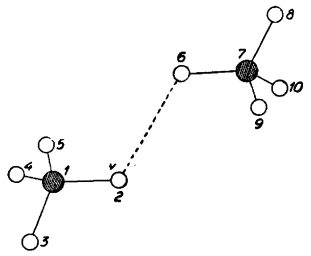


Fig. 3. One ClO_4^--tetrahedron connected with another by a hydrogen bond. Numbers refer to Table 1. Open circles = oxygen atoms. Shaded circles = chlorine atoms.

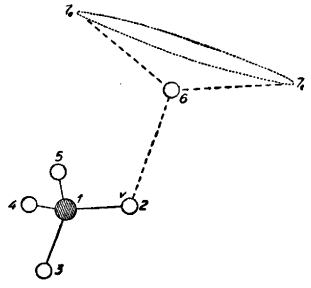


Fig. 4. A ClO_4^--tetrahedron connected with the oxygen atom of a water molecule by a hydrogen bond. The broken circle refers to the possibilities of placing the next oxygen atom. Numbers refer to Table 2.

are placed in trans positions when possible. This is in agreement with the results of earlier investigations on sulphuric and phosphoric acids in aqueous solutions [7,8], and is also assumed to be advantageous from energy considerations. For the angle v of Fig. 3 we find the value 130° to agree best with the experimental results. This large value is probably caused by a repulsion between two ClO_4^--tetrahedra. The angle v of Fig. 4, however, is approximately 109.5°, the tetrahedral angle. The oxygen atom 7, lying somewhere on the circle shown in perspective in Fig. 4, may belong to a water molecule or a perchloric acid molecule. Anyway, the distance between the atoms 2 and 7 is approximately 4.65 Å. This is also a prominent distance occurring in the structure of liquid water [6]. The structures of Figs. 3 and 4 give the distances in Tables 1 and 2, respectively.

Table 1. Distances between the atoms of Fig. 3.

Atoms	Distance in Å	Atoms	Distance in Å
1—2	1.49	4—7	5.65
2—3	2.43	4—9	5.86
2—6	2.85	5—9	6.34
1—6	3.98	3—7	6.40
4—6	4.27	4—8	6.60
3—6	5.24	3—8	7.65
1—7	5.28		

Table 2. Distances between the atoms of Fig. 4.

Atoms	Distance in Å	Atoms	Distance in Å
1—2	1.49	4—6	3.77
2—3	2.43	2—7	4.65
2—6	2.85	3—6	5.03
1—6	3.63		

As shown by Table 1, the Cl—Cl distance has no corresponding distinct maximum on the σ (r)-curves. However, the minimum on the distribution curve for water in this range is considerably more pronounced than for perchloric acid. The following considerations concerning the close packing of molecules will also show that very few distances are found around 5.3 Å.

Of the three possibilities for close packing of two molecules, the packing of water molecules around a perchloric acid molecule will no doubt be most

important for the concentrations studied here. The resulting Cl—O distance may vary from 3.4 to 4.7 Å. Judging from the σ (r)-curves, 4.2 Å is the preferred value. No definite values can be assumed for the resulting O—O distances, which may vary between 3.2 and 5.5 Å. A close packing of two perchloric acid molecules is unlikely, but packing of two water molecules may to some degree be responsible for the ascent of the curves at about 3.2 Å.

Next we want to investigate the distances between two molecules packed with a third one between them, or, as we shall call it, second order packing of molecules. Of the six possibilities for this second order packing, our concentrations eliminate three, leaving the following: water-perchloric acid-water, perchloric acid-water-perchloric acid and perchloric acid-water-water.

The first of these eventualities occurs most frequently. However, this packing can take place in numerous ways, leading to distances from 3.2 to 8.4 Å. Judging from this broad range and the fact that the weight of such a distance is small, the chance for any specific influence on the distribution curves is small.

The second of the above possibilities implies the distance between two molecules of perchloric acid. This distance has a considerable weight and explains the maximum between 7.3 and 7.4 Å on our σ (r)-curves. This conclusion is supported by results obtained in investigations of liquids with spherical molecules [1,9–11]. Calling the distance of the first order packing r_1 and that of the second order r_2, both experimental and theoretical results show that the ratio $\frac{r_2}{r_1}$ has a value between 1.8 and 2.0, varying a little from liquid to liquid. In the present case the situation is different. Even by using the approximation of spherically symmetrical molecules, the radii are not the same for the two sorts. Nevertheless, the value of the analogous ratio cannot be far from 1.8, and the value 1.75 obtained here is reasonable.

The third possibility for second-order packing is perchloric acid-water-water. From the above discussion of the water-water packing, it is not likely that the corresponding distances give a maximum on the distribution functions. However, they may to a certain degree account for the ascent of the σ (r)-curves between 6 and 7 Å. This possibility is greatest for the most diluted of our solutions.

SUMMARY

The distance Cl—O in the ClO_4^--tetrahedron has been found to be about 1.49 Å. Hydrogen bonds connect the molecules of the solutions, leading to the structures of Figs. 3 and 4. A close packing of molecules is also assumed and discussed in some detail.

The author wants to express his gratitude to *Fridtjof Nansens fond* for financial aid and to Professor Chr. Finbak and Dr. O. Bastiansen for good advice during the work.

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Short Communications

Studies on Lignin

XV *. Nitrobenzene Oxidation of Lignin-Resorcinol Condensation Products, Methylated with Diazomethane

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In Part VI[1] it was shown that when spruce lignin or lignin sulphonic acids are heated with resorcinol in acid solution, the yield of vanillin obtained on subsequent oxidation with nitrobenzene and alkali is reduced, the decrease varying with the degree of sulphonation and the time of heating with resorcinol.

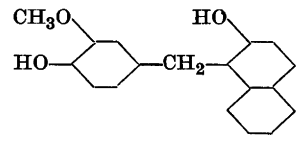
However, the yield of vanillin could not be reduced to less than about 35 % of the yield obtained from fresh spruce wood even on prolonged heating at pH 0.5. This behaviour may be explained in a number of ways [2], the most reasonable being that, owing to the instability of the resorcinol nuclei under the oxidation conditions, part of those vanillin-yielding elements which are attached to such nuclei still yield vanillin.

The latter explanation is supported by the fact that suitable model compounds containing resorcinol nuclei also give 30–35 % of the "theoretical" yield of vanillin and that resorcinol itself is completely destroyed under the standard oxidation conditions [3].

* Part XIV. *Svensk Papperstidn.* In the press.

It was then desirable to study lignin-phenol condensation products containing more stable phenolic nuclei. Two ways have been tried; substitution of resorcinol for another phenol (2-naphthol) and methylation of the lignin-resorcinol condensation products with diazomethane.

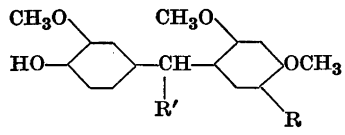
The former method gave results comparable to those obtained with resorcinol. Thus the compound I, obtained from vanillyl alcohol and 2-naphthol, gave 36 % of the theoretical yield of vanillin and the product obtained by heating spruce wood with 2-naphthol at pH 0.5 (12 hours, 135°) gave 38 % of the "normal" yield obtained from spruce wood on nitrobenzene oxidation.



I

In order to obtain information about the behaviour of methylated phenolic nuclei under the standard oxidation conditions, a number of model compounds, obtained from vanillyl alcohol, were studied (*cf.* experimental part). On the basis of the analytical results and known analogies, these compounds were considered to have structures II–IV.

On oxidation with nitrobenzene and alkali, the compounds gave the following yields of vanillin (in per cent of the "theoretical" yield): II 5.6; III 6.6; IV 0. When resorcinol dimethyl ether was subjected to the same treatment, at least



II R = H; R′ = H
III R = OCH_3; R′ = H
IV R = OCH_3; R′ = $C_6H_2(OCH_3)_3$ (2,4,5)
(Szeki [4])

80 % of the material was recovered unchanged.

As a consequence of these results, various methylated lignin-resorcinol condensation products were subjected to nitrobenzene-alkali oxidation. The yields of vanillin are given in in Table 1, as compared with the yields obtained from methylated lignin preparations not heated with resorcinol and acid. The methylations were made with diazomethane in dioxan and repeated until a constant methoxyl content was obtained. In the methylations, no loss of material was observed, and thus the yields of vanillin and veratric acid are calculated on the basis of the methoxyl content of the material before methylation. The yields of vanillin are also given in per cent of the "normal" yield obtained from spruce wood (30.5 %).

It is evident that the methylation causes a decrease in the yield of vanillin by up to 30 %, compared with the corresponding figures for unmethylated lignin-resorcinol condensation products [1]. However, methylation of untreated spruce wood causes a decrease in the yield of vanillin by 15—20 %, probably caused by the methylation of free phenolic hydroxyl groups in the vanillin-yielding elements, as shown by the occurrence of veratric acid among the oxidation products.

The figure 15—20 % conforms very well with the total content of phenolic hydroxyl groups in lignin [5,6], indicating that these groups are fairly evenly distributed among vanillin-yielding and non-vanillin-yielding elements in lignin. It is therefore reasonable to assume that most of the decrease in the yield of vanillin resulting on methyl-

Table 1. Yields of vanillin and veratric acid from lignin preparations, methylated with diazomethane.

Material	Heating with resorcinol		S/OCH_3 prior to methylation	OCH_3 after methylation	Veratric acid %	Vanillin %	
	Time, h	pH				based on OCH_3 in unmethylated material	based on "normal" yield
Extracted	0	—		8.6	1.1	25.7	84.3
	6	1.4		12.9	—	9.1	29.8
spruce	12	1.4		13.4	—	7.1	23.4
	6	0.5		15.6	0.5	4.8	15.7
wood	12	0.5		16.6	0.3	1.7	5.6
"Low sulphonated lignin"	0	—	0.34	15.4	1.5	24.1	79.0
	12	1.4	—	12.3	—	9.3	30.5
	12	0.5	0.33	14.6	—	3.8	12.4
"Further sulphonated lignin"	0	—	0.62	14.8	—	25.0	82.0
	12	0.5	0.59	13.0	—	18.4	60.3

ation of the condensation products is caused by stabilization of resorcinol nuclei attached to vanillin-yielding elements, and not simply by methylation of phenolic hydroxyl groups in such elements.

In Part VI [1] it was shown that "low sulphonated lignin" a preparation obtained by sulphonation of spruce wood at pH 5.3, gives only slightly higher yields of vanillin than wood, after reaction with resorcinol. On the other hand, lignin sulphonic acids of higher degrees of sulphonation give appreciably higher yields, approaching those from untreated wood. These results were explained by assuming that the sulphonic acid groups introduced at a fairly high pH, such as pH 5.3 (sulphonation of groups A [7]), are mainly located in non-vanillin-yielding elements, while a large proportion of the rest of the sulphonatable groups (groups B [7]) belong to vanillin-yielding elements. The sulphonation of the latter groups would then prevent the introduction of resorcinol nuclei into a large number of the vanillin-yielding elements.

This hypothesis is strongly supported by the above results, spruce wood and "low sulphonated lignin" giving only small yields of vanillin after reaction with resorcinol and methylation — yields comparable to those obtained from the corresponding model compounds referred to above.

In other words, practically all the vanillin-yielding elements contain, at the α-position, groups reactive towards resorcinol (and other phenols) in acid solution, and most of these groups seem to be of the B type.

EXPERIMENTAL

1-(4-Hydroxy-3-methoxybenzyl)-2-naphthol (I). 2-Naphthol (5 g) was dissolved in 25% ethanol (50 ml) and conc. hydrochloric acid (2 ml) added. To the boiling solution, vanillyl alcohol (2 g), dissolved in ethanol (10 ml), was added and the mixture boiled for one minute. After a few seconds an oil precipitated. On cooling, crystals were obtained (1.2 g, 33%). Recrystallization from 50% ethanol gave colourless plates of m.p. 166—166.5°. (Found OCH_3 11.0; $C_{17}H_{13}O_2OCH_3$ requires OCH_3 11.1.)

4-Hydroxy-3',4',5'-trimethoxydiphenylmethane (II). To a boiling solution of resorcinol dimethyl ether (9 g) in ethanol (75 ml) and conc. hydrochloric acid (2 ml), vanillyl alcohol (3 g), dissolved in ethanol (20 ml), was added dropwise during a period of *ca.* 15 minutes. Then the mixture was boiled for 30 minutes, water added and the mixture extracted with ether. The ethereal solution was dried over sodium sulphate, the ether distilled off, and the residue distilled *in vacuo*. First, the excess resorcinol dimethyl ether went over (95—102°) and then at 236—238° (12 mm) a product distilled (2.5 g, 47%) which crystallized on cooling and scratching. The product was recrystallized from benzene-light petroleum. Colourless prisms of m.p. 72.5—73.5°. (Found OCH_3 33.8; $C_{13}H_9(OCH_3)_3$ requires OCH_3 33.9.)

The above compound was reacted with benzoyl chloride in anhydrous pyridine, yielding a *benzoate* melting at 109—110°, after recrystallization from acetic acid. (Found OCH_3 24.3; $C_{20}H_{13}O_2(OCH_3)_3$ requires OCH_3 24.6.)

4-Hydroxy-3,2',4',5'-tetramethoxydiphenylmethane (III). Hydroxyhydroquinone trimethyl ether (3 g) and vanillyl alcohol (1 g) were reacted as described above. The ether extract was distilled *in vacuo*, yielding the excess hydroxyhydroquinone trimethyl ether as a yellow oil. The distillation residue crystallized on trituration with benzene (1 g, 51%). Recrystallization from light petroleum yielded colourless, coarse prisms of m.p. 110—111°. (Found OCH_3 41.0; $C_{13}H_9(OCH_3)_4$ requires OCH_3 40.8.)

Methylation of lignin-resorcinol condensation products with diazomethane. The methylations were carried out using a dioxan suspension of the finely pulverized lignin material. The lignin sulphonic acids were employed in the form of their barium salts.

In a typical run the following procedure was used:

A product obtained on heating extracted spruce wood powder with resorcinol at pH 0.5 for 10 hours at 135° (10 g) was suspended in dioxan (75 ml). A solution of diazomethane (*ca.* 3 g) in dioxan (150 ml) was added and the mixture left overnight. Then the mixture was filtered and the solid residue washed with dioxan and acetone. The procedure was repeated until a constant methoxyl content was obtained, as illustrated in Table 2.

In general 3 to 4 methylations were required.

Table 2. Variation of methoxyl content with number of methylations.

Material	Weight, g	OCH_3, %
Unmethylated material	10.0	4.1
After 1st methylation	10.0	10.7
» 2nd »	9.9	15.4
» 3rd »	9.7	16.8
» 4th »	9.7	16.6

The nitrobenzene oxidations and the estimations of vanillin and veratric acid were carried out as described in previous communications [8,9].

The author wishes to express his thanks to Miss I. Malmström for skilful assistance, and to Statens Tekniska Forskningsråd for financial support.

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A new Solubilization Effect

PER EKWALL, INGVAR DANIELSSON and STURE HENRIKSON.

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When hydrocarbons are solubilized by aqueous association colloid solutions the conductance is very little altered. When the solubilized substance is a polar compound such as a long-chain primary alcohol or fatty acid, a decrease in the conductance is frequently noted. The solubilization of the glycol, 1,10-decanediol, however, leads to a marked increase in the conductance. This latter phenomenon has not been described previously.

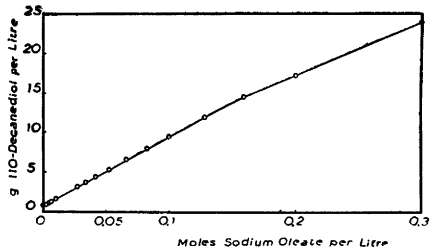


Fig. 1. The solubility of decanediol in sodium oleate solutions at 20° C.

One of us has shown that sodium oleate solutions are able to solubilize fairly large amounts of 1,10-decanediol [1] and the saturation capacity of oleate was preliminarily estimated at about 66 g diol per mole oleate at 20° C. We have now conducted a more detailed study of the solubilization of decanediol. The solubility values were determined with a Zeiss-Löwe interferometer for liquids. The solubility in pure water and in salt solutions of low ionic strength is only 720—730 mg per liter at 20° C and 1 400 mg per liter at 40° C. These values are lower than those previously used and hence the amounts solubilized are actually greater. Fig. 1 shows the relation between the solubility of the diol at 20° C and the oleate concentration. Decanediol does not seem to be solubilized by oleate solutions below 0.0015 *M*. The increase in the solubility of decanediol is linear over a fairly wide oleate concentration range up to about 0.17 *M*. In this range the saturation capacity of the micellar oleate is 82 g decanediol per mole oleate, *i.e.* 0.5 mole decanediol per mole oleate.

As already mentioned, the solubilized decanediol brings about a marked increase in the conductance of the oleate

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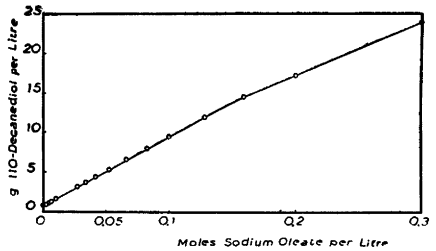


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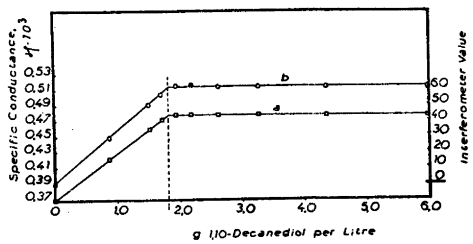


Fig. 2. a. The influence of decanediol on the specific conductance of a 0.0125 M sodium oleate solution.
b. The influence of decanediol on the interferometer value of a 0.0125 M sodium oleate solution.

solutions. Fig. 2 a shows that the specific conductance increases linearly with the amount of solubilized diol up to a maximum value. As Fig. 2 b shows, this value is attained when the solution is saturated by diol. After this point a further addition of diol has no effect on the conductance. The excess of the diol does not dissolve.

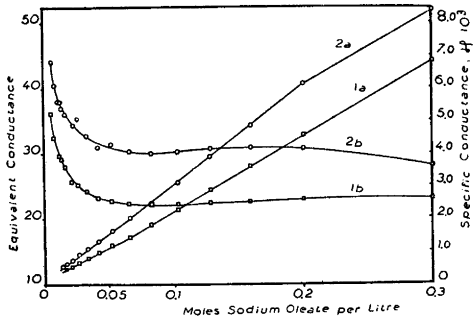


Fig. 3. The conductance of sodium oleate solutions at 20° C.
Curve 1 a: The specific conductance of pure oleate solutions.
Curve 1 b: The equivalent conductance of pure oleate solutions.
Curve 2 a: The specific conductance of oleate solutions saturated with decanediol.
Curve 2 b: The equivalent conductance of oleate solutions saturated with decanediol.

The variation of the specific and equivalent conductances in pure oleate solutions is shown in Fig. 3, curves 1 a and 1 b and that of oleate solutions saturated with decanediol in Fig. 3, curves 2 a and 2 b. The relative increase in conductance becomes rapidly greater at first until it attains a value of 33—39% at oleate concentrations between 0.03 and 0.2 *M*. Above the latter concentration the relative increase again diminishes.

We have found that the solubilization of decanediol by solutions of other paraffin chain salts also leads to an increase in the conductance. This is the case with solutions of sodium myristate, laurate and lauryl sulphate (*e.g.* in a 0.1 *M* solution of the latter the increase in the specific conductance is 58%), and hexadecyltrimethylammonium bromide (increase 41—50% in 0.005—0.02 *M* solutions). In these cases the solutions saturated with decanediol contain about 0.4—1 mole diol per mole micelle-forming substance. Decanediol is also solubilized by sodium cholate and desoxycholate solutions, but here no increase in conductance is observed and the molar ratio at saturation is lower.

The observed increase in conductance is not due to the presence of impurities in the diol nor can it be that the latter itself forms ions. The pH values of the solution are very little altered. Thus the increase must depend on changes in the micellar state of the association colloid. It seems possible that the solubilization of decanediol effects a rearrangement of the micelles by which the micelles become smaller and their number increases and the interionic forces between the micelles and gegenions diminish in strength.

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The Carbohydrates in Birchwood

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In a recently published paper Gustafsson *et al.*[1] have determined the carbohydrate composition of 20 different wood species, all of which were found to contain galactan, glucosan, mannan, araban and xylan. Shortly afterwards Jones and Schoettler[2] found L-rhamnose in a hydrolyzate of Trembling Aspenwood. Quite recently Björkqvist and Jörgensen[3] found small amounts of galactose and rhamnose in hydrolyzates of *Betula pubescens*, *Betula verrucosa* and *Populus tremula*.

We have repeated our work concerning the two above mentioned birch species and investigated samples from trees grown in widely different locations. The samples consisted of normal wood as well as of tension wood. Extracted wood flour was hydrolyzed according to Hägglund et al.[4]. The hydrolyzates were deacidified with ion exchangers and evaporated to a smaller volume in vacuo after which the sugars in the hydrolyzates were examined by means of paperpartition chromatography. The chromatograms were quantitatively evaluated according to a photometric method developed by Gustafsson *et al.*[5]. Some of the determinations were checked by extracting the chromatographically separated sugars with water and determining the sugar content of the extracts using Somogyi's reagent. The agreement between the different methods was good. The relative pentose destruction was observed according to Sundman *et al.*[6] and the results expressed as polysaccharides in percentage of the total amount of carbohydrates are given in Table 1.

In normal wood the carbohydrate composition seems to be fairly constant and is the same for *B. pubescens* and *B. verrucosa*. The high content of xylan is characteristic. Tension wood contains more galactan and glucosan but less xylan than normal wood. This difference between normal wood and tension wood has previously been shown for *Eucalyptus regnans*[7], and according to Jayme *et al.*[8] tension wood of poplar contains less pentosans and more cellulose than normal wood of the same species. Considering that birch may contain considerable amounts of tension wood, analyses of average samples, representative of a whole birch trunk, often give lower xylan and higher glucosan contents than the above mentioned figures for normal wood.

In a separate series of experiments birch wood was subjected to a mild hydrolysis. In the hydrolyzates the above mentioned

Table 1.

Trunk N:o	Species	Kind of wood	Galactan %	Glucosan %	Mannan %	Araban %	Xylan %
1	Betula	pubescens Normal wood	2.2	59.8	0.7	0.8	36.5
2	»	» »	2.6	57.6	0.9	0.9	38.0
3	»	» »	2.5	55.2	3.0	1.0	38.3
3	»	» Tension wood	11.6	73.5	0.4	Traces	14.5
4	»	» Normal wood	2.5	54.1	2.5	0.9	40.0
4	»	» Tension wood	9.6	71.6	0.5	Traces	18.3
5	»	verrucosa Normal wood	2.3	56.7	1.5	0.9	38.6
6	»	» »	2.0	58.8	2.0	0.9	36.3
7	»	» Tension wood	8.0	69.0	0.7	0.2	22.1

sugars and small amounts of rhamnose and some other not yet identified compounds could be detected.

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The Structure of Dibromo*cyclo*hexane of M.P. 49°

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When 1,4- or 1,3-*cyclo*hexanediol is treated with HBr, two solid substances are obtained, one melting at 112°, the other at 49°. It is established beyond doubt that the former is the »*trans*»-1,4-derivative with both bromine atoms in $\varkappa$ positions. Regarding the structure of the dibromide of m.p. 49°, however, conflicting viewpoints have been put forward. On the basis of rather unconvincing chemical arguments, it has been suggested that the substance is 1,2- or 1,3-dibromo*cyclo*hexane [1], 1-bromomethyl-3-bromo*cyclo*pentane [2] and 1,4-»*cis*»-dibromo*cyclo*hexane [3]. In 1938 the substance was investigated in this laboratory, using the visual method of electron diffraction. The diffraction data made it seem probable that the substance was 1$\varkappa$3$\varkappa$-dibromo*cyclo*hexane [4]. We have recently re-examined the substance, now using the sector method of electron diffraction. Considerable experimental difficulties were encountered and the diagrams obtained were not quite satisfactory. However, they seem to indicate that our earlier suggestion may be incorrect and that the substance probably is 1$\varkappa$4 ε-dibromo*cyclo*hexane. The experimental $\frac{\sigma(r)}{r}$ - curve is reproduced in Fig. 1, and appears to exhibit peaks due to both ε- and $\varkappa$-bonded bromine atoms. To account for the position of the maxima IV—VII, it is, however, necessary to assume certain deformations in the ideal 1$\varkappa$4ε structure. The dotted curve in Fig. 1 and the interatomic distances given below have been computed from a model in which the carbon ring of the ideal 1$\varkappa$4ε structure is flattened by increasing the tetrahedral angles $C_4-C_3-C_2$ and $C_4-C_5-C_6$ by 5°, the other valency angles being kept unchanged. Deformations are found also in other compounds containing ε-bonded halogen atoms, and are believed to be due to repulsion between these atoms and the nearest ε-bonded hydrogen atoms. The general agreement between experimental and theoretical curves is satisfactory, but maximum VII is much lower than should be expected. In the calculations a C—C distance of 1.54 Å and a C—Br distance of 2.01 Å were used. The maxima are interpreted as follows:

Position of max.	(in Å)	Calc. interatomic distances	(in Å)
I	1.53	C_1-C_2	1.54
II	2.02	C_1-Br_1	2.01
III	2.90	$C_1-Br_{2\varkappa\varepsilon}$	2.91
IV	3.48	$C_1-Br_{3\varepsilon}$	3.45
V	4.24	$C_1-Br_{3\varkappa}$	4.30
		$C_1-Br_{4\varepsilon}$	4.11
VI	4.85	$C_1-Br_{4\varkappa}$	4.87
VII	5.47	$Br_1\varkappa-Br_{4\varepsilon}$	5.52

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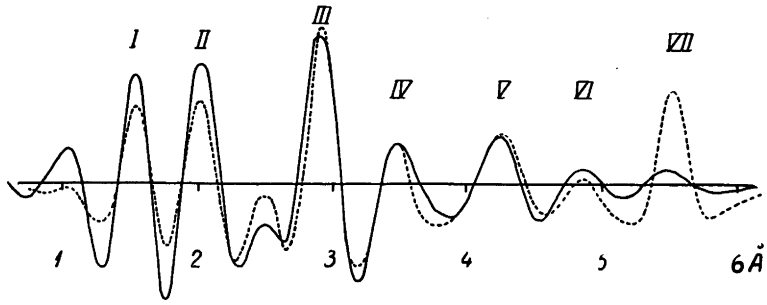


Fig. 1. Experimental and theoretical $\frac{\sigma(r)}{r}$*-curves for di-bromo-cyclohexane of m.p. 49°. The theoretical curve (dotted) corresponds to a deformed molecule 1α4ε-dibromo-cyclohexane.*

The theoretical curve for 1α3α-dibromo-*cyclo*hexane does not account for the maximum IV, and should have a maximum at 5.78 Å corresponding to the distance $Br_{1\alpha}-Br_{3\alpha}$. The possibility that the molecule is 1-bromomethyl-3-bromo-*cyclo*pentane cannot definitely be ruled out from this experiment, as an orientation of the bromine atom in the methyl group can be found, which gives a theoretical curve similar to that for 1α4ε, although the agreement does not appear to be quite so good.

Preparative work recently carried out in our laboratory using pure isomers of the 1,3- and 1,4-diols and phosphorous tribromide did not give conclusive evidence regarding the positions of the bromine atoms in the molecule.

We have also examined crystals of the substance by X-ray methods. The crystals are monoclinic, with $a = 12.26$ Å, $b = 5.45$ Å, $c = 12.24$ Å, and $\beta = 106.5°$. The higher melting isomer, 1α4α-dibromo*cyclo*-hexane, has previously been studied by X-ray methods [5,6], and is also found to be monoclinic, with $a = 12.21$ Å, $b = 5.57$ Å, $c = 6.03$ Å, and $\beta = 107°$. Space group $P2_1/n$. It will be seen that the two substances have nearly the same cell dimensions, apart from the doubling of the c axis. In fact, the two structures must be closely related, as the intensities of the $h0l$ and $0k0$ reflexions are very similar. We have not yet been able to interpret these observations. However, as was to be expected, the Fourier projection along the b axis is very similar for both substances, and seem to indicate that also the isomer melting at 49° is an 1,4-derivative of *cyclo*-hexane, in agreement with the conclusions drawn above from electron diffraction measurements.

We wish to thank Dr. W. Rigby of Birkbeck College, London, for sending us very pure samples of the substance, and Siviling. E. Risberg for taking the electron diffraction diagrams.

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Electrolytic Desalting of Amino Acid Solutions with Electronegative and Electropositive Membranes, and the Conversion of Arginine into Ornithine

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Arginine was found by Stein and Moore[1] to undergo conversion into ornithine during desalting in the apparatus of Consden, Gordon and Martin [2]. We have confirmed this observation with an improved apparatus [3].

Experiments performed to disclose the reason for this conversion pointed to the formation of ornithine at the mercury electrode used as a cathode. In the said apparatus [3] dilute H_2SO_4 streams over the surface of the dialysing membrane at the anode and removes the ions passing through the membrane, while the mercury cathode comes into direct contact with the solution undergoing desalting. It was therefore tried to replace this mercury electrode with an electrode similar to the anode, but with dilute NaOH streaming over the surface. In this manner any interference of the mercury electrode with the amino acid solution during the electrolysis could be avoided. The complete apparatus therefore consisted of two electrodes of the construction mentioned above placed in a small beaker containing the amino acid solution, and separated by a glass plate placed loosely between the electrodes in order to produce an even distribution of the electric current through the solution. Through one of the electrodes (the anode) 0.1 *N* H_2SO_4 was passed and through the second (the cathode) 0.1 *N* NaOH.

Solutions of amino acids containing about 0.5 mg N per ml were prepared in physiol. NaCl. Nitrogen was determined by micro-Kjeldahl before and after desalting. During the electrolysis a great loss of water from the solution occurred. This was compensated by adding suitable amounts of H_2O when needed. Complete desalting (of 10 ml solution) was accomplished in about 35 mins. during which time the current decreased from 0.7 amps. to 0.1 amps. The solutions were then removed, filled up to an appropriate volume and the amount of N estimated in an aliquot.

Pieces of cellophane (from Visking Nojax casings, size 36/32) were used as membrane for the anode. In experiments where this material was used also at the cathode a great loss of amino acids resulted. This was true especially in the case of neutral and basic amino acids, which disappeared almost completely, while the acidic amino acids were retained to some extent.

In the conventional construction of the desalting apparatus, where an electronegative membrane is used solely at the anode, no losses of amino acids are encountered, excepting probably a slight loss in the case of the strong acid taurine [3]. We have therefore prepared an electropositive membrane for use at the cathode by impregnating cellophane with a phenylenediamine resin as described by Gilbert and Swallow[4].

Experiments with these membranes showed a considerable improvement in recovery of the amino acids, and in the case of acidic amino acids complete recovery was approached. Typical results are presented in Table 1. The percentage of recovery of the amino acids varied with the quality of the membrane prepared and could be increased to 50 per cent or more in the case of arginine as representing a basic amino acid. In experiments where the content of arginine was estimated after Albanese and Frankston [5] and ornithine after Chinard [6,1,3] it was found that no conversion of arginine into ornithine had occurred. This result greatly supports the idea that the conversion observed in the con-

Table 1. Desalting of amino acids with either an electronegative (−) or an electropositive (+) membrane at the cathode. Results presented as mg N per 2 ml of solution before and after desalting.

	Before desalting:	After desalting: (−)	(+)
Glycine	1.06 mg N	0.03 mg N	0.26 mg N
Glutamic acid	1.01 mg N	0.75 mg N	0.97 mg N
Arginine	0.98 mg N	0.05 mg N	0.20 mg N

ventional apparatus is caused by a process taking place at the surface of the mercury cathode.

The results here obtained lead to the conclusion that complete recovery of arginine and any other amino acid might probably be obtained in an apparatus of the proposed construction provided a suitable electropositive membrane could be developed. It might be possible by using such a membrane at the cathode to obtain just as satisfactory results as those produced at the anode by the electronegative cellophane.

This investigation was supported by "Det teknisk-videnskabelige Forskningsraad", Copenhagen.

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On the Question of a Separation in a Field of Stationary Ultrasonic Waves

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A well established fact is that stationary ultrasonic waves in a liquid suspension will cause the suspended matter to move into planes separated by half sound wave lengths. It has been shown that the disperse phase will accumulate in the velocity nodes when it is of a less density than the solvent and vice versa [1,2].

Could this separating effect of the ultrasonic waves be reproduced even on a molecular scale? — No doubt it would be of a great interest if it were possible to establish permanent concentration changes in a true solution subjected to stationary ultrasonic waves. In the literature there are some indications that this might be true but as yet definite evidence is lacking.

PREVIOUS OBSERVATIONS

Young and Kersten obtained ripples on the cathode in the electrolytic deposition in an ultrasonic field [3]. The distances between these ripples corresponded to half sound wave lengths. The authors concluded that a possible explanation is that concentration differences are set up in the ultrasonorated electrolyte. In a separate study the present author, however, has tried to show that this suggestion is not very plausible and that the effect in question may be looked upon as a pure electrochemical phenomenon [4].

Ernst made an interesting observation when he determined the sound velocity in some binary solutions according to a striation method [5]. A solution of 80 weight % paraffin oil in chloroform did not give the usual line structure but showed a definite brick-stone pattern. At this concentration there was also a distinct maximum in the sound velocity. The most interesting thing, however, is that the structure was frozen in for several hours after the ultrasonic treatment had been interrupted.

Partly analogous results were obtained by Frei and Schiffer who worked with aqueous glycerol solutions and a solution of hexane in paraffin oil [6]. In their experiments the "Toepler's Schlieren" in the nodal planes were recognized up to 24 hours after the ultrasonic treatment.

Frei and Schiffer as well as Ernst state that their results seem to indicate a separating action of the ultrasonic waves. It is also pointed out by the above authors that this is contradictory to theoretical considerations by Herzfeld.[7]

However, this is apparently not true. The separating effect treated by Herzfeld and found to be negligible does not seem to be of the permanent type here dealt with (Herzfeld's considerations refer to the possibility of temporary concentration changes within a volume element with the same frequency as the ultrasonic waves; the time mean value of these rapid variations, however, being constant and equal to the initial value. This effect is thus seen to be analogous with the wellknown Debye effect. — In the permanent separation, on the other hand, it is assumed a resulting uneven concentration distribution in the acoustic field, which should be detectable even after the interruption of the sound.)

Summing up, there seems to be no theoretical account on the type of permanent separation which is the subject of this paper. The cited observations on the other hand give some circumstantial evidence that the effect in question can take place. These reasons and the importance of the effect if real were the motives for the following study.

THERMODYNAMIC CALCULATION

In the following treatment we restrict ourselves to the conditions in the proper nodal and antinodal planes. (With nodes we will mean the velocity nodes, *viz.* where the particle velocity always is zero but pressure and temperature oscillate with maximum amplitudes). We may thus represent the system by two thin plane compartments, one for the nodal and the other for the antinodal plane, which are connected with a small channel in order to permit changes in concentration.

The liquid mixture is made up of the two components M_1 and M_2, where this symbol also stands for the molecular weight. Consider the process 1 mole M_2 being transferred from the node to the antinode.

$$M_{2n} \rightarrow M_{2a} \tag{1}$$

Of the two additional subscripts n stands for the node and a for the antinode.

In order to keep the volume of the system constant the corresponding volume of M_1 must simultaneously be transferred from the antinode to the node.

$$q\ M_{1a} \rightarrow q\ M_{1n} \tag{2}$$

Partial molar quantities are approximated with molar quantities throughout in this preliminary treatment, and thus $q = V_2/V_1 = M_2\,\varrho_1/M_1\,\varrho_2$, where V refers to the molar volume and ϱ to the density of the pure components.

The proper process will be the sum of the two processes (1) and (2)

$$M_{2n} + q\ M_{1a} \rightarrow M_{2a} + q\ M_{1n} \tag{3}$$

In the state of equilibrium the free energy change, ΔF, of the process is zero, or

$$\Delta F = \mu_{2a} + q\ \mu_{1n} - \mu_{2n} - q\ \mu_{1a} = 0 \tag{4}$$

where μ designates the time mean values of the corresponding chemical potentials per mole under the prevailing conditions in the ultrasonic field. It is convenient to define the standard conditions as the unit concentration of 1 mole/cm^3, and the pressure P_s and temperature T_s where P_s and T_s are the time mean values of these quantities in the actual ultrasonic field. It is to be noted that the P_s and T_s as defined here are constant during any one experiment but may have other values for different experiments. Furthermore we impose the condition that the particle velocity is zero.

Let us first examine the free energy change which will accompany the transformation of P_s and T_s into the corresponding oscillating variables. The changes of state in the ideal acoustic wave are processes that occur at constant entropy. It can easily be shown that the appropriate thermodynamic formulae are [10]

$$\left(\frac{\partial F}{\partial T}\right)_S = \frac{V \cdot C_p}{T \cdot \left(\frac{\partial V}{\partial T}\right)_P} - S \cdot \text{and} \left(\frac{\partial F}{\partial P}\right)_S = V - \frac{S \cdot T}{C_p} \cdot \left(\frac{\partial V}{\partial T}\right)_P \tag{5}$$

From these expressions is inferred that the derivatives $\left(\frac{\partial F}{\partial T}\right)_S$ and $\left(\frac{\partial F}{\partial P}\right)_S$ may be assumed to be very near constant in this ideal case; the pressure and temperature variations amount to some atmospheres and some tenths of a degree centigrade respectively. As the equations thus will be linear there is a negligible change in the free energy when we change the stationary conditions, P_s and T_s, into oscillating ones with the same time mean values. Furthermore P_s and T_s are assumed to be the same in the nodes and the antinodes (the mean pressure equals the static pressure plus the sound pressure). It is now recognized that with the chosen set of standard conditions the pressure and temperature fluctuations in the ideal case will not affect the chemical potentials in question. — It remains, however, to obtain expressions for the influence of the particle velocity in the antinodes.

The displacements of the particles are considered to be reversible and thus the increase in the free energy of the substance is equal to the work done upon it. This quantity is here negative since work is done on the surroundings of the oscillating particles. If x is the displacement and ϱ is the density we can write for 1 cm^3 of the substance in the antinode

$$dF = \varrho \cdot \frac{\partial^2 x}{\partial t^2} \cdot dx = \varrho \cdot \frac{\partial^2 x}{\partial t^2} \cdot \frac{\partial x}{\partial t} \cdot dt \tag{6}$$

The acoustic displacements are written under the form

$$x = A \cdot \sin\ (\omega \cdot t) \tag{7}$$

where A is the amplitude. Accordingly we obtain the derivatives

$$\frac{\partial x}{\partial t} = A\omega \cos(\omega t) \text{ and } \frac{\partial^2 x}{\partial t^2} = -A\omega^2 \sin(\omega t) \tag{8}$$

Insertion of these expressions gives

$$dF = -\frac{1}{2} \cdot \varrho \cdot A^2 \cdot \omega^3 \cdot \sin(2\omega t) \cdot dt \tag{9}$$

which on integration yields

$$\Delta F = \frac{1}{4} \cdot \varrho \cdot A^2 \cdot \omega^2 \cdot (\cos(2\omega t) - 1) \tag{10}$$

The time mean value of the increase in free energy can then easily be shown to be

$$\Delta F = -\frac{1}{4} \cdot \varrho \cdot A^2 \cdot \omega^2 \tag{11}$$

the absolute value of which thus is seen to be equal to either the kinetic or the potential part of the mean energy density in the acoustic field.

The parallelism between this "acoustic potential" and the centrifugal potential may be pointed out for the sake of clarity. If a particle is transferred from any position where it is at rest to some position in a centrifugal field the increase in the free energy is also negative as in this case [10]. As a matter of fact the movement in the acoustic field constitutes the one dimensional projection of the two dimensional movement in the centrifugal field.

The free energy increase *per cm* ^{3} is divided between the two components in the quantities

$$\Delta F_1 = -\frac{1}{4} \cdot c_{1a} \cdot M_1 \cdot \omega^2 \cdot A^2 \text{ and } \Delta F_2 = -\frac{1}{4} \cdot c_{2a} \cdot M_2 \cdot \omega^2 \cdot A^2 \tag{12}$$

where c stands for the concentration in moles/cm^3.

The acoustic intensity, J, can be expressed by $J = \frac{1}{2} \cdot \varrho \cdot \omega^2 \cdot A^2 \cdot v$, where v is the sound velocity. (The formula is strictly valid for progressive waves. In a stationary field, however, the concept of intensity seems to be unclear. In this work it is rather convenient to define J also for the resonance field according to the given progressive field relation.) Utilizing this it is possible to write the free energy increase *per mole* as

$$\Delta F_1 = -\frac{1}{2} \cdot \frac{J}{v} \cdot \frac{M_1}{\varrho} \text{ and } \Delta F_2 = -\frac{1}{2} \cdot \frac{J}{v} \cdot \frac{M_2}{\varrho} \tag{13}$$

The appropriate chemical potentials are now computed to be

$$\begin{aligned}
\mu_{2n} &= \mu_2^0 + RT \ln c_{1a} \\
\mu_{1a} &= \mu_1^0 + RT \ln c_{1a} - \frac{1}{2} \cdot \frac{J}{v} \cdot \frac{M_1}{\varrho} \\
\mu_{2a} &= \mu_2^0 + RT \ln c_{2a} - \frac{1}{2} \cdot \frac{J}{v} \cdot \frac{M_2}{\varrho} \\
\mu_{1n} &= \mu_1^0 + RT \ln c_{1n}
\end{aligned} \tag{14}$$

Applying the equilibrium condition that the free energy change should be zero (4), gives as a result

$$RT \ln \frac{(c_{1n}) q \cdot (c_{2a})}{(c_{1a}) q \cdot (c_{2n})} = \frac{1}{2} \cdot \frac{J}{v} \cdot \frac{M_2}{\varrho} (1 - \varrho_1/\varrho_2) \tag{15}$$

Insertion of $\Delta c_2 = c_{2a} - c_{2n}$ and repeated approximations lead to the more convenient expression

$$\Delta c_2 = \frac{1}{2} \cdot \frac{J}{v} \cdot \frac{1}{RT} \cdot \frac{M_2}{\varrho} \cdot \frac{c_1 c_2}{c_1 + q^2 \cdot c_2} \cdot (1 - \frac{\varrho_1}{\varrho_2}) \tag{16}$$

As a practical example we may consider an aqueous glycerol solution with 50 vol% glycerol at the (rather high) acoustic intensity of 10 W/cm^2. Insertion of the numerical values gives for this case

$$\Delta c_2 = 3 \cdot 10^{-10} \text{ or } \frac{\Delta c_2}{c_2} = 5 \cdot 10^{-8}$$

This separation corresponds to a change in the refractive index of $5 \cdot 10^{-9}$, which is too small to be observed by the present "Schlieren" methods (the resolution of which may be put equal to $5 \cdot 10^{-7}$). In passing it can be noted that the value for the relative *temporary* separation in a case comparative to the above example is computed to be of the order of $5 \cdot 10^{-12}$ by means of Herzfeld's formula [7].

The approximate treatment performed above has led to small values for the separation too small to be detected by experiments *e. g.* of the kind described by Frei and Schiffer [5] and Ernst [6]. The computed effects are, however, not of an infinitesimally small magnitude.

Here it has to be remembered that there often is a very poor accordance between ultrasonic parameters as determined by experiments and calculated with the aid of the present theories. For example the experimental absorption coefficients are in some cases higher than the "theoretical values" by a factor of 1 000. These circumstances clearly indicate that in an ultrasonic field processes might occur about which at present little is known. Therefore it still seemed worth while trying some experimental approaches to this problem in

spite of the negative result of the calculation. This especially as the published results referred to above constitute a rather strong support to the idea of a separation.

EXPERIMENTAL DETERMINATIONS

The direct way of measuring any concentration changes set up in the ultrasonic field is to take out thin layers of the liquid column in the stationary ultrasonic field and analyse them. This could be done by means of a special technique: the separation cell was equipped with a small pile of filter papers; after the ultrasonic treatment the papers were separated and analysed.

Necessary for this to work well is that there is a negligible absorption of sound in the filter paper pile. In separate experiments this was demonstrated to be true; *e. g.* a pile of 20 papers in water showed a value for the transmission of 91 $\pm$ 2 % at 700 kc/s, 1.5 W/cm^2, 25 °C and a dry weight of 8.15 mg/cm^2 per paper. Since in the experiments reported here the pile consisted of 5—6 papers it is realized that absorption and reflection in the pile may not have caused any influence on the results.

The general experimental arrangements were the same as described in [4]. The utilized separation cell is shown in Figure 1. The paper pile covers the region between the reflector node and the nearest antinode 1/4 λ from there. Thermostated oil is circulated not only around the cell but also in the reflector assembly in order to obtain as good a temperature control as possible within the essential part of the cell.

For the choice of the analytical procedure one has to consider the following main requirements. Since evaporation from the papers is the most important source of error the analytic method must be very rapid. It is also wished that the result will be received in terms of concentration and not as absolute amounts. The refractometric method could fulfill these requirements.

A special feature with the refractometric method was that it was possible to perform the measurements directly on the filter paper. — A strip was snipped off from the filter paper and placed between the prisms of the Abbe refractometer. The borderline between the dark and light fields in the ocular was of course not so sharp as in measurements on a proper liquid object. The error obtained in the described procedure, however, was found to be less than the error when one pressed some liquid from the paper and made the measurements on this liquid sample.

A typical experiment was conducted in the following way. Before the experiment the filter papers were soaked in the experiment solution and the whole put under the vacuum for 3—4 hours in order to remove the gas in the papers and in the solution. The proper number of degassed papers were then quickly placed in position on the reflector and the assembly inserted in the cell. The cell was filled with some more solution and mounted on its place on the frame above the piezo quartz. — After the ultrasonic treatment this procedure was repeated in the reverse order. The cell was inverted to permit the surplus solution to run away and then dismounted. After removal of any adhering surplus solution with a strip of filter paper the papers in the pile were taken away one by one by means of sharp tweezers and put into separate small weighing glasses with covers.

The refractometric analyses were performed in a special order and at a controlled speed in order to obtain mean values as free from non-statistical error as possible. (The analyses

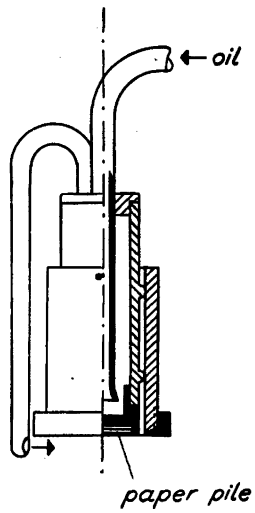


Figure 1. Separation cell with a thin acoustic window of stainless steel.

were made in the order paper No. 1, 2, 3 ... 3, 2, 1. Thus two strips were snipped off from each paper; on every strip 10 single readings were made.)

The experiments were performed on aqueous glycerol solutions, about 50 % glycerol, so as to fit with the experiments of Frei and Schiffer [6] referred to above; and on ferric nitrate solutions, 80 mg Fe^{+++}/ml, so as to compare with the electrochemical phenomena [3]. The ultrasonic treatments were performed at varying times and acoustic intensities, max. being 400 minutes at $\sim$ 3W/cm^2, most often 100 minutes at $\sim$ 7W/cm^2. These experiments were supplemented with quite analogous runs but with no sound.

No separation could, however, be detected. The experimental error amounted in the case of the glycerol solution to $\pm$ 0.0002 in the refractive index or $<$ 0.2 % in the concentration, and in the iron solution to $\pm$ 0.0005 in the refractive index or $<$ 2 % in the concentration.

DISCUSSION

From the above results one must conclude that the electrochemical phenomena are not caused by separation but must depend on some other influence on the electrode potential, as also could be inferred from a separate study on this [4]. The effects observed *e. g.* the production of ripples in electrolysis should namely, if separation were the cause, be connected with a high degree of separation, far above the experimental error in the direct concentration determinations.

Concerning the glycerol solutions we must confine ourselves to noting the experimental result that the separation, if real, must be very small and $<$ 0.2 % in these experiments. There is however still the possibility to be discussed that separation might occur to a smaller degree and be observed by Schlieren methods as has been reported [5,6]. The calculations, however, show

that the separation should be less than what is detectable even in this way. The reported results [5,6] therefore probably depends either on some unknown effect promoting separation or on structure changes in the liquid.

Contrary to a statement in the literature [6] it does not seem probable that separation by ultrasonic waves might constitute an alternative to the other methods for separation, *e.g.* thermal diffusion.*

SUMMARY

An approximate formula for the permanent separation is derived where is utilized the concept of an acoustic potential, which is partly analogous with the thermodynamic centrifugal potential. The separation effect as calculated with this formula is found to be less than what seems possible to detect by experiments. The performed experiments which were made in spite of the result of this calculation have given negative results.

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* Added in proof: The same conclusions are reported in a paper, which appeared after this paper was submitted to the Editor, Boyd, Ch., and Zentner, R. *Phys. Rev.* **83** (1951) 1059.

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A Study of Some Electrochemical Effects in a Field of Stationary Ultrasonic Waves

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An interesting electrochemical experiment with ultrasonic waves has been reported by Young and Kersten [1]. Electrolysis in a field of stationary ultrasonic waves was found to produce ripples on the cathode (which was arranged parallel with the direction of sound propagation). The distances between these ripples corresponded to half sound wave lengths. As an explanation of the phenomenon it was suggested that concentration differences are set up in the acoustic field. In connection with work on the possibility of a permanent separation in a field of stationary ultrasonic waves [2] a study was therefore undertaken of this and adjacent phenomenona.

EXPERIMENTAL DETAILS

Apparatus

The ultrasonic apparatus was a commercial unit, Crystalab Ultrasonorator Model SL 520, giving the frequencies 400, 700, 1 000 and 1 500 kc/s. In the experiments reported here only the frequencies 400 and 700 kc/s were used because of the need for rather long wavelengths.

In order to fit our purpose it was necessary to make a modification of the sound emitter unit. The crystal is here mounted in a comparatively small vessel which is placed above the frame of the Tesla transformer in its oil bath. The oil in the crystal vessel is thermostated by circulation through a cooling bath and a heater coil (max. pump velocity 7 liters/min). The fast rate of circulation permits a rapid temperature control quick in response.

The necessity of arranging the reaction vessel in a fixed and defined position has been pointed out in the literature [3,4]. In Fig. 1 is shown how this was attained in these experiments. By means of the three screws the ring was adjusted parallell with and at the desired distance from the quartz plate. The accuracy in the position and parallelism was not very high and amounted to some 0.01 mm. In the ring there are turned notches which fit with the separation cell and keep it in position. Since the separation cells were all of the same size this ring and frame could be used throughout in the experiments.

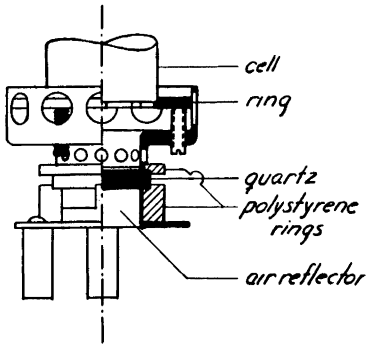


Fig. 1. Arrangement for keeping the separation cell in a fixed position.

In order to obtain a good stationary wave system it was necessary to know the sound velocity in the actual transformer oil so as to be able to compute an appropriate value for the position of the ring. The sound velocity in question was therefore determined, $v_{25°C} = 1\,416$ m/s, $dv/dt = -3.7$ m/s. · °C, by means of a simple and reliable method utilizing thermistors, as has been described elsewhere [5]. Now it has to be pointed out that the adjustment of the stationary wave system is not critical. Instead it is found that the states of non-resonance are very distinct and sensitive.

Acoustic window

The cells in which the separation were to take place will be described in a following section. However, concerning the two fundamental parts, the acoustic window and the reflector, some general remarks will be given here.

The reflectors were made with the non-resonant thickness of 1/4 λ, and furthermore the upper surface was in contact with air. The reflection is for this arrangement very near 100 %.

An acoustic window is made either as thin as possible or with the resonant thickness of 1/2 λ. The two alternatives show some differences in their properties.

A "thick acoustic window" permits a defined and stable design and under suitable conditions the sound transmission may be very high. Especially suited materials for the thick windows are polystyrene or plexiglass, which have rather low values for the acoustic resistance. At higher acoustic intensities, however, there are some complicating factors. The production of heat in the window will increase its temperature and thereby change the sound velocity in it. In this way the window more or less will come out of resonance with an accompanying lower transmittance. Furthermore the thickness of the window is a cause of poor heat transference from the reaction vessel and thus makes the temperature control more difficult.

Of these reasons the author has preferred to use thin metal windows in these experiments except for one special case.

DECREASE OF SOUND INTENSITY BY LIQUID STREAMING

Another very important problem in connection with these general experimental considerations deserves mention, especially as the phenomenon in question does not seem to have been observed before.

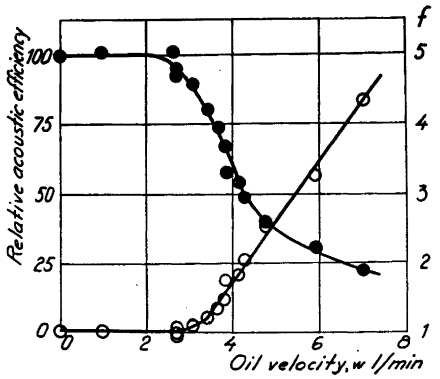


Fig. 2. Decrease of sound by liquid streaming. The points refer to the relative acoustic intensity; the circles to the factor $f = (E_S)_{W=0}/(E_S)_W$.

In order to secure good thermostating and to carry away gas bubbles formed by cavitation the experimental setup was usually so arranged that part of the circulating oil was sweeping through the interspace between the quartz and the window of the separation cell. It was not at first thought that this could disturb the sound transmission. However, during the course of the investigation it was realized that the liquid streaming had a very marked influence on the acoustic intensity as measured within the cell. The very interesting thing revealed by the diagram in Fig. 2 is that at high rates of oil circulation the sound in the cell is reduced to less than one quarter of the value for a stationary liquid. (The relative acoustic intensities have been determined by measurements of the sound pressure with the aid of a conical reflector suspended from a weighing balance.) This effect is somewhat surprising as there are very great differences between the sound velocity in the oil and the streaming velocities in question.

The phenomenon, however, probably depends on the fact that the circulating oil will carry with it part of the energy accumulated in the acoustic field. Preliminary considerations on this lead to the formula

$$f = 1 + w/c \cdot V \tag{1}$$

where f is the ratio between the maximum value for the integrated acoustic energy density at the streaming velocity $w = 0$ and the value at the actual velocity; V is the considered volume of the interspace between the quartz and the cell; and c is a constant which is closely connected with the coefficient of sound absorption.

This function is demonstrated in Fig. 2 where, as an alternative ordinate, f is given as a function of w. It is seen that above a certain value for the oil velocity the factor f is a linear function of w. The reason for the "induction period" which does not come out in the given theoretical relation, is to be found in the actual experimental conditions. The reflector by means of which the acoustic intensity was determined was of a less diameter, = 2.1 cm, than the acoustic field, the diameter of which was about 3.0 cm. The decrease in the energy density will set in at the side where the fresh liquid is coming. Under the actual experimental conditions therefore a certain threshold value for the oil velocity is necessary in order that the zone with a less energy density will come within the range of the reflector.

To avoid the influence of the phenomenon which has been described here a compromise solution had to be applied. The diameter of the separation cell was made a little less than

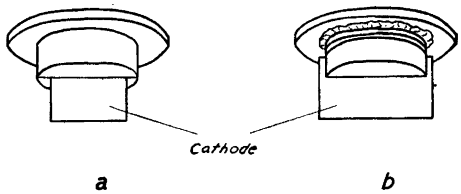


Fig. 3. a) The cathode is inserted in a slit in a massive and isolated metal reflector. b) The reflector consists of a rubber membrane in contact with air.

the acoustic field and the oil velocity was moderated as to give sufficient chilling and to carry away gas bubbles, however, without affecting the acoustic energy transmitted to the cell.

ELECTROCHEMICAL EXPERIMENTS

Ripples on electrolysis

Young and Kersten's experiments [1], in which ripples were obtained on electrolysis in an ultrasonic field, seemed worth further study in order to find out whether the ripples were caused by real concentration changes or could be explained as a pure electrochemical phenomenon.

It is of interest to know whether the preferred metal ion discharge does take place in the nodes or in the antinodes. For this purpose the arrangement described in Fig. 3 was utilized. Here the cathode is applied on the reflector in a fixed position. In a) there will be a node in the reflector plane and in b) an antinode. By measurements of the distances between the ripples and the reflector it was found that the preferred metal ion discharge took place in the antinodal planes which so far was in harmony with the separation observed in suspensions.

The first experiments on this were made with iron much in the same way as described by Young and Kersten. However, in this case the conditions did not seem quite defined depending on simultaneous hydrogen evolution. Such complications may come out also in the other formerly reported cases, so it was decided to perform some experiments under coulometer conditions. A copper coulometer electrolyte was chosen for this purpose ($CuSO_4 \cdot 5$ aq, 125 g/l; H_2SO_4 conc., 50 g/l; C_2H_5OH, 50 g/l; current density 0.02 A/cm^2; temperature $\sim$ 50° C). The cathodes were of polished steel or of copper. In these experiments there was no detectable gas evolution and distinct ripples were formed even at such low ultrasonic intensities as 0.1—0.2 W/cm^2. Furthermore it was observed that the ripples became visible simultaneously with the first appearance of the electrolytic discharge. (The sound and the electrolysis were started on the same time.) The relaxation time for the process leading to ripples thus seemed to be less than thirty seconds. — A separation, however, should occur by diffusion and convection and presumably

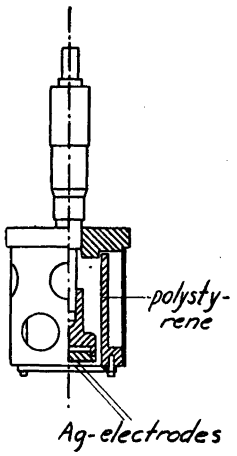


Fig. 4. Cell for measuring "ultrasonic emf:s". All metallic parts within the cell are covered with a film of polystyrene.

take a longer time. — When electrolyzing small quantities of copper on the stainless steel cathode it was also convincingly demonstrated that the ripples really corresponded to differing quantities of discharged material and not to variations in structure and appearance.

Emf measurements

It seemed reasonable that some study of electrode potentials in the ultrasonic field might give further information concerning the origin of the ripple formation.

Temporary changes in the electrode potentials with the same frequency as the applied sound have been studied by Nikitin [6]. Such variations have also been shown to occur at a hydrogen electrode, the cause, however, here being the rapid changes of state in the hydrogen gas [7].

Marinesco has observed a more *permanent* change of the electrode potential. He noticed that the emf of an element with one electrode subjected to ultrasonic waves changed so as to correspond to an increased ionization tendency of the ultrasonorated electrode [8]. On the other hand Schmid and Ehret reported that no change could be observed on the equilibrium potential of a copper electrode in 0.1 *M* $CuSO_4$ solution [3]. Their negative result may be due to that the conditions for forming stationary waves were not good in the utilized experimental arrangements; the sound was focussed by means of a kind of funnel on to the electrode.

The present investigation was chosen to study the influence on silver electrodes in silver nitrate solutions.

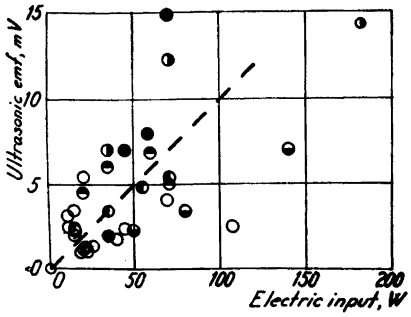


Fig. 5. "Ultrasonic emf:s" as a function of the electric input to the h.f.-generator. 400 kc/s; 0.1 M $AgNO_3$; and 25 °C.

For most of these experiments a cell was used, the principles of which appear in Fig. 4. The upper electrode is here attached to the reflector and is therefore situated in a node; the other electrode, which simultaneously functions as a thin acoustic window, is arranged 1/4 λ from there, *viz.* in the nearest antinode. Smaller adjustments in the acoustic distance between the quartz and the cell are obtained by varying the oil temperature and within the cell by altering the reflector position with the micrometer screw. It was hoped to match the acoustic properties of the system in this way in order to obtain as good stationary waves as possible and a maximum in the observed potential difference between the nodal and the antinodal electrode. However, this approach was not very successful. — Among the many factors, which make it difficult to obtain reproducible results, is to mention the heterogeneity in the acoustic field itself as emitted from the quartz and the fact that the geometry in the experimental apparatus was not ideal; optical parallelism was probably necessary. Difficulties of this kind are not easy to overcome even by a most careful design of the experiments as can be inferred from other partly analogous work on ultrasonic stationary waves where furthermore the need for quantitative results has been of first class importance [9,10].

The silver electrodes were cleaned with diluted nitric acid and rinsed in distilled water before use. The solutions were degassed in vacuum; the cell was also put under the vacuum after the filling in order to remove any air bubbles which might adhere to the electrodes. The emf was registerred by means of a 10 mV printing potentiometer (depending on the instability of the emf it was necessary to couple all the six measuring circuits of the used potentiometer in parallel, thus the instrument worked as a single function writer).

The results for some runs at 400 kc/s, 25° C and 0.1 *M* $AgNO_3$ are given in Fig. 5. Accurate values for the actual acoustic intensity within the cell are very difficult to obtain. Therefore in the diagram the electric input fed to the h.f. generator is given. However, from some single experiments in which the sound pressure within the cell was measured a conversion factor was computed; by multiplication with 0.03 the electric input is converted to give an approximate value for the acoustic efficiency in W/cm ^{2} within the cell.

Some probable reasons for the bad reproducibility which appears in the diagram have been mentioned above. From the diagram, however, it is

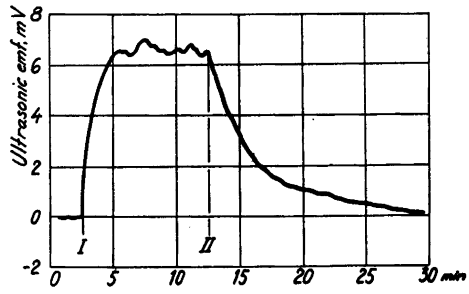


Fig. 6. "Ultrasonic emf" as a function of ime. I: start of sound; II: interruption of sound.

clearly seen that potential differences are set up, the value of which increase with increasing sound intensity. The electrode in the antinodal position is more positive which is in harmony with the results of the ripple experiments. The magnitude of the effect in these experiments is seen to be of the order of 3 ± 2 mV per 1 W/cm ^{2}.

Ultrasonic experiments are always complicated by the production of heat which accompanies sound absorption. Therefore it was necessary to learn if the resulting emf:s could be caused by thermal effects, depending on the temperature inequalities in the cell.

In separate experiments the temperature coefficient of the element in question was determined to be ~ -0.15 mV/°C (the warmer electrode is more negative). In order to obtain a value for the actual temperature differences that might occur, some experiments were performed with a special cell, provided with thermocouples, one in the $^1/_2 \lambda$ window of polystyrene and the other in the reflector. This cell was placed on a warm plate and the temperature difference and the emf of the cell were observed. The "apparent temperature coefficient" was in this way found to be ~ -0.03 mV/°C; thus $0.03/0.15 = 20$ % of the temperature difference between the thermocouples appeared between the electrodes and was measured as a "thermal emf". On the other hand the temperature differences which were observed on ultrasonic treatment at 20 W electrical input were of the order of some 0.1 °C, whereas the emf-values amounted to some mV. The "thermal emf" in this case may thus be ascertained to be less than 1 % of the actual value.

Kinetic study

As a possible way to find the cause of the ultrasonic emf:s a study of the kinetics of the electrode processes in question was undertaken.

In Fig. 6 a typical run is reproduced as it comes out on the recorder's strip chart. It is recognized that a suitable part for a kinetic analysis is the back trend to the initial value after the sound has been shut off. Now the question is whether this process is a back diffusion of ions between layers of different concentration, or a restoring of the normal conditions in the electrode double-layer. In the latter case the action of the sound is assumed to be some

influence restricted to the double-layer region itself. In order to decide between these two alternatives it is suitable to deduce theoretical expressions for the kinetics of the two cases.

The following treatment of the kinetics of the electrode reaction is performed in terms of the theory of absolute reaction rates [11]. — At the surface of the electrode two processes occur, the discharge process and the reverse reaction, the ionization process. When the rates of these processes are equal there is no net flow of current and the electrode potential has the equilibrium (static) value. In the case considered here the applied sound has caused the static value to change. After removal of the sound the potential will trend back to its original value. During this change there is a net flow of current between the electrode and the electrolyte but this electricity transport is restricted to this region and serves only to alter the charge density of the electrode capacitor.

The rate of the forward reaction, the discharge process, is given as

$$k_1 = a_+ \frac{kT}{h} \exp(-\Delta F_1^{\ddagger}/RT) \exp(\alpha VF/RT)$$

and for the ionization process

$$k_2 = \frac{kT}{h} \exp(-\Delta F_2^{\ddagger}/RT) \exp(-(1-\alpha)VF/RT$$

where a^+ is the activity of the metal ion in the bulk of the electrolyte, $\Delta F_1^{\ddagger}$ and $\Delta F_2^{\ddagger}$ the activation energies associated with the two processes, V the electrode potential (the sign of which is chosen in accordance with the Lewis & Randall convention) and α is the fraction of the electrode potential which will facilitate the forward reaction (a fuller account of this can be found in the literature [E.g.11].

If the sound has caused the electrode potential to be more positive, the resulting process after removal of the sound will be the discharge reaction, $k_1 - k_2 > 0$. The potential difference, referred to the normal equilibrium value, V_e, is represented by E, in this case $E\ (= V - V_e) > 0$. Furthermore the electric current, i amperes, from the solution to the electrode over 1 cm^2 of the electrode layer is for the case of univalent ions

$$i = F \cdot (k_1 - k_2)$$

where F stands for 1 Faraday.

The electrode layer may as usual be looked upon as a plate condensator with the capacitance C. Accordingly we may write

$$i = -\frac{\partial V}{\partial t} \cdot C \quad \text{and} \quad \frac{\partial V}{\partial t} = -\frac{F}{C} \cdot (k_1 - k_2)$$

Insertion of the appropriate expressions and rearranging results in

$$\frac{\partial E}{\partial t} = \frac{F}{C} \frac{kT}{h} [\exp(-FE/RT) - 1] \cdot [\exp(\alpha F(V_e + E) - FV_e - \Delta F_2^{\ddagger})/RT]$$

Under the conditions that $E \ll V_e$ the second exponential will vary much less with E than the first one, so we may put approximately

$$\frac{\partial E}{\partial t} \sim c \cdot [\exp(-FE/RT) - 1] \tag{2}$$

The value of the constant c is inferred from the more general formula. The solution to the simplified equation is

$$c \sim \frac{1}{t_2 - t_1} \left[E_1 - E_2 + \frac{RT}{F} \ln \frac{\exp(-FE_1/RT) - 1}{\exp(-FE_2/RT) - 1}\right] \tag{3}$$

In this case it is justified to use the approximation $e^x \sim 1 + x$, thus

$$c \sim \frac{0.059\ {}^{10}\log(E_1/E_2) + E_1 - E_2}{t_2 - t_1} \tag{4}$$

At small values for E it is realized that the function will turn into a true exponential one.

$$c \sim \frac{0.059\ {}^{10}\log\ E_1/E_2}{t_2 - t_1} \tag{5}$$

The kinetics of the other, above mentioned, alternative, where diffusion is assumed to be the rate-determining stage, is not possible to elucidate in a satisfying way because of the lack of knowledge of the primary concentration distribution. A very approximate treatment, however, yields the result

$$\frac{{}^{10}\log\ (E_1/E_2)}{t_2 - t_1} \sim \text{constant} \cdot D \tag{6}$$

where D is the diffusion coefficient of the dissolved salt.

The two cases yield approximately the same result (first order kinetics). In spite of this it is realized, however, that *e.g.* by changing the viscosity of the medium and thereby the diffusion coefficient it should be feasible to distinguish between the two treated alternatives.

Besides ultrasonic experiments on this were some other experiments performed for the sake of comparison. Two silver electrodes were arranged in a vessel with silver nitrate solution. (The electrodes had to be wholly immersed in the electrolyte and the connecting wires isolated in order to avoid the simultaneous contact between electrode, electrolyte and air). In the experiment the one electrode was placed in aq. dest. for some 10 minutes and thereafter put back in the electrode vessel. The emf of the cell was then registered in the same way as in the ultrasonic experiments. The kinetics of the emf change was found to be analogous to that which was observed in the ultrasonic experiments.

The mean values for the kinetic constant c that were obtained in these experiments (altogether 60 runs) are given in Table 1; all experiments were performed at 25° C. The values are seen to be of the same magnitude throughout, about 0.7, within the limits of experimental error. An important result

Table 1. Kinetic experiments.

	Electrolyte	Type of experiment	$c \cdot 10^4$
a	0.1 *M* $AgNO_3$	Ultrasonic treatment	0.57 ± 0.11
b	—»—	Immersing in aq. dest.	0.79 ± 0.10
c	1.0 *M* $AgNO_3$	Ultrasonic treatment	0.71 ± 0.08
d	—»—	Immersing in aq. dest.	0.63 ± 0.07
e	—»— with 50 % glycol	Ultrasonic treatment	0.80 ± 0.09
f	—»— » 50 » »	Immersing in the corresponding solvent	0.72 ± 0.13

is that the process does not seem to behave as to be diffusion controlled, which can be inferred from c) and e). The viscosities of these solutions differ by a factor of ~ 4 and therefore the diffusion coefficient D in e) should roughly be one quarter of that in c). The approximate accord between c) and e) therefore proves that the observed kinetics is not dependent on any diffusion process in the solution. This is in harmony with the result of the direct separation experiments reported in [2]. — In b), d) and f) the electrode after the treatment with the solvent carried with it a thin film of the pure solvent when it was immersed in the electrode vessel. Even in these experiments there seems to be no influence of diffusion, which, however, must depend on the rough experimental procedure.

Concerning the nature of the ultrasonic influence on the electrode potential it can be concluded that in the studied case the electrode layer after the ultrasonic treatment seems to be rebuilt in much the same way as after treatment with the pure solvent. It may also be added that the accord between a), b) and c), d) is not in harmony with the given theoretical treatment of the electrochemical alternative. The value of the kinetic constant should here be direct proportional to the silver ion activity. As this is not the case one must draw the conclusion that the rate-determining stage is one not involving silver ions but some other species, which are in about the same concentration in all the experiments.

The fact that the electrode potential in the node will be more positive and thus less noble may also be compared with the author's observation [12] that the oxidizing action of the ultrasonic waves is located to the nodal planes. (In the latter case cavitation is held responsible for the effect). However, it does not seem possible at present to draw any firmer conclusions concerning these highly complicated and uncertain phenomena.

The high experimental error was afterwards found to depend on an ageing effect. In Fig. 7 values are given for $^{10}\log c$ as a function of the logarithm of the

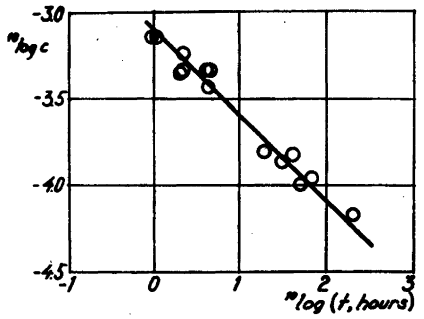


Fig. 7. The ageing effect.

time after the cleaning of the electrode with diluted nitric acid. The disturbance of the electrode layer was attained by immersing in distilled water. (This diagram constitutes also another proof that diffusion is not a rate factor in b), d) and f).) Because of the ageing effect, however, some experiments of the type a) and b) have been performed with the same electrode and at about the same time. The accord was now found to be better, within 10 % in single determinations.

SUMMARY

The preferred metal ion discharge in electrolysis in a field of stationary ultrasonic waves is found to take place in the antinodes. The potential of a silver electrode is changed so as to be less noble in the nodal position. A kinetic treatment of the phenomenon is performed.

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A Kinetic Study on the Decomposition of Propiolic Acid in Aqueous Solution

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It has long been known [1] that propiolic (propargylic) acid and its alkali salts decompose in water according to the following equations:

$$CH \vdots C \cdot COOH \rightarrow CH \vdots CH + CO_2 \quad (1)$$

$$2\ CH \vdots C \cdot COO^{-} + H_2O \rightarrow 2\ CH \vdots CH + CO_2 + CO_3^{--} \quad (2)$$

The purpose of the present work was to investigate the kinetics of these reactions, on which there is no information in the literature. The work is a continuation of a previous investigation, which concerned the thermal decomposition of acetylenedicarboxylic acid in aqueous solution [2].

EXPERIMENTAL

Preparation and properties of propiolic acid. — Propiolic acid was prepared by decomposing the potassium hydrogen salt of acetylenedicarboxylic acid [3]

$$HOOC \cdot C \vdots C \cdot COO^{-} \rightarrow CH \vdots C \cdot COO^{-} + CO_2,$$

obtained by the method given in Organic Syntheses [4] starting from fumaric acid. 50 g of the salt in 700 ml of water was kept on a boiling water bath and the evolution of the gas watched. After about 1 ½ hours, when the gas began to contain large amounts of acetylene, the flask was taken off the water bath. After cooling, the solution was acidified with sulphuric acid, saturated with ammonium sulphate and extracted about 20 times with ether. The ethereal solutions obtained from four such decompositions were united and dried with calcium chloride. The ether was distilled off and the residue fractionated at reduced pressure. At the third distillation a large (32 g) constant-boiling (b.p. 64.5°/15 mm) fraction was obtained. From this fraction the physical constants of the acid were determined, and it was also used in the kinetic investigations.

The sodium salt of propiolic acid was prepared by adding propiolic acid in slight excess [5] to a 5 per cent sodium ethylate solution. The separation of the sodium salt was

brought about by adding dry ether. The sodium salt, as colourless needles, was filtered off by suction and dried in a desiccator containing sulphuric acid and solid paraffin. This method of preparation was used, since the salt is decomposed by water and thus a product prepared in aqueous solution contains sodium carbonate. Sodium propiolate turns gradually reddish.

The physical properties of the purest propiolic acid obtained were: m.p. 17.6° (18° [6]); b.p. 64.5°/15 mm (62–63°/15.5 mm [6]); d_4^{25} 1.1308 (d_4^{25} 1.1325 [6], d_{25}^{25} 1.301 [3a]); refractive index n_D^{20} 1.43316, n_D^{25} 1.43100; molar refraction [R] 16.034 (calc. 15.588); surface tension (by double capillary method) γ^{25} 36.28 dyn/cm; parachor [P] 152.0 (calc. 158.4). To test the purity of the fraction for which the above data were obtained, a weighed amount of the acid was titrated with 0.0194 N $Ba(OH)_2$. Mean of four determinations: M = 70.20 (70.05 calculated from the formula). The acid is very hygroscopic. Propiolic acid is readily soluble in benzene, toluene, light petroleum, hexane, and carbon tetrachloride, and rather soluble in n-octane and carbon disulphide.

Method. The kinetic experiments on propiolic acid were carried out in Pyrex glass ampoules. A series of identical samples was quickly pipetted into the ampoules, which were sealed and placed in a thermostat. At appropriate times ampoules were taken from the bath, cooled quickly with cold water, opened, and, after removing the carbon dioxide and acetylene formed by the reaction (1) by suction, the contents were titrated with a 0.01 N baryta solution, cresol red being used as indicator. The rate constants were calculated by the first order formula in the form

$$k = \frac{2.303}{t} \log \frac{100}{100-p} \tag{3}$$

where p is the percentage change, obtained from the volumes of the standard base used at the times t and zero.

In the first experiments the initial concentration of the acid was 0.1 mole/l. However, this concentration is obviously too great, for as the reaction proceeded, the rate constants tended to decrease, which may, at least in part, be accounted for by a side reaction, since, especially at higher temperatures, the solution gradually became brownish. Therefore the later experiments were carried out with 10 ml samples of a 0.01 M solution. In these cases the above formula always gave a good constancy for k. The following run at 90.50° C. may serve as an illustration:

t mins.	570	690	840	1033	1258	1930	2190
p	20.5	24.2	28.5	33.8	40.2	54.0	58.6
$10^6 k$ sec.$^{-1}$	6.69	6.70	6.67	6.65	6.71	6.70	6.71

The experiments with the sodium salt were conducted by the following method. Into a 100 ml round-bottomed flask with a ground neck 100 ml of water saturated with acetylene was measured by pipette and the flask placed in a thermostat. After temperature equilibration, 0.4602 g or 0.05 moles of sodium propiolate weighed in a micro decanter glass was introduced into the flask and finally the micro decanter was dropped into the water. The gas formed in the reaction (2) was passed through an absorption apparatus containing 50 per cent potassium hydroxide solution, saturated with acetylene, for the absorption of the carbon dioxide. The gas leaving the absorption apparatus was acetylene. It was collected in a gas burette in a pneumatic trough. Water saturated with

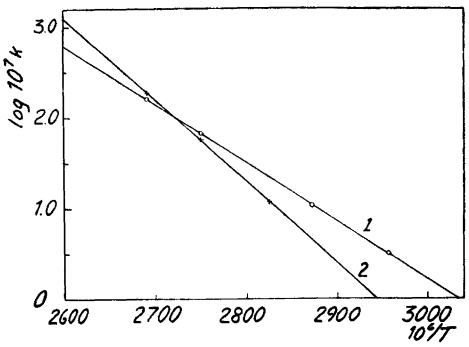


Fig. 1. Plot of log k *vs. 1/*T. *1. Propiolic acid. 2. Its sodium salt.*

sodium chloride and acetylene was used in the trough. At appropriate times the volume of the acetylene was measured and reduced to NPT. The percentage change was obtained from this gas volume and from the calculated volume corresponding to the completed reaction. The rate constants were calculated by the formula (3). To save space only the series in Table 1 will be given in detail.

Table 1. Decomposition of sodium propiolate in water. Initial concentration 0.05 mole/l. Temperature of thermostat 80.79° C. Volume of acetylene corresponding to the reaction when completed 112.07 ml. Barometric pressure b, temperature of the gas t.

Time mins.	b mm	t° C.	V ml	V_0 ml	p %	$10^6 k$ sec.$^{-1}$
1 725	752.0	24.8	14.8	13.0	11.6	1.19
2 820	753.0	24.8	24.2	21.3	19.0	1.24
3 840	758.8	24.2	31.0	27.6	24.6	1.23
4 665	768.3	22.4	36.2	32.7	29.2	1.23
5 685	769.5	23.8	41.8	37.8	33.7	1.21
6 675	770.6	23.0	44.6	40.6	36.2	1.12
7 275	770.5	23.0	46.8	42.5	38.0	1.09
10 995	770.4	21.0	66.0	60.6	54.1	1.18
12 480	760.1	28.8	70.8	63.6	56.7	1.12
					Mean	1.18

The water thermostats used could be held constant within about 0.02° C. To prevent evaporation of the water, its surface was covered with a layer of liquid paraffin. The thermometers were checked against a normal thermometer calibrated by the U.S.A. National Bureau of Standards.

RESULTS AND DISCUSSION

The results of the kinetic determinations are collected in Table 2. The linear relation between log k and $1/T$ to be expected from the Arrhenius equation $k = Ae^{-E/RT}$ was well obeyed (Fig. 1). The activation energy E and

the frequency factor A were calculated by the method of least squares. The values of the entropy of activation $\triangle S^*$, obtained by the formula

$$A = e \frac{kT}{h} e^{\triangle S^*/R}$$

are given for the temperature 90° C.

Table 2. Values of the kinetic quantities.

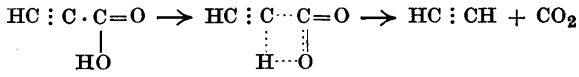
Reactant	10^7 k sec.$^{-1}$					E cal	A	$\triangle S^*$ E.U.
	65.00°	75.00°	80.79°	90.50°	98.38°			
CH ⋮ C · COOH	3.17	10.8		66.9	160	29 400	$2.98.10^{12}$	−3.8
CH ⋮ C · COONa			11.8	58.5	186	41 000	$2.58.10^{19}$	+27.8

A comparison of the values of the rate constants with those obtained for the decomposition of acetylenedicarboxylic acid and its sodium hydrogen salt [2] shows that the decomposition of propiolic acid and its sodium salt is much slower than these reactions, *e.g.*, the values of 10^7k for the four reactions for the temperature 75° C., calculated by the Arrhenius equation, are: 3 290, 2 510, 11.0, and 4.56 sec.$^{-1}$, respectively. Thus we can understand that propiolic acid can be profitably prepared by heating the sodium or potassium hydrogen salt of acetylenedicarboxylic acid in aqueous solution.

Although the values of k for the decomposition of propiolic acid and its sodium salt do not differ greatly, the activation energy and the frequency factor are very different in these two cases. The activation energy for the decomposition of the ion has the very high value of 41 000 calories, *i.e.* nearly 12 000 calories higher than that for the decomposition of the acid. It is very striking, too, that for the ion the frequency factor is 10^7 times greater than for the acid. These great differences unquestionably compel us to assume a different reaction mechanism in the two cases. The mechanisms proposed by Hall [7] for the decarboxylation of malonic acid and its acid ion, and assumed also for the decomposition of acetylenedicarboxylic acid and its acid ion [2], are obviously applicable in the present case, and, in fact, the evidence for them is now much greater than in those previous reactions.

For the decomposition of the free acid the frequency factor is $3 \cdot 10^{12}$, a value which is of a magnitude very usual in unimolecular reactions. The entropy of activation is slightly negative (−3.8 E.U.) and thus indicates that the transition state has a somewhat more rigid structure than the initial molecule; probably this is an indication of an incipient bond formation between

the hydrogen and carbon. Solvation also may influence the entropy of activation, in which case a negative entropy of activation means that the transition state is more solvated than the reactant molecule; however it is not very likely that the negative entropy of activation is here due to solvation, because it is almost certain that the transition state is not more polar than the reactant molecule. At any rate, solvation of the reactant molecules is necessary for the reaction, since the decarboxylation of propiolic acid does not occur in toluene. A 0.1 *M* solution of the acid in toluene was kept two weeks at 90° C., but no change could be observed, nor did the reaction proceed with a measurable speed at 119° C. (At 126.6° C. a slow change occurred, but probably represents another reaction.) However, the very great differences in the values of *E* and *A* between the decomposition of the acid and the ion show that ionization is not involved in the reaction. It must be assumed that the presence of the water molecules only loosens the requisite bonds of the molecule, thus making it capable of reacting. Thus the mechanism of the decarboxylation of propiolic acid is presumably



Transition state

The activation energy of the decarboxylation of $HOOC \cdot C \vdots C \cdot COOH$ (30 200 cal)[2] is about 800 calories greater than that of $HC \vdots C \cdot COOH$, and its frequency factor ($2.97 \cdot 10^{15}$) is greater by a factor of 1 000. By making the carbon atom on the other side of the triple bond more positive, the undissociated carboxyl group reduces the attraction (or increases the repulsion) between this carbon atom and the hydrogen atom of the adjacent carboxyl group and thus increases the energy of activation. The increase of the frequency factor may be accounted for in several ways. Both carboxyl groups of the molecule are equally capable of undergoing the reaction, the reactant is more polar and accordingly more solvated than the transition state, and the transition state may have a loose structure. All of these could tend to raise the frequency factor.

For the decomposition of the propiolate ion the mechanism seems undoubtedly to be

$$HC \vdots C \cdot COO^- \rightarrow HC \vdots C^- + CO_2 \qquad \text{(slow)}$$

followed by

$$HC \vdots C^- + H_2O \rightarrow HC \vdots CH + OH^- \qquad \text{(fast)}$$
$$OH^- + CO_2 \rightarrow HCO_3^- \qquad \text{(fast)}$$
$$2\ HCO_3^- \rightarrow H_2O + CO_3^{--} + CO_2 \qquad \text{(fast)}$$

The net result is reaction (2). Evidence for the first stage of this mechanism lies in the unusually high values of E and A; the later stages can be deduced on the ground of the reaction products. The decarboxylation must clearly be facilitated if the hydrogen atom is replaced by a substituent which is more strongly electron-attracting than hydrogen. The undissociated carboxyl is such a group, and thus $HOOC \cdot C : C \cdot COO^-$ should split off CO_2 more easily than does $HC : C \cdot COO^-$. This is in agreement with experiments. As was seen above, at 75° the former reaction is 550 times faster than the latter. For the latter the energy of activation is 41 000 cal, for the former 30 000 cal.[2] There are several reasons [2] for presuming that the reaction is $HOOC \cdot C : C \cdot COO^- \rightarrow HOOC \cdot C : C^- + CO_2$ and not $HOOC \cdot C : C \cdot COO^- \rightarrow HC : C \cdot COO^- + CO_2$.

The frequency factor is very large in both cases (for the acetylenedicarboxylate ion $A = 1.70 \cdot 10^{15}$), and accordingly the entropy of activation highly positive, 9 and 28 E.U., respectively. This indicates that in the transition state the bond which is to be broken must be greatly stretched, which is quite obvious if we think of the nature of the reaction. Especially in the case of the propiolate ion where energy of activation is so large the bond must be drastically stretched and the distribution of the charge far advanced before the critical state is reached. In addition, owing to the distribution of the charge, the transition state is less polar, and accordingly less solvated, than the reactant, the propiolate ion; this effect, too, will result in an increase of A and E.

According to the theory of absolute reaction rates, the specific rate of a chemical reaction can be expressed also by the equation

$$k = \frac{\mathbf{k}T}{\mathbf{h}} e^{-\triangle G^*/\mathbf{R}T}$$

where $\triangle G^* = \triangle H^* - T\triangle S^*$ is the standard free energy of activation. $\triangle H^* = E - \mathbf{R}T$ is the standard heat of activation. The values of $\triangle G^*$ for the two reactions considered here are close together and thus the velocities do not differ much. The entropies of activation are only slightly dependent on the temperature. For the decarboxylation of free propiolic acid the free energy of activation is nearly independent of the temperature, showing only a very small increase with temperature, whereas in the case of the propiolate ion it decreases rapidly with increasing temperature. The values for some temperatures are:

	$HC : C \cdot COOH$					$HC : C \cdot COO^-$			
t	25°	50°	75°	100°	t	25°	50°	75°	100°
$\triangle S^*$ E.U.	−3.4	−3.6	−3.8	−3.9	$\triangle S^*$ E.U.	+28.2	+28.1	+28.0	+27.9
$\triangle G^*$ kcal	29.8	29.9	30.0	30.1	$\triangle G^*$ kcal	32.0	31.3	30.6	29.9

At 94.5° C. $\triangle G^*$ is equal for both reactions; consequently, at this temperature they occur with equal velocities.

Propiolic acid is a strong organic acid, its dissociation constant being about $5 \cdot 10^{-3}$ (unpublished experiments), and its solutions contain, besides undissociated molecules, a remarkable concentration of ions. Thus *e.g.* at low temperatures, where the decomposition of the acid is faster than that of the ion, a greater initial concentration a or an addition of a strong acid should favour the reaction. This is really the case, as is shown by the following results at 75° C:

a_{Acid}	moles/liter	0.01	0.1	0.01	0.01	0.1
a_{HCl}	» »	0	0	0.01	0.02	0.1
$10^6 k$	$sec.^{-1}$	1.08	1.30	1.27	1.41	1.53

However, in these cases k has a tendency to decrease as the reaction proceeds (see above). It is also possible that HCl molecules are added to the triple bond [1a]. At any rate, these observations confirm the results presented and discussed above.

SUMMARY

The kinetic of the decomposition of propiolic acid and its sodium salt in water were investigated. The results are discussed.

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Über die Theorie der Selbstdiffusion in fluiden Stoffen und Mischungen

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EINLEITUNG ÜBER DAS ZWEIKOMPONENTENSYSTEM

Die Selbstdiffusion kann bekanntlich mittels isotopen Indizierungen in oft sehr guter Annäherung der Beobachtung zugänglich gemacht werden. Sie ist prinzipiell vom Mischungsverhältniss der »identischen» Komponenten unabhängig und bedeutet daher bei reinen Substanzen eine Materialkonstante. Gegenstand der vorliegenden Untersuchung ist in erster Linie das Dreikomponentensystem, das aus zwei »identischen» und einer »fremden» Komponente zusammengesetzt ist. Ehe wir auf die Theorie desselben eingehen scheint es zweckmässig, einige Grundtatsachen über das Zweikomponentensystem zu erörtern. Es wird zudem auf frühere Arbeiten über eine Verallgemeinerung der osmotischen Diffusionstheorie hingewiesen, siehe speziell Ref.[1], auch [2–6]. Die betreffende Theorie ist für Selbstdiffusion und für differenzielle Diffusion (d.i. Diffusion mit kleiner Konzentrationsdifferenz) in beliebigen Mischungsverhältnissen der Komponenten exakt gültig, wenn die letzteren im thermodynamischen Sinne unzweideutig sind und die Mischung mechanisch normal ist. (Die letztgenannte Bedingung ist für s.g. Quellungsdiffusion nicht erfüllt.) J. Stefan [7] dürfte als erster sich über eine symmetrische Behandlung der Diffusion von gelöster Substanz und Lösungsmittel geäussert haben, d.i. das Problem der »Rückdiffusion des Lösungsmittels». Zur Orientierung sei hervorgehoben, dass die Weiterführung dieser Gedanken eine Klärung der Frage der Unterscheidung von Diffusion und Konvektion herbeiführen musste, wenn diese gleichzeitig auftreten. Es geschah dies durch die Einführung der partiellen Molvolumina durch Onsager und Fuoss [8], vgl. die unabhängige Arbeit Salin's [9].

In dem Ausdruck für den Diffusionskoeffizienten

$$D_{12} = RTB_{12} \frac{n_1 n_2}{\varphi_{12}\ (n_1 + n_2)} \tag{1}$$

ist

$$B_{12} = 1 + \frac{\partial \ln f_1}{\partial \ln N_1} \equiv 1 + \frac{\partial \ln f_2}{\partial \ln N_2} \tag{2}$$

Für die Selbstdiffusion erhält man daraus, da B_{12} bei zweckmässiger Wahl des Molekulargewichtes gleich eins ist,

$$\varphi_{12} = \frac{RT}{D_{12}} \frac{n_1 n_2}{n_1 + n_2} \tag{3}$$

Hier sind n_1 und n_2 die Konzentrationen in Mol/cm³, N_1 und N_2 die Molenbrüche. Auf das letzte Konzentrationsmass beziehen sich die Aktivitätskoeffizienten f_1 und f_2, mit deren Hilfe der Faktor B_{12} ausgedrückt ist. φ_{12} ist der Reibungskoeffizient pro cm³ zwischen den Komponenten.

Die Wahl des richtigen Multipels des Grundmolekulargewichtes ist für die Theorie von prinzipieller Bedeutung. Allgemein geht aus Gleichung (2) hervor, dass $B_{12} = 1$ sobald der Aktivitätskoeffizient der einen (und damit auch der anderen) Komponente von dessen Molenbruch unabhängig ist. Dies gilt in irgend einer Lösung wenn die Konzentration, sagen wir N_2, sehr klein wird, bei entsprechender Wahl des Standardzustandes im Sinne Lewis. Um in der Diffusionsgleichung (1) in verdünnter Lösung $B_{12} = 1$ setzen zu können, muss also a) dieser Standardzustand der Komponente 2 praktisch erreicht sein * und b) das entsprechende — wirklich vorhandene — Molekulargewicht bei Berechnung von n_2 gewählt werden. (Wenn n_2 klein ist, wird Gleichung (1) von n_1 unabhängig).

Als einführendes Beispiel diene die Diffusion von O_2^{16} (Komponente 2) in H_2O^{16} bei gewöhnlichen Bedingungen von Temperatur und Druck. Trotzdem n_2 klein ist, wird die Dissoziation von O_2^{16} nicht beobachtbar sein, so dass $M_2 = 32$ zu setzen ist, damit $B_{12} = 1$. (Es ist nicht einzusehen, dass eine Einstellung des Austuschgleichgewichtes zwischen O_2^{16} und H_2O^{16} hierin eine Änderung bewirken sollte, eine Reaktion die allerdings im gewählten Beispiel sehr träge ist. Wir können von solchem Austausch zwischen i s o t o p e n-

* Es ist dies in qualitativem Sinne zu verstehen. Der besprochene Zustand ist ja ein gedachter, in welchem die Konzentration gleich eins ist. — Die Formulierung der Bedingung a) setzt stillschweigend einen assymptotischen Verlauf des Aktivitätskoeffizienten für $N_2 \to 0$ voraus.

gleichen Atomen im folgenden schon aus prinzipiellen Gründen völlig absehen).

Gilt dagegen etwa die Diffusion von O_2^{18} in O_2^{16} so muss bei rascher Einstellung des Austauschgleichgewichtes $M_2 = 18$ gesetzt werden, damit in verdünnter Lösung $B_{12} = 1$. O_{18} liegt sozusagen atomar vor, nämlich als $O^{18}O^{16}$, und die Assoziation mit O^{16} ist für die Wahl des Molekulargewichtsmultipels ebensowenig von Belang wie im ersten Beispiel eine etwaige Assoziation des O_2 zu $O_2 \cdot H_2O$ das Komponentenmolekulargewicht beeinflussen kann. Die Frage wird im nächsten Abschnitt an Beispielen durchgerechnet.

Bei mässiger Einstellungsgeschwindigkeit solcher Gleichgewichte muss die Diffusionstheorie Geschwindigkeitskonstanten der chemischen Reaktionen enthalten.

Da in der identischen Mischung der Diffusionskoeffizient von der Konzentration nicht abhängt, so gilt $B_{12} = 1$ in jedem Mischungsverhältniss, wenn die Molekulargewichte entsprechend festgestellt worden sind. In unschlüssigen Fällen ist es natürlich wichtig, die zur Berechnung von v, n_2 usw. verwendeten Molekulargewichte anzugeben.

Der thermodynamische Faktor. Zwei nur isotopenungleiche Doppelmoleküle mögen I_2' und I_2'' sein, entsprechend Komponente 1 und 2 der Mischung. Die Symbole seien zugleich Konzentrationen in Mol/cm³. Wir nehmen inneres Gleichgewicht an, haben also

$$I_2' + I_2'' \rightleftharpoons 2\ I'I'' \tag{4}$$

Die statistische Gleichgewichtskonstante ist 4. B_{12} und die Aktivitätskoeffizienten f_1, f_2 der Komponenten sollen berechnet werden. Die Aktivitätskoeffizienten der Molekelgattungen sind sehr nahe 1 * und werden durchwegs gleich eins gesetzt. Die Aktivität der Komponente 2 kann dann gemäss Gleichung (5) mittels der Konzentration I_2'' ausgedrückt werden; die einzige aber sehr wesentliche Bedingung ist, dass die Molekulargewichte der Komponenten dadurch als die doppelten I-Gewichte festgelegt worden sind. Wir schreiben:

$$a_2 = f_2 N_2 = \text{Konst. } I_2'' \tag{5}$$

Das Massenwirkungsgesetz gibt

$$I_2'' = \frac{n_2^2}{n_1 + n_2} = \frac{N_2^2}{v} \tag{6}$$

* Bekanntlich machen einfache Verbindungen der ersten Plejade (HDT) hierin eine Ausnahme.

und also

$$a_2 = \frac{\text{Konst.}}{v} \cdot N_2^2 \; ; \; f_2 = \frac{\text{Konst.}}{v} \cdot N_2 \tag{7}$$

und, gemäss der Definition, Gleichung (2)

$$B_{12} = 2 \tag{8}$$

Wir können dies dahin erweitern, dass Dissoziation der Doppelmoleküle berücksichtigt wird, durch die Gleichungen $(I')^2 = \mathrm{K}\, I_2'$, $(I'')^2 = \mathrm{K}\, {}_2''I$ und dann zwangsweise $I' \cdot I'' = I'I''\ \mathrm{K}/2$. Die Komponentenaktivität kann nun ausser durch Gleichung (5) auch mittels Einfachmoleküle definiert werden. Der erste Weg führt wieder zu $f_2 = \text{Konst.}\, N_2$, der zweite zu $f_2 = \text{Konst.}$, und B_{12} wird 2 bezw. 1. Wir können also konstatieren, dass die betrachtete binäre Lösung (4) wenn das Molekulargewicht entsprechend den Doppelmolekülen gewählt wird, als nichtideal angesehen werden muss, da die Aktivität nicht proportional zu N_2 ist. Die Mischung muss einfachmolekular aufgefasst werden, damit letztgenannte Bedingung erfüllt sein soll. Es gilt dies auch wenn die Konzentration der Einfachmoleküle verschwindend klein ist. Entsprechend wird nur bei letztgenannter Auffassung $B_{12} = 1$.

Wir wollen nun auch den Fall besprechen, dass die Gleichgewichtskonstante des Ausdrucks (4) von dem identisch-statistischen Wert 4 auf 4 $(1 + \delta)$ erweitert wird. Damit wird die chemische Ungleichheit der isotopindizierten Moleküle berücksichtigt, jedoch unter Beibehaltung der Annahme dass die Aktivitätskoeffizienten der drei Molekelgattungen gleich eins gesetzt werden können.

Bei kleinem δ lassen sich leicht durch einmalige succesive Approximation die folgenden Ausdrücke herleiten. Die Molekulargewichte wurden entsprechend den Doppelmolekülen gewählt:

$$f_2 = \text{Konst.}\ N_2\ (1 - \delta\ N_1^2) \tag{9}$$

$$B_{12} = 2(1 + \delta\ N_1 N_2) \tag{10}$$

Da durch Permutierung auch f_1 erhalten wird so ist leicht zu sehen, dass der Gibbs-Duhem'schen Beziehung $N_1\ \mathrm{d}\ \ln f_1 + N_2\ \mathrm{d}\ \ln f_2 = 0$ beim Verwenden des approximativen Ausdrucks (9) ebenfalls nur — ausser wenn $N_1 = N_2$ — approximativ genügt wird. Der prinzipiellen Bedeutung wegen wurde deshalb kontrolliert, dass bei Verwendung der exakten Gleichungen der Gibbs-Duhem'schen Beziehung genau genügt wird. — Mit der Ausnahme gewisser einfacher, im Wasserstoff indizierten Verbindungen, dürfte die Genauigkeit der Gleichung (10) für Diffusionszwecke ausreichen.

Über das Verfahren, die Aktivität durch eine ausgewählte Molekelart zu representieren, sei folgendes hinzugefügt. Bei der Konstruktion einer Anordnung zur reversiblen Überführung einer Komponente, würde man sich dieser Molekelart bedienen. Es ist deshalb natürlich, dass die Molekelart komponentenrein sein muss, also nicht Mischmoleküle gleich $I'I''$ ausmachen kann *.

DAS DREIKOMPONENTENSYSTEM

Theorie für drei Komponenten, wovon zwei identisch sind. Wir indizieren die drei Komponenten 1, 2 und 3 und lassen 1 und 2 identisch sein. Gelegentlich wird dies auch als ein zweikomponentiges System betrachtet. Für solche Fälle brauchen wir auch einen Index unter welchem 1 und 2 als eine Komponente gesammelt betrachtet werden kann, und wählen dafür 0. Unser Ausgangspunkt ist (vgl. Ref.[3]) die Differentialgleichung

$$\frac{\partial n_1}{\partial t} = -RT\,\frac{\partial}{\partial x}\left[\frac{n_1}{\bar{v}_{123}\,\Sigma\varphi\varphi}\left\{[\varphi_{23}(Y_2+Y_3)+\varphi_{31}\,Y_2]\,B_2\,\frac{\partial N_2}{\partial x}+\right.\right.$$
$$\left.\left.+\,[\varphi_{23}(Y_2+Y_3)+\varphi_{12}\,Y_3]\,B_3\,\frac{\partial N_3}{\partial x}\right\}\right] \qquad (11)$$

Hier sind t Zeit und x Abstandskoordinate in der Diffusionsrichtung, n_i sind Konzentrationen in Mol/cm³, N_i Molenbrüche, Y_i Volumenbrüche $(= n_i\bar{v}_i)$, $\bar{v}_i$ partielle Molvolumina und φ_{ij} Reibungskoeffizienten zwischen je zwei Komponenten, auf die im cm³ enthaltenen Substanzmengen der Komponenten i und j bezogen. Weiter ist $\bar{v}_{123}$ das Molvolumen ** der Mischung, definiert als

$$\bar{v}_{123} \equiv N_1\bar{v}_1 + N_2\bar{v}_2 + N_3\bar{v}_3 \equiv \frac{1}{n_1+n_2+n_3} \qquad (12)$$

Gleichung (11) enthält auch den Reibungsausdruck

$$\Sigma\varphi\varphi \equiv \varphi_{12}\varphi_{23} + \varphi_{23}\varphi_{31} + \varphi_{31}\varphi_{12} \qquad (13)$$

sowie die thermodynamischen Faktoren

$$B_j \equiv 1 + \frac{\partial \ln f_i'}{\partial \ln N_i} \qquad (14)$$

Die Aktivitätskoeffizienten f_i sind auf Molenbrüche bezogen.

---

* Es sollte allerdings möglich sein, als Komponenten $I'I''$ und z.B. I'' zu wählen, eine Alternative der aber kein reales Interesse zukommen dürfte.

** Bekanntlich ist das Gesamtvolumen $V = m_1\bar{v}_1 + m_2\bar{v}_2 + m_3\bar{v}_3$, wo m_i die Anzahl Mole sind. Lässt man diese in n_i übergehen, wird $V = 1$. Division mit $n_1 + n_2 + n_3$ gibt Gleichung (12).

Es gilt nun, Gleichung (11) zu vereinfachen. Die Versuche sollen so ausgeführt werden, dass n_3 in der Diffusionssäule vom Anfang an konstant ist. Da die Komponenten 1 und 2 identisch sind, kann auch während des Versuches kein Gradient in Komponente 3 entstehen, so dass u.a.

$$\frac{\partial n_3}{\partial t} = 0, \quad \frac{\partial N_3}{\partial x} = 0 \tag{15}$$

Weiter ist $B_1 = B_2 = 1$, da bei konstantem N_3 f_1 bezw. f_2 von N_1 bezw. N_2 unabhängig ist *. Gleichung (11) wird dadurch:

$$\frac{\partial n_1}{\partial t} = - RT \frac{\partial}{\partial x} \left[\frac{n_1}{\bar{v}_{123} \Sigma \varphi\varphi} \left[\varphi_{23}(Y_2 + Y_3) + \varphi_{31} Y_2 \right] \frac{\partial N_2}{\partial x} \right] \tag{16}$$

Ausserdem kann $\frac{\partial N_2}{\partial x}$ durch $- \frac{\partial N_1}{\partial x}$ ersetzt werden. Übrigens, da in unserem Falle $n_1 + n_2 + n_3$ konstant ist, gilt

$$\frac{\partial N_1}{\partial x} = \frac{\partial n_1}{\partial x} \frac{1}{n_1 + n_2 + n_3} = \bar{v}_{123} \frac{\partial n_1}{\partial x} \tag{17}$$

Aus (16) wird also

$$\frac{\partial n_1}{\partial t} = RT \frac{\partial}{\partial x} \left[\frac{n_1}{\Sigma \varphi\varphi} \left[\varphi_{23}(Y_2 + Y_3) + \varphi_{31} Y_2 \right] \frac{\partial n_1}{\partial x} \right] \tag{18}$$

Dies ist eine Wiener — Boltzmann'sche Differentialgleichung

$$\frac{\partial n_1}{\partial t} = \frac{\partial}{\partial x} \left(D_1 \frac{\partial n_1}{\partial x} \right) \tag{19}$$

mit dem Diffusionskoeffizienten

$$D_1 = RT \frac{n_1}{\Sigma \varphi\varphi} \left[\varphi_{23}(Y_2 + Y_3) + \varphi_{31} Y_2 \right] \tag{20}$$

Wegen der Identität der Komponenten 1 und 2 muss $D_1 \equiv D_2$ sein, was leicht durch Permutierung der Indecis zu kontrollieren ist. Aus Symmetriegründen führen wir deshalb die Bezeichnung D_0 für die Eigendiffusion ein. Aus statistischen Gründen gilt

$$\frac{\varphi_{31}}{\varphi_{23}} = \frac{n_1}{n_2} \tag{21}$$

* Für die Wahl des Molekulargewichtsmultipels gelten ganz ähnliche Betrachtungen wie für das Zweikomponentensystem. Zweckmässig ist, an dem Standardzustand festzuhalten, der dem sehr verdünnten Zustande entspricht.

Diese Gleichung, sowie $Y_1/Y_2 = n_1/n_2$, ermöglichen die weitere Vereinfachung

$$D_0 = RT \frac{n_1\varphi_{23}}{\Sigma\varphi\varphi} \equiv RT \frac{n_2\varphi_{31}}{\Sigma\varphi\varphi} \tag{22}$$

Für $n_3 \to 0$ wird dies zu 0/0; wird Gleichung (21) in die Reibungssumme, Gleichung (13), eingeführt, erhalten wir jedoch

$$D_0 = \frac{RT\ n_1n_2}{\varphi_{12}(n_1 + n_2) + \varphi_{31}n_2} \tag{23}$$

Wichtig ist zu sehen, ob in Gleichung (23) die Reibungskoeffizienten einzeln bestimmt werden können. Wenn wir 1 und 2 als *eine* Komponente betrachten, haben wir diese mit 0 indiziert. Ausser Gleichung (21) gilt also

$$\varphi_{31} + \varphi_{23} = \varphi_{03} \tag{24}$$

Durch Eliminierung von φ_{31} aus (23) wird schliesslich die symmetrische Gleichung erhalten:

$$D_0 = \frac{RT}{\varphi_{12}\left(\frac{1}{n_1} + \frac{1}{n_2}\right) + \varphi_{03}\frac{1}{n_1 + n_2}} \tag{25}$$

Durch differentielle Diffusionsmessung an dem System (0,3) bei der Konzentration n_3 (und also $n_0 = n_1 + n_2$) wird φ_{03} erhalten, vgl. Gleichung (1):

$$D_{03} = \frac{RT\ B_{03}}{\varphi_{03}\left(\frac{1}{n_0} + \frac{1}{n_3}\right)} \tag{26}$$

Endlich kann also auch φ_{12} aus (25) berechnet werden. Die Aufgabe, die in die Theorie eingehenden Reibungskoeffizienten zu bestimmen, ist also vollständig lösbar; siehe auch unten über molare Reibungen.

Elektrolyte erfordern spezielle Erwägungen. Man observiere nämlich, dass beim Verwenden von Gleichung (26) in solchem Falle elektrische Kräfte wirksam sind, die bei der Eigendiffusion gemäss Gleichung (25) nich auftreten.

In Anlehnung an Gleichung (3) schreiben wir auch für (25)

$$\varphi_{12} = \frac{n_1n_2}{n_1 + n_2}\left[\frac{RT}{D_0} - \frac{\varphi_{03}}{n_1 + n_2}\right] \tag{27}$$

Molare Reibungen. Jeder Reibungskoeffizient φ_{ij} (pro cm³) lässt sich durch Division mit der molaren Konzentration n_i in Reibung pro Mol, $\Phi_{i(j)}$, verwandeln. Diese bedeutet den Reibungskoeffizienten zwischen i und j in einem

Volumen, dass 1 Mol der Komponente i enthält. Gleichung (25) bezw. (26) kann also geschrieben werden

$$D_0 = \frac{RT}{\Phi_{1(2)} + \Phi_{2(1)} + \Phi_{0(3)}} \tag{28}$$

bezw.

$$D_{03} = \frac{RT\,B_{03}}{\Phi_{0(3)} + \Phi_{3(0)}} \equiv \frac{RT\,N_3\,B_{03}}{\Phi_{0(3)}} \tag{29}$$

Es gibt also die folgenden zwei Wege, die Selbstdiffusion in Abhängigkeit von der Konzentration einer fremden Komponente zu interpretieren: durch Angabe von D_0 oder $\Phi_{1(2)} + \Phi_{2(1)}$. Letztere Grösse ist also, wie D_0, von der Indizierungskonzentration unabhängig. Dagegen sind diese Φ-Werte einzeln genommen, wie auch φ_{12}, nicht nur von N_3 sondern auch von den Konzentrationen der identischen Komponenten abhängig. Diese Abhängigkeit ist durch Gleichung (27) gegeben, die sowohl φ_{12} gibt wie auch, nach Division mit n_1 bezw. n_2, auch $\Phi_{1(2)}$ und $\Phi_{2(1)}$.

Es ist möglich, aber völlig unbewiesen, dass Gleichung (28) auch bei Gegenwart von mehreren fremden Komponenten gilt, wobei $\Phi_{0(3)}$ als Summe zu betrachten wäre für die Reibung gegen alle fremden Komponenten, also durch $\Phi_{0(3)} + \Phi_{0(4)} \ldots$ zu ersetzen wäre.

Die charakteristische Reibungssumme. Die Grössen Φ haben wir molare Reibungen genannt, was nur eine kürzere Bezeichnung von Reibungskoeffizienten ist. Wenn nur zwei Komponenten diffundieren, wie z.B. in Gleichung (29) vorausgesetzt ist, könnte der zweite, eingeklammerte Index zum Wegfall kommen, entspechend der Praxis der früheren Arbeiten. Da die Summen $\Phi_{1(2)} + \Phi_{2(1)}$ bezw. $\Phi_{0(3)} + \Phi_{3(0)}$ für die Theorie typisch sind, schlage ich für deren Bezeichung den Namen charakteristische Reibungssummen vor. Letztere sind, wie die φ-Werte, symmetrische (gegenseitige) Eigenschaften, die mit $\Sigma\Phi_{ij} \equiv \Sigma\Phi_{ji}$ bezeichnet werden können. Eine solche Grösse representiert den Reibungskoeffizienten zwischen den Komponenten i und j in dem »charakteristischen Volumen» $\frac{1}{n_i} + \frac{1}{n_j}$ cm³. In dieser Bezeichnungsweise werden (28) und (29) also

$$D_0 = \frac{RT}{\Sigma\Phi_{12} + \Phi_{0(3)}} \equiv \frac{RT}{\Sigma\Phi_{12} + N_3\Sigma\Phi_{03}} \tag{30}$$

$$D_{03} = \frac{RT\,B_{03}}{\Sigma\Phi_{03}} \tag{31}$$

Allgemein gesehen, bringen die Reibungskoeffizienten — wie in der klassischen osmotischen Theorie — zum Ausdruck, dass die Diffusion als stationäre Bewegung aufgefasst werden kann, indem die Komponentengeschwindigkeit proportional dem Gradienten im chemischen Potential als treibende Kraft gesetzt worden ist. Dass eine Molekulartheorie der Erscheinung direkt an diesen Koeffizienten einsetzt scheint weder ausgeschlossen noch notwendig. Zur Zeit gehen die Diffusionstheorien der niedrigmolekularen Zustände meistens andere Wege, und die Ergebnissen — von Gasen und verdünnten Elektrolytlösungen abgesehen — sind, der grossen Schwierigkeiten wegen, im gewissen Sinne zu dimensionsmässigen Schätzungen begrenzt.

Da in gewissen Fällen eine Direktbestimmung von $\Phi_{0(3)}$ gemäss Gleichung (29) Schwierigkeiten bereitet, so fragt sich ob es möglich wäre, diese Grösse durch Messung der Selbstdiffusion des Mediums zu bestimmen. Es würde dies eine selbständige Theorie von $\Sigma\Phi_{12}$ in Gleichung (30) voraussetzen. Die erste, approximative Lösung erhält man u.a. durch den Ansatz

$$\varphi_{12} = Kn_1n_2 \tag{32}$$

wobei K von n_3 unabhängig sein soll und gemäss Gleichung (3) berechnet wird:

$$K = \frac{RT}{D_0^0\ (n_1 + n_2)^0} \tag{33}$$

Index ^{0} zeigt das reine Medium an ($n_3 = 0$) und $(n_1 + n_2)^0$ ist das invertierte Molvolumen v_0^0 des Mediums. Da $\Sigma\Phi_{12} = \varphi_{12}\left(\frac{1}{n_1} + \frac{1}{n_2}\right)$, wird

$$\Sigma\Phi_{12} \eqsim \frac{RT}{D_0^0}\ \frac{n_1 + n_2}{(n_1 + n_2)^0} \equiv \frac{RT}{D_0^0}\ n_0v_0^0 \tag{34}$$

Schliesslich wird gemäss Gleichung (30) die molare Reibung der gelösten Substanz

$$\Phi_{3(0)} \eqsim \frac{n_0}{n_3}\left(\frac{RT}{D_0} - \frac{RT}{D_0^0}\ n_0v_0^0\right) \tag{35}$$

(wobei eine grosse Genauigkeit in der Diffusionsmessung erforderlich ist). Die Verwendbarkeit der Gleichung ist durch Experimente zu entscheiden. Besonders nachteilig erscheint dass $\Phi_{3(0)}$ keinen anwendbaren Limeswert für $n_3 \to 0$ hat, ausser wenn die Komponenten identisch werden.

Drei identische Komponenten. Sobald Komponente 3 mit $1 \equiv 2$ identisch wird, muss auch $D_0 = D_{03}$ sein, so dass

$$\Sigma\Phi_{12} = N_0\Sigma\Phi_{03} \tag{36}$$

Dies bedeutet, dass die charakteristische Reibungssumme für die Selbstdiffusion in einem herausgemerkten Teile eines Mediums (entsprechend dem Molenbruch N_0) eben N_0 mal der für das ganze Medium geltenden charakteristischen Reibungssumme ist. — Dass die letzte Gleichung auch aus Gleichung (34) erhalten werden kann, ist leicht einzusehen.

Dissoziierende bezw. austauschende Systeme. Die Selbstdiffusion einer einfachen Salzschmelze wie NaCl ist davon abhängig, ob beim Na oder beim Cl indiziert wird. Analoge Fälle müssen auch bei nicht-elektrolytisch dissoziierenden Systemen beachtet werden, z.B. beim Jodwasserstoff, also überhaupt wenn die im Dissoziationsgleichgewicht befindlichen Teile ungleichartig sind, welch letztere Bedingung also hier zum Unterschied vom früher behandelten Beispiel /Gleichgewicht (4)/ neu hinzukommt. Dieselbe Komplikation ist bei rasch austauschenden Systemen wohl denkbar, wobei also keine für den Prozess massgebende Dissoziation vorzuliegen braucht. Was speziell das Wasser betrifft, so scheint es fraglich, ob ein messbarer Unterschied bei (ideal-gedachter) Indizierung am H bezw. am O vorliegt. Die Messungen und Diskussion von Wang [10] beschäftigen sich mit dieser Frage. Ein spezieller Diffusionsmechanismus etwa der Protone welcher von merklicher Bedeutung wäre, ist übrigens mit den elektrischen Leitfähigkeitseigenschaften des Wassers nicht vereinbar. Es muss vorläufig dahingestellt werden, ob es andere reine Flüssigkeiten gibt, bei denen Austausch und genügend freie Molekularrotation derartig zur Diffusion beiträgt, dass die Selbstdiffusion in dem betreffenden Sinne mehrdeutig wird.

Jedenfalls kommt bei dissoziierenden Substanzen die Theorie des Dreikomponentensystems wieder zur Anwendung. Nehmen wir als Beispiel das System Wasserstoff und Jod bei erhöhter Temperatur, also mit glatt eingestelltem Gleichgewicht $H_2 + J_2 = 2\ HJ$. Der oben besprochene Spezialfall mit zwei identischen Komponenten lässt sich auf die folgenden Systemen anwenden: H_2, H_2*, J_2 und J_2, J_2*, H_2. Die zwei Selbstdiffusionskoeffizienten des Jodwasserstoffes kommen als Spezialfall für molare Proportionen zwischen Wasserstoff und Jod hervor. Die letzte Bedingung, $n_1 + n_2 = n_3$, kommt übrigens in den Gleichungen nicht zum Vorschein.

Oberflächlich gesehen, scheint es wohl möglich, einfache Schmelzelektrolyte in derselben Weise zu behandeln (z.B. nach dem Schema Na, Na*, Cl und Cl, Cl *, Na), obwohl die Ionen nicht eigentliche Komponenten sind. Ohne eine Spezialuntersuchung kann jedoch die Berechtigung dieses Verfahrens nicht beurteilt werden, da die Theorie ganz auf dem Komponentenbegriff basiert ist. Ohne Zuhilfenahme von Leitfähigkeitsdaten ist die Aufgabe, die Reibungskoeffizienten zu bestimmen, in solchen Fällen nicht vollständig lösbar.

Da der Hauptgegenstand dieser Untersuchung die Selbstdiffusion einer Substanz in Gegenwart einer Fremdkomponente (Zusatz) ist, sei zuletzt die Aufmerksamheit auf die Möglichkeit von Anomalien durch Austausch zwischen der Substanz und dem Zusatz gerichtet. Für eine Verbindung wie Wasser kann gesagt werden, das deren Selbstdiffusion bei Indizierung am H bezw. am O prinzipiell verschieden ausfallen muss, wenn Austauschgleichgewicht mit dem Zusatz nur in dem einen Falle vorliegt. Es wird auf solche Erscheinungen hier nur hingedeutet.

ZUSAMMENFASSUNG

Solche Fälle der Selbstdiffusion wurden behandelt, welche die Gleichungen der Diffusion des dreikomponentigen Systems zu fordern scheinen. Hierher gehört teils die Selbstdiffusion unkomplizierter Gase oder Flüssigkeiten bei Gegenwart einer fremden Komponente, teils die Selbstdiffusion dissoziierender, ev. auch austauschender reiner Stoffe. Es wurde gezeigt, von welcher Reibungsgrösse der Selbstdiffusionskoeffizient abhängt und die Bedingungen diskutiert, unter denen die experimentelle Bestimmung der in dieser Reibungsgrösse eingehenden Reibungskoeffizienten (durch Diffusionsmessung) möglich ist.

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Studies on the Absorption Spectrum of Leghemoglobin, especially of Leghemiglobin

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While examining in this laboratory the chemical nature of leghemoglobin, the chromoprotein present in root nodules, we tried to isolate it in the pure state and to investigate in detail its composition and properties. The pigment is easily removed even from whole excised nodules, if they are steeped in water for some time, but the removal is accomplished most rapidly if the nodules are first crushed. In this latter case other substances present in the nodules are brought to solution in quantities greater than if the whole nodules are soaked. Leghemoglobin can be purified to a great extent by ammonium sulphate precipitation. By this method we usually obtained a preparation of leghemiglobin whose iron content was $\sim$ 0.27 % of dry matter [1], and only once [2] did we obtain a preparation with 0.34 % Fe, *i.e.* the same iron content as in blood hemoglobin and myoglobin. Ellfolk and Virtanen [3,4] have electrophoretically divided the leghemiglobin preparation with 0.27 % Fe into two components, the faster one containing about 0.35 % Fe and the slower one about 0.17 % Fe. The molecular weight of the former is about 17 000 and it contains one hemin group. It is probably pure leghemiglobin. The molecular weight of the slower component is much greater and the said authors presume it to be an absorption compound of an iron-free protein and leghemiglobin.

In this paper we shall present results of determinations of absorption spectra of leghemoglobin. Most of the determinations were made with preparations which contained 0.27 % iron. Their purity, judged by their iron and hemin content, was about 80 % leghemoglobin, the remainder being hemin-free protein. Our determinations were made in 1948—1949 but we were unable to publish the results earlier, as the composition of the preparations was not defined. The results of Ellfolk and Virtanen now justify their presentation. These authors also state that the spectrum of the low-hemin (slower) compo-

nent with trivalent iron closely corresponds to that of the pure leghemiglobin if the absorption is calculated on the basis of the hemin content of the preparation. For pure leghemiglobin, the faster component, separated by electrophoresis, we determined the spectrum only at pH 7.10.

Kubo [5] determined the absorption maxima of unpurified hemoglobin isolated from the root nodules of soybean, using the oxy-, carboxy-, cyanide-, and fluoride compounds. Later, Keilin and Wang [6] determined the absorption between 630 and 490 mμ of a hemoglobin preparation also isolated from soybean. Calculated on the basis of its hemin content the preparation contained about 40 % hemoglobin. The determinations included both oxy- and carboxyhemoglobin. Using raw preparations of leghemoglobin from soybean Little and Burris [7] also determined the absorption of its different derivatives.

We have been particularly interested in the absorption spectrum of leghemiglobin since the chemical composition of globin and the manner of linkage of the hemin group to globin seem to affect more strongly the spectrum of the hemi-form.

EXPERIMENTAL

The chromoprotein brought into solution by the action of water on the nodules contained its iron chiefly in trivalent form. Thus in the first place it was possible to isolate leghemiglobin from the nodules. By passing carbon monoxide into the water suspension of nodules both before and during crushing, solutions were obtained which contained much carboxy-leghemoglobin together with leghemiglobin. Leghemiglobin has been present in large or small quantities in all preparations, which were made by adding sodium azide to the solution before crushing the nodules. Therefore we isolated chromoprotein for our spectral determinations without adding any oxidation-preventing substances, *i.e.* in the form of leghemiglobin. The preparation thus obtained was purified by repeated precipitations with ammonium sulphate. The leghemiglobin content of the preparations was determined by comparing their absorption values with the corresponding values for blood hemiglobin at the point of intersection of the extinction curves, at 523 and 519 mμ. Similarly a comparison was made between the absorption maxima of the pyridine hemochromogen of leghemoglobin preparations and of blood hemoglobin. Reduced leghemoglobin, CO- and NO-leghemoglobin were prepared from the purified leghemiglobin preparation. It was difficult to prepare the O_2-leghemoglobin compound, since oxidation of iron to the trivalent form took place to some extent while the reduced leghemoglobin took up O_2. Only once did we obtain an O_2-leghemoglobin compound of such high purity that no leghemiglobin was present when judged by the spectrum. This was when a leghemiglobin solution containing much hemin-free protein was allowed to stand in a refrigerator after addition of dithionite. This preparation contained only about 40 % leghemoglobin and 60 % hemin-free protein.

All the spectral determinations presented in this paper were made using the Beckman Quartz Spectrophotometer. The sample compartment of the apparatus was equipped with a thermospacer, hence the temperature of the solution under test could be maintained approximately constant. The slit of the spectrophotometer was very narrow for all determinations. The width of the spectral band was about 1 mμ within the wavelengths

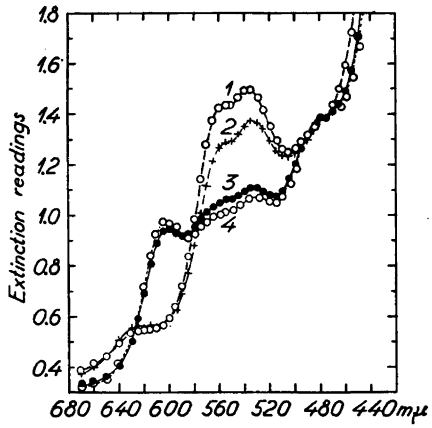


Fig. 1. Absorption spectra of crushed soybean nodules. Plants young, before flowering, nodules red. Determinations made about 1 hour after crushing the nodules. pH 6.5. 30—31 Aug., 1948.

1) 2.5 g nodules + 10 ml H_2O;
2) 2.5 » » + 10 » » + 0.4 mg $K_3Fe(CN)_6$;
3) 2.5 » » + 10 » » + 0.2 g NaF;
4) 2.5 » » + 10 » » + 0.2 » » + 0.4 mg $K_3Fe(CN)_6$.

of 600—450 mμ. The lengths of the absorption cells were each 1.00 cm. The extinction (density) values were determined usually at every 5th mμ, but near the absorption maxima as frequently as at every 2nd mμ. The curves were drawn to pass through every determination point. — All pH determinations were made immediately after the spectral determinations, using the glass electrode.

RESULTS AND DISCUSSION

Spectrum of leghemiglobin. Fig. 1 shows the spectrum of the water extract of the root nodules of soybean, both with and without potassium ferricyanide. The crushing and centrifugation were carried out rapidly. It appears from the spectrum that the chromoprotein brought into solution is almost entirely leghem*i*globin, since the potassium ferricyanide does not essentially affect the shape of the absorption curve. The spectrum also shows that the distinct maximum at 630 mμ, which is a characteristic of blood hem*i*globin, is faint for the leghem*i*globin solution isolated from the nodules and appears at about 620 mμ.

Addition of sodium fluoride to the water extract of crushed root nodules produces an absorption maximum at about 605 mμ thus showing the presence of leghem*i*globin. This maximum was formed regardless of whether or not ferricyanide was used as an oxidant (Fig. 1).

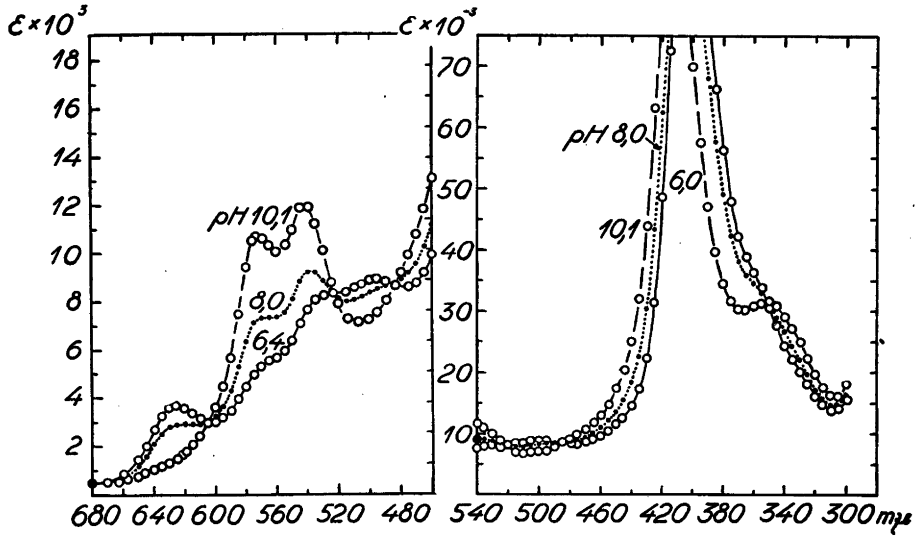


Fig. 2. Absorption spectra of purified leghemiglobin at pH 6.02, 6.40, 8.01, and 10.06, ionic strength in each solution 0.100. (Absorption curves practically the same at pH 6.02 and 6.4). Leghemiglobin concentration 0.66 mg/ml. Buffer: Na_2HPO_4-KH_2PO_4 (+NaCl) at pH 6.02—8.01; Na_2CO_3—$NaHCO_3$ (+NaCl) at pH 10.06, t = 22° C. The extinction curves intersect at 670, 605, 523, 484, about 410 (uncertain), and 352 mμ. 25 July, 1949.

The spectrum of the purified leghemiglobin preparation at different pH is shown in Fig. 2. For comparison, the spectrum of blood hemiglobin is presented in Fig. 3.

Purified leghemiglobin has a distinct maximum at 626 mμ. Hence, the diffuse band of the water extract of crushed nodules at about 620 mμ has

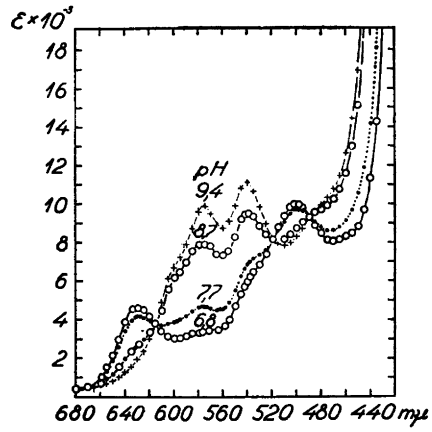


Fig. 3. Absorption spectra of blood hemiglobin (cow) at pH 6.41, 7.72, 8.70 and 9.43. O_2-hemoglobin oxidized with $K_3Fe(CN)_6$ with an amount not affecting the spectrum. Hemiglobin concentration 0.66 mg/ml. Buffer: Na_2HPO_4-KH_2PO_4 at pH 6.41—7.72, NaOH-glycine from pH 8.70 to 9.43. The extinction curves intersect at 670, 616, 519, 490, 414, and about 355 (uncertain) mμ. 22 Nov., 1948.

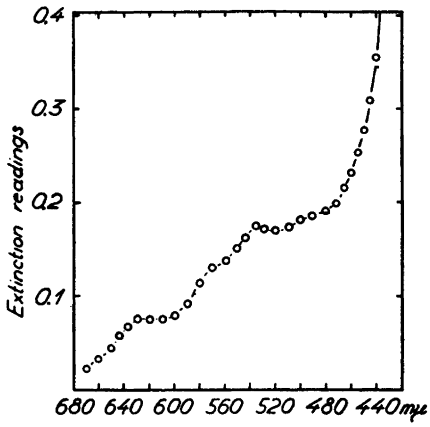


Fig. 4. Spectrum of electrophoretically purified leghemiglobin, faster component with I.P. 4.4, at pH 7.10 (Ellfolk and Virtanen). 5 May, 1950.

become denser and is shifted somewhat toward the red after the removal of foreign substances. In any case, the maximum, even with the purified leghemiglobin, is weaker than with blood hemiglobin.

Pure leghemiglobin isolated electrophoretically (the faster component with I.P. 4.4 and iron content 0.35 %) gave the spectrum illustrated by Fig. 4 according to the determinations of Ellfolk and Virtanen at pH 7.10. Due to an oversight no determinations were made with the preparation at other pH values.

The curve shows a great similarity between the spectrum of pure leghemiglobin and that of leghemiglobin of 80 % purity. First of all the curve confirms

Table 1. Intersection points of extinction curves of blood hemiglobin, leghemiglobin, and myohemiglobin determined at different pH values.

Authors	Source of hemiglobin	Concentration of the solution mg/ml	Intersection points of extinction curves * mμ
Austin and Drabkin [8]	blood	0.72	660 616 521
Hari [9]	»	?	616 518 487
Haurowitz [10]	»	0.19	619 519 490
Hicks and Holden [11]	»	?	418 358
Horecker [12]	»	2	672 617 521 488
Kubowitz [13]	»	?	680 621 521 490 414 355
Sternberg and Virtanen	»	0.96	670 616 519 490 ~ 414 ~ 355
Sternberg and Virtanen	root nodules	0.66	~670 605 523 484 ~ 410 ~ 352
Theorell and Ehrenberg [14]	muscle		626 523 495

* Calculated from published results or curves.

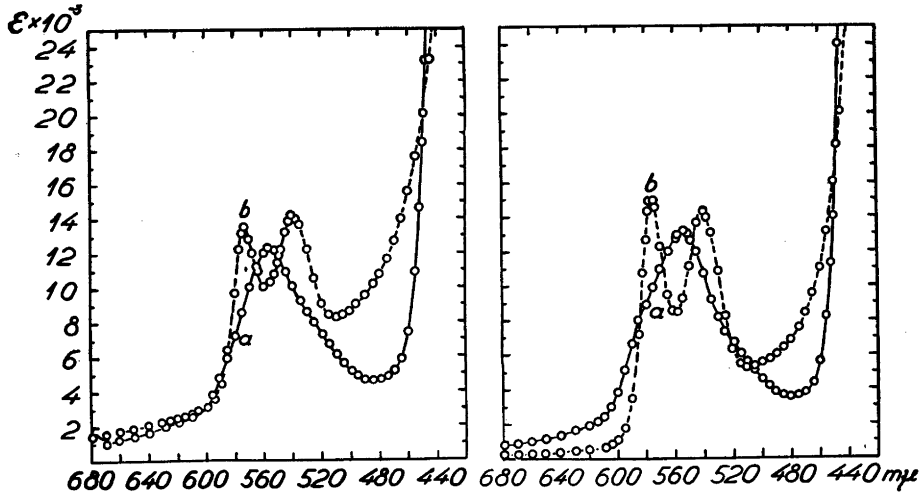


Fig. 5. Spectra of leghemoglobin (a) and legoxyhemoglobin (b), 0.65 mg/ml, 25 July. 1949 and 14 Oct., 1948.

Fig. 6. Spectra of bovine blood hemoglobin (a) and oxyhemoglobin (b), 0.65 mg/ml, 11 Nov., 1948.

the comparative weakness of the maximum at 626 mμ compared with the maximum of blood leghemiglobin at 630 mμ. The great dependence of the spectrums of hemiglobins on the pH of the solution makes it possible to compare the extinction curves of different hemiglobins only if they are determined at the same pH. Table 1 records the intersection points of the extinction curves of blood hemiglobin calculated from the values or curves of different investigators and the corresponding points for leghemiglobin on the basis of our own determinations.

Spectrum of leghemoglobin and legoxyhemoglobin. Leghemiglobin was reduced with sodium dithionite to leghemoglobin and the absorption curve determined (Fig. 5).

The purity of the legoxyhemoglobin preparation was as stated above and its spectrum is illustrated in Fig. 5. For comparison the spectra were also determined for blood oxyhemoglobin (cow) and for hemoglobin prepared from it (Fig. 6).

Spectrum of CO-leghemoglobin. Fig. 7 represents the spectrum of the carbon monoxide compound of leghemoglobin.

Spectrum of NO-leghemoglobin. Fig. 8 shows the spectra of the NO-compounds of leghemoglobin and blood hemoglobin.

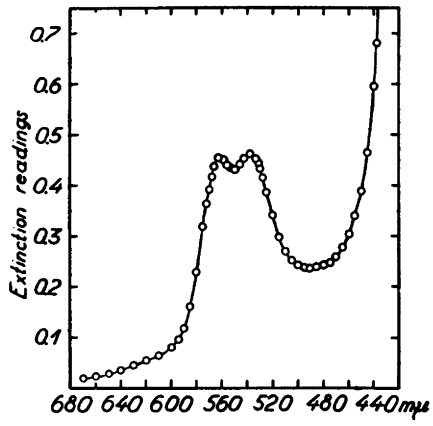


Fig. 7. Spectrum of CO-leghemoglobin, about 0.6 mg/ml, 2 Aug., 1949.

Spectrum of pyridine hemochromogen from leghemoglobin and blood hemoglobin. The curve in Fig. 9 shows the identity of the spectra of the pyridine hemochromogens prepared from both hemoglobins.

Spectrum of the green pigment. Virtanen [15] and Virtanen *et al.*[16] have found that leghemoglobin is changed to a green pigment when nitrogen fixation ceases. This pigment, legcholeglobin, easily gives biliverdin in acetic acid solution and has no marked absorption maxima between 600 and 420 mμ.

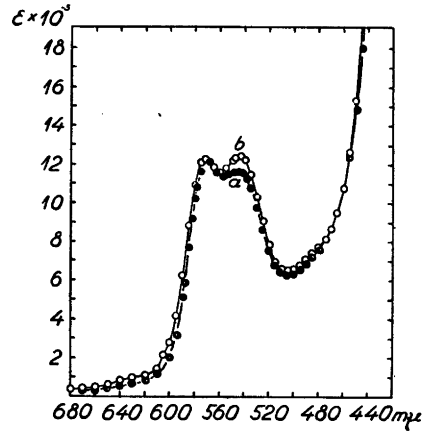


Fig. 8. Spectra of NO-leghemoglobin (a) and NO-hemoglobin of bovine blood (b), 0.65 mg/ml, 27 July, 1949 and 25 Nov., 1948.

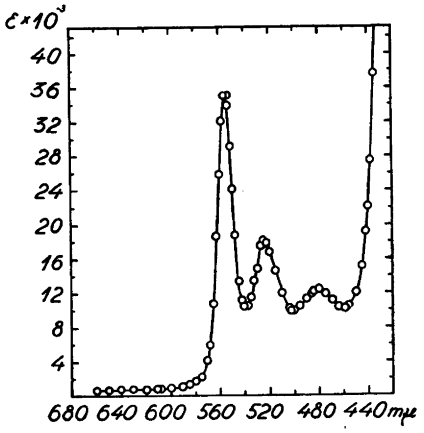


Fig. 9. Spectrum of pyridine hemochromogen from leghemoglobin (26 July, 1949) and blood hemoglobin (16 Nov., 1948). Concentration corresponds to 0.73 mg hemoglobin per ml.

Table 2.

Compound	Absorption maxima and minima of leghemoglobin and its compounds, mμ							Author
	Max.	Min.	Max.	Min.	Max.	Min.	Max.	
O_2-leghemoglobin	575		540					Kubo [5]
»	574		540					Keilin & Wang [6]
»	575		540					Little & Burris [7]
»	573	559	540	510				This paper
Leghemoglobin	555							Kubo
»	557							Keilin & Wang
»	555							Little & Burris
»	555	485						This paper
CO-leghemoglobin	570		535					Kubo
»	564		538					Keilin & Wang
»	565		539					Little & Burris
»	562	551	538	492				This paper
NO-leghemoglobin	570	557	545	504				This paper
Globin hemochrome	555		525					This paper
Pyridine leghemochrome	557		530					Kubo
» »	555	539	525	501	481	460		This paper
Leghem*i*globin, pH 7	625		563		530			Kubo
»	627							Little & Burris
» » 6.02	626	602			496	477		This paper
» » 7.10	625	610	570?		535	520		Ellfolk & Virtanen [3]
» » 8.01			570?		537	514		This paper
» » 10.06			572	560	541	508		This paper
CN-leghem*i*globin	540							Kubo
»	541							Little & Burris
» pH 6.48	540	504					417	This paper
F-leghem*i*globin	610							Kubo
»	605							Little & Burris
»	605							This paper

Table 2 summarizes the earlier determinations of the absorption maxima and minima of leghemoglobin compounds and also those determined in this work.

Equilibrium constant of acid and alkaline leghemiglobin. The pronounced change in the spectrum of hem*i*globin solutions which accompanies the pH-change is due to the amount of acid and alkaline hem*i*globin at different pH values. Spectrophotometric studies of Austin and Drabkin [8] with blood hem*i*globin have shown that at intermediate pH values (from 6.0 to 9.4) the conversion from the acid to the alkaline form closely conforms to the equation

$$\mathrm{pH} = \mathrm{p}K + \log \frac{Hi \text{ alkaline}}{Hi \text{ acid}} \qquad (1)$$

Using the equation

$$\frac{1 + 10^{pK-pH_1}}{1 + 10^{pK-pH_2}} = \frac{\Sigma |E_2 - E_0|}{\Sigma |E_1 - E_0|} \qquad (2)$$

where E_0 is the extinction value of the acid form, and E_1 and E_2 the extinction values at pH_1 and pH_2, all measured at the same wavelength, we obtain directly the weighted average of pK within the wavelength region examined (presuming that the limit of error is approximately the same at every E-value). $\Sigma|E_2-E_0|$ is the sum of all absolute E_2-E_0 values. Such E-values must be excluded, which measured at the same wavelength (near the intersection points of the curves), possibly give values of opposite signs for E_2-E_0 and E_1-E_0. Using the equation (2), the pK of leghemiglobin was calculated from the extinction values of the leghemiglobin solutions examined (99 values at 33 different wavelengths within 650—490 mμ) and the value 8.261 was obtained assuming that at pH 6.02 leghemiglobin is exclusively in acid form. This, however, is not exactly the case. According to equation (1) using the pK value 8.261 it can be calculated that alkaline leghemiglobin is present at different pH values in the following percentages: at pH 6.02 = 0.57 %, at pH 8.01 = 36.0 % and at pH 10.06 = 98.4 %.

Considering these values we obtain the corrected pK value 8.254 $\pm$ 0.04 which, in consequence, is the equilibrium constant for acid and alkaline leghemiglobin at 22° C and ionic strength of 0.100.

With a leghemiglobin preparation of lower purity (about 40 %) the pK value of 8.18 was found at an ionic strength of 0.050 and 13° C. By employing the equation introduced by Austin and Drabkin [8] and Coryell *et al.*[17] for the dependence of the pK value of blood hemiglobin on the ionic strength (μ): $\Delta\ pK = 0.59 \cdot \Delta \sqrt{\mu}$ we obtain 8.235 for the pK value of 40 % leghemiglobin at an ionic strength 0.100, *i.e.* practically the same as for 80 % leghemiglobin. The effect of temperature on the pK of hemiglobin is unknown.

For the hemiglobin of dog's blood Austin and Drabkin found pK = 8.12 $\pm$ 0.01 and for bovine hemiglobin Coryell *et al.* found pK = 8.07 $\pm$ 0.02, at 22—26° C both at the ionic strength 0.100. Theorell and Ehrenberg [14] have recently found for ferrimyoglobin (myohemiglobin) pK = 8.95 at ca 20° C and at ionic strength 0.10. The pK of leghemiglobin is much closer to the pK values of blood hemiglobin than to those of myohemiglobin. The dependence of the pK of leghemiglobin on the ionic strength is, according to our determinations, of the same order as that determined by Austin and Drabkin with blood hemiglobin and hence, much greater than that of myohemiglobin according to Theorell and Ehrenberg. It must be borne in

mind, however, that our determinations were made with leghemiglobin preparations of varying purity (80 % and 40 %) and at different temperatures.

The noticeable parallelism of the leghemoglobin content of root nodules with their nitrogen-fixing ability is apt to draw special attention to this pigment. Our knowledge of the structure of leghemoglobin has substantially increased of late. The protein component of leghemoglobin is very different from that of the hemoglobin of blood, as revealed by the great difference in the amino acid composition and isoelectric points. On the contrary, there is a great similarity between the absorption spectra. Pronounced differences have, however, been noted in the spectra of blood hemiglobin and leghemiglobin. Not only the absorption maxima but also the positions of the intersection points of the absorption curves where the absorption is independent of pH are partly different (Table 1).

The pK values of leghemiglobin and blood hemiglobin do not seem to differ much from each other. Thus leghemiglobin would noticeably differ from myohemiglobin. The linkages between iron and protein can be presumed to be different, though nothing definite can be said in this connection on the basis of our results. On the other hand, it is questionable whether the differences noted in the spectra of leghemiglobin and blood hemiglobin are entirely due to the different structure and molecular weight of the corresponding proteins or whether even then the different linkage of iron is in question. The identical spectra of pyridine hemochromogens of leghemoglobin and blood hemoglobin confirm the finding of Kubo that the hemins of leghemoglobin and blood hemoglobin are identical.

Another leghemoglobin compound whose spectrum differs considerably from the spectrum of the corresponding blood hemoglobin compound is carboxyleghemoglobin. The maxima of CO-leghemoglobin are at 562 and 538 mμ, the corresponding maxima of CO-hemoglobin of blood being, according to our estimations, at 569 and 539 mμ. The maxima of O_2-leghemoglobin and blood (bovine) O_2-hemoglobin are at 574, 540 mμ, and at 576, 540 mμ respectively. The distance of the α-maxima of O_2- and CO-leghemoglobin in Å (Span) is 110, and for blood (bovine) hemoglobin it is 80. Keilin and Wang [6] have found the value of 100 for the Span of a leghemoglobin preparation of about 40 % purity.

SUMMARY

Determinations of absorption spectra were made with purified leghemoglobin preparations, which, with the exception of the O_2-compound, contained about 20 % foreign protein. Particular attention was given to the absorption spectra of leghemiglobin at different pH values. The absorption curve of

leghem*i*globin differs noticeably from the corresponding absorption curves for blood hem*i*globin and myohem*i*globin. The pK value of acid and alkaline leghem*i*globin lies near the corresponding pK value for blood hem*i*globin but differs considerably from that for myohem*i*globin. The ionic strength, too, has a distinctly different effect on the pK values of leghem*i*globin and myohem*i*globin.

Since the amino acid composition of leghemoglobin is essentially different from that of blood hemoglobin and myohemoglobin the structural differences of globin may cause some differences in the absorption spectra. A different linkage of globin to the hemin group is also possible as suggested by the considerable differences noted in the pK values of leghem*i*globin and myohem*i*globin. Whether both or only one of these factors is concerned cannot be decided on the basis of our experiments.

Addition concerning the nomenclature. In this paper as well as in the previous ones from this laboratory the nomenclature suggested by Lemberg for hemoglobin and its derivatives has been used because of its brevity. However, differentiation of *o* and *i* by italics is very unwieldy and unclear. The nomenclature introduced by Pauling and Coryell, from which the valency of iron distinctly appears, is perhaps preferable (*e.g.* methemoglobin (Keilin), hem*i*globin (Lemberg), ferrihemoglobin (Pauling)).

A. I. Virtanen.

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Acid and Enzymic Hydrolysis of β-Glucosides. II

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In a recent investigation Guggenheim and Wiseman [1] have measured the velocity of inversion of sucrose by strong acids and have found the velocity to be strictly first order, the first order velocity constant k_1 being related to the molarity of strong acid c by the formula

$$k_1/\text{min}^{-1} = 6.95 \cdot 10^{-3} \cdot c \cdot 10^{Bj \cdot c},$$

where B_j is a constant determined by the anion. For hydrochloric acid they found $B_j = 0.28$. If the acid contains neutral uni-univalent salts the expression

$$k_1/\text{min}^{-1} = 6.95 \cdot 10^{-3} \cdot c_H \cdot 10^{\Sigma Bj \cdot cj},$$

where c_H denotes the molarity of strong acids and c_j the molarity of each anion, was found valid, each B_j having the same value as in the formula for single acids. The formula is in accordance with Brønsteds principle of specific interaction of ions [2].

Guggenheim and Wiseman compared their results with those of other workers, *e.g.* an investigation by Leininger and Kilpatrick [3], and found good agreement within the experimental accuracy.

Previously Veibel and Frederiksen [4] investigated the acid and the enzymic hydrolysis of β-alkylglucosides. They found the velocity constants dependent on the concentration of the acid and, using some values for the activity of hydrogen ions in hydrochloric acid of molarity ranging from 0.1 *M* to 5 *M* given by Duboux [5], they found the velocity constants proportional to the activity of hydrogen ions in the solution examined. They, too, compared their results with the above mentioned investigation [3]. The American authors found at 0° $k_{1.0\ M\ HCl}/k_{0.5\ M} = 2.79$ as compared with 2.75 calculated from Duboux' values of activity of hydrogen ions. This agreement was by Veibel and Frederiksen taken as a corroboration of the dependency of k on the activity of hydrogen ions.

It seems, however, more likely that the dependency of k on the molarity of the acid is governed by the principle of specific interaction of ions than by the activity of hydrogen ions in the solution. We therefore recalculated the results of Veibel and Frederiksen, making use of the formula proposed by Guggenheim and Wiseman. From experiments with 1.0 M and 0.5 M HCl we found at 25° $B_{j\mathrm{Cl}} = 0.26_2$, not very different from the value 0.28 given by Guggenheim and Wiseman at 24.7°. From Leininger and Kilpatricks experiments is found at 25° $B_{j\mathrm{Cl}} = 0.20_2$.

Most of the experiments of Veibel and Frederiksen are made at temperatures ranging from 60° to 90°, the total range of temperature investigated being from 0° to 90°. Leininger and Kilpatrick found B_j to be dependent on the temperature. They indicate $B_j = 0.2230$ at 10°, 0.1806 at 40°. We found it possible to express the dependency on the temperature by the formula $B_j = B_{jo} + \beta \cdot T$, T being the absolute temperature. For chlorine ion we find $B_{jo\mathrm{Cl}^-} = 0.710$ and $\beta = -0.0015$. From the figures given by Leininger nad Kilpatrick we calculate $B_{jo\mathrm{Cl}^-} = 0.619$ and $\beta = -0.0014$.

In a single experiment Veibel and Frederiksen examined the influence of the addition of an uni-univalent salt on the velocity constant. They compared the hydrolysis of dimethyl-ethyl-carbinol-β-D-glucoside at 40° with 0.5 N HCl and with a solution being at the same time 0.5 N in HCl and KCl. From the formula (1) given beneath we calculate $\log(k_{0.5N\ \mathrm{HCl} + 0.5N\ \mathrm{KCl}}/k_{0.5N\ \mathrm{HCl}}) = 0.12$ or $k_{0.5N\ \mathrm{HCl} + 0.5N\ \mathrm{KCl}}/k_{0.5N\ \mathrm{HCl}} = 1.32$. Experimentally was found $k_{0.5N\ \mathrm{HCl} + 0.5N\mathrm{KCl}} = 51.9 \cdot 10^{-4}$ and $k_{0.5N\ \mathrm{HCl}} = 39.6 \cdot 10^{-4}$. The relation between these two constants is 1.31 in excellent agreement with the calculated value 1.32.

Guggenheim and Wiseman found k_1 for 0.1 N HCl to be linear in the concentration of sucrose, the constants increasing some 5 % with an increase in sucrose concentration from 0.1 M to 0.2 M. Veibel and Frederiksen, on the other hand, found no significant dependency of k on the concentration of glucoside, and if a dependency could be found it was a slight decrease in k with an increase of glucoside concentration from 0.1 M to 0.2 M.

In table 1 we summarise all the experiments of Veibel and Frederiksen, calculated according to the formula

$$k_1 \cdot 10^4/\mathrm{min}^{-1} = a \cdot c_{\mathrm{HCl}} \cdot 10^{(0.710 - 0.0015T)c\mathrm{HCl}} \qquad (1)$$

In these experiments only alkyl-β-D-glucosides were examined. We have extended the investigation to phenol- and *o*-cresol-β-D-glucoside, and Table 1 summarises also the results of the new experiments.

The experimentally found velocity constants used in the calculations are underlined. For temperatures different from the temperature of the underlined experiment the k_1-values are calculated from the expression log (k_{T_1}/k_{T_2})

Table 1. Velocity constants for the acid hydrolysis of β-glucosides,

$$log\ k/min^{-1} = log\ a + log\ c_{HCl} + B_j \cdot c_{HCl}.$$
$$B_j = B_{jo} + \beta \cdot T;\ B_{jo} = 0.710,\ \beta = -0.0015.$$

Glucoside	log a	Q cal	60° $k \cdot 10^4$		70° $k \cdot 10^4$		80° $k \cdot 10^4$		90° $k \cdot 10^4$	
Methyl			exp	calc	exp	calc	exp	calc	exp	calc
1.0 *M* HCl	0.9852−4	32520	0.91	0.97	3.93	3.92	14.6	—	53.5	50.6
0.5 *M* HCl				0.38	1.51	1.57	6.01	5.95		
Propyl										
1.0 *M* HCl	0.1275−3	32520	1.20	1.35	5.27	5.45	20.3	—	69.0	70.3
0.5 *M* HCl					1.86	2.18	7.8	8.3		
Isopropyl										
1.0 *M* HCl	0.3502−3	31800	2.06	2.39	8.42	9.37	33.9	—	113.5	114.0
0.5 *M* HCl					3.09	3.74	13.1	13.8		
Diethyl-carbinol			50°		60°		70°		80°	
1.0 *M* HCl	0.5022−3	31800	0.74	0.80	3.08	3.40	13.4	13.3	48.1	—
0.5 *M* HCl							5.05	5.31	19.0	19.5
Trimethyl-carbinol			20°		30°		40°		50°	
1.0 *M* HCl	0.1750−3	30340	0.85	1.00	4.97	5.37	26.0	—	116.3	113.7
0.5 *M* HCl							9.5	9.9	42.8	44.0
Dimethyl-ethyl-carbinol			0°		20°		30°		40°	
1.0 *M* HCl	0.8081−3	30340	0.098	0.101	4.25	4.29	23.0	23.1	111.7	—
0.5 *M* HCl							8.1	8.6	39.6	42.4
Neopentyl			60°		70°		80°		90°	
1.0 *M* HCl	0.2333−3	32520	1.74	1.72	6.44	6.95	25.9	—	89.5	89.6
1.0 *M* HCl		30340		2.07		7.61		—		82.3
Phenol										
1.0 *M* HCl	0.8099−3	30340	7.51	7.81	27.2	28.7	97.7	—		
1.0 *M* HCl		32520		6.48		26.2		—		
0.5 *M* HCl		30340		3.07	12.4	11.5	40.7	39.7		
0.5 *M* HCl		32520				10.5		39.7		
			40°		60°		80°			
o-Cresol	0.7625−3									
1.0 *M* HCl		30340	0.36	0.40	6.59	7.00	87.6	—		
1.0 *M* HCl		32520		0.26		5.81		—		
0.5 *M* HCl		30340	0.13	0.15	2.63	2.75	36.7	35.6		
0.5 *M* HCl		32520		0.10		2.28		35.6		

$= Q(T_1-T_2)/2.303 \cdot R \cdot T_1 \cdot T_2$, the activation energy Q being calculated from two experiments with as great temperature difference as possible, *viz.* 40°. It is obvious from the table that it is not permissible to use the same value of Q over the whole range of temperature examined. Leininger and Kilpatrick [3] have for the inversion of sucrose determined the variation of Q with the temperature. They found Q decreasing with increasing temperature, $\Delta Q/\Delta T$ being approximately -70 cal pr. degree at $T = 283°$, decreasing with increasing temperature. Moelwyn-Hughes [6] found the larger value -94 cal pr. degree at $T = 307°$. The accuracy of the determinations of Veibel and Frederiksen not being sufficient to allow the calculation of the temperature dependency on Q we have used the average value for the temperature range examined, but the discrepancy between calculated and found values is considerably greater at the lower end of the temperature range than at the upper end, in agreement with the statement that the activation energy increases with decreasing temperature.

Leininger and Kilpatrick found the activation energy dependent on the concentration of hydrochloric acid, Q decreasing some 500 cal pr. unity of molarity in the range 0 M to 3 M hydrochloric acid. This means for activation energies about 30 000 cal that k will decrease some 30 % when 0.5 M HCl is used in stead of 1.0 M HCl. From Table 1 is seen that we too find an influence of the concentration of acid on k, but not so great as that stated by Leininger and Kilpatrick.

Table 2. Acid hydrolysis of some β-D-glucosides. HCl 1.0 N *if not otherwise stated.*

	$k \cdot 10^4/\text{min}^{-1}$				Q
	61.2°	60°	77°	80°	
Methyl-β-D-glucoside					
Kuhn and Sobotka [7]			4.6	(6.8)	
0.5 *N* HCl					
Moelwyn-Hughes [8]		1.0		18.2	33 370
Phenol-β-D-glucoside					
Kuhn and Sobotka [7]					
0.5 *N* HCl			24.3	(35.0)	
Scheiber [9]	9.4	(8.0)			
o-Cresol-β-D-glucoside					
Scheiber [9]	7.8	(6.7)			

(The values in brackets are calculated assuming the Q-values found in this investigation.)

The acid hydrolysis of some β-D-glucosides has been investigated previously by Kuhn and Sobotka [7], by Moelwyn-Hughes [8], and by Scheiber [9]. Table 2 summarises the results.

By comparison with Table 1 it is seen that the values found by Veibel and Frederiksen for methyl-glucoside and the values found by us for phenol- and *o*-cresol-glucoside agree tolerably well with the values found by other investigators. The accuracy obtained by Scheiber cannot be regarded as sufficient as there is no guarantee for the temperature being kept really constant (the only indication is: boiling chloroform) and, moreover, only two observations are used for the calculation of k.

More recently Heidt and Purves [10] have investigated the acid hydrolysis of some glycosides, among which were methyl-β-D-glucoside and phenol-β-D-glucoside. Using roughly 0.1 N HCl and temperatures from 55° to 96° the American authors found the velocity constant proportional to the concentration of hydrochloric acid, and calculated values for half life time at 60° in 0.05 N HCl were indicated. Besides, they indicate the activation energy and the constant B in the Arrhenius-equation. Table 3 gives their figures and the value of $k \cdot 10^4$ calculated from the half life time indicated. Using formula (1) we have from these data calculated $k \cdot 10^4$ in 0.5 N HCl at 60°.

Table 3. Heidt and Purves' values for acid hydrolysis of glucosides.

	Half life time 0.05 N HCl 60° min	$k \cdot 10^4$ 0.05 N HCl 60°	Q	B	$k \cdot 10^4$ 0.5 N HCl 60°
Methyl-β-D-glucoside	104 000	0.029	33 460	18.07	0.36
Phenol-β-D-glucoside	11 500	0.26	32 200	18.20	3.2

Both for methyl-glucoside and for phenol-glucoside the values calculated for 0.5 N HCl agree pretty well with the values found by us.

Veibel and Frederiksen have for the constant B in the Arrhenius equation calculated values approximately 16.0 for glucosides of primary and secondary alcohols and 17.5 for glucosides of tertiary alcohols. From Table 1 is seen that the corresponding Q-values are 32 520, 31 800, and 30 340 cal. respectively.

In Table 4 we have calculated the B-values for phenol- and for *o*-cresol-β-D-glucoside from the formula $\log k = \frac{-Q}{2.303\ RT} + B$, using for k values with the second as unit of time. It is seen that the average value of B is 15.2

for the arylglucosides, *i.e.* considerably lower than the values for alkylglucosides.

From the formula the influence of variation of Q and of B on k may be calculated. When B is kept constant a decrease in activation energy from 32 520 cal to 30 340 cal means an increase in k by a factor 25, and when Q is kept constant a decrease of B from 17.5 to 15 means a decrease of k by a factor 315.

Table 4. Values of k, Q, *and* B *for arylglucosides at different temperatures.* k *is calculated with the second as unit of time and with natural logarithms.*

Aglucon	1.0 *N* HCl				0.5 *N* HCl			Enzymic			
	t	$k \cdot 10^5$	*Q*	*B*	$k \cdot 10^5$	*Q*	*B*	*t*	$k_3 \cdot 10^3$	*Q*	*B*
Phenol 0.1 *M*	60°	2.88	29600	14.89	—	—	—	30°	2.44	11980	6.03
	70°	10.4	29600	14.88	4.76	29600	14.54	20°	1.24		
	80°	37.4	29600	14.90	15.6	29600	14.52				
				14.9			14.5				6.0
Phenol 0.05 *M*	40°	0.14	29600	14.82	—	—	—				
	60°	3.02	29600	14.91	1.34	29600	14.56				
	70°	10.8	29600	14.89	4.61	29600	14.52				
	80°	36.7	29600	14.89	15.4	29600	14.52				
				14.9			14.5				
o-Cresol 0.1 *M*	40°	0.13	30520	15.44	0.05	30520	15.02	30°	13.4	10790	5.92
	60°	2.53	30520	15.43	1.01	30520	15.03	20°	7.3		
	80°	33.6	30520	15.43	14.1	30520	15.05				
				15.4			15.0				5.9
o-Cresol 0.05 *M*	40°	0.13	30520	15.44	0.05	30520	15.03				
	60°	2.52	30520	15.43	1.01	30520	15.03				
	80°	33.9	30520	15.43	13.7	30520	15.04				
				15.4			15.0				

A comparison of Table 1 and Table 4 shows that the rapid hydrolysis of glucosides of tertiary alcohols is due to the combined effect of a relatively small activation energy and a great constant of action. For the arylglucosides examined Q has the same value as for glucosides of tertiary alcohols but the B-value is considerably lower than the B-value of glucosides of tertiary alcohols and even of primary and secondary alcohols. In Table 1 we have tentatively calculated the velocity constants for arylglucosides at different temperatures, assuming for Q first the value found for glucosides of tertiary alcohols and next the value found for glucosides of primary alcohols. It is seen that the agreement between calculated and found values is considerably better when

the value for glucosides of tertiary alcohols is used than with the value for glucosides of primary alcohols.

For neopentylglucoside the velocity of enzymic hydrolysis is of the same order of magnitude as for glucosides of tertiary alcohols, the velocity of acid hydrolysis of the same order of magnitude as for glucosides of primary and secondary alcohols. The B-value is for acid hydrolysis somewhat lower than the B-values for glucosides of primary and secondary alcohols. In Table 1 we have calculated the velocity constants at different temperatures, assuming first the Q-value of glucosides of primary alcohols and next the Q-value of glucosides of tertiary alcohols. It is seen that here the best agreement is obtained by assuming the Q-value of glucosides of primary alcohols whereas Veibel and Frederiksen for the enzymic hydrolysis of neopentylglucoside found a Q-value approximately the same as for glucosides of tertiary alcohols.

As mentioned above the agreement between the velocity constants found by Heidt and Purves and by us is tolerably good. Considering the Q- and B-values there is, however, disagreement, most pronounced for phenol-β-D-glucoside. The American authors have found for phenolglucoside a Q-value slightly lower than the Q-value for methylglucoside, the B-values being practically identical. We, on the other hand, find the Q-value for phenolglucoside considerably lower than the Q-value for methylglucoside and the B-value somewhat lower than the value of B for glucosides of primary alcohols. But the B-values found by Heidt and Purves are 18.07 and 18.20 whereas we find the values 16.0 and 15.2. From this difference in B we should expect the velocity constants found by Heidt and Purves to be 300—400 times higher than the constants found by us. We are not able to give an explanation to this discrepancy, the experimental evidence given by Heidt and Purves not allowing a recalculation of their Q- and B-values. The tolerance given by them for their values seems to exclude experimental error as an explanation.

From the velocity constants for the enzymic hydrolysis of phenol- and o-cresol-β-D-glucoside at 30° and at 20° we calculated the heat of activation and, with the same reservation as in the above mentioned investigation of the enzymic hydrolysis of alkylglucosides [4], the B-values in the Arrhenius-equation. Besides,we have determined the affinity constants K_m and K_{m2} for the compounds enzyme-phenol-β-D-glucoside and enzyme-phenol respectively so that the k_3-value for the hydrolysis of phenol-β-D-glucoside may be calculated and compared with the k_3-value for the hydrolysis of o-cresol-β-D-glucoside previously determined. The results are summarised in Table 5.

It is seen from Table 4 that by acid hydrolysis phenol-β-D-glucoside is hydrolysed somewhat faster than o-cresol-β-D-glucoside. By enzymic hydrolysis catalysed by almond emulsin o-cresol-β-D-glucoside is hydrolysed considerably

Table 5. Enzymic hydrolysis of aryl-β-D-glucosides. Sweet almond emulsin. Glucoside 0.0400 M. Sal.f. = 0.60. Phosphate-citrate buffer, pH 4.4.

		Phenolglucoside	o-Cresolglucoside
K_m 30°		0.10	0.013
K_{m_2} 30°		0.10	0.020
$10^2 \cdot k_{obs}/e$ (Sal.f.)	30°	43.6	756
	20°	22.6	414
$10^2 \cdot k_3$	30°	6.37	34.8
	20°	3.24	18.9
$k_{obs\ cresol}/k_{obs\ phenol}$	30°	17.3	
	20°	18.4	
$k_{3\ cresol}/k_{3\ phenol}$	30°	5.4	
	20°	5.8	

faster than phenol-β-D-glucoside, the k_3-value for o-cresol-β-D-glucoside being 5.5 times greater than the corresponding value for phenol-β-D-glucoside, whereas the factor for the k_{obs}-values is 18. The effect of the methyl group in o-position to the glucosidic linkage is consequently partly due to an increase in the affinity between enzyme and substrate and partly to an increase in the velocity of dissociation of the enzyme-substrate compound into enzyme, glucose, and aglucon.

Table 6. Acid hydrolysis.

Phenolglucoside 0.05 *M*. Hydrochloric acid 1.0 *N*. 80°

t min	*a*	*x*	*c*−*x*	$k \cdot 10^4$ *	*x'*	*v*	$k \cdot v$
0	−1.40	—	2.09	—	—	—	—
10	−0.99	0.41	1.68	94.9	0.196	0.0607	5.77
20	−0.66	0.74	1.35	95.0	0.354	0.0793	7.53
30	−0.38	1.02	1.07	96.9	0.488	0.0760	7.37
50	−0.02	1.38	0.71	93.8	0.660	0.0542	5.08
70	0.24	1.64	0.45	95.3	0.785	0.0308	2.94
100	0.47	1.87	0.22	97.8	0.895	0.0108	1.05
120	0.59	1.99	0.10	110.0	0.952	0.0030	0.33

mean value 97.7 $\qquad \frac{\Sigma\, k \cdot v}{\Sigma\, v} = 95.5$

* In this and the following tables k' means constants calculated from zero time to time of sample-taking, whereas k means constants calculated from point to point. x' is degree of hydrolysis.

EXPERIMENTAL

Substrate. Phenol-β-D-glucoside and *o*-cresol-β-D-glucoside were prepared by current methods and showed the physical constants reported in the litterature.

Enzyme. The β-glucosidase used in this investigation was prepared from sweet almonds by Dr. Shih-Lin Yang, see the following paper. The preparation had a Sal.f. 0.60, corresponding to a β-glucosidase value 2.00.

Methods. For the acid hydrolysis the technique indicated by Veibel and Frederiksen [4] was employed, for the enzymic hydrolysis that indicated by Veibel and Lillelund [11,12]. The following tables are examples from the laboratory journal. In experiments with acid hydrolysis the constants are calculated as indicated by Christiansen [13] who stresses the importance of giving more weight to the range 20—60 % reaction than to the 0—20 % and 60—100 % ranges, due to the greater reading accuracy obtainable in the preferred range.

Table 7. Acid hydrolysis.

o-Cresolglucoside 0.10 *M*. Hydrochloric acid 0.5 *N*. 40°.							
t min	*a*	*x*	*c*−*x*	$k \cdot 10^4$	*x'*	*v*	$k \cdot v$
0	−2.68	—	4.02	—	—	—	—
1440	−2.52	0.16	3.86	0.12_2	0.040	0.0162	0.00198
2880	−2.36	0.32	3.70	0.12_5	0.080	0.0305	0.00381
4320	−2.18	0.50	3.52	0.13_4	0.124	0.0443	0.00591
5760	−2.03	0.65	3.37	0.13_3	0.162	0.0563	0.00749
7200	−1.87	0.81	3.21	0.13_6	0.202	0.0622	0.00844
8640	−1.72	0.96	3.06	0.13_7	0.239	0.0686	0.00942
10080	−1.60	1.08	2.94	0.13_5	0.269	0.0726	0.00979

mean value 0.13_2 $\qquad \frac{\Sigma\, k \cdot v}{\Sigma\, v} = 0.13_4$

From the values given in Table 8 k_3 is calculated, using the expression

$$k_3 = k_{obs}\,(K_m + c + (K_m/K_{m1} + K_m/K_{m2} - 1)c_x/e(\text{Sal.f.}),$$

k_{obs} being the constant calculated from point to point, c_x the mean concentration of the products of hydrolysis in the points used for the calculation of k_{obs}, and K_m, K_{m1}, K_{m2} the dissociation constants of the compounds enzyme-*o*-cresolglucoside, enzyme-glucose, and enzyme-*o*-cresol respectively. These values are at pH 4.4 0.013, 0.20, and 0.020. The k_3-values found are at 30° $34.8 \cdot 10^{-2}$, at 20° $18.9 \cdot 10^{-2}$, $k_{3_{30}}/k_{3_{20}} = 1.84$. $k_{3_{30}}$ was previously (Veibel and Lillelund [7]) found to be $37.5 \cdot 10^{-2}$.

Table 8. Enzymic hydrolysis. 30° and 20°. o-Cresolglucoside 0.0394 M. *Phosphate-citrate buffer pH 4.4. Emulsin 0.0056 g in 50 ml (20°) or 0.0028 g in 50 ml (30°). Sal.f. 0.60.*

$a_{beg} = -1.305°$, $a_{end} = +0.610°$, $a_{emulsin} < 0.005°$.

	30°				20°			
t min	a	x	$c-x$	$k' \cdot 10^3$	a	x	$c-x$	$k' \cdot 10^3$
0	−1.305	—	1.915	—	−1.305	—	1.915	—
10	−0.800	0.505	1.410	13.3	−0.760	0.545	1.370	14.5
20	−0.445	0.860	1.045	13.2	−0.395	0.910	1.005	14.0
30	−0.180	1.125	0.790	12.9	−0.110	1.195	0.720	14.2
40	0.000	1.305	0.610	12.4	0.095	1.400	0.515	14.3
60	0.285	1.590	0.325	12.8	0.315	1.620	0.295	13.5
90	0.460	1.765	0.150	12.3	0.490	1.795	0.120	13.3
120	0.545	1.850	0.065	12.2	0.565	1.870	0.045	13.6
		mean value		12.7		mean value		13.9
		k/e (Sal.f.)		$= 756 \cdot 10^{-2}$		k/e (Sal.f.)		$= 414 \cdot 10^{-2}$

$k_{30}/k_{20} = 1.83$

The corresponding values for phenol-β-D-glucoside are $k'_{obs_{30}}/e$(Sal.f.) $= 43.6 \cdot 10^{-2}$, $k'_{obs_{20}}/e$(Sal.f.) $= 22.6 \cdot 10^{-2}$, $k'_{obs_{30}}/k'_{obs_{20}} = 1.93$, $k_{3_{30}}/e$(Sal.f.) $= 6.37 \cdot 10^{-2}$, $k_{3_{20}}/e$(Sal.f.) $= 3.24 \cdot 10^{-2}$, $k_{3_{30}}/k_{3_{20}} = 1.97$. For the determination of the affinity constant for β-glucosidase-phenol-β-D-glucoside the velocity constants of hydrolysis for solutions of the glucoside, 0.0100, 0.0200, 0.0300, 0.0400, 0.0600 and 0.0800 M, were determined. Table 9 and Fig. 1 give the result.

Table 9. Determination of K_m.

Phenol-β-D-glucoside 30°. Phosphate-citrate buffer pH 4.4. $e = 0.0119$. Sal.f. $= 0.60$.

$c_{glucoside}$	$k' \cdot 10^4$	$\frac{10^2 \cdot k}{e \text{ (Sal.f.)}}$	e(Sal.f.)$/k'$	$k'(k_m + c) \times 10^2$	$10^2 \cdot k_3$
0.0100	39.8	55.7	1.79	6.13	5.90
0.0200	35.4	49.6	2.02	5.95	5.88
0.0300	31.8	44.5	2.25	5.79	5.83
0.0400	29.9	41.9	2.39	5.86	5.95
0.0600	27.4	38.4	2.61	6.14	5.99
0.0800	24.0	33.6	2.98	6.05	5.94
			mean value	5.99	5.92

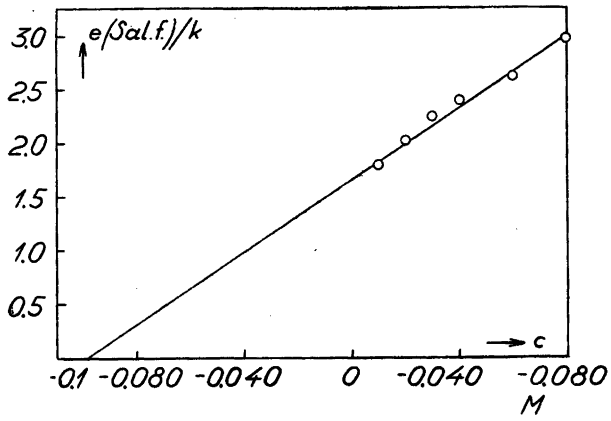


Fig. 1. Determination of K_m. *Phenol-β-D-glucoside.*

From Fig. 1 K_m is determined to 0.10. In the 5. column in Table 9 k_3 is calculated from the simplified expression $k_3 = k'(K_m + c)$, in column 6 the values of k_3 are calculated from the more accurate expression indicated above. The experiment recorded in Table 10 gives for K_{m2} the value 0.10, and for K_{m1} the value 0.20 found previously [12] is used. It is seen that the difference between the two series of k_3-values does not exceed 4 %.

Finally, we summarise in Table 11 all results obtained with enzymic hydrolysis of phenol-β-D-glucoside.

Table 10. Determination of K_{m_2}.

Phenol-β-D-glucoside 30°. Phosphate-citrate buffer pH 4.4. e = 0.0069, Sal.f. = 0.60.

c_{Phenol}	$k \cdot 10^4$	$k/k_{ph}-1$	$\frac{k/k_{ph}-1}{c_{ph}}$
0.00	16.1	—	—
0.01	14.7	0.095	9.5
0.02	13.7	0.175	8.8
0.04	12.8	0.255	6.4
0.06	11.7	0.375	6.3
0.08	10.6	0.520	6.5

mean value 7.5

$$K_{m_2} = \frac{K_m \cdot c_{ph}}{(K_m + c)\ (k/k_{ph}-1)}$$

$$= \frac{0.10}{0.14 \cdot 7.5} = 0.10$$

Table 11. Summary of results obtained with enzymic hydrolysis of phenol-β-D-glucoside.

	$c_{glucoside}$	c_{phenol}	$10^2 \cdot k'$ e/(Sal.f.)	$10^2 \cdot k$ e/(Sal.f.)	$10^2 \cdot k_3/e$ (Sal.f.)
30°	0.0400	0.00	43.6	43.6	6.39
	0.0100	0.00	55.7	53.1	5.90
	0.0200	0.00	49.6	48.6	5.88
	0.0300	0.00	44.5	43.6	5.83
	0.0400	0.00	41.9	41.7	5.95
	0.0600	0.00	38.4	36.7	5.99
	0.0800	0.00	33.6	32.4	5.94
	0.0400	0.00	38.9	38.6	5.48
	0.0400	0.01	35.5	34.8	5.27
	0.0400	0.02	33.1	32.4	5.22
	0.0400	0.04	30.9	30.0	5.44
	0.0400	0.06	28.3	28.5	5.73
	0.0400	0.08	25.6	26.1	5.77
	mean value (for k and k' only underlined experiments)		41.5	41.3	5.75
20°	0.0400	0.00	22.6	22.1	3.24

SUMMARY

The acid hydrolysis of phenol- and *o*-cresol-β-D-glucoside has been investigated. The former glucoside is hydrolysed by acids somewhat faster than the latter, whereas when hydrolysed with β-glucosidase from sweet almonds as catalysator *o*-cresol-glucoside is hydrolysed some 18 times as fast as phenol-glucoside.

The velocity constants of acid hydrolysis are of the same order of magnitude as for glucosides of primary and secondary alcohols but inferior to the velocity constants for glucosides of tertiary alcohols by 2—3 orders of magnitude.

The heat of activation for the acid hydrolysis of phenol- and *o*-cresol-β-D-glucoside is closer to the heat of activation for the hydrolysis of glucosides of tertiary alcohols than to the heat of activation for the hydrolysis of glucosides of primary and secondary alcohols.

The constants of action for the hydrolysis of the aryl-β-D-glucosides examined are lower than the constants of action for the hydrolysis of glucosides of not only tertiary but also primary and secondary alcohols. This is in disagreement with the findings of Heidt and Purves, who for phenol- and methyl-β-D-glucoside indicate constants of action identical within the limit of error.

The heat of activation and the "constants of action" for the enzymic hydrolysis of the two aryl-β-D-glucosides are closer to the corresponding values for the enzymic hydrolysis of glucosides of primary and secondary alcohols than to those of tertiary alcohols. The difference in velocity of enzymic hydrolysis of *o*-cresol- and phenol-β-D-glucoside is due partly to the greater affinity of the β-glucosidase to *o*-cresol-β-D-glucoside than to phenol-β-D-glucoside, partly to a greater k_3-value for the former glucoside than for the latter.

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Acid and Enzymic Hydrolysis of β-Glucosides. III

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Scheiber [1] examined the acid and the enzymic hydrolysis of different aryl-β-D-glucosides in order to determine the influence of different substituents in the aromatic nucleus on the velocity of hydrolysis. It was found that the acid hydrolysis was not much influenced by the introduction of a single methyl group or by two methyl groups, one in *o*-, the other in *p*-position to the hydroxyl group, but the introduction of two methyl groups in *o,o'*-position caused an increase of the velocity constant to 4 times the value for the unsubstituted or the monosubstituted phenol-β-D-glucoside.

For the enzymic hydrolysis, on the other hand, the effect of the introduction of methyl groups was quite different. With sweet almond emulsin as enzyme the velocity constant for *o*-cresol-β-D-glucoside was found 13 times as great, for *p*-cresol-β-D-glucoside only 1/3 as great as for phenol-β-D-glucoside, and the value for *o,p*-xylenol-β-D-glucoside is only 1/3 of the value for *o*-cresol-β-D-glucoside. The effect of two methyl groups in *o*-position to the glucoside linkage was a retardation of the velocity of hydrolysis, the constant being only 1/3—1/4 of the value for phenol-β-D-glucoside, slightly inferior to the value for *p*-cresol-β-D-glucoside.

This effect reminds of the effect of methyl groups on the hydrolysability of alkyl-β-D-glucosides, examined by Veibel and Frederiksen [2] and by Veibel and Lillelund [3]. For the acid hydrolysis it was found that the constants for methyl- and propyl-β-D-glucoside were nearly equal. The velocity constant for ethyl-β-D-glucoside will presumably be very similar to the two other constants. For isopropyl-β-D-glucoside the velocity constant is about twice as great as for methyl and propyl-glucoside, whereas the constant for trimethylcarbinol-

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β-D-glucoside is superior to the other constants by 2—3 orders of magnitude. For the enzymic hydrolysis, on the other hand, the velocity constants for ethyl-, isopropyl-, and trimethylcarbinol-β-D-glucoside were $5.3 \cdot 10^{-2}$, $16.9 \cdot 10^{-2}$, and $0.02 \cdot 10^{-2}$ respectively, *i.e.* an increase by a factor 3 for one methyl group and a decrease by 2—3 orders of magnitude for two methyl groups.

Scheiber does not mention the hydrolysis of mesitol-β-D-glucoside in which the phenol is substituted by two methyl groups in *o*-position and one in *p*-position. In order to see if the retarding influence of the *p*-methyl group is sufficient to cause a decrease in velocity of hydrolysis of mesitol-β-D-glucoside as compared with *o,o'*-xylenol-β-D-glucoside we prepared the two glucosides and examined the acid and the enzymic hydrolysis.

Scheiber indicates that he has used 0.052 *M* solutions of the glucosides investigated both for the acid and for the enzymic hydrolysis. The mesitol-glucoside is, however, so slightly soluble in water that a 0.007 *M* solution is nearly saturated at 30°. For the enzymic hydrolysis this solution, therefore, means the upper limit of concentration and in order to obtain comparable results we used this solution for the experiments with acid hydrolysis too.

The result of the investigation is that the velocity constants for the acid hydrolysis of the two glucosides are very nearly equal, that for mesitol-glucoside being slightly greater than that for the other. For the enzymic hydrolysis the inverse is found. In both instances are the differences, however, hardly greater than the experimental error.

EXPERIMENTAL

Substrates. *o,o'*-Xylenol-β-D-glucoside was prepared as indicated by Helferich and Scheiber [1] from *o,o'*-xylenol. This latter substance may be prepared easily according to Carlin and Landerl [4].

Mesitol-β-D-glucoside: On a steam bath a mixture of 20 g of β-pentaacetylglucose and 14 g of mesitol was melted, and 0.26 g of *p*-toluenesulfonic acid was added. The homogenous melt was heated on the steam bath for 5 hours, the resulting dark brown sirup was dissolved in 250 ml of benzene, mesitol and *p*-toluenesulfonic acid was removed by washing the refrigerated solution with 2 *N* sodium hydroxide and with water. After drying over calcium chloride the benzene solution was evaporated in vacuo and the crystalline residue was freed from a sirupous substance by recrystallising it from methanol till the m.p. remained constant. Tetraacetyl-mesitol-β-D-glucoside, m.p. 164.5—165°, $[\alpha]_{20}^{D}$ −14.8° (chloroform, $c = 1.016$, $l = 2$, $\alpha = -0.30°$), was obtained as colourless needles in a yield of 65%.

$C_{23}H_{30}O_{10}$ (466.5)	Calc.	C	58.88	H	6.02
	Found	»	59.21	»	6.48

Deacetylation produced the mesitol-β-D-glucoside, colourless prisms recrystallised from water. M.p. 222.5—223.5°, $[\alpha]_{20}^{D}$ —6.07° (water, $c = 0.989$, $l = 2$, $\alpha = -0.12°$).

$C_{15}H_{22}O_6$ (298.3)	Calc.	C	60.37	H	7.44
	Found	»	60.70	»	6.95

Enzyme. The β-glucosidase used was prepared from sweet almonds. 1 kg of sweet almonds were pressed in a hydraulic press, the dry presscake (about 500 g) was milled to a coarse powder from which the β-glucosidase was extracted essentially according to the indications of Helferich [5]. During the extraction the solution was stirred mechanically. For all centrifugations a refrigerated centrifuge from *International Equipment Co., Boston, Mass.*, was used. We take the opportunity to thank the *Carlsberg Foundation* for a grant which enabled one of us (S.V.) to obtain the centrifuge. Yield of β-glucosidase 5.26 g with a Sal.f. of 0.60 (β-glucosidase value 2.0), somewhat higher than indicated by Helferich (β-glucosidase value 1.25). The standardisation of the β-glucosidase was made with o-cresol-β-D-glucoside as substrate (Veibel and Lillelund [6–8]). Table 1 gives the result.

Table 1. Standardisation of β-glucosidase.

o-Cresol-β-D-glucoside 0.0400 *M*. $\alpha_{beg.} = -1.325°$, $\alpha_{end} = +0.620°$. Emulsin: 2 ml of a solution containing 0.0074 g in 20 ml dissolved to 50 ml. $e = 0.00074$. $\alpha_{Emulsin} = 0°$. Phosphate-citrate buffer pH 4.4. 30°.

$$K' = K_m/K_{m_1} + K_m/K_{m_2} - 1 = -0.28$$

t min	α	c—x	$10^4 \cdot k_{obs}$	$K' \cdot x$	$10^2 \cdot k_3$/Sal.f.
0	—1.325	1.945	—	—	—
10	—1.220	1.840	24.1	0.0003	(17.2)
20	—1.100	1.720	29.3	0.0010	20.6
30	—0.970	1.590	34.1	0.0017	23.6
40	—0.840	1.460	37.0	0.0024	25.3
60	—0.620	1.240	35.5	0.0034	23.7
90	—0.360	0.980	34.1	0.0048	22.2
120	—0.170	0.790	31.5	0.0060	20.0
				mean value	22.6

For k_3 Veibel and Lillelund [6] found $37.5 \cdot 10^{-2}$ (phosphate-citrate, pH 4.4). Sal.f., therefore, is $\frac{22.6}{37.5} = 0.60$ and β-glucosidase value $= \frac{\text{Sal.f.}}{\log 2} = 2.0$.

Methods. In previous investigations in this laboratory the polarimetrical method for following the hydrolysis was used. The mesitol-β-D-glucoside is, however, so slightly soluble in water that at 30° the most concentrated solution is about 0.007 *M*. The total change of rotation for complete hydrolysis being only 0.11—0.12° we had to use another method than the polarimetrical, and the Hagedorn-Jensen method for determination of glucose, as modified by Hanes [9], was considered the most reliable.

The enzymic hydrolysis was followed by taking samples of 5 ml at intervals, suppressing the enzymic action by adding the sample to 1 ml of 20% potassium carbonate and

determining the glucose content in 5 ml of the mixture. For the acid hydrolysis the method described by Veibel and Frederiksen [2] was used. Here, too, the hydrolysis was followed by Hagedorn-Jensen-determination of the glucose content of the samples, not polarimetrically. The acid hydrolysis was interrupted by adding 5 ml samples to 2 ml 20% potassium carbonate in experiments with *N* acid, to 2 ml 10% potassium carbonate in experiments with 0.5 *N* acid. The results are given in Tables 2—4. It is seen from the tables that there is no significant difference between the velocity constants for the two glucosides.

Table 2. Enzymic hydrolysis.

Glucoside 0.007 *M*. Emulsin 0.0043 g in 50 ml. Sal.f. = 0.60. Phosphate-citrate buffer pH 4.4. 30°. Use of 0.0129 *N* $Na_2S_2O_3$ by complete hydrolysis 11.71 ml ~ 5.25 mg glucose.

A. *o,o'*-Xylenol-glucoside				B. Mesitol-glucoside			
t min	ml $Na_2S_2O_3$	$c_{end} - c_t$ mg glucose	$k \cdot 10^4$	*t* min	ml $Na_2S_2O_3$	$c_{end} - c_t$ mg glucose	$k \cdot 10^4$
0	—	5.25	—	0	—	5.25	—
15	0.20	5.16	5.00	15	0.18	5.17	4.47
30	0.33	5.10	4.20	30	0.29	5.12	3.63
60	0.65	4.94	4.42	60	0.60	4.97	3.97
90	0.76	4.89	3.46	90	0.73	4.91	3.23
135	1.12	4.74	3.29	135	1.09	4.75	3.22
195	1.73	4.48	3.53	195	1.69	4.50	3.44
285	2.42	4.17	3.51	285	2.37	4.19	3.44
		mean value	3.9			mean value	3.6
	k/e (Sal.f.) $= 15 \cdot 10^{-2}$				k/e (Sal.f.) $= 14 \cdot 10^{-2}$		

Table 3. Acid hydrolysis. 1.0 N HCl.

Glucoside 0.007 *M*. 60°. Complete hydrolysis 10.22 ml $Na_2S_2O_3$ ~ 4.50 mg glucose

A. *o,o'*-Xylenol-glucoside				B. Mesitol-glucoside			
t min	ml $Na_2S_2O_3$	$c_{end} - c_t$ mg glucose	$k \cdot 10^4$	*t* min	ml $Na_2S_2O_3$	$c_{end} - c_t$ mg glucose	$k \cdot 10^4$
0	—	4.50	—	0	—	4.50	—
15	0.50	4.28	14.5	15	0.51	4.27	15.2
30	1.04	4.03	16.0	30	1.23	3.95	18.9
50	1.80	3.70	17.0	50	1.94	3.63	18.7
80	1.96	3.63	11.7	80	3.13	3.10	20.2
120	2.47	3.40	10.0	120	3.28	3.03	14.3
180	2.90	3.21	(8.0)	180	3.73	2.83	(11.2)
		mean value	13.8			mean value	17.5

Table 4. Acid hydrolysis. 0.5 N HCl.

Glucoside 0.007 *M*. 60°. Complete hydrolysis 10.22 ml $Na_2S_2O_3 \sim 4.50$ mg glucose.

A. *o, o'*-Xylenol-glucoside				B. Mesitol-glucoside			
t min	ml $Na_2S_2O_3$	$c_{end} - c_t$ mg glucose	$k \cdot 10^4$	*t* min	ml $Na_2S_2O_3$	$c_{end} - c_t$ mg glucose	$k \cdot 10^4$
0	—	4.50	—	0	—	4.50	—
15	0.23	4.40	6.5	15	0.29	4.37	8.5
30	0.47	4.29	6.9	30	0.51	4.27	7.6
60	0.86	4.12	6.4	60	0.98	4.06	7.5
90	1.17	3.98	5.9	90	1.24	3.95	6.3
150	1.87	3.66	6.0	150	1.97	3.62	6.3
210	2.65	3.32	6.3	210	2.58	3.26	6.7
300	3.16	3.09	5.4	300	3.04	3.14	5.2
		mean value	6.2			mean value	6.9

In Table 5 we have collected the velocity constants found by Scheiber [1], recalculated by us so that they are comparable with the constants found here and in previous papers by Veibel *et al.* [7,10,11]. Scheibers constants for the acid hydrolysis are determined at 61.2° (boiling chloroform). Veibel *et al.* have determined the velocity constants at different temperatures and have calculated the heat of activation, so that the values for phenol- and *o*-cresol-*β*-D-glucoside at 61.2° may be calculated. For *o,o'*-xylenol- and mesitol-*β*-D-glucoside, too, we have calculated the velocity constants at 61.2°, using the same heat of activation as found for phenol- and *o*-cresol-glucoside.

Scheibers constants for the enzymic hydrolysis are determined at 30° in acetate buffered solution at pH 5.0. The constant for *o*-cresolglucoside is directly comparable with the constant determined by Veibel and Lillelund [7], as the value found by them for a 0.040 *M* solution may be recalculated to the concentration 0.052 *M* used by Scheiber by application of the formula $k(K_m + c) = \text{const.}$, K_m being the Michaelis-constant, 0.027 (acetate, pH 5.0). Veibel, Møller and Wangel [10] have found the value $18.1 \cdot 10^{-2}$ for the velocity constant of hydrolysis for phenolglucoside, using a phosphate-citrate buffer at pH 6.0. According to the findings of Veibel and Lillelund [7] the velocity constant in acetate buffer at pH 5.0 is some 30 % higher than in phosphate-citrate buffer at pH 6.0. Veibel and Hjorth [11], on the other hand, found for 0.0400 *M* solutions in phosphate-citrate buffer at pH 4.4 $k = 43.6 \cdot 10^{-2}$, $K_m = 0.10$, from which is calculated $k = 40.0 \cdot 10^{-2}$ for 0.052 *M* solutions.

Table 5. Velocity constants for the hydrolysis of different β-D-glucosides.

Aglucon	Enzymic, 30°		Acid		
	Scheiber	Veibel	Scheiber 61.2°	Veibel 60°	Veibel recalc. 61.2°
	$10^2 \cdot k/e$ (Sal.f.)		$10^4 \cdot k$	$10^4 \cdot k$	$10^4 \cdot k$
Phenol	26.2	$24<k<40$	9.4	7.5	8.7
o-Cresol	343	567 (0.052 *M*) 1320 (0.007 *M*)	7.8	6.6	7.8
m-Cresol	—	—	8.3	—	—
p-Cresol	8.6	—	8.3	—	—
o,o'-Xylenol	7.4	15.3	35.3	13.8 6.2	16.5 1.0 *N* HCl 7.4 0.5 *N* HCl
o,p-Xylenol	113	—	8.1	—	—
o,o', p-Mesitol	—	14.3	— —	17.5 6.9	20.6 1.0 *N* HCl 8.2 0.5 *N* HCl

The velocity constant in acetate buffer at pH 5.0 is practically identical with the velocity constant in phosphate-citrate buffer at pH 4.4 (Veibel and Lillelund [7]). We, therefore estimate the value to be compared with Scheibers value to be somewhere between $24 \cdot 10^{-2}$ and $40 \cdot 10^{-2}$, the latter value being the more probable of the two.

Table 5 shows that when our values for the enzymic hydrolysis of *o*-cresol- and *o,o'*-xylenol-β-D-glucoside are compared with Scheibers, approximately the same ratio is found, even if the single values found by us are nearly twice as great as Scheibers values. If, however, we calculate a $k_{o\text{-cresol}}$-value for 0.007 *M* solution we find $k = 1320 \cdot 10^{-2}$ (for 0.0100 *M* solution Veibel and Lillelund [7] found experimentally $k = 1139 \cdot 10^{-2}$), and then we find the ratio *o*-cresol/*o,o'*-xylenol nearly twice as great as that found by Scheiber. In both cases a significant retarding effect of the second methyl group is found, a retarding effect more pronounced than the accelerating effect of the first methyl group ($k_{\text{phenol}} > k_{o,o'\text{-xylenol}}$).

The value for mesitol-β-D-glucoside is within the limit of error identical with the value for *o,o'*-xylenolglucoside. The retarding effect of a methyl group in *p*-position found by Scheiber for *o*-cresol-/*o,p*-xylenol-glucoside is not found for the couple *o,o'*-xylenol-/mesitol-glucoside, but on the other hand no trace of the accelerating effect found in the couple ethyl-/isopropyl-glucoside seems to exist.

For the acid hydrolysis the values for phenol- and *o*-cresol-β-D-glucoside found by Scheiber and by Veibel and Hjorth [11] are identical within the limit

of error. For *o,o'*-xylenol-β-D-glucoside Scheiber finds the velocity constant twice as great as found by us. Here, too, Scheiber has used the concentration 0.052 *M*. Veibel and Hjorth have, in experiments with 0.05 *M* and 0.1 *M* glucoside solutions, found the velocity of acid hydrolysis proportional to the concentration of glucoside, but minor deviations from the proportionality may be responsible for the difference found for 0.052 *M* and 0.007 *M* solutions.

The acid hydrolysis is found proportional to the concentration of hydrogen ions, the constants calculated for 0.5 *M* HCl as indicated by Veibel and Hjorth[11] being 6.0 and 6.9 respectively, as compared with the experimentally found values 6.2 and 6.9.

The accelerating effect of a second *o*-methyl group found by Scheiber is to a minor degree found by us, too. Only a slight retarding influence of a methyl group in *p*-position is found for the couples phenol-/*p*-cresol-, *o*-cresol-/*o,p*-xylenol-, and *o,o'*-xylenol-/mesitol-glucoside.

In the aliphatic series the effect of the second methyl group is much greater than found here in the aromatic series.

In the first paper in this series [2] we have discussed the possibility of steric hindrance being responsible for the slow enzymic hydrolysis of glucosides of tertiary alcohols. The voluminous enzyme molecules cannot attach themselves to the glucoside linkage when three alkyl groups are linked to the carbon atom responsible for the glucoside linkage. The acid hydrolysis, on the other hand, is catalysed by hydrogen ions which on account of their smaller volume are not being hindered in attacking the glucoside linkage.

A determination of the constant *B* in the Arrhenius-equation did not, however, support this idea. We, therefore, have not continued the investigation in this direction.

Lack of affinity between enzyme and substrate might be responsible for the slow enzymic hydrolysis of *o,o'*-xylenol-β-D-glucoside. For trimethylcarbinol-β-D-glucoside Veibel [12] found that the extremely slow hydrolysis was partially due to failing affinity, the Michaelis-constant being 1.46. For *o*-cresol-β-D-glucoside we have, as indicated above, found the value 0.027 for the Michaelis-constant. A value for the Michaelis-constant for *o,o'*-xylenol-glucoside may be estimated from the velocity constants for 0.052 *M* (Scheiber) and 0.007 *M* solutions. From the equation $k(K_m + c) = \text{const.}$ the value for K_m is calculated to 0.036, *i.e.* an affinity nearly as great as that between the enzyme and *o*-cresolglucoside. Evidently, lack of affinity is not responsible for the slow hydrolysis of *o,o'*-xylenolglucoside.

Further investigations seem necessary before the question of the mechanism of hydrolysis of glucosides can be solved.

SUMMARY

The acid and the enzymic hydrolysis of *o,o'*-xylenol- and mesitol-β-D-glucoside have been investigated and the results compared with results obtained previously with phenol-, *o*-, *m*-, *p*-cresol-, *o,o'*- and *o,p*-xylenol-β-D-glucoside.

In experiments with enzymic hydrolysis an accelerating effect of one methyl group in *o*-position to the glucosidic linkage is found, but if two methyl groups are introduced in *o*-position to the glucosidic linkage the acceleration is transformed into a retardation.

In experiments with acid hydrolysis the effect of one methyl group is a slight retardation, that of two methyl groups in *o*-position to the glucosidic linkage an acceleration of the velocity of hydrolysis.

The effect of a methyl group in *p*-position is for enzymic hydrolysis a retardation of the velocity of hydrolysis. For acid hydrolysis a hardly discernable retardation is found.

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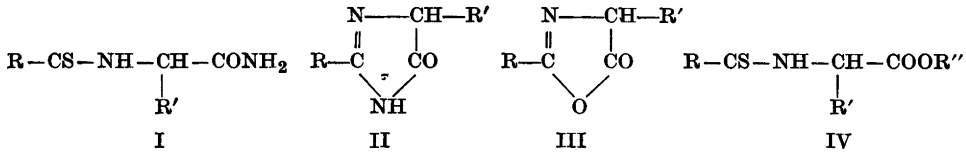
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Thioacylamido Acid Amides and Their Reactions with Mercuric Acetate

ANDERS KJÆR

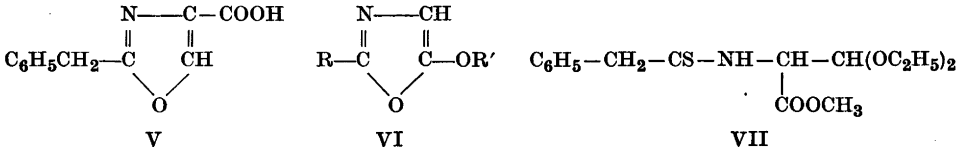
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In previous papers [1,2] the preparation of various N-thioacylated amino acid amides was reported. The present communication describes some additional substances of a similar type and is concerned also with the action of mercuric acetate on these compounds (I), a study originally initiated with the prime objective of providing a new method of synthetising 5-imidazolones (II).



I II III IV

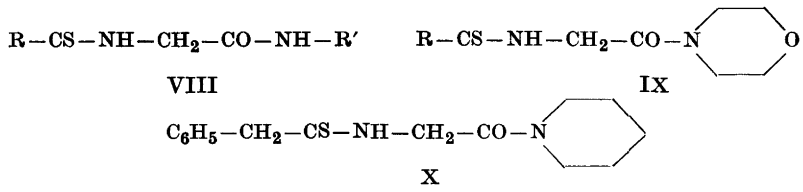
In recent years several syntheses of heterocyclic rings have been reported utilising the elimination of hydrogen sulphide from an acyclic thioamide function. For example, the important method of closing imidazole-rings developed by Todd *et al.*[3] and the successful synthesis of desthiobenzyldihydropenillamine by the Upjohn group [4] rest on this principle. The formation of 5-oxazolones (III) upon treatment of α-thioacylamido acids (IV,R″ = H) with silver salts [5] was discovered during the work on penicillin chemistry. This also contributed valuable new routes to 2-benzyloxazole-4-carboxylic acid [6] (V) and 5-alkoxy-oxazoles [7] (VI) by cyclisation of methyl thiobenzylpenaldate diethyl acetal (VII) and α-thioacylamido esters (IV, R″ = alkyl) respectively, under the influence of silver salts. Apparently, however, no attempts to extend this reaction to α-thioacylamido acid amides (I) have been recorded.



V VI VII

The requisite thioacylamides (I) were produced as previously described [1,2], the ammonolysis of the thioacylamido esters generally being preferred to the direct thioacylation of α-amino amides most of which are rather tedious to prepare. Thiohippuramide (I, R = C_6H_5, R′ = H) and thiophenaceturamide (I, R = $C_6H_5CH_2$,R′ = H) however, were conveniently obtained by the latter method [1,2] owing to the ready availability of glycinamide [8]. In performing the thioacylations of the amino esters, duplicate runs were made in pyridine solution with triethylamine, following the directions recently given by Crawhall and Elliott [9]. This modification, however, did not in the present cases constitute any improvement over the original method, neither with regard to time nor results. High yields of thiobenzoylalanine methyl ester (IV,R = C_6H_5,R′ = R″ = CH_3) and phenylthioacetylalanine methyl ester (IV,R = $C_6H_5CH_2$,R′ = R″ = CH_3), obtained upon treatment of the corresponding acids with diazomethane, demonstrated the uncomplicated reactions with this reagent. The ethyl esters of L-aspartic acid, L-glutamic acid, L-leucine, L-tyrosine, DL-methionine and DL-phenylalanine were submitted to thiobenzoylation, yielding oily reaction-products of which only the last two crystallised spontaneously. No efforts were made to induce crystallisation of the oily products which were aminolysed without further purification. N-Phenylthioacetyl-DL-alanine methyl ester was readily obtained as colourless crystals from reaction of the ester with carboxymethyl dithiophenylacetate [2] in aqueous solution.

During the ammonolysis experiments a rather striking difference in reactivity was noticed. Whereas all the esters studied reacted quantitatively at room temperature in the course of about twenty-four hours with a methanolic solution of dry ammonia, regardless of the nature of the alkyl in the ester grouping, the same substances were hardly affected under similar circumstances after treatment with ammonia dissolved in ethanol. This influence of the medium presents no novelty in the ammonolysis of esters (see *e.g.*[10]) but in the present case the effect appears to be extraordinarily pronounced. The resulting amides containing the thiobenzoyl-grouping (I,R = C_6H_5) are crystalline compounds with yellow colours of varying intensity (see Table 1). For the products derived from L-aspartic and L-glutamic esters, analysis indicates that both ester functions have been exchanged with ammonia producing the compounds (I,R = C_6H_5,R′ = CH_2CONH_2) and (I,R = C_6H_5,R′ = $CH_2CH_2CONH_2$) respectively. In cases where R in (I) is benzyl, the amides are colourless substances, melting considerably lower than the corresponding thiobenzoyl-derivatives.

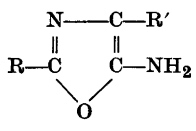


VIII IX

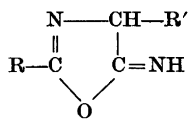
X

On submitting ethyl thiohippurate to reaction with ethylamine in methanol, N-ethyl-thiohippuramide (VIII, $R = C_6H_5$, $R' = C_2H_5$) was obtained as colourless crystals which showed signs of existing also in a yellow form as previously discussed in an analogous case [1]. Again, benzylamine yielded the intensely yellow N-benzylamide (VIII, $R = C_6H_5$, $R' = C_6H_5CH_2$) whereas with morpholine the yellow thiohippuromorpholide (IX, $R = C_6H_5$) was produced. Piperidine reacted abnormally with the ester, a small amount of the benzylamine salt of thiohippuric acid being the sole crystalline constituent which could be isolated from the complex reaction mixture. No reaction could be induced with aniline, even under forced conditions. Thiohippurylglycine (VIII, $R = C_6H_5$, $R' = CH_2COOH$) was produced for the first time following the directions previously given [1]. The corresponding ethyl thiophenaceturate yielded a crystalline N-benzylamide (VIII, $R = R' = C_6H_5CH_2$), morpholide (IX, $R = C_6H_5CH_2$) and piperidide (X). Again, aniline failed to react.

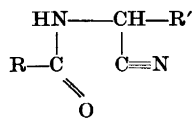
Attention was next turned to the reaction of the amides (I) with mercuric acetate. On mixing equimolecular amounts of the reactants dissolved in absolute ethanol an instantaneous reaction took place, indicated by the separation of a precipitate, ranging in colour from white to yellow — presumably a mercuric mercaptide — darkening within a few seconds and changing into precisely one molecular proportion of mercuric sulphide. The filtrates on evaporation deposited crystalline, colourless products, possessing elementary compositions indicating the loss of the elements of hydrogen sulphide, and devoid of notable basic properties. The formulation of the products as 5-imidazolones (II) was excluded by their low melting points, their insolubility in aqueous alkali, and also by direct comparison with an authentic specimen of the representative compound (II, $R = C_6H_5$, $R' = CH_3$), prepared according to Cornforth and Huang [11]. Alternative formulations as 5-aminoöxazoles (XI) or the tautomeric 5-iminoöxazolines (XII) were ruled out, partly because of the lack of basic properties (which may, however, be rather feeble in such structures) but mainly on account of the ultra-violet absorption spectra being incompatible with the oxazole-structure.



XI



XII



XIII

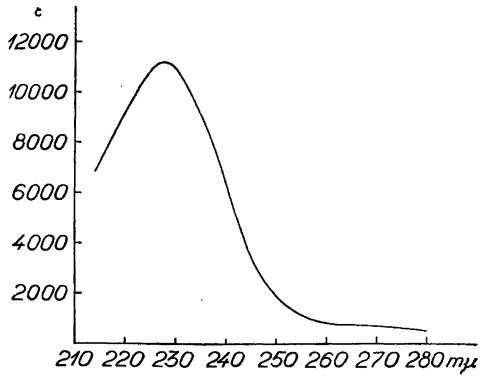


Fig. 1. Ultra-violet absorption spectrum in 95% ethanol of (XIII, R = C_6H_5, R′ = H), typical in its general form for all compounds listed in Table 2 with R = C_6H_5.

As inferred from the rather scant literature [12,13], strong absorption would be expected at *ca.* 300 mμ for the 5-aminoöxazoles (XI or XII, R = C_6H_5, R′ = alkyl). However, most reaction products listed in Table 2 showed typical benzamide-absorption [14] with a high peak at 228 mμ and a plateau at about 270 mμ as seen from Fig. 1, whereas the analogous compounds containing the benzyl grouping possessed absorption data (Fig. 2) agreeing with those of phenylacetamide [15]. Confirmatory evidence for the correctness of formulating the reaction products as acylated α-aminonitriles (XIII) was obtained by comparison of (XIII, R = C_6H_5 or $C_6H_5CH_2$, R′ = H) with authentic samples of hippuronitrile and phenaceturonitrile [16]. Furthermore, (XIII, R = C_6H_5, R′ = CH_3) agrees in melting point with the known α-benzamidopropionitrile [17].

Only a few acylated α-aminonitriles (XIII) are recorded in the literature, all prepared by acylation of the corresponding α-aminonitriles. The present reaction proceeds from α-amino acids and may be of some value in the synthesis of α-acylamidonitriles, especially those containing complicated side chains, particularly because it obviates the preparation of the often difficultly accessible aldehydes needed in the traditional Strecker-synthesis of the corresponding α-aminonitriles.

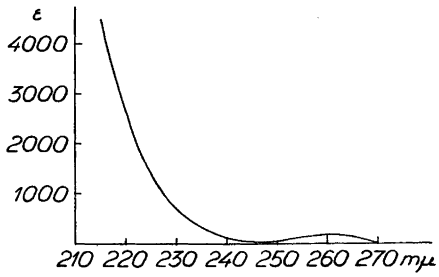
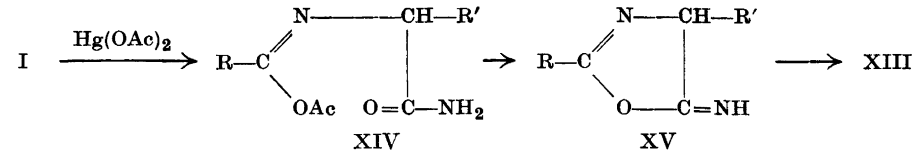


Fig. 2. Ultra-violet absorption spectrum in 95% ethanol of (XIII, R = $C_6H_5CH_2$, R′ = H or CH_3).

The reactions leading to the acylamidonitriles may be envisioned as proceeding through the following stages:



the postulation of (XIV) as an intermediate being purely hypothetical. The smooth interconversion of (XV) to (XIII) is conceivable, being paralleled in various analogous compounds [12,13]. This formulation is in accord with the observation made during the present study that N-substituted thioacylamides (VIII) undergo simple desulphurisation to the corresponding oxygen-analogues, the intermediates (XIV) or possibly (XV) suffering hydrolytic fission during the isolation procedure. Thus, on treatment with mercuric acetate, the compounds (VIII, R = C_6H_5, R′ = $C_6H_5CH_2$) and (VIII, R = R′ = $C_6H_5CH_2$) gave N-benzylhippuramide and N-benzylphenaceturamide respectively, both being produced in low yield and identified by comparison with synthetic specimens. Again, (X) similarly treated afforded phenaceturopiperidide, whose identity was proved by independent synthesis. In all other cases where N-substituted thioacylamides were submitted to treatment with mercuric acetate, exactly one molecular proportion of mercuric sulphide was formed, but only impure oily reaction products were obtained, indicating the complex nature of the reaction.

EXPERIMENTAL *

α-Thioacylamido esters (IV)

Prepared according to the procedure previously described [1] or by the method of Crawhall and Elliott [9]. Yields of the crude oily reaction products, which were used in the following step without further purification, amounted to 75—90 % regardless of which conditions were selected.

*N-Thiobenzoyl-*DL*-methionine ethyl ester* (IV, R = C_6H_5, R′ = $CH_3SCH_2CH_2$, R″ = C_2H_5). The crude reaction product crystallised spontaneously. Yellow, rhombic platelets from light petroleum, m.p. 82—83°.

$C_{14}H_{19}O_2NS_2$ (297.4)	Calc.	C 56.54	H 6.44	N 4.71	S 21.56
	Found	» 56.33	» 6.22	» 4.91	» 21.49

*N-Thiobenzoyl-*DL*-phenylalanine ethyl ester* (IV, R = C_6H_5, R′ = $C_6H_5CH_2$, R″ = C_2H_5). Separated as clusters of yellow prisms from light petroleum, m.p. 74—76°.

$C_{18}H_{19}O_2NS$ (313.4)	Calc.	C 68.98	H 6.11	N 4.47	S 10.23
	Found	» 69.01	» 6.07	» 4.69	» 10.41

* All melting points are uncorrected and have been determined in capillary tubes in an electrically heated block.

*N-Thiobenzoyl-*DL*-alanine methyl ester* (IV, R = C_6H_5, R′ = R″ = CH_3). Upon treatment of 2.07 g (0.01 mole) of N-thiobenzoyl-DL-alanine, dissolved in methanol, with an ethereal solution containing 0.025 mole of diazomethane, 1.72 g (77 %) of the crystalline ester was produced. Recrystallised from dilute methanol, m.p. 99—100°. (Ref.[1] 100—101°).

*N-Phenylthioacetyl-*DL*-alanine methyl ester* (IV, R = $C_6H_5CH_2$, R′ = R″ = CH_3). Obtained by methylation of the corresponding acid with diazomethane as described above, in 72 % yield after one recrystallisation from dilute methanol. An additional recrystallisation from chloroform-petroleum ether yielded an analytical sample as colourless leaflets, m.p. 71—73°.

$C_{12}H_{15}O_2NS$ (237.3) Calc. C 60.73 H 6.37 N 5.90 S 13.51
Found » 60.70 » 6.40 » 5.67 » 13.46

Phenylthioacetylation of DL-alanine methyl ester gave a 71 % yield of the ester, m.p. 70°.

α-Thioacylamido amides (I)

As a general procedure the thioacylated amino esters above were treated with 7—8 times their weight of dry methanol, saturated at 0° with dry ammonia. After standing at room temperature in tightly stoppered vessels for 24 hours, the solvent was removed and the remaining yellow solids were recrystallised from appropriate amounts of 95 % ethanol, if necessary diluted with water. The experimental data are presented in Table 1.

N-Substituted thioacylamido amides (VIII)

N-Ethyl-thiohippuramide (VIII, R = C_6H_5, R′ = C_2H_5). A mixture of 1.85 g of ethyl thiohippurate and 2.0 ml of anhydrous ethylamine in 5 ml of methanol was left at room temperature overnight. After removal of the solvent *in vacuo* the residue crystallised readily on scratching with dry ether. Recrystallised from 50 % ethanol as practically colourless prisms. Yield 1.36 g (74 %), m.p. 116—117°. An analytical sample was pro-

Table 1.

α-Thioacylamido Acid Amides

R—CS—NH—CH(R′)—CO—NH_2

	R′	Yield %	M.p.	Formula	Analyses: Carbon Calc.	Carbon Found	Hydrogen Calc.	Hydrogen Found	Nitrogen Calc.	Nitrogen Found	Sulphur Calc.	Sulphur Found
H_5	CH_2CONH_2 [a,b]	59	174—175	$C_{11}H_{13}O_2N_3S$	52.56	52.98	5.22	4.85	16.72	16.55	12.76	12.61
H_5	$CH_2CH_2CONH_2$ [a,c]	61	186—187	$C_{12}H_{15}O_2N_3S$	54.32	54.55	5.70	5.62	15.84	15.79	12.09	12.15
H_5	$CH_2CH(CH_3)_2$ [a,d]	63	163	$C_{13}H_{18}ON_2S$	62.37	62.24	7.25	7.02	11.20	11.21	12.81	12.77
H_5	$CH_2CH_2SCH_3$	88	151—152	$C_{12}H_{16}ON_2S_2$	53.69	53.59	6.01	6.25	10.44	10.42	23.88	23.96
H_5	$CH_2C_6H_5$	83	156—157	$C_{16}H_{16}ON_2S$	67.58	67.71	5.67	5.55	9.85	10.00	11.28	11.06
H_5CH_2	CH_3 [e]	77	144—145	$C_{11}H_{14}ON_2S$	59.41	59.21	6.35	6.59	12.60	12.50	14.42	14.22

a L-Configuration.
b $[\alpha]_D^{23}$ + 5.9° (*c* 0.51, dioxane).
c $[\alpha]_D^{23}$ + 6.0° (*c* 0.50, dioxane).
d $[\alpha]_D^{23}$ + 31.0° (*c* 2.55, ethanol).
e Colourless crystals.

duced by an additional crystallisation from aqueous ethanol, m.p. 117°. The solutions in various solvents were intensely yellow and once an apparently unstable yellow modification was brought to crystallisation.

$C_{11}H_{14}ON_2S$ (222.3)	Calc.	C	59.41	H	6.35	N	12.60	
	Found	»	59.57	»	6.27	»	12.69	

Attempts to determine the sulphur according to the Zimmermann-procedure [18], usually employed in this laboratory, led to violent explosions in the reaction with metallic potassium.

N-Benzyl-thiohippuramide (VIII, R = C_6H_5, R′ = $C_6H_5CH_2$). Prepared in 81 % yield from ethyl thiohippurate and benzylamine by the procedure just described. Thin, spear-shaped yellow plates were obtained by recrystallisation from 65 % ethanol, m.p. 123—124°.

$C_{16}H_{16}ON_2S$ (284.4)	Calc.	C	67.57	H	5.67	N	9.85	S 11.28
	Found	»	67.30	»	5.80	»	10.02	» 11.01

Thiohippuromorpholide (IX, R = C_6H_5). A solution of 1.98 g of ethyl thiohippurate and 2.0 ml of morpholine in 3 ml of methanol was heated for 3 hours on a steam bath, cooled and scratched after addition of dry ether. 1.07 g (46 %) of yellow crystalline material were obtained, sparingly soluble in ethanol. Recrystallisation from a large volume of ethanol afforded beautiful yellow prisms, m.p. 160°.

$C_{13}H_{16}O_2N_2S$ (264.3)	Calc.	C	59.07	H	6.10	N	10.60	S 12.13
	Found	»	59.23	»	6.07	»	10.76	» 11.97

N-Benzyl-thiophenaceturamide (VIII, R = R′ = $C_6H_5CH_2$). After heating a mixture of 2.02 g of oily ethyl thiophenaceturate, 2.0 ml of benzylamine and 2 ml of ethanol on the steam bath for 2 hours, addition of ether precipitated 1.94 g (76 %) of a pale yellow crystalline product, m.p. 130—132°. Very pale yellow needles were produced upon recrystallisation from dilute methanol, m.p. 132—133°.

$C_{17}H_{18}ON_2S$ (298.4)	Calc.	C	68.41	H	6.08	N	9.39	S 10.74
	Found	»	68.63	»	5.91	»	9.45	» 10.53

Thiophenaceturomorpholide (IX, R = $C_6H_5CH_2$). A poor yield (about 15 %) of the thiomorpholide was obtained employing the procedure just described. Clusters of flat, colourless prisms separated from 50 % ethanol, m.p. 120—121°.

$C_{14}H_{18}O_2N_2S$ (278.4)	Calc.	C	60.41	H	6.52	N	10.07	S 11.52
	Found	»	60.39	»	6.73	»	10.14	» 11.76

Thiophenaceturopiperidide (X). The conditions just described gave in poor yield (about 30 %) the crystalline piperidide, separating from 50 % ethanol in small, colourless needles, m.p. 119—120°.

$C_{15}H_{20}ON_2S$ (276.4)	Calc.	C	65.17	H	7.30	N	10.14	S 11.60
	Found	»	65.13	»	7.13	»	10.24	» 11.53

Thiohippurylglycine (VIII, R = C_6H_5, R′ = CH_2COOH). Following the directions given in previous papers [1,2] an 84 % yield of crude product was obtained. From water a colourless or a yellow form can be produced, apparently depending on the rate of cooling. Small, pale yellow needles separated from ethyl acetate-petroleum ether. M.p. 153° with decomposition, after preliminary sintering from about 146°.

$C_{11}H_{12}O_3N_2S$ (252.3)	Calc.	C	52.36	H	4.80	N	11.11	S 12.71
	Found	»	52.36	»	5.03	»	11.27	» 12.72

α-Acylamidonitriles (XIII)

The following general procedure was employed: equimolecular amounts of the thioacylamido amide and mercuric acetate were separately dissolved in the minimum amounts of lukewarm absolute ethanol. The two solutions were mixed at once and a white to yellow precipitate immediately formed. Within a few seconds this turned dark and, after standing at room temperature for 24 hours, the mercuric sulphide was filtered off on a tared glass filter. In numerous experiments conducted as just described the weight of mercuric sulphide formed never deviated more than 5% from the calculated amount. The yellow to orange filtrates were concentrated *in vacuo*, yielding crystalline residues often contaminated with oily by-products. Recrystallisation from dilute methanol, if necessary with addition of decolourising charcoal, followed by a crystallisation from appropriate solvents yielded the analytically pure and colourless products listed in Table 2.

Table 2.

α-Acylamidonitriles

$$R-CO-NH-\underset{\displaystyle R'}{\underset{|}{CH}}-C\equiv N$$

R	R′	Yield %	M.p.	Formula	Analyses Carbon Calc.	Carbon Found	Hydrogen Calc.	Hydrogen Found	Nitrogen Calc.	Nitrogen Found
C_6H_5	H	60[a]	142[b]	$C_9H_8ON_2$	67.48	67.56	5.04	4.98	17.50	17.44
C_6H_5	CH_3	80[a]	108–109[c]	$C_{10}H_{10}ON_2$	68.95	69.23	5.79	5.91	16.09	16.21
C_6H_5	$CH_2CH(CH_3)_2$	58[d]	96–97	$C_{13}H_{16}ON_2$	72.19	72.38	7.46	7.43	12.95	12.97
C_6H_5	$CH_2CH_2SCH_3$	71[d]	118–119	$C_{12}H_{14}ON_2S$[e]	61.50	61.65	6.02	5.92	11.96	11.95
C_6H_5	CH_2CONH_2	57[f]	203–204[g]	$C_{11}H_{11}O_2N_3$	60.81	61.06	5.11	5.23	19.35	19.39
C_6H_5	$CH_2CH_2CONH_2$	59[f]	170–171	$C_{12}H_{13}O_2N_3$	62.33	62.22	5.67	5.69	18.18	18.37
C_6H_5	$CH_2C_6H_5$	68[d]	150	$C_{16}H_{14}ON_2$	76.79	76.91	5.64	5.51	11.20	11.06
C_6H_5	(*p*)-$CH_2C_6H_4OH$	78[a]	200–202	$C_{16}H_{14}O_2N_2$	72.16	72.16	5.30	5.23	10.53	10.59
$C_6H_5CH_2$	H	68[f]	91[h]	$C_{10}H_{10}ON_2$	68.95	68.92	5.79	5.88	16.09	15.89
$C_6H_5CH_2$	CH_3	88[a]	87–88	$C_{11}H_{12}ON_2$	70.18	70.03	6.43	6.45	14.88	15.20

[a] Recrystallised from dilute ethanol.
[b] Ref.[16] 142°.
[c] Ref.[17] 108°.
[d] Recrystallised from chloroform-petroleum ether.
[e] Sulphur analysis: Calc. 13.68, Found 13.60.
[f] Recrystallised from water.
[g] Decomposition.
[h] Ref.[16] 90.5°.

Reactions between N-substituted thioacylamido amides (VIII)–(X) and mercuric acetate

N-Benzyl-hippuramide. When the general method above was applied to N-benzylthiohippuramide, an orange-coloured oil was obtained yielding, after addition of water and concentration *in vacuo*, a small amount of crystalline material. Two subsequent recrystallisations from benzene and dilute ethanol, yielded colourless leaflets, m.p. 160–161.5°.

$C_{16}H_{16}O_2N_2$ (268.3)	Calc.	C 71.63	H 6.01	N 10.45
	Found	» 71.39	» 6.23	» 10.43

An authentic sample of N-benzylhippuramide was produced from hippuric acid azlactone and benzylamine in the usual way. M.p. 161°, not depressed on admixture with the substance above.

Found C 71.69 H 6.20 N 10.39

The literature lists a non-analysed product with m.p. 162—162.5°[19].

N-Benzyl-phenaceturamide. On recrystallisation from methanol of the crude oily product, resulting from the reaction between (VIII, R = R′ = $C_6H_5CH_2$) and mercuric acetate, a small amount of crystalline material was isolated. After an additional crystallisation from ethanol, colourless, rhombic platelets were obtained, m.p. 176—177°.

$C_{17}H_{18}O_2N_2$ (264.3) Calc. C 72.33 H 6.43 N 9.92
Found » 72.64 » 5.99 » 9.92

For the purpose of identification, authentic N-benzyl-phenaceturamide was prepared from methyl phenaceturate and benzylamine. Colourless plates, m.p. 177—178°, unchanged on mixing with the preparation just described.

Found C 72.44 H 6.60 N 10.11

In the Penicillin Monograph two non-analysed preparations are reported with m.p. 178—179° and 175—176° respectively [20].

Phenaceturopiperidide. Reaction of (X) with mercuric acetate afforded a 20% yield of a crystalline substance which, after two recrystallisations from dilute ethanol, appeared as clusters of fine, colourless needles with m.p. 156—158°, not depressed on admixture with the authentic material described below.

$C_{15}H_{20}O_2N_2$ (260.3) Calc. C 69.20 H 7.75 N 10.76
Found » 69.46 » 7.89 » 10.60

An authentic specimen of phenaceturopiperidide was prepared from methyl phenaceturate and piperidine, m.p. 157°.

Found C 69.18 H 7.60 N 10.82

Again, a non-analysed preparation was reported previously [21] with m.p. 161—162°.

Upon treatment of the following thioacylamido amides with mercuric acetate under the usual conditions, the theoretical amount of mercuric sulphide rapidly separated but only yellow oily products were obtained from the filtrates, resisting all attempts to induce crystallisation: thiohippurylglycine (VIII, R = C_6H_5, R′ = CH_2COOH), N-ethyl-thiohippuramide (VIII, R = C_6H_5, R′ = C_2H_5), thiohippuromorpholide (IX, R = C_6H_5), thiophenaceturomorpholide (IX, R = $C_6H_5CH_2$) and N-phenylthioacetyl-L-asparagine [2] as a representative of the β-thioacylamido amides.

SUMMARY

A series of amides and substituted amides of thioacylated α-amino acids are described.

Reaction of the former with mercuric acetate in ethanol was shown to yield acylated α-aminonitriles, whereas the N-substituted amides were partly transformed into the corresponding acylated α-amino acid amides.

The microanalyses have been performed in this laboratory by Mr. A. Grossmann.

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Potentiometric and Spectrophotometric Studies on 8-Quinolinol and Its Derivatives. V. Ionization of 8-Quinolinol-5-Sulfonic Acid and 7-Iodo-8-Quinolinol-5-Sulfonic Acid in Aqueous Solution

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For the investigation of the metal compounds of 8-quinolinol-5-sulfonic and 7-iodo-8-quinolinol-5-sulfonic acids the ionization constants of the acids were required. The earlier determinations are, however, few and ionic strength and temperature are not specified. Therefore a reinvestigation was necessary.

These acids are dibasic and their ionization can thus be represented formally by

$$H_2A \rightleftharpoons HA^- + H^+ \quad (1)$$

$$HA^- \rightleftharpoons A^= + H^+ \quad (2)$$

The law of mass action gives

$$(H^+)\ [HA^-]/[H_2A] = K_1' \quad (3)$$

and

$$(H^+)\ [A^=]/[HA^-] = K_2' \quad (4)$$

where (H^+) is the activity of the hydrogen ion and the symbols in brackets are concentrations. For the determination of K_1' and K_2' the familiar method was used in which sodium hydroxide was added in such an amount that the quotients $[HA^-]/[H_2A]$ or $[A^=]/[HA^-]$ were nearly unity. The pH measured is then approximately the pK required. In this case we can write with sufficient accuracy

$$[HA^-]/[H_2A] = (c_B + [H^+])/(c - c_B - [H^+]) \quad (5)$$

and

$$[A^=]/[HA^-] = (c_B - c)/(2c - c_B) \quad (6)$$

where c and c_B are the stoichiometric concentrations of the acid and sodium hydroxide added. The more exact equations can easily be derived [1] but are here unnecessary.

The experimental procedure was the same as previously in this series [2]. All reagents used were the best obtainable.

RESULTS

Table 1 includes the results on the first ionization of the acids investigated. Each value for the ionization constant is a mean value of four measurements. The deviation of two identical measurements was never greater than 0.01 for pK'. The relatively poor solubility in water does not admit the use of higher concentrations than about 0.003 moles per litre for 8-quinolinol-5-sulfonic acid and 0.005 moles per litre for 7-iodo-8-quinolinol-5-sulfonic acid. The ionic strength is calculated according to the equation $I = 1/2\sum z_i^2 c_i$, where c_i are molarities. In equation (5) the hydrogen ion concentration is of considerable significance, particularly in the case of the iodine derivative. The hydrogen ion concentration was obtained from the pH value measured

Table 1. Determination of the first ionization constants of 8-quinolinol-5-sulfonic acid and 7-iodo-8-quinolinol-5-sulfonic acid in aqueous solution of potassium chloride at 25° C.

8-quinolinol-5-sulfonic acid				7-iodo-8-quinolinol-5-sulfonic acid			
$\sqrt{I}$	pH	pf_H+	pK'_1	$\sqrt{I}$	pH	pf_H+	pK'_1
0.040	4.131	0.020	4.081	0.061	2.947	0.026	2.487
0.307	4.050	0.080	3.992	0.312	2.942	0.080	2.393
0.748	4.085	0.070	4.023	0.748	2.967	0.070	2.461
1.050	4.160	0.018	4.115	1.050	2.982	0.018	2.551
1.460	4.317	−0.100	4.289	1.480	3.006	−0.106	2.702
$c = 3.00 \cdot 10^{-3}$; $c_B = 1.50 \cdot 10^{-3}$.				$c = 5.00 \cdot 10^{-3}$; $c_B = 2.50 \cdot 10^{-3}$			

using the activity coefficient values of the hydrogen ion according to Näsänen, Lumme and Mukula [2].

These results can be represented by the Debye-Hückel equation

$$pK'_1 = pK_{1,0} - \frac{0.509\sqrt{I}}{1 + a\sqrt{I}} + BI \qquad (7)$$

For the 8-quinolinol-5-sulfonic acid the method of least squares gave

$$pK_{1,0} = 4.092. \qquad a = 1.44. \qquad B = 0.207. \qquad (25° \text{ C}).$$

The values for 7-iodo-8-quinolinol-5-sulfonic acid, because of its considerable strength, are not sufficiently accurate for the calculation of the parameters in equation (7) according to the method of least squares. Therefore the thermo-

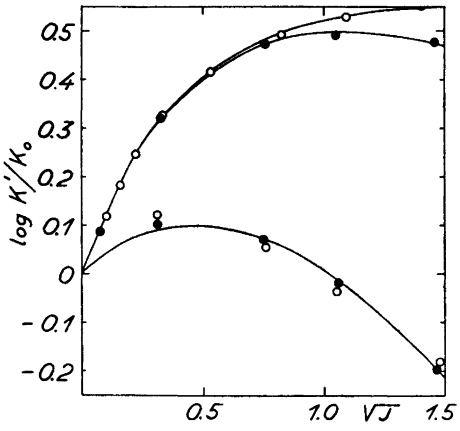


Fig. 1. Ionization constants of 8-quinolinol-5-sulfonic acid and 7-iodo-8-quinolinol-5-sulfonic acid as functions of ionic strength. First ionization: lower curves. Second ionization: upper curves.

● *8-quinolinol-5-sulfonic acid.*
○ *7-iodo-8-quinolinol-5-sulfonic acid.*

dynamic constant was calculated by means of equation (7) using the values of α and B obtained for 8-quinolinol-5-sulfonic acid. For this constant the mean value

$$pK_{1,0} = 2.514 \pm 0.009 \quad (25° \text{ C})$$

was obtained. The manner of calculation seems to be justified, for in Fig. 1, where the first ionization constants are represented as functions of ionic strength, we see that the effect of ionic strength is in both cases almost equal within the experimental error.

The results concerning the second ionization are summarized in Table 2. In this case the Debye-Hückel equation

$$pK'_2 = pK_{2,0} - \frac{1.527\sqrt{I}}{1 + \alpha\sqrt{I}} + BI \qquad (8)$$

was used. The method of least squares gave the values of Table 3.

Table 2. Determination of the second ionization constants of 8-quinolinol-5-sulfonic acid and 7-iodo-8-quinolinol-5-sulfonic acid in potassium chloride solution at 25° C.

8-quinolinol-5-sulfonic acid		7-iodo-8-quinolinol-5-sulfonic acid	
$\sqrt{I}$	pK'_2	$\sqrt{I}$	pK'_2
0.0662	8.690	0.0963	7.303
0.312	8.455	0.148	7.237
0.744	8.305	0.216	7.172
1.042	8.290	0.331	7.089
1.447	8.300	0.523	7.004
		0.815	6.928
$c = 3.00 \cdot 10^{-3}$		1.081	6.890
$c_B = 4.50 \cdot 10^{-3}$		1.402	6.865
		$c = 5.00 \cdot 10^{-3}$	
		$c_B = 7.50 \cdot 10^{-3}$	

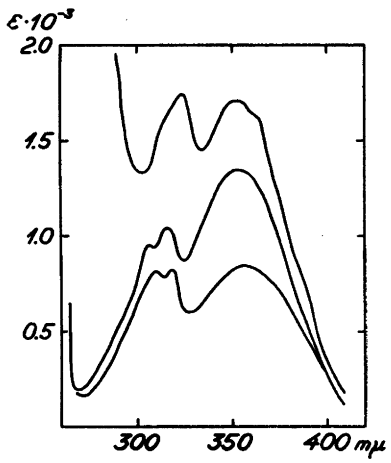


Fig. 2. Molar extinction coefficients of the neutral constituents of 8-quinolinol-5-sulfonic acid (middle curve) and 7-iodo-8-quinolinol-5-sulfonic acid (upper curve) compared with that of H_2Ox^+ *(lower curve).*

Table 3.

	8-quinolinol-5-sulfonic acid	7-iodo-8-quinolinol-5-sulfonic acid
$pK_{2,0}$	8.776	7.417
α	1.62	1.71
B	0.088	0.0386

In Fig. 1 the second ionization constants are represented as functions of ionic strength. The effect of temperature on pK_2' was preliminary investigated over the range 15°—45° C. For both acids the decrease of pK_2' was, on average, 0.095 for one degree rise in temperature.

DISCUSSION

van Urk [3] has investigated the indicator properties of 7-iodo-8-quinolinol-5-sulfonic acid. Feldman and Powell [4] have determined colorimetrically the ionization constants of this acid; the ionic strength and temperature are not given. From their values we obtain $pK_1' = 2.44$ and $pK_2' = 7.15$. These values are in good agreement with our values at ionic strength 0.05 (25° C), for which we obtained $pK_1' = 2.44$ and $pK_2' = 7.17$.

Phillips and Merritt [5] have determined spectrophotometrically the ionization constants of 8-quinolinol-5-sulfonic acid and have obtained $pK_1' = 4.004$ and $pK_2' = 8.86$ of which the first constant is in relatively good agreement with our value but the second constant differs somewhat more. Temperature and ionic strength are not stated. No other determinations seem to have been made.

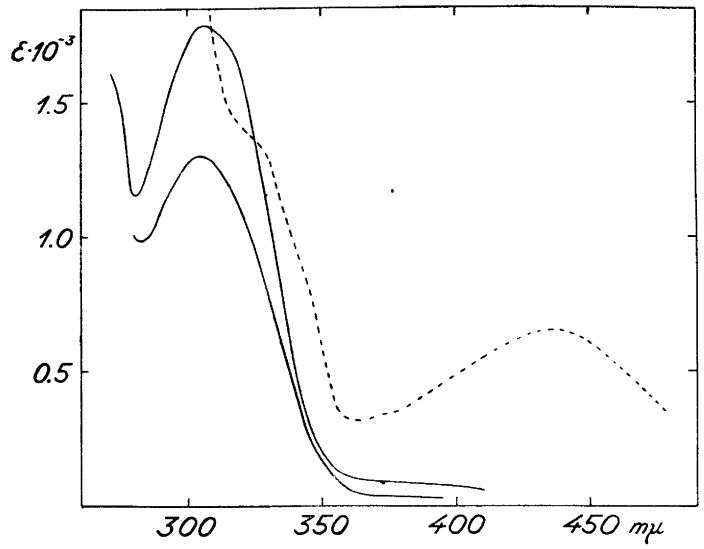
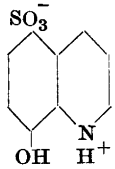


Fig. 3. Molar extinction coefficient of the HA^- *ions of 8-quinolinol-5-sulfonic acid (upper full line) and 7-iodo-8-quinolinol-5-sulfonic acid (dotted line) compared with that of* HOx *(lower full line).*

The earlier authors describe these compounds as typical ampholytes. Thus in acidic solution the reagents should exist as positive ions. The titration curve shows, however, that in moderately acidic solutions the reagents exist as neutral molecules. Because of the great acidic strength of the sulfonic group it is very probable that the reagents exist in acidic solutions as zwitterions like



For the same reason the importance of ions like H_3A^+ seems to be slight. The effect of ionic strength on ionization also shows that the compounds in question are dibasic acids. The limiting theoretical slope of the Debye-Hückel equation is namely for $pK_1' + 0.509$ and for $pK_2' + 1.527$ in the case of the sulfonic acid derivatives but -0.509 and $+0.509$ respectively for 8-quinolinol [2].

In figures 2—4 the ultraviolet spectra of the three constituents H_2A, HA^-, $A^=$ of the acids investigated are compared with the spectra of the corresponding constituents of the parent compound 8-quinolinol: H_2O^+, HOx, Ox^-.

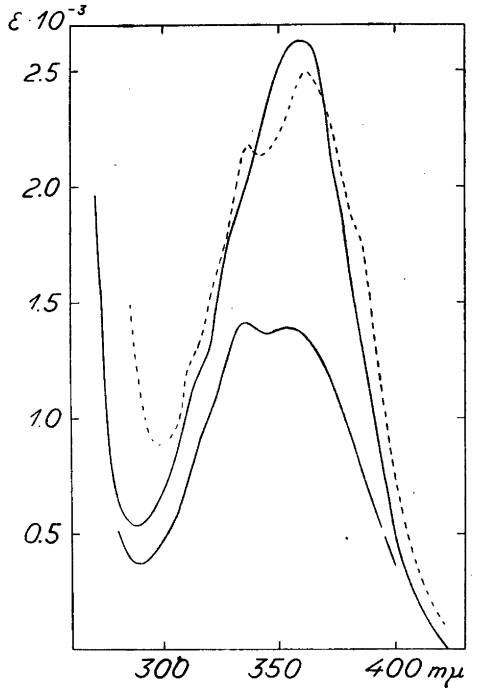
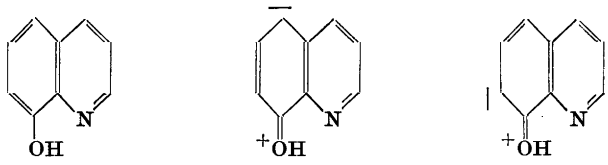


Fig. 4. Molar extinction coefficients of the $A^{=}$ *ions of 8-quinolinol-5-sulfonic acid (upper full line) and 7-iodo-8-quinolinol-5-sulfonic acid (dotted line) compared with that of* Ox^{-} *(lower full line).*

These spectra can be obtained directly. The spectrum of H_2A was obtained using a solution with a slight excess of a strong acid and the spectrum of $A^{=}$ using an excess of strong hydroxide. The spectrum of HA^{-} was obtained using a buffer solution with a pH corresponding to the first equivalent point. At this pH the constituents H_2A and $A^{=}$ have negligible effect on the spectra. In Fig. 2 the spectra of H_2A and H_2Ox^{+} are represented. About the same maxima appear in all cases. The effect of the substituents is, however, distinct. The maximum at 355—360 mμ (which can be related to the polar structure II of 8-quinolinol)



I II III

has been strengthened in the sulfonic acid derivatives. Also the double maximum of the sulfonic acid derivatives at 305—315 mμ is stronger than in the spectrum of H_2Ox^{+}, especially that of the 7-iodo-8-quinolinol-5-sulfonic acid.

In the latter case a shift to the longer wavelength appears. This maximum at 325 mμ also occurs as a little shoulder in the spectrum of the constituent HA^- of 7-iodo-8-quinolinol-5-sulfonic acid, as we see in Fig. 3, where the spectra of HA^- and HOx are represented, but is hardly noticeable in the spectrum of $A^=$ represented in Fig. 4. We further see from Fig. 3 that the constituent HA^- of the iodine derivative has a maximum at 435 mμ. This maximum does not appear in the spectra of the two other constituents of this derivative, probably because of the greater importance of the polar structures in these cases. From Fig. 4 we see that the constituent $A^=$ of 8-quinolinol-5-sulfonic acid has a very strong maximum at 355 mμ which corresponds to the structure II, but that the maximum at 335 mμ has degenerated to a shoulder. The constituent $A^=$ of the iodine derivative still has a relatively strong maximum also at 335 mμ. The spectra of $A^=$, like Ox^- display shoulders at 305—315 mμ. The spectra thus show that the substituent SO_3^- increases the importance of the quinoid structure II and the substituent I the importance of the polar structure III as expected. Both substituents raise the acid strength considerably as expected.

SUMMARY

The ionization of 8-quinolinol-5-sulfonic acid and 7-iodo-8-quinolinol-5-sulfonic acid has been investigated. The first and second ionization constants of these dibasic acids, previously treated as typical ampholytes, have been determined. The effect of ionic strength on the ionization has been investigated in potassium chloride solution. The ultraviolet spectra of the three constituents of these acids in aqueous solution have been measured and compared with those of 8-quinolinol. The structures and the effect of substitution on the ionization have been discussed.

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On the Interpretation of Dielectric Results on Sodium Thymonucleate. I. On the Relations Between Increment, Dipole Moment and Molecular Weight

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In recent years, dielectric measurements have become of increasing importance for the elucidation of the structure and mode of action of various substances. Special difficulties are, however, encountered in applying dielectric methods to physiologically important substances that are often poorly soluble in non-polar media. These difficulties are mainly of two kinds. Firstly, these substances often possess too high an electric conductivity that necessitates the use of special methods for the determination of the dielectric constant (DC). Secondly, the interpretation of the results is made more difficult by the fact that no generally applicable theory can be put forward that gives an absolute measure of the polarity of the molecules for solutions in polar solvents.

The object of the present and subsequent studies in this series is to correlate the dielectric results obtained on sodium thymonucleate, in the following denoted as DNA, with the conceptions obtained by other methods. This is all the more necessary since the structure of DNA, although very simple in principle, is of a very individual nature. Except for the nucleic acids it does not appear to be closely analogous to any other substances. The dipolar structure of DNA differs very considerably from that assumed for molecules in dielectric theories, owing to a pronounced molecular asymmetry with moment-bearing groups (in combination with electrolytic properties) superficially located. We shall, however, confine ourselves to a few simple aspects that appear to be of interest for the understanding of DNA in aqueous solution.

In our work on polynucleotides in this laboratory, we have chosen the ellipsoid method of R. Fürth [1]. We refer to our other publications [2,3] for the choice of method, the technique of measurement as well as the results. The method is a force method and was elaborated for use at a number of vary-

ing frequencies in the 0.03—38 Mc/s range. In principle, the method is fairly independent of the electric conductivity of the solution and its application to strongly conducting solutions is mainly limited by the heat convections. In some experiments a special technique has been used on solutions with relatively high electric conductivity [4].

The majority of studies were made on DNA from calf thymus prepared according to Hammarsten's procedure [5]. It is the best-defined thymonucleic acid preparation available, although it is not altogether ideal owing to some low protein content. Nevertheless, other specimens, produced with different methods and of varying origin, all proved to have the same dielectric regularity. Thus, the empirical relations found should be generally applicable to thymonucleic acid.

THE DIELECTRIC INCREMENT OF DNA

There is some contrast between the difficulties of calculating the dipole moment *in vacuo* from measurements in an aqueous solution and the simplicity and regularity often found in dielectric measurements. There is often very good agreement between various substances in a similar group of substances. The systematic study of those groups that can be varied in a definite way, for example by polymerization, was therefore found to be a suitable procedure for the investigation of the relation between the dielectric properties and the molecular structure, etc. The most appropriate investigations appear to be those made by J. Wyman Jr. and co-workers on amino acids and peptides. The conformity found has been of fundamental importance for all dielectric research, in particular for substances in aqueous solution. It is beyond the scope of the present paper to survey the results obtained by Wyman's school. We refer instead to the monograph "Proteins, Amino Acids and Peptides" by Cohn and Edsall (1943).

In aqueous solution, DNA shows an extremely high increment [2,3,6]. The increment is very dependent on the concentration and even in solutions of less than a few hundredths per cent, it deviates from linearity. Different preparations behave differently in this respect, and in extremely dilute solutions an increment is obtained, characteristic for the degree of polymerization of the specimen of DNA. We explain the deviation from linearity as an "association" in the general meaning of the term. This will be discussed further in a subsequent paper in the present series.

Table 1 gives some figures both for the increment per gram per litre and for the molar increment. Some of the results obtained by Wyman and coworkers are included for comparison. This shows, *inter alia*, the interesting relations between the increment and the molecular weight.

Table 1.

$M = n \cdot M_0$	n	I_g	δ	μ_K	$\mu_K \cdot n^{-x}$	μ_D	$\mu_D \cdot n^{-x}$
Amino acids and peptides		Temp. 25° C			$x = ½$		$x = ½$
Glycine 75	1	0.30	22.6	15.7	15.7	0.61	0.61
Di-glycine 132	2	0.53	70.6	28	19.7	0.91	0.64
Tri-glycine 189	3	0.60	113	32	20.2	1.04	0.60
Tetra-glycine 246	4	0.65	159	43	21.5	1.08	0.54
Penta-glycine 303	5	0.71	215	48	21.4	1.13	0.50
Hexa-glycine 360	6	0.65	234	50	20.4	1.14	0.47
Hepta-glycine 417	7	0.70	290	56	21.2	1.17	0.44
β-alanine 89	(2)	0.39	34.6	19.4	13.5	0.64	0.45
γ-aminobutyric acid 103	(3)	0.50	51	23.8	11.9	0.83	0.48
δ-aminovaleric acid 117	(4)	0.54	63	26.2	13.1	0.88	0.44
ε-aminocaproic acid 131	(5)	0.59	77	29.0	13.0	0.94	0.42
ς-aminoheptylic acid 145	(6)	0.60	87	30.1	12.5	0.97	0.39
Desoxyribo-nucleate		Temp. 20° C			$x = 1$		$x = 1$
DNA, from thymus [1]							
in $1.5 \cdot 10^{-3}$ N KCl 31 000	94	9.2	$2.9 \cdot 10^5$	1 750	18.7	76	0.81
in $8 \cdot 10^{-4}$ N NaCl 53 000	160	17.4	$9.2 \cdot 10^5$	3 150	19.7	137	0.85
in $4 \cdot 10^{-4}$ N NaCl 81 000	245	27	$2.2 \cdot 10^6$	4 850	19.8	205	0.84
in pure water 120 000	360	45	$5.3 \cdot 10^6$	7 600	21.3	315	0.88
in pure water 180 000	545	70	$1.3 \cdot 10^7$	11 700	21.6	495	0.90
DNA, from salmon [2]							
in pure water 390 000	1 200	160	$6.2 \cdot 10^7$	26 000	22.0	1 100	0.92
in pure water 540 000	1 600	210	$1.1 \cdot 10^8$	35 000	21.5	1 500	0.92
DNA, from thymus [3]							
in pure water 610 000	1 850	240	$1.5 \cdot 10^8$	40 000	21.4	1 700	0.92
DNA, from thymus [4]							
in pure water 840 000	2 500	350	$2.9 \cdot 10^8$	56 000	22.3	2 400	0.94

[1] Prepared according to Hammarsten (1924).

[2] Prepared according to Mirsky and Pollister (1942).

[3] Prepared according to Gulland, Jordan and Threlfall (1947).

[4] Recent measurements by Allgén (unpublished) for a specimen prepared by Signer and Schwander (1949) in 0.0025% aqueous solution.

The δ-values for amino acids and peptides are taken from Cohn and Edsall's review and refer to Wyman's measurements. The calculations and measurements on DNA are published by I. Jungner [3].

M is the molecular weight, M_0 the weight of the "monomer", n is the degree of polymerization (or number of carbon atoms between the charged groups). I_g is the dielectric increment per gram per litre (Edsall's $\Delta D_0/g$) and δ the molar increment. μ_K and μ_D are the dipole moments calculated according to Kirkwood ($\mu_K = 3.3 \cdot \sqrt{\delta}$) and Debye respectively.

For glycine peptides, Wyman found that the molar increment (δ) is proportional to the number (n_p) of the constituent amino acids. For the different monoaminomonocarboxylic acids (AA) the molar increment is proportional to the number of carbon atoms (m_{CH_2}) between the momentbearing groups.

This gives the following equations

$$\delta_p = const. \cdot n_p \qquad \delta_{AA} = const. \cdot m_{CH_2} \qquad (1a, 1b)$$

i.e.

$$\delta_p = const. \cdot M \qquad (2)$$

As a result, a solution of a certain concentration in weight/volume of peptides gives approximately the same increment irrespective of whether the amino acids are coupled as peptides or not, *i.e.* the increment per gram per litre (I_g) is constant. It must be pointed out that, in the case of the peptides, the increment was calculated from the DC of the solvent, whereas for DNA it was computed according to $\frac{(\varepsilon_l - \varepsilon_h)}{c}$ where ε_l and ε_h are the DC at respectively lower and higher frequencies than the actual dispersion range at the concentration c. Extrapolation to zero concentration according, for example, to Hedestrand [7] was not made, since the measurements can be made in such dilute solutions that the difference can be disregarded.

The relation given by Wyman between the increment and the molecular weight in peptides appears to be fairly generally applicable. Approximately the same order of magnitude for the increment is also obtained for proteins with considerable molecular weights and very appreciable differences in geometric shape. It would be tempting to draw conclusions from this fact in respect of the length of the chain of polypeptides in a protein molecule. Nevertheless, this connexion is presumably considerably more complicated than implied by the apparent simplicity and, in any event, more difficult to interpret than in the case of DNA.

The conditions for DNA are in contrast to these data obtained for analogous amino acids and peptides. For the former we find

$$I_{DNA} \simeq const \cdot M \qquad (3)$$

For DNA, the increment per gram per litre is thus a direct function of the molecular weight. This can be utilized in studying, for example, enzymatic degradation [3,8] and is in good agreement with the fact that the dielectric increment per gram per litre follows the increment of viscosity.

It is difficult to establish the connexion experimentally down to very low molecular weights. A solution of lowmolecular nucleotides has a very consider-

able electric conductivity which appreciably limits the possibility of obtaining exact values. Moreover, with a decreasing degree of polymerization, the increment becomes very low. On the other hand, the relation is established in a large molecular weight range from approximately 30 000 to 800 000. The regularity of the dielectric increment definitely implies a corresponding regularity in the structure which presumably applies to the whole DNA molecule.

There is found the following formula for the molar increment of DNA

$$\delta_{\mathrm{DNA}} \simeq const \; M^2 \tag{4}$$

THE DIPOLE MOMENT OF DNA

Debye's theory and computation of permanent dipole moments, founded on the equation of Clausius and Mosotti for the relation of molecular polarization, is actually only valid for gases but possibly applicable to cubic crystals as well. Experience has nevertheless shown that it can also be used for solutions of permanent dipoles in non-polar media. On the other hand, calculations with Debye's formula do not give the correct order of magnitude for the dipole moment in a polar solvent and particularly not for such a strongly polar solvent as water.

The best-known of the attempts to extend the applicability of Debye's theory is that of Onsager [9]. The pre-requisites for Onsager's theory, *i.e.* a spherical molecule with a centrally located dipole moment, are not fulfilled by DNA, but the conception of a dielectric reaction field must be borne in mind. The theory has been of considerable importance for the interpretation of the empirical relation for the dielectric increment. It has found a widespread use, for example, in the study of proteins. Certain of the conclusions drawn from the theory will therefore be discussed in the following.

Wyman interprets his results obtained on amino acids and peptides to imply that a simple relation also exists between the dipole moment and the increment. This is also apparent to some extent from Debye's theory in the expression for the dipole moment, μ_{D}, (T = the absolute temperature, c = the weight fraction, d = the density, k = Boltzmann's constant, N = Avogadro's number):

$$\mu_{\mathrm{D}}^2 = \frac{9 \cdot k \cdot T \cdot M}{4 \cdot \pi \cdot N \cdot c \cdot d} \left(\frac{\varepsilon_l - 1}{\varepsilon_l + 2} - \frac{\varepsilon_h - 1}{\varepsilon_h + 2} \right) \quad \text{e.s.u.} \tag{5 a}$$

$$\mu_{\mathrm{D}} = 0.0128 \cdot 10^{-18} \cdot \sqrt{\frac{M \cdot T \cdot 3 \; (\varepsilon_{\mathrm{l}} - \varepsilon_{\mathrm{h}})}{c \cdot d \cdot (\varepsilon_{\mathrm{l}} + 2) \; (\varepsilon_{\mathrm{h}} + 2)}} \quad \text{e.s.u.} \tag{5 b}$$

in which, as a good approximation, we can insert

$$\mu_{\mathrm{D}} \simeq const \cdot \sqrt{\frac{M \cdot (\varepsilon_{\mathrm{l}} - \varepsilon_{\mathrm{h}})}{c}} \tag{6}$$

As pointed out in the foregoing, the increment per gram per litre (I_g) for DNA is proportional to the molecular weight. From it we find the important relationship

$$\mu_{\text{DNA}} \simeq const. \cdot M \tag{7}$$

In comparison with this, Wyman's result implies that

$$\mu_{\text{peptide}} \simeq const. \cdot M^{\frac{1}{2}} \tag{8}$$

Under ideal conditions, the dipole moment can be determined in absolute units. Since, according to the definition, $\mu = e \cdot l$, this is an expression both of the size of the charge (e) and of the dipole length (l), *i.e.* the distance between the centroids of the positive charges and the corresponding negative charges. The mutual relationship between e and l cannot be deduced from the dipole moment. Whereas a polar solvent can be expected to have an effect on the charge that is hard to determine, a simple relation between the dipole length and the dipole moment can be anticipated. If μ_s denotes the dipole moment calculated according to Debye for a substance in a polar solvent and e_{eff} denotes the effective charge, and, correspondingly, l_{eff} the effective dipole length, we can write as a generalization

$$\mu_s = l_{\text{eff}} \cdot e_{\text{eff}} < \mu_{\text{vac}} \tag{9}$$

where μ_{vac} denotes the dipole moment valid for the vacuum.

For analogous substances we can assume that e_{eff} is influenced in a uniform way by a given solvent. The relation found by Wyman [10] for peptides thus implies that the dipole length of a polypeptide is not the sum of the dipole lengths of the constituent amino acids. Nor will the distance between charges of the different monoamino-monocarboxylic acids be the greatest possible (*i.e.* as in an extended chain). Nor is this to be anticipated, since an attraction between opposite poles must be taken into consideration. The relations given by W. Kuhn [11] and Eyring [12] are attempts to find a measure of the effective dipole length on a statistical basis or from theoretical computations. We shall not enter into these relations which are not of importance for DNA, but only note that they show good agreement with empirical data.

It is shown in Fig. 1 how the effective dipole lengths arise in principle and the conditions are compared in dipoles placed side-by-side and end-to-end. Since the dipole length of a polymerized molecule is proportional to the molecular weight, the relation between the dipole moment and the molecular weight in polymeric substances can be expressed generally as

$$\mu = a \cdot M^x \tag{10}$$

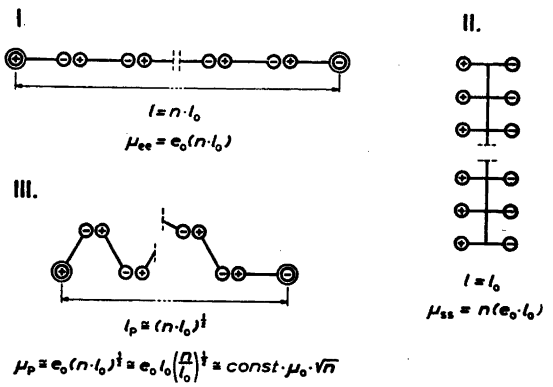


Fig 1. Dipole length in different kinds of polymerization
I. End-to-end with a completely extended chain
II. Side-by-side (as in DNA)
III. End-to-end with a folded chain (as in peptides).

where a is a constant and the exponent x can vary between 0 and 1. $x = 0$ then corresponds to a ring formation in which the partial dipole moments cancel each other, whereas $x = 1$ corresponds to a fully stretched chain of dipoles or one in which the dipoles are linked side-by-side polarly. For peptides and different monoamino-monocarboxylic acids, $x \simeq \frac{1}{2}$.

There is no reason to assume that the exponent for amino acids and peptides should be altogether constant at different dipole lengths. A deviation can be noted for both low and high glycine peptides [13]. It is interesting to note that the exponent for DNA lies in the immediate vicinity of 1. The exponent falls under the influence of various agents but only a small deviation can be noted on variation of the molecular weight by means of minimal quantities of salts.

From a dielectric aspect, it is irrelevant whether a dipole moment *in vacuo* is composed of an entirely straight chain of similar dipoles (end-to-end) or whether the dipoles lie side-by-side. The exponent x equals 1 in both cases. To sum up, it can thus be noted that, *as regards DNA, the calculations of the dipole moments show that the theoretical extreme case is present with the dipoles parallel and in the same direction.*

It must be pointed out that the formula (10) for the generalized relation between the dipole moment and the molecular weight corresponds to that given by Staudinger and co-workers [14] for the viscosity of long chain molecules. Dielectric measurements show on the whole many conditions that are analogous to the viscosity. The dependence of the increment (and thus also of the dipole moment) on the concentration appears to be of particular interest. As Huggins and co-workers [15] pointed out in respect to viscosity, a formula such as that given is usually an expression of whether the molecule is coiled into a ball-shape or not. By analogy, the formula for the dipole moment would then mainly be an expression of the effective dipole length.

The simple conditions of the dipole length in DNA indicate a possibility of ascertaining the effect of the solvent on the charges in the individual dipoles. For glycine, a dipole moment of 0.6 D (Debye units) in an aqueous solution is found by a calculation according to Debye. This is only a fraction of the dipole moment of approximately 15 D that could be established by Kirkwood [16] both from the structural formula and experimentally, although indirectly.

From the results obtained for DNA, we get a dipole moment of approximately 0.9 D per average mononucleotide. If the conditions were the same as for glycine, the individual average mononucleotide would have a dipole length of only approximately 4 Å. There is, however, a considerable difference between the placing of the charges in DNA and, for example, a polypeptide. In DNA, all the moment-bearing groups are in more or less intimate contact with the solvent, whereas in the polypeptide only the extreme charges can be affected by it. The fact that the dipole moment of DNA is relatively small in comparison with, for example, glycine, although the effect of the solvent should be relatively greater indicates that it should be possible, as a generalization, to express the effect of the solvent as a decrease in the charge (and the dipole moment). We can assume that the function for this effect includes a dependence both on the dipole moment of the solute (μ_1) and of the solvent (μ_2), as well as on the effect of the Na ions (α_{Na}), the intensity of the outer field and other factors. If, on the basis of this simple assumption, we compare the dipole moment μ_{ss} of substances with dipoles arranged side-by-side and μ_{ee} of dipole molecules arranged end-to-end, we find that

$$\mu_{ss} = n \cdot \mu_0 - n \cdot f(\mu_1 \cdot \mu_2 \cdot \alpha_{Na}) = n\mu_0 - n\Delta\mu \tag{11}$$

$$\mu_{ee} = n\mu_0 - \Delta\mu \tag{12}$$

When $\mu_{ss} < \mu_{ee}$, it is explicable on the basis of this conception that the dipole moment computed according to Debye for DNA will be relatively low even in comparison to other dipoles in aqueous solution.

In this connexion, we can mention attempts to determine the dipole moment *in vacuo* from determinations on aqueous solutions according to other methods of calculation. Wyman's formula for molecular polarization, P,

$$P = \frac{M}{d} \cdot \frac{\varepsilon + 1}{g} \qquad \text{cm}^3 \tag{13}$$

has been of great importance. The factor g is obtained empirically. It results in the following expression for the dipole moment (μ_w) of a substance in a solution

$$\mu_w = const \cdot \sqrt{\frac{M \cdot T}{c \cdot d} \cdot \frac{\varepsilon_l - \varepsilon_h}{g}} \tag{14}$$

This empirical relation has been borne out by Onsager's theory. The value of the factor g must, however, be determined. Wyman, on the basis of a large number of substances, found 8.5 to be the most useful mean figure, although with considerable variations. The relatively low figure of 5.8 was obtained for amino acids (Oncley [17]).

A prerequisite for the calculation according to Wyman is an approximate knowledge of the dipole moment *in vacuo* of some substance within the intended group. A value for g obtained in this way can then be used for similar substances. The true dipole moment for DNA can only be estimated very roughly and with a number of assumptions from the structural formula for the mononucleotides. Provided that the mononucleotide can be interpreted as a simple dipole ion with a charge that does not differ essentially from the elementary charge, the moment will depend on the length of the dipole. From the structural formula, the distance from the oxygen atoms in phosphoric acid to, for example, N_3 can be estimated as approximately 10 Å. The distance does not presumably fall below 7—8 Å nor exceed 16 Å. Under these conditions — which thus require a number of assumptions — the dipole moment can be estimated as between 35 D and 80 D with a value of approximately 50 D as the most probable.

If these values are tested in Wyman's formula, very low values are obtained for g, 50 D corresponding to approximately 2. Such a figure deviates very considerably from all others and gives reason for much doubt. If, in order to obtain an idea of the physical implication of such a value of g, the value for the moment *in vacuo* is tested according to Onsager's theory, the "internal refractive index" is found to be less than 1. The test shows that the assumptions are not valid. Since a mononucleotide presumably has a high dipole moment of the stated order of magnitude, the testing indicates that DNA — as could be anticipated — does not correspond to the prerequisites for Onsager's theory.

Kirkwood [16] in a general statistical theory of dielectric polarization, extended Onsager's theory. He then used a dipole moment, μ_K, which also includes the effect of a moment induced by the adjacent molecules. The theory leads approximately to the expression

$$\mu_K = 3.30 \cdot \sqrt{\delta} \qquad \text{e.s.u.} \qquad (15)$$

This gives very good agreement with the values of, for example, peptides. A similar dipole moment as with Wyman's calculation is, however, obtained for DNA. Other empirical relations, for example according to Cohn [18] also give the same low figures of the order of magnitude of 20 D. For the present we refrain, in accordance with the views put forward by Arrhenius [19], from

attempts to calculate a dipole moment that is correct as regards the order of magnitude as given by Wyman, Onsager and others and confine ourselves to the calculation according to Debye. The results of the different theories only differ essentially in the attempts to obtain absolute values for the dipole moment. The calculation according to Debye is serviceable for obtaining *relative* values.

THE DIELECTRIC METHOD FOR THE DETERMINATION OF THE MOLECULAR WEIGHT

The molecular weights used for DNA were calculated, as were the dipole moments, from the dielectric dispersion curve. In principle, the way in which the molecular weights were obtained is of no importance. As, however, the measuring technique is thus common to both the values compared, there is reason to discuss the extent to which the calculations are mutually dependent.

There is obviously some uncertainty when it is necessary to deviate from the simple principles for the computation of molecular weights. In the case of amino acids and known polypeptides, these are obtained with great accuracy from their chemical structure. The accuracy in these cases depends mainly on how well defined the preparation is. In the case of DNA, it is a question of obtaining — in addition to the determination of the polarity — as exact a value as possible for the molecular weight with the use of a cumbersome method. The dielectric method for the determination of molecular weight of DNA is based on the following formulae

$$M = \frac{N \cdot k \cdot T \cdot \lambda_c}{3 \cdot v \cdot \eta \cdot 2\pi\bar{c} \cdot \psi_2} \cdot \frac{\varepsilon_h + 2}{\varepsilon_l + 2} = const. \frac{\lambda_c (\varepsilon_h + 2)}{(\varepsilon_l + 2)} \tag{16}$$

(N = Avogadro's number, $6.02 \cdot 10^{23}$, k = Boltzmann's constant, $1.38 \cdot 10^{-16}$ ergs per molecule, $\bar{c}$ = velocity of light, $3 \cdot 10^{10}$ cm/sec., ψ_2 = Perrins' shape-factor, 4/3, η = viscosity of water, 0.01 Poise, v = specific volume of DNA = 0.50 cm^3/g).

The random errors in the determination thus derive essentially from the error in λ_c since the variations in the expression $\left(\frac{\varepsilon_h + 2}{\varepsilon_l + 2}\right)$ are of minor importance.

From equation (5) we can derive

$$\mu = const. \cdot \sqrt{\frac{M \cdot (\varepsilon_l - \varepsilon_h)}{(\varepsilon_h + 2)(\varepsilon + 2)}} \tag{17}$$

for the dipole moment. The insertion of the expression for the molecular weight from the dielectric data then gives

$$\mu = const. \sqrt{\frac{\lambda_c \cdot (\varepsilon_1 - \varepsilon_h)}{(\varepsilon_1 + 2)^2}} \simeq const. \sqrt{\lambda_c \cdot I} \tag{18}$$

It is then evident that the dielectric molecular weight mainly depends on λ_c, *i.e.* the position of the dispersion curve along the axis of frequency, whereas the dipole moment is dependent on $\sqrt{\lambda_c \cdot I}$. Thus, two different conditions are compared. The characteristic of DNA is the expression $\frac{\mu}{n}$, as is seen from the following

$$\mu = const. \sqrt{M \cdot I} = const. \sqrt{n \cdot I} \tag{19}$$

$$\frac{\mu}{n} = \bar{\mu}_N = const. \sqrt{\frac{I}{n}} \tag{20}$$

On the basis of this computation, the determination of the dipole moment per average mononucleotide ($\bar{\mu}_N$) is made, this being a very good characteristic of the polarity of the molecule as a kind of "specific" polarity.

It must be pointed out in this connexion that the dielectric method for the determination of the molecular weight of DNA gives considerably lower values than the methods generally used. Owing to the uncertainty of Perrin's form factor, used as a correction for deviations from the spherical shape of the molecule, the method cannot be used as an absolute method for globular molecules. It should, however, be stressed that, for globular proteins, the use of molecular weights obtained in other ways gave form factors that were in agreement with the results in respect to the molecular shape. The uncertainty in the form factor is eliminated in the case of thread molecules. The method gives the molecular weight in absolute measures from the specific volume and the dielectric data alone. The determination should refer to the hydratized molecule. We assume, however, that DNA is moderately hydratized and that only a fairly insignificant number of water molecules accompanies it on orientation.

We refer the discrepancies in the results as compared with those by ultracentrifuge, diffusion measurements, streaming birefringence, etc. to the difficulty of following the individual molecule in an isolated state in the case of extreme thread molecules with high "specific" dipole moments.

As will be discussed in more detail in a later paper, the exchange energy of DNA increases so considerably with the degree of polymerization that the

mutual interaction becomes too strong even at considerable distances. We therefore feel that the dielectric method for the determination of the molecular weight, based on the effect of an external electric field on dipoles, gives a more accurate magnitude in the case of the individual molecule. Moreover, the method allows a variation in the solvent's content of, *inter alia*, electrolytes, although to a limited extent. This permits a study of the molecular weight under various conditions [3] which is very informative.

It is well known that, for example, the sedimentation *constant* varies with the concentration as an expression of the mutual interaction. The dielectric method also shows a peculiar relation to the concentration [3,6]. With a falling concentration, the molecular weight rises, whereas we should rather have anticipated a very slight shifting of the critical wave-lengths towards higher frequencies. This effect is also certainly caused by a dipole interaction and is explained by the fact that the free orientation of the molecules is prevented in stronger solutions.

An uncertainty in the dielectric method for the determination of the molecular weight appears to be the use of the macro-viscosity of the solvent. The structure of DNA is such that it readily allows hydrogen bonds with the surroundings. We do not in DNA, as for example, in carboxyhaemoglobin [19], find the theoretical relation in solutions containing glycerol. It is true that in the case of DNA (unpublished) it is difficult to obtain exact values with the method used, but it is evident that the use of the macroviscosity is not altogether correct. If this systematic error were considerable, it would nevertheless imply that the molecular weights found were too high. The deviation from the results of other methods is thus not due to the viscosity of the solvent, which presumably gives rise to an error of minor importance. This also appears reasonable since DNA is a long and stiff rod somewhat flattened at the sides and very large in relation to the surrounding water molecules.

The general conception of the shape and appearance of the molecule corresponds to the regularity shown for DNA by the dielectric method. The comparison with, for example, the viscosity is particularly informative. The details of the theoretical calculations are also in very good agreement with experiences with X-ray crystallography and streaming birefringence. They show the reliability of the dielectric method for the determination of the molecular weight and also that the use of a relative value for the dipole moment according to Debye is justified.

Its high increment and marked dielectric regularity appear to render DNA a suitable and in principle simple model substance for dielectric studies under conditions that are theoretically difficult to handle.

SUMMARY

The results of dielectric studies on sodium thymonucleate (DNA) are reported, mainly for comparison with the relations found for peptides and amino acids, in particular by Wyman's school. It is found that the dielectric increment per gram per litre of DNA is directly proportional to the molecular weight instead of being approximately constant, as is the case in peptides and mono-amino-monocarboxylic acids of the type $NH_2(CH_2)_n \cdot COOH$.

As a result, the dipole moment calculated according to Debye shows a direct proportionality to the molecular weight. This is interpreted as an expression of the fact that DNA has a polar structure with simple dipoles (mononucleotides) placed side-by-side in conformity with the generally accepted view. The dependence of the dipole moment on the molecular weight is found to be the theoretically extreme case, implying the maximum of polarity for a multipole.

The results and the comparison with the conditions in amino acids and peptides give reason to generalize the connexion between the dipole moment (μ) and the molecular weight (M) in the following form

$$\mu = const. \cdot M^x$$

where $1 \geqq x \geqq 0$. The value for $x = 1$ is thus obtained for DNA, whereas in peptides $x \simeq 1/2$.

This relation is completely analogous to Staudinger's relation between the intrinsic viscosity and the molecular weight. The exponent x varies with the shape of the molecule, *i.e.* with the resulting length of the dipole.

The possibility is discussed of expressing the dipole moment in absolute values with the use of an empirical factor g, in accordance with Wyman's suggestion. For DNA, g would then be of the order of magnitude of 2. This is a very low figure and therefore gives rise to considerable doubt. The calculation according to Debye is therefore accepted instead and the value for the dipole moment is considered to be a relative value.

The calculated dipole moment for an average mononucleotide in poly-DNA is approximately 0.9 Debye. This is low and is explained by the fact that the moment-bearing groups are superficially located and are considerably influenced by the polar solvent.

Finally, the dielectric method for the determination of the molecular weight of stiff thread molecules is discussed. The conclusion is reached that the dielectric method can be expected to give more correct values for thread molecules with high transverse dipole moments than methods in which the mutual dipolar effect plays a considerable rôle and the conditions in the individual

molecule are harder to determine. The possibility of varying the properties of the solvent, particularly as regards its content of electrolytes, makes the method more advantageous in some respects than a number of others.

The results of the measurements in aqueous solution of poly-DNA are in accordance with the general conception of the substance. They therefore support the usefulness of the dielectric method for the determination of the molecular weight and indicate possibilities of using it in fields inaccessible with other methods.

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On the Interpretation of Dielectric Results on Sodium Thymonucleate. II. The Dipole Association of Highly Polymerized Molecules

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Dielectric studies of sodium desoxyribonucleate (DNA) in aqueous solution have revealed that the interaction of the molecules plays a greater rôle than could be assumed *a priori* in such dilute solutions as a few hundredths per cent.

It is a generally valid fact that the association tendency increases with the dipole moment. As DNA in aqueous solution has a very high increment also implying a very high dipole moment, the intermolecular forces of attraction can therefore also be expected to be considerable. With many physico-chemical methods DNA evidences a number of deviations from the known rules, supposedly due to "association". The term "association" has a very wide connotation and will therefore be more closely defined later.

In dielectric studies DNA also shows anomalies [1]. In solutions with a concentration as low as 0.1 per cent, the anomalous dispersion takes place with a distinct deviation from the theoretical course. In measurements in still more dilute solutions, the dielectric dispersion appears at a lower frequency, *i.e.* it then corresponds to a longer relaxation time and a higher molecular weight. Also the dielectric increment per gram per litre increases as the concentration decreases. This fact indicates the presence of a non-polar association even in 0.1 per cent solution but that later — as expected — it decreases with dilution.

From another aspect as well DNA shows properties corresponding to association. The molecule can easily be disaggregated in extremely dilute aqueous solutions by, for example, the addition of a minute quantity of electrolytes. This takes place with the retention of the "specific" polarity of the molecule, *i.e.* the same dipole moment per mononucleotide is obtained, as opposed to

the conditions in higher salt concentrations where the polarity decreases appreciably. This aggregation-disaggregation corresponds to the variation in the polar "association". Such a preponderance of a purely polar association in extremely dilute solutions is surprising. The fact that an "association" and dipolar interaction occur to such an extent and in so distinctly different ways gave rise to this investigation.

Fürth's ellipsoid method [2] adapted for high frequency [3,4] was used for the determination of the dielectric constant (DC) of solutions with considerable electric conductivity. The method is not, however, suitable for experiments at, for example, varying temperatures nor does it permit, as would be desirable, a number of other investigations. In the present study therefore, the conditions will instead be considered theoretically and an attempt made, in conjunction with available experimental data, to understand the behaviour of such high polymerized dipoles (multipoles) as DNA in aqueous solution. The first question will then be to investigate the effect of the degree of polymerization of DNA, whose simple dipoles are the mononucleotides.

THE APPLICABILITY IN THE CALCULATION OF THE EXCHANGE ENERGY FOR DIPOLES IN SOLUTION

Referring to an earlier paper in the present series [5] the structural formulae for DNA can be replaced, from a dielectric point of view, by a simple model. DNA consists of a number of mononucleotides, packed closely above each other, with the purine and pyrimidine groups pointing in one direction and the phosphoric acid groups in the opposite direction. The mononucleotides can be interpreted as dipolar ions in which less important differences in the moment-bearing groups can be disregarded. We can therefore consider DNA as a polarly polymerized multipole in which the simple and similar dipoles lie side-by-side. The resulting transverse dipole moment, μ, which caused the dielectric dispersion, will then be

$$\mu = \mu_0 \cdot n = n \cdot e \cdot l \tag{1}$$

where μ_0 is the dipole moment of the individual average mononucleotide, n is the number of mononucleotides, *i.e.* the degree of polymerization, e is the charge ($+e$ and $-e$ respectively) of the momentbearing groups and l is the distance between the positive and negative centres of gravity in each mononucleotide.

With greater distances between the molecules, corresponding to the extremely dilute solutions that will mainly be studied, it can be anticipated that the electrostatic forces will essentially outweigh the induction and disper-

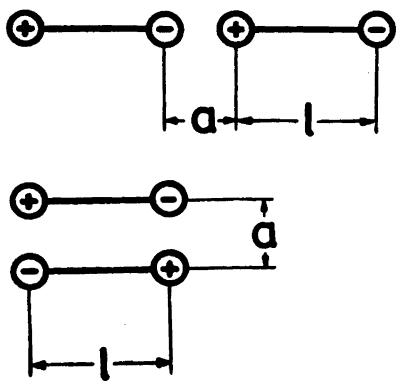


Fig. 1. Different forms of association in simple dipoles.
Upper figure: polar association.
Lower figure: non-polar association.

sion effects. In the following we shall therefore only deal with these Coulombic forces.

The mutual interaction between the dipoles is dependent on the size of the exchange energy. The greater the energy, the smaller is its activity and the smaller is the possibility for the dipoles of orientating freely in an electric field.

The attraction between two charges, $+e$ and $-e$, corresponds to an exchange energy, E which according to Coulomb's law is

$$E = -\frac{e^2}{\varepsilon \cdot r} \tag{2}$$

where ε is the DC for the intermediate medium and r the distance between the charges.

The question is then the extent to which different values for E can be compared. The "effective" charges are considered to be equal. We can assume the DC should lie around 80 in the field outside the molecule. In the immediate vicinity of the charges, on the other hand, the difference must be considerable and approach the value *in vacuo*. We can also assume a low value for the internal field within the DNA molecule despite a large number of polar groups. In the case of the field strengths between solute dipoles in dilute solutions, it is, however, only with relatively small inter-molecular distances that the lines of the forces fail to enter the solvent. If we compare the exchange energy between dipoles at considerable distances, we can thus assume that the effect of the polar solvent is constant. $\bar{\varepsilon}$ is then the expression of the effective DC that can be envisaged to arise, taking into consideration the thickness of the layers with different dielectric constants.

When the exchange energy is to be compared under conditions that are fairly similar with the exception of the distance, we are thus relatively independent of minor variations in the effective charges and the resulting DC of the solvent. We can therefore write

$$E = -\frac{e^2}{\bar{\varepsilon}} \cdot \frac{1}{r} \simeq const. \cdot r^{-1} \tag{3}$$

A comparison of the different value for E thus shows that this mainly depends on the distance.

The main forms of association to be discussed in the following are the polar and the non-polar (with anti-parallel arrangement). As can be inferred from Fig. 1, the exchange energy for simple dipoles can be calculated from the following equations:

$$\overrightarrow{\rightarrow} E = -\frac{e^2}{\bar{\varepsilon}}\left[\frac{1}{a} + \frac{1}{a+2l} - \frac{2}{a+l}\right] = -\frac{e^2}{\bar{\varepsilon}} \cdot \overrightarrow{\rightarrow} P \tag{4}$$

$$\rightleftharpoons E = -\frac{e^2}{\bar{\varepsilon}}\left[\frac{2}{a} - \frac{2}{\sqrt{a^2+l^2}}\right] = -\frac{e^2}{\bar{\varepsilon}} \cdot \rightleftharpoons P \tag{5}$$

$\overrightarrow{\rightarrow} E$ is then the energy with polar and $\rightleftharpoons E$ with non-polar association, a the shortest distance between the dipoles and l the length of the dipole. The mean distance between the dipoles, r, is thus respectively a and $(a + l)$.

A definition of the function for the distance is introduced into equations (4) and (5). This *position function*, P, evidently determines the variation in the exchange energy. Moreover with our assumptions, the relation between the position functions is the same as that between the exchange energies.

In the case of simple dipoles, Duncken [6] set up an equation for the relation, Φ, between the energies with these different forms of association

$$\Phi = \frac{\rightleftharpoons E}{\overrightarrow{\rightarrow} E} = \frac{\rightleftharpoons P}{\overrightarrow{\rightarrow} P} = \frac{2\left(\frac{1}{\sqrt{1+l^2/a^2}} - 1\right)}{\frac{2}{1+l/a} - \frac{1}{1+2\,l/a} - 1} \tag{6}$$

The value of Φ at different distances between the dipoles is given in Fig. 4 (p. 1412). It gives the impression that in the case of simple dipoles it is the relative dipole length, *i.e.* l/a, that is decisive. It can be seen that the polar association predominates at greater distances.

In order to simplify the problem, the calculations are made only for those values applicable to DNA. Thus, for the dipole length, 10 Å is taken as the most probable value for the distance between the charges within the mononucleotides. Although it is not essential for the calculations to fix this figure it is important to bear in mind that *a relatively great dipole length* must be counted on for DNA. Only in the case of extremely large distances between the dipoles, of an order of magnitude in which it is not as a rule necessary to take the exchange energy between the molecules into account, can the dipole length in relation to the distance be disregarded. For very strong dipoles such as DNA, these conditions are, however, of great interest. Simpler calculations are then very useful. We can recall that with parallel dipoles with dipole moments μ_1, and μ_2 at a distance of r, the bound energy is obtained by means of

$$E = \frac{\mu_1 \cdot \mu_2}{\bar{\varepsilon} \cdot r^3} (2 \cos \varphi_1 \cdot \cos \varphi_2 - \sin \varphi_1 \cdot \sin \varphi_2) \qquad (7)$$

where φ_1, and φ_2 are the angles between the dipole axis and the straight line between the centres. In extreme cases with polar and non-polar association,

$$\varphi_2 = 180° + \varphi_1 \ (= \alpha)$$

and the following simple relations are obtained

$$\overrightarrow{\leftarrow} E = - \frac{\mu_1 \cdot \mu_2}{r^3}$$

$$\overrightarrow{\rightarrow} E = - \frac{2 \cdot \mu_1 \cdot \mu_2}{r^3} \qquad (8a, b)$$

In the study of the effect of the degree of polymerization to be made in the following, the distance, d, between the dipoles is also required. This has been established from X-ray data to be approximately 3.4 Å [7].

THE EXCHANGE ENERGY BETWEEN POLARLY POLYMERIZED MULTIPOLES SUCH AS DNA

It is necessary, for the calculation of the exchange energy between two molecules, X and Y, to know the position functions for the individual dipoles in relation to the dipoles in the other molecule. The association forms are seen schematically in Fig. 2. The position function, P, for each pair of simple dipoles is obtained as follows. $P_{1,1}$ denotes the position function for the terminal simple dipoles in each molecule, and $P_{1,2}$ the position function for

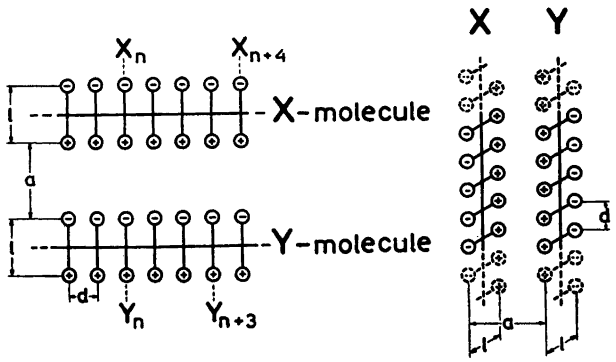


Fig 2. Schematic representation of polar (on the left) and non-polar association of two multipoles with the same structure as DNA.

the terminal dipole to the neighbour of the corresponding dipole in the other molecule, etc. We then obtain the following expression

$$\vec{P}_{1,1} = \frac{1}{a} + \frac{1}{a+2l} - \frac{2}{a+l}$$

$$\vec{P}_{1,2} = \frac{1}{\sqrt{a^2+d^2}} + \frac{1}{\sqrt{(a+2l)^2+d^2}} - \frac{2}{\sqrt{(a+l)^2+d^2}}$$

$$\vec{P}_{1,3} = \frac{1}{\sqrt{a^2+4d^2}} + \frac{1}{\sqrt{(a+2l)^2+4d^2}} - \frac{2}{\sqrt{(a+l)^2+4d^2}}$$

As a generalization, this therefore gives the following for *polar* association

$$\vec{P}_{1,n} = \frac{1}{\sqrt{a^2+(n-1)^2d^2}} + \frac{1}{\sqrt{(a+2l)^2+(n-1)^2d^2}} - \frac{2}{\sqrt{(a+l)^2+(n-l)^2d^2}} \qquad (9)$$

$\rightleftharpoons$ In the same way, the following general expression for the position function, P, is deduced for *non-polar* association

$$\overset{\rightleftharpoons}{P} = \frac{2}{\sqrt{a^2+(n-1)^2d^2}} - \frac{2}{\sqrt{a^2+l^2+(n-1)^2d^2}} \qquad (10)$$

It can easily be seen that the exchange energy, E_n, for two multipoles with the degree of polymerization n for the simple dipoles is obtained from the position functions

$$-\frac{\varepsilon}{e^2} \cdot E_n = n \cdot P_{1,1} + 2(n-1) \cdot P_{1,2} + 2(n-2) \cdot P_{1,3} + \ldots . 2 \cdot P_{1,n} \qquad (11)$$

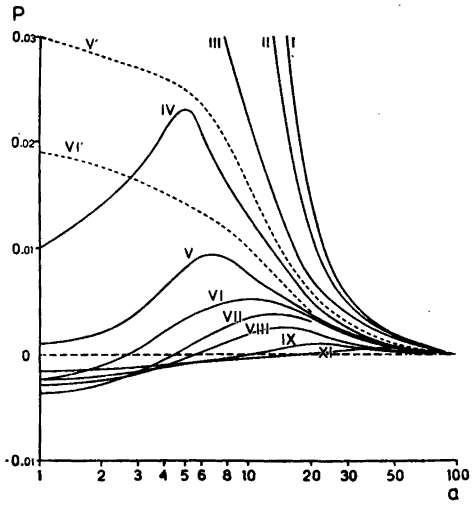


Fig. 3. The variation in the polar position functions for different distances between multipoles (see text). By way of comparison the non-polar position functions $P_{1,5}$ and $P_{1,6}$ are included (dotted lines).

No difficulties are encountered in the calculation of the position functions although it is a laborious procedure. Since the component variables are of the same order of magnitude and must be varied within a very wide range, it is necessary to calculate a large number of position functions and thence to obtain a relative value for the exchange energy.

It is convenient to use substitution with trigonometrical functions. It is then found that

$$\overrightarrow{P} = t \cdot [\cos \alpha + \cos \beta - 2 \cos \gamma] \tag{12}$$

$$\rightleftharpoons P = 2\ t \cdot [\cos \alpha - \cos \delta] \tag{13}$$

where

$$\tan \alpha = a \cdot t;\ \tan \beta = (a + 2l) \cdot t;\ \tan \gamma = (a + l) \cdot t;\ \tan \delta = t \cdot \sqrt{a^2 + l^2} \text{ and } t = \frac{1}{(n-1) \cdot d}$$

The cosine functions can then be obtained directly from the tangent values with suitable tables.

The series of which the exchange energy is composed converges slowly and a relatively large number of terms must be included and estimated. It is however, possible to calculate the first and most important terms and then to estimate the remainder by means of the integral calculus. The error in the sum of the integrals is then low.

The position functions obviously decrease with increasing distances but this change takes place in different ways. With non-polar association, the

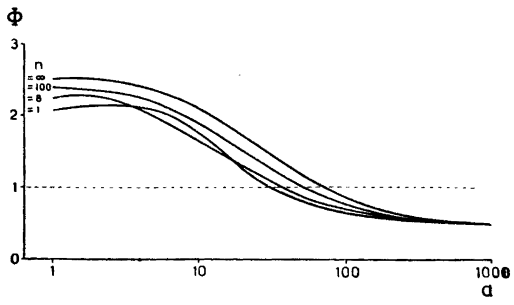


Fig 4. The relation between the interaction with non-polar and polar association (Φ) and distance between the dipoles (a) with different degrees of polymerization (n).

decrease in $\overrightleftharpoons{P}$ is regular. For $\overrightarrow{P}$, on the other hand, even $\overrightarrow{P}_{1,4}$ reaches a distinct maximum. From $\overrightarrow{P}_{1,6}$ and onwards, a resulting repulsion instead of attraction takes place at short distances between the multipoles. The repulsion is maximal for $\overrightarrow{P}_{1,7}$ and $\overrightarrow{P}_{1,8}$ depending on the distance between the multipoles but then decreases rapidly. Fig. 3 shows this variation in the position functions. It should, however, be noted that the absolute values for the exchange energy are not directly comparable for small distances between the multipoles. They have been included in order to follow the course in principle and are only of qualitative significance.

The numerical values for some $\overrightleftharpoons{P}$ and $\overrightarrow{P}$ at various distances and degrees of polymerization are given in Table 1. It is found, as anticipated, that the position functions at distances that are very large in comparison with the dipole length vary very insignificantly with moderate degrees of polymerization. This makes it possible to give a simple approximate expression for the dependence on the degree of polymerization. If we insert in equation (11) the value for the position function P as constant and equal to $\overline{P}$, we obtain

$$-\frac{\varepsilon}{e^2} \cdot E = \overline{P}\ [n + 2(n-1) + \ldots\ldots 2] = \overline{P} \cdot n^2 \qquad (14)$$

We can write, as a generalization,

$$E = -const. \cdot n^x \qquad (15)$$

in which the exponent x deviates little from the value *1* with a low n and a but rapidly approaches the borderline value 2 at greater distances. We can therefore conclude that *the degree of polymerization has a relatively greater effect at larger distances*, a fact that is of importance for the understanding of DNA in very dilute aqueous solutions. It then also follows that the exchange energy for molecules such as DNA can be very considerable as a result of high

degree of polymerization, even at distances where the dipolar exchange energy can usually be disregarded.

The relation between the exchange energies in non-polar and polar associations is of particular interest. This is shown in Fig. 4. As seen, the effect of the degree of polymerization throughout is such that the non-polar association can be expected to predominate. This effect is greatest at moderate distances and only at distances of 100 Å and more is the effect of the degree of polymerization less. It is interesting to note that in comparison with the conditions for simple dipoles, as calculated by Dunken, the distance at which equilibrium is attained, from the energy view point is displaced from approximately 30 Å to approximately 100 Å. This implies that at all distances at which a firmer and actual association combination occurs, this always has a decided tendency to be non-polar.

DISCUSSION AND CONCLUSIONS

The result of the calculations of the exchange energy with polar and non-polar association has a number of consequences for the conception of DNA. A very strong tendency to non-polar arrangement must be assumed with short distances between the dipoles. On the other hand, the dielectric experiments show the occurrence of molecular aggregates whose formation is entirely polar. Results obtained with other methods show in addition a pronounced thread shape. It is necessary to reconcile these findings. In addition to the explanation by means of molecules assembled end-to-end, we can envisage the formation of aggregates by a polar linking of projecting parts. Such a molecule would be approximately thread-shaped and correspond to all available data. The foregoing calculations nevertheless definitely indicate that such an interpretation is incorrect. We must therefore conclude that the formation of larger aggregates takes place end-to-end despite the fact that considerable electrostatic forces oppose such a polar polymerization.

As a result, we must envisage other forms of linking between the molecules to form a larger aggregate. We must assume that the union between two basic molecules is brought about by relatively strong short-distance forces. Since the slight disaggregation with electrolytes likewise indicates that it is predominantly electrostatic, the nature of this linking is fairly well defined. This problem will be treated in a later paper.

The effect of electrolytes on DNA is reversible so that the same aggregate weight is obtained after dialysis [3]. It could be expected that the aggregation, under the influence of the strongly dipolar interaction would not regain a high degree of polar structure. The reversibility therefore appears to require another mechanism in addition to the specific connecting forces.

Table 1. Some values of the position functions ($P_{n,n+m}$).

Distance, a (in Å)	1	3	5	8	10	20	50	100	1000
Position Functions *Polar* Arrangement									
$P_{1,1}$	.866	.222	.107	.050	.033	.009	$95 \cdot 10^{-5}$	$15 \cdot 10^{-5}$	$19 \cdot 10^{-8}$
$P_{1,2}$	.173	.123	.079	.042	.029	.008	$95 \cdot 10^{-5}$	$15 \cdot 10^{-5}$	
$P_{1,3}$	.045	.044	.040	.028	.022	.007	$92 \cdot 10^{-5}$	$15 \cdot 10^{-5}$	
$P_{1,4}$	.010	.017	.023	.016	.013	.005	$87 \cdot 10^{-5}$	$15 \cdot 10^{-5}$	
$P_{1,5}$	.001	.004	.008	.009	.008	.005	$82 \cdot 10^{-5}$	$14 \cdot 10^{-5}$	
$P_{1,6}$	−.002	.000	.003	.004	.005	.004	$76 \cdot 10^{-5}$	$14 \cdot 10^{-5}$	
$P_{1,7}$	−.004	−.001	.000	.002	.002	.003	$68 \cdot 10^{-5}$	$13 \cdot 10^{-5}$	
$P_{1,8}$	−.003	−.002	−.001	.000	.002	.002	$61 \cdot 10^{-5}$	$13 \cdot 10^{-5}$	
$P_{1,9}$	−.002	−.002	−.001	.000	.000	.001	$53 \cdot 10^{-5}$	$13 \cdot 10^{-5}$	
$P_{1,10}$	−.002	−.001	−.001	−.001	.000	.000	$45 \cdot 10^{-5}$	$12 \cdot 10^{-5}$	$19 \cdot 10^{-8}$
Non-polar Arrangement									
$P_{1,1}$	1.801	.475	.221	.094	.059	.011	$78 \cdot 10^{-5}$	$10 \cdot 10^{-5}$	$10 \cdot 10^{-8}$
$P_{1,2}$	.383	.260	.161	.074	.052	.009	$78 \cdot 10^{-5}$	$10 \cdot 10^{-5}$	
$P_{1,3}$	.130	.112	.090	.049	.040	.007	$76 \cdot 10^{-5}$	$10 \cdot 10^{-5}$	
$P_{1,4}$	.057	.053	.046	.033	.026	.006	$73 \cdot 10^{-5}$	$10 \cdot 10^{-5}$	
$P_{1,5}$	.030	.027	.027	.020	.017	.004	$70 \cdot 10^{-5}$	$10 \cdot 10^{-5}$	
$P_{1,6}$	.018	.016	.014	.012	.010	.004	$66 \cdot 10^{-5}$	$10 \cdot 10^{-5}$	
$P_{1,7}$	.011	.011	.010	.009	.009	.004	$62 \cdot 10^{-5}$	$9 \cdot 10^{-5}$	
$P_{1,8}$	.007	.007	.007	.007	.006	.004	$58 \cdot 10^{-5}$	$9 \cdot 10^{-5}$	
$P_{1,9}$	.006	.006	.005	.005	.004	.002	$53 \cdot 10^{-5}$	$9 \cdot 10^{-5}$	
$P_{1,10}$	.004	.004	.004	.003	.003	.002	$48 \cdot 10^{-5}$	$9 \cdot 10^{-5}$	$10 \cdot 10^{-8}$

We consider that a satisfactory explanation is provided by according greater significance to the dipole directive forces discussed in this paper. It is only necessary to complement the views put forward by Bernal and Fankuchen [8] and Ferry [9] and others by stressing the *orientation* of the molecules. We then interpret DNA in dilute aqueous solutions as a quasi-crystalline structure. (*cf.* Girard and Abadie [10]). For it is characteristic of thread molecules that they have a tendency in solution to form longitudinal parallel streaks with a hexagonal formation in the cross-section. Fig. 5 shows how this conception is envisaged. It must be emphasized that the stability of this structure in solution is essentially dependent on the very large transverse dipole moment. Such an interpretation of the conditions in DNA appears to explain the

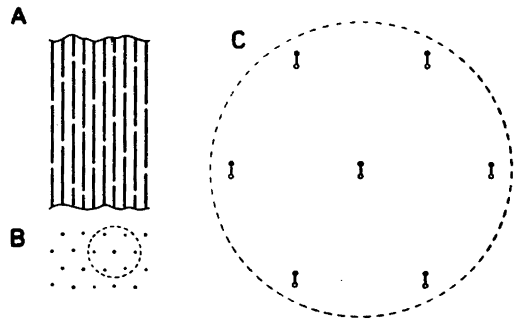


Fig. 5. Structural formation of thread molecules in solution.
A shows the parallel positions of the thread molecules.
B cross-section of the structure.
C enlargement of the encircled part of B, showing the orientation of the thread molecules.

reversibility. *It is the orientating effect of the surrounding molecules that permits the smaller units to be polarly — end-to-end — joined on re-polymerization and to overcome the Coulombic forces emanating from the basic molecules.* The fact that if disaggregation is very advanced it becomes more difficult to obtain reversibility is thereby explained. The lower units cannot form a sufficiently pronounced quasi-crystalline structure to permit a spontaneous reformation of larger aggregates.

In DNA, disaggregation and aggregation appear to be the expression of an unusually pronounced *one-dimensional reactivity* (*v.* Hammarsten's discussion[11]). This property can be thought to appear under physiological conditions. *In vivo*, the dipole orientation by, for example, high polar proteins may correspond to the orientating effect of the neighbouring molecules *in vitro*.

In analogy to the other dielectric measuring results, the conception of the quasi-crystalline structure of DNA in dilute aqueous solutions explains the fact that the anomalous dispersion range is displaced towards higher frequencies at rising concentrations. The smaller the intermolecular distance becomes, the more is the free orientation prevented as a result of the dipolar exchange effect. Such an explanation for aqueous solutions is extremely schematic but nevertheless appears warranted.

Finally, the term "association" must be more closely defined. In DNA, as in similar thread molecules, nucleohistone and possibly in viruses and many other substances both a true molecular association between individual molecules and a dipolar interaction at greater distances must presumably occur. These phenomena are observed in the same way in dielectric measurements as a relatively too low value for the dielectric increment. In the case of high-molecular and high-polar substances, the conditions in solution are often hard to define and DNA, for example, often shows different kinds of association simultaneously. Even if it is not considered possible in extreme cases to differentiate clearly between these forms, it is nevertheless suitable for

practical purposes to make use of association as a common term. It is thought to be incorrect to deny the existence of associated aggregates of large molecules. The conditions found in DNA are almost certainly to be encountered in other high-molecular substances as well. Briefly, we prefer to use the term association, although it is applied in its widest connotation.

SUMMARY

A calculation is made of the exchange energy between associated polymeric multipoles with the probable dimensions of sodium thymonucleate (DNA). Strictly speaking, such a calculation is only valid for the conditions *in vacuo*. It is, however, found that, with considerable distances between the dipoles, comparable values are also obtained for solutions in polar media.

The exchange energy increases with the degree of polymerization. This increase is most rapid when the distance between the dipoles is large, and thus under conditions that most nearly correspond to extremely dilute solutions. Presumably, only multipoles with very high transverse dipole moments show interaction at such large distances between the dipoles as are usually found in dilute solutions.

The relation between the exchange energy in polar and non-polar association, calculated for simple dipoles by Dunken, is displaced on polymerization to the advantage of non-polar association. At short distances between the dipoles, a considerable predominance of the non-polar aggregate of association can be anticipated.

The results are discussed and lead to the postulation of a quasi-crystalline structure for solutions of thread molecules. The importance of large transverse dipole moments is stressed. Certain effects in dielectric measurements of DNA are explained on the basis of the structural formation.

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Reactions of Ethyl Disulphide and Alkyl Iodides in Presence of Mercuric Iodide

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INTRODUCTION

The purpose of this work was partly to clarify reactions of simple compounds containing sulphur and having analogy to wool keratin, and partly to attempt transferring these reactions to wool. The wool keratin has a unique position among the proteins owing to the high sulphur contents, which amount to 3—4 % and are of the greatest importance for the properties of wool.

In keratin the main peptide chains are linked together laterally by sulphur in the cystin. The bridges between the chains, the disulphide bonds, are relatively easily attacked. If therefore the disulphide bond could be transformed into a more stable bond by chemical means, an improvement in the resistance of wool to attack might result. Several attempts have been made but so far with only limited success.

In the first part of the investigations ethyl disulphide was used, the reactions with alkyl iodide and alkylene diiodide in presence of mercuric iodide being examined.

THEORETICAL

Hilditch and Smiles [1] have described reactions of ethyl disulphide and ethyl iodide in presence of mercuric iodide and state that they have been able to isolate some unknown intermediate reaction products. In spite of a most careful investigation it has been impossible to reproduce these compounds.

The reaction of ethyl disulphide and ethyl iodide gives the following products in 2—3 months:

$$C_2H_5S-SC_2H_5 + 4C_2H_5J = 2(C_2H_5)_3SJ + J_2 \quad (1)$$

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The said authors suppose that the reaction passes through the following stage:

$$C_2H_5S-SC_2H_5 + C_2H_5J = (C_2H_5)_2(C_2H_5S)SJ \quad (2)$$

and that the thioethyl group subsequently is split off and replaced by an ethyl group with liberation of iodine. The intermediate product — diethyl thioethyl sulphonium iodide — should be impossible to isolate in this case as it is rapidly converted into triethyl sulphonium iodide.

If, however, mercuric iodide is present in the reaction mixture, the velocity of the addition is considerably increased, and by interrupting the reaction after a certain time, the authors are of the opinion that they had isolated diethyl thioethyl sulphonium iodide as a double salt with mercuric iodide (3). By prolonged action Hilditch and Smiles found triethyl sulphonium iodide with one or two molecules mercuric iodide, (5) and (4), and liberated iodine.

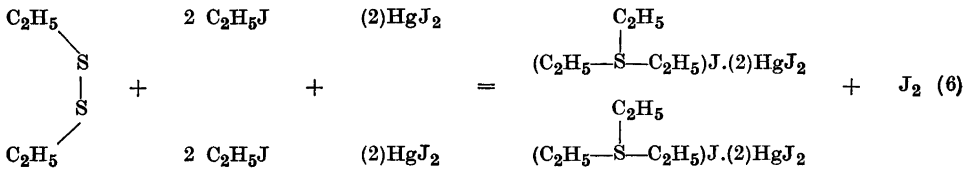
Reagents	*Time*	*Main product*	
Equimolecular proportions of ethyl disulphide and mercuric iodide	12 hours	$(C_2H_5)_2(C_2H_5S)SJ \cdot 2HgJ_2$	(3)
with excess of ethyl iodide.	3 days	$(C_2H_5)_3SJ \cdot 2HgJ_2$	(4)

Some of the experimental data from the paper are compiled, *viz.:* the melting points and the combustion analyses of the alleged intermediate (3) and the final products (4) and (5), and the theoretical values for carbon and hydrogen in the said compounds.

(3)	$C_6H_{15}Hg_2J_5S_2$	(1187)	M.p.	104° C	Calc.	C	6.07	H 1.274
					Found	»	5.88	» 1.19
(4)	$C_6H_{15}Hg_2J_5S$	(1155)	M.p.	115—116° C	Calc.	»	6.239	» 1.309
					Found	»	6.44	» 1.31
(5)	$C_6H_{15}HgJ_3S$	(700)	M.p.	110° C	Calc.	»	10.29	» 2.14
					Found	»	10.40	» 2.03

By calculating the discrepancy between the values found for (3) and the theoretical values for (4) in percent, it gives the differences: C = −5.75 %, H = −9.09 %. Due to the low contents of carbon and hydrogen the possibility can not be excluded that the authors have analysed an impure triethyl sulphonium compound.

A thorough investigation by the present author has shown that it is impossible to isolate the intermediate diethyl sulphonium iodide even in presence of mercuric iodide. It appears most probable that the sulphur-sulphur bond in the disulphide is split and that the thioethyl groups immediately form the triethyl sulphonium iodide (6).



The experiments were carried out with ethyl disulphide, mercuric iodide and the following iodides: 1) Ethyl iodide; 2) methyl iodide; 3) *n*-propyl iodide; 4) *n*-butyl iodide; 5) trimethylene diiodide. All the monoiodides reacted according to equation (6). The velocity of reaction varied with the different iodides. With methyl iodide the velocity was greatest, in 2—3 hours the mercuric iodide was dissolved, and the first crystals could be isolated after only 1 hour. With ethyl iodide crystals were isolated after 10—14 hours, whereas the reaction with *n*-propyl iodide took considerably more time.

The symmetrical triethyl sulphonium iodide was most readily obtained as crystals. From the reaction mixture with methyl iodide as well as from that with *n*-propyl iodide yellow oils first separated. These oils crystallized after being kept for some time, and the crystalline products were easily purified. The product of the reaction with *n*-butyl iodide did not crystallize — even on sharp cooling. With trimethylene diiodide a yellow oil was first precipitated, from this a yellow powder separated. The powder was completely insoluble in all common solvents and could accordingly not be recrystallized.

In the cases where crystalline compounds were formed, analyses of the products were made. In every case it was shown that the crystals first formed were identical with those precipitated at a later stage. In none of the experiments a thioethyl compound could be isolated, but respectively:

$(C_2H_5)_3SJ \cdot HgJ_2$	melting	point	110° C
$(C_2H_5)_3SJ \cdot 2HgJ_2$	»	»	115—116° C
$(CH_3)_2(C_2H_5)SJ \cdot HgJ_2$	»	»	84—85° C
$(n\text{-}C_3H_7)_2(C_2H_5)SJ \cdot HgJ_2$	»	»	74—75° C

With trimethylene diiodide the reaction takes a course different from that of the monoiodides. A comparison between the found composition (see Exp.) and some theoretical values shows a poor agreement. The high contents of iodine found indicate that — at least to some extent — only one of the iodine atoms of the trimethylene diiodide has reacted, possibly in conjunction with some chain or ring formation. The high melting point — the product did not melt at 350° C — may likewise be taken as suggesting a high molecular weight.

EXPERIMENTAL

1) *Ethyl iodide.* Equivalent portions of ethyl disulphide and mercuric iodide were weighed and about three times the calculated amount of ethyl iodide was added, acetone being used as a solvent. In some cases the excess of ethyl iodide used was sufficient to act as a solvent in itself.

The mixture was shaken continuously at room temperature and the time of reaction varied from a few hours to several days. In all cases the reaction mixture turned brown

after a few hours, the reaction proceeding most rapidly with ethyl iodide as a solvent. Undissolved mercuric iodide was filtered off and the brown solution which contained iodine was decolourized with charcoal. On addition of ether yellow needles were precipitated, these were recrystallized in different ways: a) By cooling a hot, saturated acetone solution; b) by precipitating from an acetone solution by means of ether; c) by precipitating from an acetone solution by means of water; d) by cooling a hot, saturated solution in ethyl alcohol; e) by cooling a hot, saturated solution in methyl alcohol.

In all cases the melting point of the product was 110° C, whether the mixture had reacted for 10—14 hours or for several days. This product should be the compound: $(C_2H_5)_3SJ \cdot HgJ_2$ (5), and the analysis corroborates this.

By using two equivalents of mercuric iodide in relation to the ethyl disulphide, the crystals separating were of a stronger yellow hue. They were easily decomposed by treatment with ether, and were therefore purified by concentrating the acetone solution *in vacuo*. The substance melted at 115—116° C with separation of mercuric iodide. These crystals correspond to the composition: $(C_2H_5)_3SJ \cdot 2HgJ_2$ (4).

The crystals most readily formed, whether produced in a short or a long time, melt at 110° C, irrespective of the way in which they have been purified. Pure crystals of m.p. 104° C have been impossible to obtain. With different combinations of (4) and (5) the melting points are lower than for either of them. A 1 : 1 mixture melted at 90° C whereas all other mixtures gave melting points between 90 and 115° C.

Iodine was estimated according to Baubigny-Chavanne [2].

Mercury was estimated in two ways: 1) By conductometric titration with sodium sulphide. The substance was dissolved in acetone and water added till near precipitation. A calculated amount of silver nitrate was added and the very slightly soluble silver iodide was filtered off. The solution was diluted with water to appr. 200 ml and titrated with 0.5 *N* sodium sulphide. The method gives distinct equivalent points. 2) By digestion and weighing as mercuric sulphide according to Rupp-Nöll [3].

(4) $C_6H_{15}Hg_2J_5S$ (1155) M.p. 115—116° C	Calc.	Hg	34.74			J	54.95
	Found	»	34.80	(method 2)		»	54.96
(5) $C_6H_{15}HgJ_3S$ (700) M.p. 110° C	Calc.	»	28.64			»	54.35
	Found	»	28.70	(method 1)		»	54.43
	»	»	27.96	(method 2)		»	

The experimental methods as described for ethyl iodide were also used in the case of the other iodides.

2) *Methyl iodide.* By precipitating with ether a yellow oil first separated which crystallized in the course of a few minutes.

After purification the crystals were needleshaped, m.p. 84—85° C.

$C_4H_{11}HgJ_3S$ (672)	Calc.	C	7.14	H	1.65	Hg	29.80		J 56.61
	Found	»	7.25	»	1.58	»	28.60	(method 1)	» 56.55
						»	29.50	(method 2)	

3) n-*Propyl iodide.* By precipitating with ether a yellow oil separated and from this oil large crystals were slowly formed.

They were easily purified by recrystallization, m.p. 74—75° C.

$C_8H_{19}HgJ_3S$ (728)	Calc.	C	13.19	H	2.63	Hg	27.54		J 52.25
	Found	»	12.43	»	2.66	»	26.82	(method 1)	» 52.70
						»	27.93	(method 2)	

4) n-*Butyl iodide.* It was impossible to make the yellow oil formed crystallize and for this reason an analysis was not undertaken. The velocity of reaction according to

equation (6) was, however, determined in the same manner as for the other alkyl odides.

5) *Trimethylene diiodide.* From the yellow oil precipitated on the addition of ether a yellow powder separated on standing. The product could not be recrystallized, because once precipitated, it could not be redissolved. It was thoroughly washed with acetone before the analysis was carried out.

Some theoretical values are calculated and compared with the found composition.

(a)	$C_5H_{11}HgJ_3S$	(684)	Calc.	C	8.78	H	1.62	Hg	29.30	J 55.62
(b)	$C_5H_{11}Hg_2J_5S$	(1139)	»	»	5.28	»	0.97	»	35.22	» 55.71
(c)	$C_{10}H_{22}HgJ_3S_2$	(914)	»	»	13.1	»	2.42	»	21.93	» 55.50
(d)	$C_8H_{17}HgJ_5S$	(980)	»	»	9.8	»	1.75	»	20.46	» 64.73
			Found	»	12.1	»	2.16	»	21.7	» 61.5

In (a), (b) and (c) are supposed that both, in (d) only one of the iodine atoms in trimethylene diiodide have reacted. The agreement is best for (c). But the high value of iodine contents as compared with the theoretical one in (a) and (b), may be taken to indicate that not all the iodine atoms in the trimethylene diiodide have reacted, and that some reaction according to (d) may have occurred.

Determination of the velocity of reaction of ethyl disulphide, mercuric iodide and alkyl iodides

From the equation:

$$C_2H_5S\text{-}SC_2H_5 + 4\ \text{alkyl-J} + n HgJ_2 = 2\ (\text{alkyl})_2(C_2H_5)SJ.nHgJ_2 + J_2 \qquad (7)$$

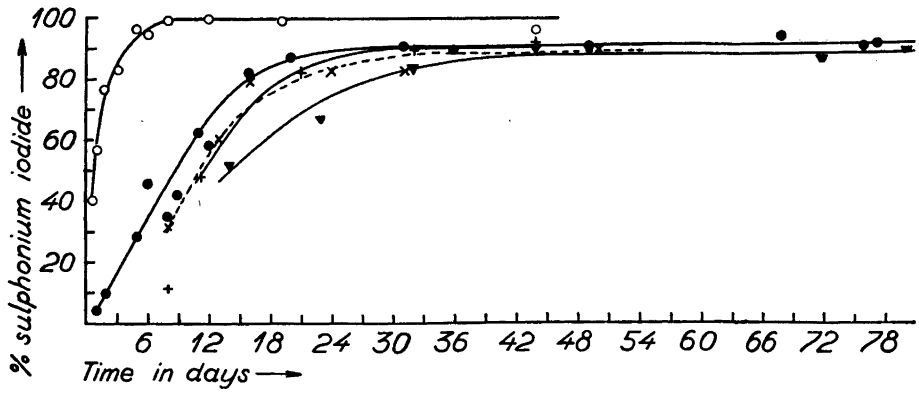
it is seen that iodine is liberated in an amount equivalent to the sulphonium salt formed, and by determination of the iodine the amount reacted can be estimated. The iodine was determined by titration with standard sodium thiosulphate solution. As mentioned above, it turned out that the reaction proceeded most rapidly when alkyl iodide was used in such an excess that it acted as a solvent. During the reaction the mixtures were shaken continuously.

1 g ethyl disulphide and 7.5 g mercuric iodide were weighed and then mixed with 4—6 times the calculated amount of alkyl iodide in ground glass-stoppered flasks. The samples withdrawn were diluted with acetone and titrated with 0.1 *N* sodium thiosulphate solution (with samples containing much iodine, 0.2 *N* solution was used).

In the graph the results of the velocities may be compared.

Hilditch and Smiles[1] state that the reaction between ethyl iodide and dimethyl sulphide in presence of mercuric iodide is complete in 15 minutes. In comparison the reaction of ethyl iodide and ethyl disulphide appears rather slow, indicating that the reaction is of an other kind. Though the first crystals could be isolated after 10 hours reaction, the yield in 30 days was only 90 %.

Though the value for the liberated iodine are hardly sufficiently accurate to allow an exact analysis of the reaction, it is seen that the curves found can



Velocity of reaction by the formation of the five different sulphonium iodides, all of them with complex bound mercuric iodide.

—○— *Dimethyl ethyl sulphonium iodide.*
—●— *Triethyl sulphonium iodide.*
—+— *Di-n-propyl ethyl sulphonium iodide.*
—▼— *Di-n-butyl ethyl sulphonium iodide.*
—×— *Product from trimethylene diiodide.*

not easily be reconciled with the assumption of the formation of a comparatively stable intermediate product. Were namely a diethyl thioethyl sulphonium compound first formed this reaction should take place without liberation of iodine, and the velocity of iodine formation should consequently increase from zero up to a certain point. In other words, a twostage reaction with a stable intermediate product should be indicated by a curve with a point of inflection.

Some of these reactions were carried out on wool, but in this case other groups, for instance the amino groups, can also react and cause complications. Investigations on the supercontraction of the wool fibers made it, however, possible to show some change in the disulphide links. The supercontraction of the fibers compared with increase in weight indicated that the disulphide bonds had reacted to some extent. Following cases were examined: a) Untreated wool; wool treated with b) mercuric iodide; c) methyl iodide and mercuric iodide; d) trimethylene diiodide and mercuric iodide; e) methyl iodide; and f) trimethylene diiodide.

SUMMARY

The intermediate compound diethyl thioethyl sulphonium iodide mercuric iodide described by Hilditch and Smiles could not be reproduced. By treating ethyl disulphide with 1) ethyl iodide in presence of mercuric iodide, only

triethyl sulphonium iodide with one and two molecules mercuric iodide were isolated. The formation of triethyl sulphonium iodide liberates iodine as stated by Hilditch and Smiles. According to equation (6) the reaction liberates one equivalent of iodine, which makes it possible to determine the velocity of the reaction. The present author made also experiments with ethyl disulphide and the following alkyl iodides: 2) Methyl iodide; 3) *n*-propyl iodide; 4) *n*-butyl iodide; and 5) trimethylene diiodide. The alkyl monoiodides give dialkyl ethyl sulphonium iodide mercuric iodide. The products from methyl iodide and from *n*-propyl iodide were — like those from ethyl iodide — crystalline, and they were all analyzed. The product from *n*-butyl iodide did not crystallize.

It was impossible to clarify the reaction mechanism between ethyl disulphide and trimethylene diiodide, but the insoluble reaction product was analyzed.

The velocities of all reactions mentioned were determined and compared. Methyl iodide reacted most rapidly.

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An X-Ray Study of the Phases in the Silver-Zirconium System

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No structure determinations of the intermediate phases in the silver-zirconium system have been published earlier. In 1929 Sykes [1] prepared silver-zirconium alloys with a zirconium content up to about 33 at. % and reported the existence of a phase with a still higher zirconium content. He further stated that the solid solubility of zirconium in silver is very small. Raub and Engel [2] in 1948 published a constitutional diagram for the silver-zirconium system up to about 50 at. % zirconium. They could not detect any solid solubility of zirconium in silver by X-ray or micrographic methods and consider it to be less than 0.1 at. % at 900° C. Raub and Engel found an intermediate phase AgZr with a small homogeneity range. The powder photograph reflections indicated a tetragonal lattice with a structure probably similar to the CuAu superlattice. In an alloy with 61.6 at. % zirconium they found another intermediate phase with unknown composition.

EXPERIMENTAL

The starting materials for the preparations were zirconium, prepared by thermal dissociation of the iodide (Foote Mineral Co., Philadelphia) and silver prepared by reduction in solution according to a method given by Barth and Lunde [3]. The finely divided metals were carefully mixed and moulded into pastils. Specimens with a weight of about 1 g were melted in zirconia crucibles in a high-frequency vacuum induction furnace [4] under a pressure of $10^{-4}-10^{-3}$ mm mercury. Since silver as well as zirconium reduces zirconium dioxide at elevated temperatures, precautions had to be taken to reduce the contact area between the pastil and the crucible and to avoid excessive reaction temperatures. Melted samples were then heat-treated in sealed, evacuated silica tubes maintained at various temperatures up to 1 100° C and quenched in cold water.

The phase analysis and structure determinations were made from powder photographs taken with CuK_α radiation in a Guinier-type camera using a curved quartz monochroma-

tor. For measurements of the high-angle reflections, additional photographs were taken with Phragmén-type focusing cameras. The intensities of the reflections were visually estimated, starting from 4:2:1 as the relative values of the intensities of CuK_α, K_{α_2}, and K_β lines. Relative values of $p|F|^2$ were calculated by means of the following expressions:

1. for the Guinier-type camera a formula calculated by Hägg [5]:

$$I = c \cdot \frac{1 + \cos^2 2\Theta \cdot \cos^2 2\Theta_m}{\sin^2 \Theta \cdot \cos \Theta} \cdot \frac{1}{\cos (2\Theta - a)} \cdot e^{-\frac{\mu \cdot t}{2 \cos (2\Theta - a)}} \cdot$$

$$\cdot \left(1 - e^{-\frac{u_f \cdot h}{\cos (2\Theta - a)}}\right) \cdot p|F|^2$$

where Θ_m = the glancing angle in the monochromator, μ_f = the linear absorption coefficient of the emulsion layer, and h = the thickness of this layer. The product $\mu_f \cdot h$ is determined experimentally.

2. for the Phragmén-type camera a formula given by Hägg and Regnström [6].

Observed and calculated values of $p|F|^2$ are given in Tables 1 and 3.

Single crystals could not be obtained of any of the intermediate phases.

THE STRUCTURE OF THE PHASES

The α Phase. For pure silver the axial length $a = 4.080$ Å ($V = 67.92$ Å^3) was determined. Powder photographs taken with alloys containing up to 50 at. % zirconium and quenched from different temperatures, showed α phase reflections whose lattice parameter did not vary. This agrees with the result obtained by Raub and Engel [2].

The β Phase. Reflections from a pure phase — the β phase — appeared in a powder photograph of an alloy containing 50 at. % zirconium which had been kept at 900° C for 10 hours. At temperatures between 900° C and 1 100° C the alloy samples evidently reacted with the silica tube. The corresponding photographs showed a few weak extra lines which could not be indexed.

The photographs indicated a tetragonal cell for the β phase. The smallest dimensions (at 50 at. % zirconium) observed were $a = 3.468$ Å, $c = 6.603$ Å, $c/a = 1.904$ ($V = 79.41$ Å^3), and the largest dimensions $a = 3.476$ Å, $c = 6.629$ Å, $c/a = 1.907$ ($V = 80.08$ Å^3). If Vegard's law is assumed to be valid within this narrow homogeneity range this implies a width of the range of less than 3 at. %. The composition closely corresponds to the formula AgZr. With four atoms in the unit cell the density calculated of the composition AgZr is 8.33. The observed value was 8.20.

The absence of the $hk0$ reflections, for which $h + k = 2n + 1$, is characteristic for the space-groups C_{4h}^3—$P\ 4/n$ and D_{4h}^7—$P\ 4/nmm$. The phase is

Table 1. Observed and calculated $p\,|F|^2$ values for the a phase.

$h\,k\,l$	$p\,\|F\|^2$		$h\,k\,l$	$p\,\|F\|^2$	
	Observed	Calculated		Observed	Calculated
001	17	16	215	0	4.7
002	0	0.1	116	0	0.1
101	0	0.6	224	17	13
110	150	169	313	0	1.3
102	250	298	321	0	0.0
111	6	6.6	304	0	0.0
003	0	2.5	007	0	1.0
112	0	0.1	206	0	0.1
103	17	20	322	30	27
200	85	70	314	30	22
201	0	3.1	107	0	4.9
004	30	24	225	0	3.0
113	0	4.9	216	17	15
202	0	0.1	323	0	2.8
211	0	0.1	117	0	3.5
104	0	0.2	305	0	1.8
212	150	124	400	6	6.8
114	50	46	401	0	0.3
203	0	2.3	315	6	6.5
005	0	2.7	402	0	0.0
213	0	8.5	411	0	0.0
105	0	4.1	324	0	0.1
220	10	16	207	0	4.1
221	0	0.8	008	0	1.2
204	17	22	226	0	0.1
115	0	5.7	330	6	9.1
222	0	0.0	412	30	36
301	0	0.0	331	0	0.5
214	0	0.1	403	0	0.9
006	0	0.0	217	10	15
310	17	21	108	0	0.2
302	17	19	306	10	13
311	0	1.0	332	0	0.0
223	0	0.7	413	0	6.7
205	0	4.0	118	0	9.4
106	0	9.4	325	0	9.5
312	0	0.0	316	0	0.4
303	0	1.8	420	30	37

Table 2. Interatomic Distances in the β Phase. (50 at. % Zirconium.)

Atom	Surrounding Atoms	Interatomic Distance, Å
Ag in 2 (*a*)	4 Ag	2.812
	4 Zr	2.952
	1 Zr	3.037
Zr in 2 (*a*)	4 Zr	3.107
	4 Ag	2.952
	1 Ag	3.037

isomorphous with γ—CuTi[6] and thus has a B 11 structure. The four atoms can be placed in only two two-fold positions 2 (*c*) : 0 $\frac{1}{2}$ z : $\frac{1}{2}$ 0 z in the space-group P^7_{4h}—P 4/*nmm*. The best intensity agreement and the best distribution in space were obtained for the values $z_{Ag} = 0.105$ and $z_{Zr} = 0.645$. Calculated and observed $p\,|F|^2$ values are recorded in Table 1, and the values of interatomic distances may be found in Table 2.

It may be well to point out that attempts were made to prepare Ag—Zr—O alloys in order to ascertain the possible existence of metallic oxides of low oxygen content, analogous to the Cu—Ti—O system where the oxide Cu_3Ti_3O [7,8] has been found. The starting materials were silver, zirconium and zirconium dioxide. At the melting point of silver a rather strong evolution of oxygen began to take place. Powder photographs proved this reduction process to be almost complete. The resulting X-ray pattern from the melt corresponded to the phases in the binary metal system. Only very weak extra lines were visible.

The γ Phase. Reflections from this phase appeared faintly in photographs taken with a specimen containing 60 at. % zirconium. But at the same time α-zirconium reflections appeared. Maximum intensity of the γ phase reflections was obtained with zirconium contents between 70 and 80 at. %. In spite of prolonged heat-treatments it was not possible to reach equilibrium or to obtain this phase pure. Photographs of an alloy with 85 at. % zirconium still contained very weak β phase lines.

The composition of the γ phase evidently corresponds closely to the ideal formula $AgZr_3$ and as a matter of fact the structure has been found to be isomorphous with the structure of $CuTi_3$ and $CuZr_3$[7], which may be considered as a tetragonal deformed $AuCu_3$ (L 12) type. The smallest and largest dimensions observed were : $a = 4.566$ Å, $c = 3.986$ Å, $c/a = 0.873$ ($V = 83.10$ Å^3)

Table 3. Observed and Calculated $p\,|F|^2$ Values for the γ phase.

$h\,k\,l$	$p\,\|F\|^2$		$h\,k\,l$	$p\,\|F\|^2$	
	Observed	Calculated		Observed	Calculated
100	1	0.5	320	0	0.0
001	0	0.2	222	10	13
110	1	0.3	113	10	12
101	1	0.4	302	0	0.0
111	150	131	321	0	0.1
200	50	51	312	0	0.1
210	0	0.2	203	0	0.0
002	17	18	400	6	4.4
201	0	0.2	213	0	0.0
102	0	0.1	410	0	0.0
211	1	0.3	401	0	0.0
112	0	0.1	331	6	6.8
220	17	22	420	6	6.8
300	0	0.1	004	0	1.7
202	50	42	402	6	6.6
221	0	0.1	313	10	12
310	0	0.1	204	6	5.1
212	0	0.1	422	10	10
301	0	0.1	511	10	9.9
311	50	42	224	6	5.4
003	0	0.0	333	6	5.5
103	0	0.0	440	0	3.6

and $a = 4.582$ Å, $c = 3.989$ Å, $c/a = 0.871$ ($V = 83.75$ Å^3), indicating a homogeneity range of approximately 3 at. %.

The space-group of the γ phase is D_{4h}^1—$P\,4/mmm$ with 1 Ag in $1(a)$: 0 0 0 and 3 Zr in $1(c)$: $\frac{1}{2}\,\frac{1}{2}\,0$ and $2(e)$: $0\,\frac{1}{2}\,\frac{1}{2}$; $\frac{1}{2}\,0\,\frac{1}{2}$. The observed and calculated $p\,|F|^2$ values for this structure are compared in Table 3. The interatomic distances of closest approach (8 coordination) are 3.030 Å and 3.038 Å, respectively at the lower and upper phase boundaries.

As the γ phase disappeared in photographs of specimens quenched from 800° C and higher temperatures this phase evidently only exists below this temperature. It was not possible to determine the exact decomposition temperature.

A survey of alloys of the composition Me(1) Me(2)_3 with Me(1) = Cu, Ag or Au and Me(2) = Ti or Zr shows an interesting correlation between the

Table 4. Alloys Me(1) Me(2)$_3$ with Me(1) = Cu, Ag or Au and Me(2) = Ti or Zr.

Alloy		c/a	$r_{Me(1)}/r_{Me(2)}$
$CuTi_3$[7]	Tetragonally deformed $AuCu_3$ type	0.86	0.88
$AgTi_3$[10]	No homogeneous phase	—	0.99
$AuTi_3$[10]	$AuCu_3$ type	1	0.99
$CuZr_3$[7]	$CuTi_3$ type	0.82	0.82
$AgZr_3$	$CuTi_3$ type	0.87	0.92
$AuZr_3$[9]	No evidence of any phase of $CuTi_3$ type	—	0.92

axial ratio c/a and the radius ratio $r_{Me(1)}/r_{Me(2)}$ as is evident from Table 4. No phase $AuZr_3$ isomorphous with $CuTi_3$ exists although the radius ratio in this case is the same as in $AgZr_3$.

Zirconium-rich Alloys. In powder photographs from specimens with compositions between 75 and 100 at. % zirconium, no reflections indicating any new intermediate phase were visible.

For pure zirconium (α—Zr) the axial lengths were found to be $a = 3.227$ Å, $c = 5.142$ Å, $c/a = 1.593$ ($V = 46.09$ Å^3). If the α-zirconium lattice is assumed to dissolved silver atoms in a solution of the substitution type, the cell volume may be expected to decrease. No such decrease was, however, noticed.

SUMMARY

The silver-zirconium system has been investigated by X-ray methods. Two intermediate phases have been found: β and γ with the ideal compositions AgZr and $AgZr_3$. The ranges are narrow.

The structure of the β phase is of the B 11 type (isomorphous with γ—CuTi) and that of the γ phase may be considered as a deformed L 12 ($AuCu_3$) type (isomorphous with $CuTi_3$ and $CuZr_3$).

No solid solubility of zirconium in silver or of silver in α-zirconium could be detected.

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Energetics of the Pasteur Effect

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Under certain specified physico-chemical conditions the overall rate of biological phenomena is approximately proportional to the molar loss of free energy (molar entropy production). It is maintained as probable that these conditions are fulfilled in growing bacteria. On this basis it is shown that the devaluation of the energetic value of glucose by transition from aerobic to anaerobic growth will automatically cause a higher rate of glucose consumption, which is the Pasteur effect. An analogy between the energetic behaviour of the living cell and the AC transformer is indicated.

An experiment illustrating the relation between metabolism of growing and resting bacteria is described.

Pasteur has discovered[1] that yeast being able to utilize glucose both aerobically and anaerobically will do so with highly different rates. The anaerobic break-down of glucose, which may lead to lactic acid or to alcohol, is a much faster process than the aerobic break-down leading to carbon dioxide and water. This phenomenon which is shown by most cells having both aerobic and anaerobic metabolism is called the Pasteur effect. Various hypotheses have been advanced to explain the apparent inhibition of the anaerobic break-down (glycolysis or fermentation) by oxygen [2,3]. In the present paper it is suggested that the rise in glucose consumption by transition from aerobic to anaerobic metabolism is a simple thermodynamic consequence of the accompanying devaluation of the energetic value of the glucose. No special regulatory mechanism seems to be necessary to explain the Pasteur effect. Contemporary ideas concerning the nature of transference of free energy from energy providing to energy requiring reactions provide a mechanism by which glucose consumption is automatically regulated by the energy requirements of the cell.

Before the demonstration of the energetics of the Pasteur effect it is necessary to have at hand two physico-chemical laws and one postulate:

1. The equivalence of loss of free energy and entropy production. This is an exact law.

2. The proportionality between rate of reaction and displacement from equilibrium. This is an approximate law which is valid near equilibrium only (generally for $-\Delta G \ll RT$).

3. A postulate of even distribution of entropy production amongst the vast variety of reactions taking place in a living cell.

We shall discuss these points in turn.

1. The majority of reactions in a cell are enzyme catalyzed reactions of the type

$$A + B \rightarrow C + D$$

Examples of such reactions are dehydrogenations, transaminations and phosphorylations [4]. Is a the concentration of the species A, b of B, etc., we may write the condition of equilibrium

$$\frac{a \cdot b}{c \cdot d} \cdot K = 1$$

This condition is of course not obeyed by reactions going on in the metabolizing cell. If it were, the rate of the reaction considered would be zero. In general we have

$$\frac{a \cdot b}{c \cdot d} \cdot K = \alpha$$

where $\alpha > 1$ if the reaction is proceeding from left to right. This gives

$$-\Delta G = \Delta\mu = T\Delta S'' = RT\ln\alpha$$

where $-\Delta G$ is the loss of free energy, $\Delta\mu$ the difference in chemical potential, and $\Delta S''$ the corresponding entropy production. This is, when motility and radiation is excluded, the only mechanism by which free energy dissipates from the cell. It is true, of course, that certain reactions apparently proceed against affinity, the free energy necessary being provided by some other reaction, but such reactions are now known to proceed in steps, each of which are enzyme catalyzed according to the principle outlined above [4]. The total loss of free energy in metabolism equals the integral entropy production.

Let us consider a bacterium growing on a medium with glucose as sole carbon source. The glucose is providing carbon for new cell material. The protein matter and other cell constituents will contain carbon which on average is slightly more reduced than glucose, so the free energy of combustion per carbon atom will be somewhat higher than in glucose. Accordingly, the

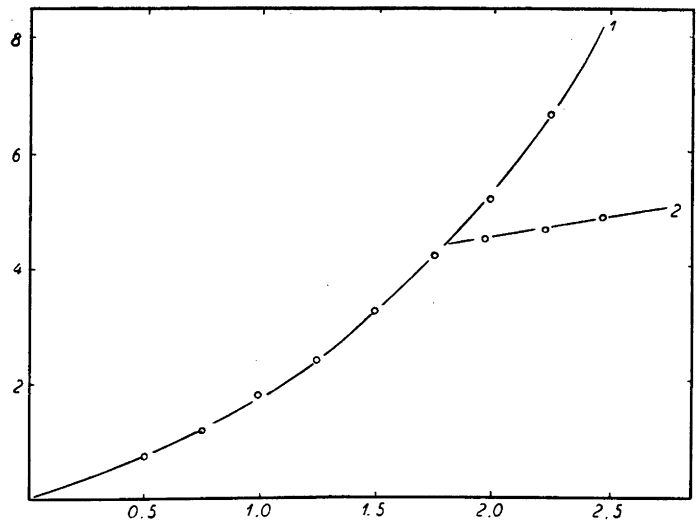


Fig. 1.

Ordinate: Oxygen consumption (mg/l), Abscissa: time (hours) 1: $C_{NH_4^+} = 5.10^{-3}$, *2:* $C_{NH_4^+} = 1.3.10^{-4}$.

carbon dioxide production during aerobic growth is higher than the oxygen consumption. The necessary free energy will be provided by the reactions:

glucose + oxygen → carbon dioxide + water. *aerobic* (*ca.* 700 kcal)
glucose → lactic acid *anaerobic.* (*ca.* 60 kcal)

Some of this free energy is as indicated invested into the cell constituents but the main part will be degraded into heat and thus entropy produced as described above. Every reaction in the cell takes part in this entropy production. If some of the reactions are stopped, their contribution to the entropy production will disappear, and the consumption of free energy must accordingly decrease.

This is demonstrated by Fig. 1. Two flasks containing a dilute air-saturated glucose medium were inoculated with equal amounts of a suitable *Bacterium lactis aerogenes* culture. One of the flasks contained a small amount of nitrogen (ammonium sulphate) only, so growth would stop after a couple of hours. The contents of the flasks were distributed amongst a number of glass stoppered bottles which were filled up into the neck so they did not contain any gas phase when stoppered. The bottles were incubated in a water thermostat at 25° C, and at suitable intervals one of each series was taken out and oxygen determined according to Winkler. Fig. 1 shows that as long as there is nitrogen available for growth the behaviour of the two series is identical. When nitrogen

was used up in the series with limited supply, the rate of oxygen consumption fell to about 16 % of the value during growth. The reason is clearly that all reactions leading to synthesis of new cell material have now ceased, and so has the contribution of these reactions to the total entropy production of the cell. Consequently, the rate of loss of free energy, and therefore the rate of oxygen consumption, must decrease.

2. Prigogine [5] and others have demonstrated that the rate of a chemical reaction proceeding with small affinity, *i.e.* near equilibrium, is proportional to the affinity. In this section we shall see that this law has an essentially better region of validity when simple enzyme reactions are considered. Let us take the reaction

$$A \rightarrow B$$

a and b being stationary concentrations of the intermediate metabolic products considered. The reaction is catalyzed by an enzyme, and let the mechanism be

$$A + X_1 \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} X_2 \qquad (\pm 1)$$

$$X_2 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} X_1 + B \qquad (\pm 2)$$

where X_1 and X_2 are enzyme and enzyme-substrate complex. It is generally assumed that the Michaélis equation

$$s = \frac{k \cdot E \cdot a}{K_m + a}$$

where $k = k_{-2}$, $K_m = \dfrac{k_{-1} + k_2}{k_1}$ and E the enzyme concentration is an adequate expression for the rate of such reactions. The assumption leading to this equation is that the first step is proceeding in both directions, the second only from left to right. This assumption can not be valid for the majority of reactions in the cell as it leads to a large displacement from equilibrium and therefore to a large entropy production per reaction, which, as we shall see in the next section, is not justified when the total loss of free energy is compared with the number of reactions going on in the cell. We must therefore take (−2) into consideration, and a steady state treatment gives:

$$s = E \frac{k_1 k_2 a - k_{-1} k_{-2} b}{k_1 a + k_{-1} + k_2 + k_{-2} b}$$

a_0 and b_0 being a set of equilibrium values of the concentrations of A and B gives:

$$k_1 k_2 a_0 = k_{-1} k_{-2} b_0$$

Keeping the concentration of B constant equal to b_0 and defining $a = a_0 \alpha$ gives:

$$s = E \frac{k_1 k_2 a_0 (\alpha - 1)}{k_1 k_0 \alpha + k_{-1} + k_2 + k_{-2} b_0}$$

α has the same thermodynamic significance as in the preceding section. Christiansen [6] has shown that in the denominator of such expressions the terms will generally be of very different order of magnitude. Assuming symmetry in A and B we have either

$$k_1 a_0 \sim k_{-2} b_0 \gg k_{-1} \sim k_2$$

or

$$k_1 a_0 \sim k_{-2} b_0 \ll k_{-1} \sim k_2,$$

Now it is well known that the rate of bacterial growth in synthetic media is generally independent of concentrations of medium constituents within very wide limits. This indicates that the rate of enzyme reactions does not change very much when concentrations of the reacting molecules are changed, so numerator and denominator would probably be influenced in the same way by change in concentrations. This rules out the second inequality, so we have

$$s = E \frac{k_1 k_2 a_0 (\alpha - 1)}{k_1 a_0 \alpha + k_{-2} b_0}$$

Fig. 2 shows the relation between s/s_0 and α, where s_0 is the limiting rate for $\alpha \to \infty$, when $k_1 a_0 = k_{-2} b_0$. Fig. 3 shows the corresponding relationship between s/s_0 and $\ln \alpha$. The function plotted is

$$s/s_0 = \frac{\alpha - 1}{\alpha + 1}$$

It is easily seen that this function is identical with

$$s/s_0 = \tanh(\tfrac{1}{2} \ln \alpha)$$

This rough treatment shows that the law

$$s = EX \ln \alpha = EX(\Delta G)/RT$$

where

$$X = k_1 k_2 a_0 / (k_1 a_0 + k_{-2} b_0)$$

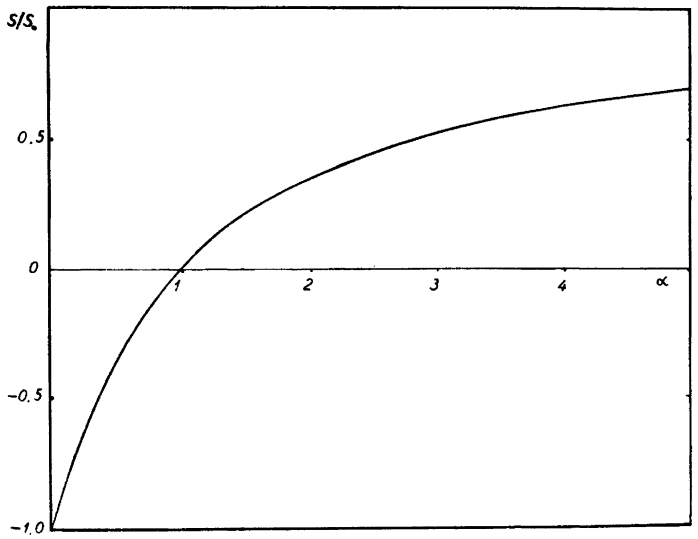


Fig. 2.

is reasonably valid up to about $\ln a \sim 1.7$ or $\Delta G = 1$ kcal. The corresponding rate is about 70 % of the limiting rate. This agreement is far better than the agreement calculated from simple noncatalytic reactions [5]. The reasons are two: Firstly our reaction proceeds in two steps amongst which the loss of free energy is distributed, and secondly the competition of A and B for the enzyme gives a rate expression which favours the agreement.

The reaction here investigated is an oversimplification of the typical cell reaction treated in the preceding section. A similar treatment of the more general reaction encounters two difficulties: The mechanism of such reactions is as yet not exactly known, and if it were, the mathematical treatment would be extremely complicated. The result would presumably be less unique than outlined above, although the general features leading to the validity of the rate-affinity law are retained. A possible source of complication is the feature that the equilibrium

$$A + B \rightleftharpoons C + D$$

has three "degrees of freedom" where

$$A \rightleftharpoons B$$

has one only. It is seen that in the simple case it is indifferent which equilibrium is taken as reference state as long as the concentrations of A and B are not very small. As the number of steps in the more general enzyme reaction is

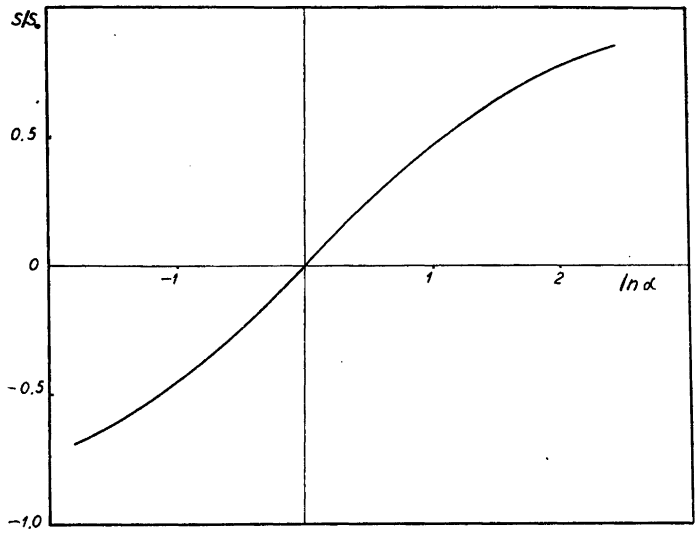


Fig. 3.

necessarily greater than two an even better region of validity might be expected. The fact that growth rate of bacteria is largely independent of concentrations of medium constituents supports the assumption of validity in the more complex case also.

We shall leave this section with the remark that the treatment of the relation between affinity and rate of enzyme reactions should be taken as an introduction to a general treatment which is in progress.

3. In this section we shall compare the total loss of free energy with the number of reactions going on in the cell. Determination of the respiration of growing bacteria shows that only a fairly small fraction of the glucose turnover is oxidized. For *Bacterium lactis aerogenes* this fraction is, according to investigations to be published later, about 1/4. The loss of free energy per mole glucose oxidized is about 700 kcals. A part of this free energy is invested in the carbon atoms of the cell material, so the total loss of free energy per mole glucose metabolized aerobically will probably be of the order 150 kcals. The fate of a glucose molecule oxidized is essentially as follows:

Glucose is through about 13 steps, the glycolytic enzyme system, converted into two molecules of pyruvic acid. After about half of these steps the glucose is split into two triose molecules, each of which must go through the last part of the sequence, so there has effectively been about 20 steps. Now pyruvic acid is oxidized to carbon dioxide in the tricarboxylic acid cycle, a number of

coenzyme molecules at the same time being reduced. It is well known that free energy is trapped out of the glycolytic reactions by the reaction

$$\text{ADP} + \text{Phosphate} \rightarrow \text{ATP}$$

(ADP = Adenosinediphosphate, ATP = Adenosinetriphosphate)

which proceeds against affinity [4,7]. There is every reason to believe that exactly the same mechanism is effective when free energy is trapped out of other energy providing reactions in cell metabolism. The tricarboxylic acid cycle is known to comprise some ten steps when phosphorylations are not considered. It is also known that a part of the free energy lost during these steps is made available to the cell, so we must necessarily assume that the reactions in this cycle include a number of as yet unknown phosphorylations and transphosphorylations. If all the known reactions provide energy, we must have three steps, *viz.* phosphate uptake before the reaction, and phosphate transference afterwards, for each reaction, or thirty steps altogether. There were two molecules of pyruvic acid, so the score is now, when glucose is oxidized to carbon dioxide, and 12 coenzyme molecules are reduced, 80 enzyme reactions of the type considered in section 1. The coenzyme molecules are re-oxidized in reactions with the flavoproteins, and through one-electron reactions, 24 electrons per glucose molecule oxidized, the hydrogen is transferred to oxygen through an unknown number of steps catalyzed by cytochromes and cytochrome oxidase. Some of the free energy lost in these steps is undoubtedly trapped out into the ADP—ATP system. The number of steps must be quite considerably. Even a small number like 5 per hydrogen atom would lead to a total of more than 200 reactions per mole glucose oxidized. It is not unlikely that the average number of steps per glucose molecule incorporated into new cell material is very similar. Most of the carbon compounds in the cell have very little structural similarity with glucose indeed. So the result of this discussion is that the average number of steps per glucose molecule metabolized is at least of the same order as the average number of kcals free energy lost through irreversibility. That means that the average loss of free energy is compatible with the law of proportionality between rate and displacement from equilibrium. The question is now whether the total loss of free energy is distributed evenly amongst the reactions of the cell. The only way we can go here is to postulate that this is the case.

This postulate gains in credibility when it is realized that it is equivalent with the assumption that the total amount of protein of the cell is distributed adequately on the various enzyme functions. It is easy to prove that if a certain amount of protein is at disposal for the formation of a number of enzymes catalyzing a sequence of reactions, then there will be a certain distribu-

tion which gives higher overall rate of the sequence than all other distributions. Such an enzyme system would be completely without "bottle necks".

The postulate here outlined should of course be taken with some reservation. Work to investigate its validity is in progress. In favour of the postulate is maybe the idea [8] that a large loss of free energy in a single enzyme reaction might be fatal to the enzyme. Further, the adaptation theory [9,10] of bacterial training, which has in some cases been proved to be definitely superior to the mutation theory, shows that when bottle necks are introduced into the enzyme machinery by addition of drugs to growth media, or by transference to media with new carbon sources, then the bacteria are able to remove these bottle necks by expansion of the enzymes, the activity of which were reduced by the drug, or which became necessary in greater amounts by a new mode of metabolism. The adaptation theory clearly supports the idea that growing bacteria endeavour a bottle neck free composition.

The arguments leading to low average affinity for enzyme reactions and therefore to proportionality between rate and loss of free energy may be extended as follows: Bacteria are able to grow logarithmically on a variety of different media with highly different growth rates [9,11]. Thus *Bacterium lactis aerogenes* will grow on a synthetic medium with glucose as sole carbon source at 40° C with a mean generation time (MGT) of 33 minutes. If this organism is transferred to a broth medium, the MGT will fall to about 18 minutes, *i.e.* the growth rate is almost doubled. The reason is, according to the points of view here advanced, that the broth medium contains a large number of ready made cell constituents, which the cell is saved to synthesize. That means that no free energy will be lost on the reactions normally leading to these substances, so more free energy is available to reactions which are still necessary. Such reactions will be displaced further from equilibrium, and therefore proceed with higher rate. The rate of these reactions will of course in the broth medium be lower than the limiting value for infinitely high affinity, and the rate in the synthetic medium will thus be lower than about half this rate. That will, if affinity is evenly distributed, be equally valid for all other reactions. Inspection of Fig. 3 shows that this is well within the region where the rate-affinity law should be applicable. We shall in the following discussion of the mechanism of the Pasteur effect assume that affinity is fairly evenly distributed, *i.e.* the rate of enzyme reactions is essentially proportional to the loss of free energy.

We shall now consider the fate of the free energy lost when glucose is converted aerobically to the pyruvic acid-lactic acid state:

$$-\Delta G = 60 \text{ kcals}$$

The exact value will of course depend somewhat on the concentrations of glucose and pyruvic acid, but is rather insensitive. A change by a factor 1 000 will only change $-\Delta G$ by about 4 kcals. $-\Delta G$ may be split up into two parts:

$$-\Delta G = m + n$$

where m is the free energy trapped out by the reaction

$$\text{ADP} + \text{Phosphate} \rightarrow \text{ATP}$$

m depends on two factors: the number of times this reactions goes on, determined by the stoichiometry of the glycolysis sequence, and the difference in chemical potential between the two sides. The building up of this chemical potential is the combined effort of all reactions providing free energy, and a steady state is created, also controlled by the processes utilizing free energy by the reversal of the reaction. The rest of the free energy, n, is lost inside the sequence, a corresponding amount of entropy simultaneously produced. This part of the free energy loss is the driving force of these reactions, and the rate of glucose utilization is according to the view advanced above proportional to n. Let the oxygen disappear. The contribution to the ATP pool from all reactions below glycolysis is ceased. ATP is in the first instance used with the same rate but the ATP concentration very soon drops, and a new steady state is created, in which the chemical potential between the two sides of the energy trapping reaction is reduced by a certain factor, *i.e.* the value of m is decreased. The inevitable consequence is that n increases, and so does the rate of attack on glucose which is proportional to n. This means that the devaluation of the energetic value of glucose caused by absence of oxygen is automatically compensated by a higher rate of glucose-consumption, which is the Pasteur effect. The rate of growth is controlled by the energetic value of ATP, and is accordingly reduced during anaerobiosis [12].

The corresponding mechanism of the phenomenon shown in Fig. 1 is of course: When growth stops because nitrogen is used up, a great number of reactions using ATP will cease and ATP will accumulate. The chemical potential between the two sides of the energy conserving reaction will increase, *i.e.* m will increase. Hence n will decrease, hence a much slower attack on glucose.

In this elucidation of the mechanism of the Pasteur effect much attention has been placed on the ADP-ATP system. The author does not claim any originality in the assumption that the glucose consumption "hangs on the ATP/ADP ratio", but he does claim that only the physico-chemical development given here justifies to explain the Pasteur effect by this assumption.

The most important feature of the treatment is that no special regulatory mechanism is necessary to explain the effect. Indeed, it might be said that if living cells able to utilize glucose both aerobically and anaerobically did not show the Pasteur effect, then there would be something fundamentally wrong in the current ideas concerning the structure of biochemistry. It should be emphasized that the attention paid to the ADP-ATP system is partly a matter of convenience in the perspicuous demonstration of the energetic mechanism. The real significance should be concentrated on the fact that the entropy production corresponding to the loss of free energy in respiration or glycolysis is distributed over all reactions going on in the cell, and the assumption that the energy is trapped out stepwise by some obligatory mechanism more or less common to all energy providing steps. All systems working in accordance with this principle will display a "Pasteur effect".

An interesting analogy to the energetic behaviour of the living cell is shown by the AC transformer. The loss of energy in the primary side is almost completely determined by the composition of the secondary circuit. It is possible to keep the state in the secondary circuit unchanged when the tension on the primary side is altered, provided the ratio of the transformer is changed simultaneously. Under such circumstances the product of tension and current, *i.e.* the loss of free energy, will remain constant. That the integral energetic value of aerobic and anaerobic utilization of glucose is also of the same order is seen from the following rough calculation: When 1 mole of glucose is converted aerobically into *Bacterium lactis aerogenes*, 1/4 is oxidized, the corresponding loss of free energy being $0.25 \times 700 = 175$ kcals. The rest of the glucose, 0.75 mole, is used for other purposes, essentially for growth. To get the same amount of bacteria anaerobically 3.5 mole of glucose is needed [12]. Of this amount 0.75 mole is invested in the same way as before, and 2.75 mole is converted into lactic acid, the loss of free energy being $2.75 \times 60 = 165$ kcals. The entropy production is not distributed in the same way between the primary and the secondary side of the chemical transformer in the two cases.

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Some Reactions of Esterified Collagen

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By modifying or inactivating specific protein groups, the function of any particular group in various reactions may be studied by comparison with the intact protein as a blank, provided that the method of altering the reactivity of the protein is specific and does not alter other protein groups to any appreciable extent and is attended by a minimum risk of degradation [1]. In an earlier investigation of the fixation of cationic chromium complexes by collagen and of the participation of its carboxylic ions [2], the latter were completely discharged by H-ions in systems with pH values in the range 1.0—1.5, employing acid-resistant olated chromium salts containing added acid of the same pH in the presence of swelling-depressing neutral salts of the same anion. Since the behaviour of such systems is more informative in the pH range 3—4, this method has only a limited usefulness. Hence, the function of the ionic carboxyl groups of collagen in the fixation of various chromium complexes has been investigated by means of intact collagen and collagen with its free carboxyl groups methylated. The esterification of collagen and the reactivity of the resulting products have recently been investigated by Bowes and Kenten[3] who employed methyl sulphate and methyl bromide; fourteen or more consecutive methylations with the former agent being required to obtain a fair degree of esterification. However, the details given in the paper of Bowes and Kenten indicate a marked breakdown of the protein during these severe treatments. These reagents as well as diazomethane and 1,2-epoxides, when used as methylating agents for globular proteins, combine not only with the carboxyl groups but also with other groups, *e.g.*, the amino groups and cause hydrolytic breakdown [4]. The same observations have been made in the present investigation on collagen, diazomethane in particular having given only a low degree of methylation of the carboxyl groups. A satisfactory, practically complete esterification of collagen with minimum of side reactions is obtained by the method of Fraenkel-Conrat and Olcott [4], originally used

by them on globular proteins with very satisfactory results. This method involves treatment of the protein with methanol, 0.05—0.1 *N* in respect to hydrochloric acid, at room temperature and has been found to work satisfactorily on collagen.

MATERIALS AND METHODS

Complete esterification of the carboxyl groups was obtained by shaking 20 g of collagen in the form of acetone-dehydrated pieces of a thin split (the middle layer of corium) of a calf skin, consisting mainly of isoelectric collagen, in 1 000 ml of methanol to which had been added 8.0 ml of 36% hydrochloric acid, intermittently for 6 days at room temperature. After methylation, the collagen was rinsed with 50 per cent aqueous methanol, and placed in one litre of 4 per cent sodium chloride solution, in order to prevent swelling and to facilitate diffusion of the protein-bound hydrochloric acid into the salt solution. The mixture was shaken and kept at pH 5 by the gradual addition of *N* sodium hydroxide. After complete neutralization, the methylated stock was shaken in several changes of water for a few minutes each time, twice dehydrated in acetone for 24 hours and finally dried in vacuum at 60° C. It is important to keep the treatment in water as brief as possible since the methylated collagen swells considerably in water and hydrolysis of the ester may occur gradually. In some experiments the skin was used in the pressed state, containing about 60 per cent of water. The resulting product, otherwise treated as described, showed a slightly lower degree of esterification (0.73 meq. OCH_3 fixed by 1 g collagen). The methylated collagen was in the dry state very similar to acetone-dehydrated intact skin collagen, although somewhat more greyish and stiffer. The most marked difference, compared to intact collagen, was its high swelling power in water.

The analytical characteristics of the intact and methylated preparations of collagen are given in Table 1. The methoxyl content of intact collagen arises from its methionine. The degree of inactivation of the acidic (carboxylic) groups is calculated from the data of the acid binding capacity to be somewhere between 85 and 90%. The acid binding capacity (HCl) of the collagens was determined by treating portions of 0.5 g of the protein in 12.0 ml of 0.1 *N* hydrochloric acid, 0.75 *M* in sodium chloride for 2, 6 and 24 hours, titrating the excess acid (methyl red)[5]. The reaction was complete in 6 hours, since 6 and 24 hours treatments gave identical results, Hence, rather surprisingly no splitting of the ester takes place at pH 1.1.

The complete inhibition of chromium fixation from solutions containing all the chromium in the form of cations (see Table 2) indicates practically complete inactivation of the carboxyl groups. The content of free carboxyls of moderately limed collagen, such as the present specimen, is generally 0.8—0.9 meq.per g collagen and the number of methoxyl groups, 0.79 meq.per g, evidently closely corresponds with the number of free acidic groups. The shrinkage temperature, T_s, was determined on freely suspended strips of substrate, using the shocking method [6], in order to eliminate as far as possible any complications due to swelling of the methylated collagen during the determination.

The total N content of the modified collagen is close to the theoretical value (17.97) calculated, assuming the methylation of the carboxyl groups. 0.79 meq. of carboxyl groups per g collagen are esterified. The acid binding gives a corresponding figure of 0.77. Of particular interest is the large decrease in the shrinkage temperature of 25° C. By complete inactivation of the acid-binding groups of collagen by means of the non-swelling

Table 1.

Type of collagen	% total N	% ash	% total N on ashfree collagen	meq. OCH_3 per g collagen	Acidbinding capacity in meq. HCl fixed by 1 g collagen	T_s in °C
Intact	18.13	0.2	18.17	0.03	0.93	62
Methylated	17.87	0.5	17.96	0.82	0.16	37

naphthalene-β-sulphonic acid, specific for ionic protein groups, the electrovalent type of crosslinks in the protein are eliminated, resulting in a lowering of T_s by 16—18° [7]. Accordingly, the very much larger lowering of the hydrothermal stability induced by the esterification of collagen apparently must be due to the rupture or weakening of some other types of cohesive forces besides the salt-links. It is likely that non-ionic crosslinks (coordinate bonds) of the hydrogen bond type are involved.

The unusually large swelling of the isoelectric methylated collagen in water also indicates diminished intermolecular cohesion of the main type of bonds present in collagen, *i.e.*, coordination crosslinks (hydrogen bonds). This view is strengthened by the results from experiments on the combining capacity of the methylated collagen for agents which react with collagen mainly by means of coordinate valency forces (non-ionic).

RESULTS AND DISCUSSION

Before entering into a discussion of the main problem of this investigation, *i.e.*, the function of the carboxyl ions of collagen in the binding of chromium complexes, the results of some experiments on the general reactivity of this modified collagen will clarify the picture.

In comparative experiments with the two types of specimens of Table 1, the fixation of a synthetic tannin of the type of a low sulphonated Novolak resin was determined. Specimens equal to 2.0 g of collagen were shaken two consecutive times in 25 ml of a 10 % solution of the Novolak resin adjusted to pH 4, in order to minimize as far as possible the ionic function of the sulphonic acid group of the Novolak in the reaction. In spite of the fact that part of the binding of the syntan by the intact collagen is due to ionic interaction, which type of acid binding is practically eliminated for the esterified collagen, the latter fixed larger amounts of the sulphonated resin than the intact collagen; the percentages, on the basis collagen, being 72.6 and 60.2 respectively. The shrinkage temperature was elevated by 14° for the intact collagen and by 39° in the case of the modified collagen. The main reactivity of the Novolak sulphonate at pH 4—5 is due to the phenolic groups, probably due to their coordination on non-ionic groups of collagen, as for example, the peptide groups. Hence, it is evident that the coordination reactivity of the esterified

collagen is considerably greater than that of collagen proper, the former possessing a larger number of coordination active sites. These probably result from the rupture of non-ionic crosslinks (hydrogen bonds) in the treatment with methanol.

This is further brought out by the data on the irreversible uptake by the two types of collagen of agents which react with collagen preponderately by means of non-ionic valency forces, such as tannic acid and mimosa tannins. These experiments were carried out with hide powder in its native form and after methylation as described, since the very high affinity of the esterified skin collagen for these natural tannins jeopardized the penetration of the tannins into the interior of the skin because of obstruction by the heavily fixed tannin. Treatments for five days in solutions of these tannins commencing with 5% strength of the solutions and finishing up in 10% solutions, in both instances containing 0.6 eq./l of sodium chloride, gave the following irreversible fixation of tannins, calculated on the weight of collagen: The intact hide powder fixed 71 per cent and the methylated hide powder 107 per cent of tannic acid at a final pH of 4.0. The corresponding figures for the mimosa fixation at a final pH of 5.0, both pH values selected in the range 4 – 5 in order to minimize hydrolytic demethylation during the extended treatment, were 62 and 109 respectively. These data provide evidence for the occurrence of secondary reactions during the esterification leading to rupture of non-ionic crosslinks of the protein making additional sites available for reaction. However, these changes do not affect the electrovalent reactions, such as those of cationic chromium complexes.

The experiments with the chromium compounds were carried out with the two types of skin collagen, employing portions of hydrated skin equal to 2 g of collagen, which were shaken for 6 hours in 50 ml of the solution of chromium salt containing 0.6 eq./l Cr, if not otherwise noted. Making the solutions 0.5 *N* in regard to neutral salts by the addition of sodium chloride to the solutions of chromium chlorides and sodium sulphate to those of the chromium sulphates, swelling of the substrate was prevented. The amounts of chromium fixed by the intact and the methylated collagens, and the increase in shrinkage temperature of the tanned specimens are given in Table 2 which also lists particulars of the nature of the compounds employed and the reaction.

The composition of the various compounds in regard to the electrochemical state of the chromium complexes was determined by the ion exchange technique using the sodium salt of Dowex 50 and the hydrochloride of Amberlite IRA-400 [8]. The increase in the shrinkage temperature, ΔT_s, determined by the shocking method is a measure of the degree of stability of the chromed protein structures, the + sign denoting improved and the – sign impaired degrees of hydrothermal stability. It is to be noted that solutions no. 1, 2, and 3 contained all the chromium as positively charged complexes, whereas the chromium in the remaining solutions existed predominantly as non-cationic complexes.

The data show that for the fixation of the cationic chromium complexes of the chloride, perchlorate and sulphate the presence of the ionic carboxyl groups of collagen is essential. By prolonging the time of reaction, a slight fixation of cationic chromium gradually takes place, probably mainly due to the hydrolytic splitting of the ester link which frees carboxyl groups available for the binding of cationic chromium. This is demonstrated by Fig. 1 which

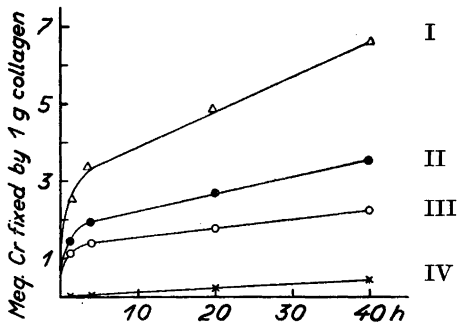


Fig. 1. The fixation of chromium by intact collagen and methylated collagen at various time of interaction.

Sulphito-chromium sulphate (no. 6 of Table 2) with 72% of its chromium in the form of non-cationic complexes:

I —△—△— *intact collagen*
II —●—●— *methylated collagen.*
Cationic complexes of 66% acid Cr-chloride (no. 1 of Table 2):
III —○—○— *intact collagen*
IV —×—×— *methylated collagen.*

shows the fixation of chromium by the two types of collagen, as a function of time, for a typical cationic agent and a typical coordination-active agent, largely composed of non-cationic chromium. The graduál, but marked, augmentation of the fixation of the sulphito-chromium sulphate is characteristic for reactions of the coordination type which require more time for the attainment of equilibrium than the ionic type of reactant, represented by the basic chromium chloride. The linear function of the slight fixation of the latter compound with time is in line with a gradual freeing of carboxylic groups by hydrolysis of the ester.

Whereas equilibrated dilute solutions of basic chromium sulphates of the type given in Table 2 (no. 3), which are composed mainly of cationic sulphato-hydroxo-chromium complexes, do not react with the esterified collagen, highly concentrated solutions of these compounds (Table 2; no. 4) possess some affinity for collagen devoid of carboxylic groups. By increasing the chrome concentration of these basic sulphates, non-ionic and anionic chromium complexes are formed; in the present instance (no. 4) the main constituent is of non-cationic nature. This latter type of complexes is thus responsible for the uptake of chrome by the methylated collagen, probably by means of coordination of these complexes on groups in the collagen other than the carboxyl groups. Such a dual nature of the chromium fixation by intact collagen from concentrated

Table 2.

No.	Type of chromium compound	Type of collagen	Complex composition cat-ionic	non-ionic	an-ionic	Final pH	meq. Cr fixed by 1 g collagen	ΔT_s in °C
1	66% acid Cr-chloride	Intact	100	0	0	2.9	1.70	+27
1	corresp. to: $Cr_2(OH)_2Cl_4$. 2NaCl	Methylated	100	0	0	2.8	0.00	−6
2	66% acid Cr-perchlorate	Intact	100	0	0	2.8	1.42	+23
2	corresp. to: $Cr_2(OH)_2(ClO_4)_4$.2 $NaClO_4$	Methylated	100	0	0	2.7	0.00	−4
3	66% acid Cr-sulphate	Intact	96	4	0	3.0	1.96	+38
3	corresp. to: $Cr_2(OH)_2(SO_4)_2$. Na_2SO_4	Methylated	96	4	0	2.8	0.04	−6
4	66% acid Cr sulphate as no 3 except	Intact	42	48	10	2.7	2.84	+38
4	5.0 eq./l Cr	Methylated	42	48	10	2.7	0.69	−4
5	33% acid Cr-chloride	Intact	32	68	0	3.5	4.24	+26
5	corresp. to: $Cr_2(OH)_4Cl_2$. 4NaCl	Methylated	32	68	0	3.5	0.38	−4
6	Sulphito-Cr-sulphate	Intact	28	50	22	4.5	3.72	+37
6	corresp. to: $Cr_2(OH)_2(SO_4)_2$. Na_2SO_4 . $2Na_2SO_3$	Methylated	28	50	22	4.5	1.65	+12
7	Phthalato-Cr-sulphate	Intact	32	68	0	4.6	3.01	+34
7	corresp. to: $Cr_2(OH)_2(SO_4)_2.Na_2SO_4.(NaOCO)_2C_6H_4$	Methylated	32	68	0	4.6	0.92	+2

solutions of basic chromium sulphate containing at least one mole of neutral sulphate per mole of chromium oxide, was indicated in earlier investigations of the reactivity of collagen with discharged carboxyl ions (at pH = 1.5). The present findings corroborate this view. The main reaction is the ionic attraction of the chromium cations to the carboxyl ions of collagen, followed by the penetration of the discharged carboxyls into the chromium complex with the formation of a covalent-coordinate link with chromium. In highly concentrated solutions an additional reaction of the non-cationic chromium complexes which does not require the ionic carboxylic groups of collagen, is shown to take place. The second type of chromium fixation is probably of coordinated nature, and does not lead to any marked stabilization of the protein lattice. Chlorides of low basicity which do not show this non-ionic type of reaction, conform with the explanation given, since they do not form any appreciable amounts of non-cationic chromium complexes.

From the solutions of basic chromium sulphates complexed by means of phthalate and sulphite which form mixed chromium complexes of non-ionic and anionic nature, the esterified collagen fixes such complexes, evidently by groups other than the carboxyl groups. Accordingly, a large part of the binding of sulphito-sulphato-chromium compounds by intact collagen must then occur through non-ionic protein groups (coordination)[9].

As a further illustration of this type of fixation of chromium complexes by collagen, the reactivity of such a sulphito compound consisting to three fourths

of non-ionic chromium and to one fourth of anionic chromium is of interest. Solutions of this compound, which contained three moles of sodium sulphite per one mole of chromium oxide, did not react with the carboxylic ions of a cation-exchanging resin, Amberlite IRC-50, equilibrated with sodium ions at pH 7. This proves that the charged carboxyl group cannot penetrate into the nucleus of these non-cationic complexes. The fixation of this compound by collagen devoid of carboxyl groups was 3.6 meq. Cr per g collagen compared with 6.3 meq. per g intact collagen. In addition to peptide bonds and cationic protein groups as potential sites for the fixation of non-cationic chromium complexes, the possible participation of hydroxy groups *), such as those of serine and hydroxyproline, in the binding of these complexes by collagen is indicated by data from interaction of modified polyamides with these chromium compounds (10).

The behaviour of collagen with its cationic as well as its anionic groups completely inactivated by means of irreversibly fixed polymetaphosphoric acid towards basic chromium salts is of interest in this connection. This polymetaphosphated collagen fixes chromium compounds irrespective of their electrochemical type. The reaction occurs probably through reactive centres of the polymetaphosphate chain held by the protein. This indirect binding of cationic chromium complexes does not lead to an improved degree of stability of the original collagen-compound or only a minor one. However, the sulphito compound mentioned elevates the shrinkage temperature by 23° C upon 4 hours' interaction. Solutions of the basic sulphate (no. 3), equilibrated at a concentration of 0.6 eq./l Cr, only slightly increases this property (4—5° C), whereas the solution of this sulphate applied *immediately* after diluting the stock solution of 6.0 eq./l Cr to the aforementioned concentration of 0.6 eq./l Cr raises the shrinkage temperature by 25° C. The directly diluted solution contained as the dominating constituent, non-ionic chromium complexes. Apparently this additional fixation involves the non-ionic protein groups, resulting in additional stabilization of the collagen lattice. These observations add further strength to the concept advanced.

Concluding Remarks. The main finding of this investigation affords an additional proof of the governing importance of the carboxyl ions of collagen for the fixation of cationic chromium complexes. This important reaction appears at the present state of our knowledge to be initially an ionic reaction between the positively charged chromium complexes and the negatively charged carboxyl groups of collagen. The discharge is followed up by the most important reaction for the stability of the resulting protein-compound formed, the penetration of the carboxyl group into the chromium complex, entering into

*) Professor John T. Edsall should be credited with the suggestion of residues of hydroxyamino acids as *loci* for the binding of tanning agents by collagen (personal communication, 1948).

covalent-coordinate linking with chromium. By attachment of two or more carboxylic groups of adjacent protein chains to two or more chromium atoms of one polynuclear chromium complex, an exceedingly stable crosslinking of the protein chains should be effected.

The high degree of stability of the chrome-carboxyl link exhibited by chrome leather tanned by means of cationic chromium compounds is also evident in the chromium compound formed between cationic chromium complexes and cation exchanging resins carrying the carboxyl ion as functional group. These are absolutely resistant towards neutral salts and only a very small part of the chromium fixed by the resin is removed upon prolonged and repeated digestions with 5 *M* solutions of hydrochloric acid. A great excess of strong complexing agent containing hydroxy and carboxylic groups leads to a gradual removal of the fixed chromium in both instances, through the mass action effect of the added complexing agent.

SUMMARY

The carboxyl group of collagen has been completely esterified by means of methanol made 0.1 *N* in respect to hydrochloric acid, following the method of Fraenkel-Conrat and Olcott. The esterified collagen contained 0.79 meq. methoxyl per g collagen and its HCl-binding capacity was reduced by 0.77 meq. H-ion. The methylated collagen commenced to shrink at 37° C, compared with 62° for the intact collagen. It swelled considerably in water.

Investigations of the reaction of various chromium compounds with intact and esterified collagens show:

1. Dilute solutions of basic chlorides, perchlorates and sulphates of chromium, containing cationic chromium complexes, have practically no affinity for collagen with its carboxyl groups completely inactivated by esterification.

2. From concentrated solutions of basic chromium sulphates of the type: $Cr_2(OH)_m(SO_4)_n \cdot Na_2SO_4$, which contain a large percentage of non-ionic and anionic chromium complexes, the esterified collagen fixes small amounts of chromium. This fixation does not appreciably improve the hydrothermal stability of the protein. The reaction is indicated to involve non-ionic protein groups by coordination. This implies a dual nature of the chrome fixation by collagen from concentrated solutions of basic chromium sulphates.

3. From solutions of sulphito-sulphato-chromium compounds and of basic chromium sulphates complexed by means of sodium phthalate, with non-cationic chromium as the main constituent, esterified collagen fixes irreversibly large amounts of these complexes, in some instances amounting to more than

half of the value of chromium fixed by intact collagen. This fixation markedly improves the hydrothermal stability of the modified collagen.

4. The results prove the dominating function of the ionic carboxyl groups of collagen in the fixation of the most important chromium compounds, *i.e.*, positively charged chromium complexes. The final result is probably a multipoint crosslinking of adjacent protein chains through the incorporation of two or more carboxylic groups of different chains into one polynuclear chromium complex with direct attachment of the carboxyl group on the chromium.

5. The similarity of chrome-collagen compounds and chrome-carboxylic resin compounds is stressed.

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On the Phase Relations of Synthetic and Natural Pyrrhotites ($Fe_{1-x}S$)

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Natural and synthetic pyrrhotites ($Fe_{1-x}S$) are distinguished by a rather large variation of the iron to sulfur ratio. Hägg and Sucksdorff [1] found that the sulfur excess causes vacant iron positions in the B-8 (NiAs) structure rather than substitution of sulfur for iron. They also found the existence of a superstructure from the stoichiometric composition FeS to $Fe_{0.953}S$ (51.2 atomic per cent S). In preparations quenched from 650° C the phase of the B-8 (NiAs) structure type was observed to exist in the composition range $Fe_{0.938}S$-$Fe_{0.802}S$ (51.6 to 55.5 atomic per cent S). Later, synthetic pyrrhotites have been subjected by Roberts [2] to thermal analysis and by Haraldsen [3–5] to X-ray and magnetic studies. Neither Hägg and Sucksdorff [1] nor Haraldsen [4,5] found any phases with other structures than the superstructure (B-8 like) and the ordinary B-8 structure. Roberts [2] mentioned, however, that he observed signs of slow structural changes from his exploratory X-ray photographs.

According to Haraldsen [5], a survey diagram as shown on Fig. 1 can be drawn concerning the transformations in pyrrhotites. The heavy lines indicate the temperatures where abrupt changes in the lattice constants and magnetic susceptibility take place. The β-transformation at 325° C is common to all pyrrhotites and likewise the α-transformation which takes place at 138° C in FeS. The α-transformation temperature is lowered with increasing sulfur content and the transformation finally becomes very sluggish. In addition, two other lines are drawn indicating transformations which vary with the sulfur content. The line starting at 150° C and 51.5 atomic per cent sulfur indicates the γ-maximum of a sudden increase in magnetic susceptibility. The preparations on the right side of the dotted line at 52.4 atomic per cent sulfur are ferromagnetic. Roberts [2] expressed the opinion that an additional transformation takes place in synthetic pyrrhotites between 325° and 550° C.

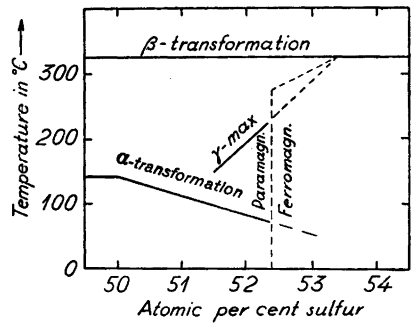


Fig. 1. Temperatures where magnetic effects take place in synthetic pyrrhotites.

Byström [6] investigated Swedish pyrrhotites by means of X-rays with the interesting result that many of them displayed a monoclinic structure while only a few of them had hexagonal structure. Others again contained a mixture of these two structures. Chemical analyses gave no connection between the sulfur content and the symmetry, and Byström suggested that the change in structure was due to a polymorphous transformation.

Buerger [7] investigated single crystals of ferromagnetic pyrrhotites from two different localities by the precession method [8]. Both crystals revealed the same hexagonal superstructure characteristics and the one from Schneeberg, Saxony, was found to have the following cell dimensions *:

$$A = 2a = 6.87 \text{ Å} \qquad C = 4c = 22.7 \text{ Å}$$

The a-axes are two, the c-axis four times greater than of pyrrhotites having the ordinary B-8 structure. The cell dimensions of this superstructure have no connection with those observed by Hägg and Sucksdorff and the phase is furthermore ferromagnetic instead of paramagnetic. Buerger states, however, that there are also some indications present that the two pyrrhotites possess either a lower hexagonal symmetry or even an orthorhombic or monoclinic symmetry. The latter possibility would agree with the results obtained by Byström.

Another new superstructure with $A' = 3a$ and $C' = 2c$ has been found by Graham [9] in single crystals of paramagnetic pyrrhotites which were formed by the action of a sodium sulfide solution on the walls of a steel bomb held at 400° C and about 1 200 bars.

Our investigation was performed in order to find out if the phases with monoclinic structure and superstructures reported to exist in natural pyrrhotites also had ranges of stability in pyrrhotites synthesized by dry methods. In addition, some Norwegian pyrrhotites were studied.

* Lattice constants taken from earlier investigations have been transformed from kX units to Å units by multiplication with the factor 1.00202.

The present investigation has confirmed many of the results obtained earlier. Thanks to the good X-ray equipment now at our disposal, the range of existence of the superstructure, detected by Hägg and Sucksdorff, and of the B-8 structure, could be determined more accurately. Furthermore, it was possible to ascertain that the iron sulfide phase with monoclinic structure is stable at temperatures below 325° C and has a higher sulfur content than the phase with B-8 structure which is stable in the same temperature region.

PREPARATION OF THE ALLOYS

The iron used for making the alloys was prepared from Fe_2O_3, Merck "Ferrum oxydatum sec. L. Brandt" by reduction with dry, purified hydrogen gas. The sulfur used had been prepared by Haraldsen[3] from sulfur c.p. by repeated vacuum distillations. The pyrrhotites were synthesized by weighing the amounts of iron and sulfur calculated for the different compositions into silica tubes which were then evacuated and sealed off. Each sample weighed approximately one gram.

After being heated at 1 000° C for 24 hours, the preparations were slowly cooled down in the furnace, then ground and homogenized at 290° C. This temperature was chosen in order to be well below the 325° C transformation. A regulator kept the furnace temperature constant to ± 3° C. After annealing for 40 days, the preparations were quenched by dropping them into ice water.

INVESTIGATION OF THE PYRRHOTITES

A. X-ray technique

X-ray photographs of the synthetic pyrrhotites were taken in a Unicam 19 cm diameter high temperature camera using an air-cooled X-ray tube with Fe anode of a Philips Geiger Counter Spectrometer. The exposure time was 20 hours at 36 kVp and 6 mA. The derived lattice constants are expressed in Å units using $\lambda = 1.935\ 97$ Å as the wavelength of $FeK\alpha_1$ radiation. The absolute errors in our lattice dimensions are less than ± 0.001 Å for values given to the fourth decimal and less than one part in two thousand when three decimals are given.

A series of room photographs were also taken of freshly annealed parts of the sample earlier prepared by Haraldsen. A cylindrical 11.4 cm diameter powder camera was used, and with the Fe-K radiation from a Siemens tube, the exposure time was 3 hours at 37 kVp and 8 mA. Powder photographs of the pyrrhotite minerals were taken in a Philips 11.48_3 cm camera utilizing the Straumanis technique of film mounting.

B. Synthetic pyrrhotites

The composition and lattice constants of the new preparations, investigated at room temperature (15—20° C) in the Unicam camera, are listed in Table 1. The lattice constant values in parentheses are arranged to bring out the orthohexagonal cell dimensions.

Table 1. Lattice constants in Å units of synthetic pyrrhotites at room temperature.

Composition	a, $(b\sqrt{3})$	b, $\left(\frac{a}{3}\sqrt{3}\right)$	c	β
$Fe_{1.020}S$	5.963_6	(3.443_1)	$2\times5.874_5$	
$Fe_{1.000}S$	5.965_9	(3.444_4)	$2\times5.879_1$	
$Fe_{0.971}S$	5.969_7	(3.446_6)	$2\times5.882_3$	
	(5.969_7)	3.446_6	6.761_2	
$Fe_{0.943}S$	(5.972_5)	3.448_2	5.760_4	
$Fe_{0.926}S$	(5.975_2)	3.449_8	5.751_4	
$Fe_{0.909}S$	(5.969_0)	3.446_2	5.736_9	
$Fe_{0.893}S$	(5.959_8)	3.440_9	5.720_2	
$Fe_{0.877}S$	5.952	3.433	5.690	90.4
$Fe_{0.833}S$	5.951	3.435	5.692	90.4

$Fe_{1.000}S$. In accordance with earlier results, the X-ray photograph of this preparation shows "superstructure" interferences. Hägg and Sucksdorff[1] derived the superstructure from the B-8 (NiAs) structure by 90° rotation in the basal plane, multiplication of the a-axis by $\sqrt{3}$, and doubling of the c-axis. Our values for the lattice constants are in good accord both with the results of Hägg and Sucksdorff[1], ($a = 5.958$ Å, $c = 11.74$ Å) and with those of Haraldsen[4], (mean values: $a = 5.97$ Å, $c = 11.76$ Å).

$Fe_{0.971}S$. This composition should lie in the range between that of the superstructure and the B-8 structure. Hägg and Sucksdorff were of the opinion that the transition between these structures was continuous while Haraldsen observed a sharp break in the lattice dimension curves at $Fe_{0.962}S$ (51.0 atomic per cent S) and therefore concluded a two phase transition. The photograph of our annealed $Fe_{0.971}S$ preparation, clearly reveals that two phases are present, the superstructure phase and a phase richer in sulfur with B-8 structure. The $Fe_{0.971}S$ preparation contains considerable amounts of the phase with B-8 structure. On the other hand Haraldsen[3,4] found that the preparations $Fe_{1.020}S$ and $Fe_{1.010}S$ belong to the two phase region iron/FeS. Thus the existence of the superstructure phase is evidently connected with the stoichiometric composition FeS.

$Fe_{0.943}S$. This preparation gives the interferences from a structure of the B-8 (NiAs) type. In addition the two strongest interferences from the superstructure phase are observed, and $Fe_{0.943}S$ is therefore a two phase preparation just as $Fe_{0.971}S$. In accordance with this, the lattice constants of $Fe_{0.971}S$ and $Fe_{0.943}S$ are found practically unchanged.

$Fe_{0.926}S$ to $Fe_{0.893}S$. In this concentration range the lattice constants show a considerable variation and a range of homogeneity no doubt exists. The

preparation $Fe_{0.893}S$ seems, however, to lie in a two phase region as it contains the strongest interferences of the preparation $Fe_{0.877}S$ richer in sulfur. The phase with B-8 structure should accordingly have a range of homogeneity between say $Fe_{0.935}S$ and $Fe_{0.900}S$ (51.7 to 52.6 atomic per cent S) *.

$Fe_{0.877}S$ to $Fe_{0.833}S$. At the composition $Fe_{0.877}S$ a marked change is observed on the photographs. Some previous interferences are split up into two or more components in a manner which is characteristic of a change to lower symmetry. It was a deformation just of this kind which Byström [6] observed on some pyrrhotite minerals. Such a low symmetric — possibly monoclinic — pyrrhotite structure has, however, not earlier been found in synthetic preparations and we suppose that annealing below the 325° C transformation temperature is essential for its formation.

Phases with B-8 like structures of low symmetry have been observed in many of the systems of transition elements with the chalcogens (S, Se, Te). In the system iron-selenium ** Hägg and Kindström [10] suggested a monoclinic indexing of the low symmetric structure. Until single crystal studies can be made on synthetic pyrrhotites we follow the scheme of Hägg and Kindström when indexing the interferences from the low symmetric structure.

No differences in the lattice constants are found which indicate a range of homogeneity between $Fe_{0.877}S$ and $Fe_{0.833}S$. In addition, FeS_2 (pyrite) interferences also show up on the $Fe_{0.833}S$ preparation. The monoclinic phase should therefore have a composition close to $Fe_{0.877}S$ and the phase can be designated as Fe_7S_8.

As already mentioned, a series of synthetic pyrrhotites, previously prepared by one of us, were studied at room temperature with X-rays. The composition, heat treatment and lattice constants of these preparations are listed in Table 2. By comparing the results in Tables 1 and 2, the agreement is seen to be very good. It is of special interest to note that also the 16 year old $Fe_{0.971}S$ preparation is found to consist of two phases after annealing. Furthermore, the deformation into lower symmetry has taken place in the $Fe_{0.870}S$ and the $Fe_{0.833}S$ preparations.

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* Whether or not a range of homogeneity exists at room temperature it cannot be concluded from the quenched preparations.

** Hägg and Kindström found that the phase with B-8 like structure has a range of homogeneity between $Fe_{1.000}Se$ and $Fe_{0.739}Se$. According to our results, not yet published, this is only the case at higher temperatures. At lower temperatures there exist two separate phases with low symmetric B-8 like structures, one at the composition $Fe_{0.870}S$ with no appreciable range of homogeneity, the other with a composition between $Fe_{0.800}Se$ and $Fe_{0.750}Se$. The structure of the former phase is very similar to that of $Fe_{0.877}S$. A phase with ordinary B-8 structure does not exist at room temperature in the system iron-selenium.

Table 2. Lattice constants in Å units of the previous preparations after annealing.

Composition	Heat-treatment		a, $(b\sqrt{3})$	b, $\left(\frac{a}{3}\sqrt{3}\right)$	c	β
	C°	days				
$Fe_{1.000}S$	300	30	5.967	(3.445)	2×5.873	
$Fe_{0.971}S$	290	40	5.967	(3.445)	2×5.871	
			(5.967)	3.445	5.752	
$Fe_{0.952}S$	300	30	(5.974)	3.449	5.780	
$Fe_{0.926}S$	290	40	(5.977)	3.451	5.760	
$Fe_{0.909}S$	300	30	(5.970)	3.447	5.738	
$Fe_{0.893}S$	290	40	(5.960)	3.441	5.721	
$Fe_{0.870}S$	300	30	5.962	3.442	5.714	90.3°
$Fe_{0.833}S$	290	40	5.953	3.438	5.702	90.4°

The existence of the phase with ordinary B-8 structure is by the present investigation found to be restricted to the narrow composition range between $Fe_{0.935}S$ and $Fe_{0.900}S$. High temperature thermal analysis carried out by Jensen [11] showed that congruent melting took place only in synthetic pyrrhotites at the composition $Fe_{0.926}S$, while FeS was found to melt over a temperature range of nearly 100° C. The only solid pyrrhotite with a definite melting point therefore has a composition within the range of homogeneity of the phase with plain B-8 structure at low temperature.

As a result of our X-ray investigation of annealed, synthetic pyrrhotites these phases were found:

1. A phase with superstructure (B-8 like) at the stoichiometric composition $Fe_{1.000}S$.
2. A phase with B-8 structure in the composition range $Fe_{0.935}S$ to $Fe_{0.900}S$.
3. A phase with structure of low symmetry and a composition close to $Fe_{0.877}S$ or Fe_7S_8.

C. Natural pyrrhotites

The pyrrhotites found in nature are of terrestrial and meteoritic origin. The terrestrial pyrrhotites generally have sulfur in excess of the stoichiometric composition and also ferromagnetic properties. Most formulas range between $Fe_{16}S_{17}$ and Fe_5S_6 (51.52 to 54.55 atomic per cent S), see *e.g.* Henglein [12]. The meteoritic types are designated as troilite and have usually compositions close to the stoichiometric ratio. Terrestrial pyrrhotites are, however, not always richer in sulfur than the meteoritic. Eakle [13] analyzed a terrestrial pyrrhotite from Del Norte County in California and reported stoichiometric

composition. On the other hand, a compilation of 31 "troilite" analyses by Buddhue [14] showed that 7—8 % of the meteoritic pyrrhotites had a sulfur excess corresponding to that of ordinary terrestrial pyrrhotites.

Norwegian pyrrhotites were placed at our disposal by the Mineralogical-Geological Museum of the University in Oslo and we should like to express our appreciation to Professor Ivar Oftedal for careful selection of the specimens. Chemical analyses were carried out on four of these pyrrhotites. The sulfur was determined by weight as $BaSO_4$, the iron as Fe_2O_3. Cobalt and nickel, if present, were precipitated and ignited with the iron. Our best thanks are due to mag. scient. Astri Tønsager for carrying out the analyses. The results are listed in Table 3 together with a spectrographic search for other elements,

Table 3. Analyses of four Norwegian pyrrhotites.

Locality	Bø, Valle Setesdal	Setregruben Østfold	Åmdal Froland	Herzog Friedrich Grube, Kongsberg
Fe	61.79 61.90	61.60 61.54	61.02 60.75	59.84 59.82
S	38.43 38.06	38.64 38.41	39.11 39.14	39.50 39.59
Insol. (Ag)	—	—	—	0.55
Sum	100.22 99.96	100.24 99.95	100.13 99.89	99.89 99.96
Formula	$Fe_{0.928}S$	$Fe_{0.916}S$	$Fe_{0.893}S$	$Fe_{0.869}S$
Ferromagnetic properties	V. weak	Weak	Medium	Strong
Spectrographic analysis	0.1 Ni 0.1 Cu	0.1 Co 0.1 Ni 0.1 Cu	0.2—0.3 Co 0.1 Ni 0.2—0.3 Cu	0.2 Co <0.1 Ni <0.5 Cu

kindly carried out by Ing. S. Rutlin, Statens Råstofflaboratorium, Oslo. By a qualitative magnetic test the ferromagnetic properties of the minerals were found to increase with increasing sulfur content. This is in accordance with the results of Haraldsen [3] on synthetic preparations, and those of Juza and Biltz [15] on decomposed pyrite.

The following pyrrhotites were investigated by X-rays and the lattice constants found are listed in Table 4.

1. *Bø, Valle in Setesdal.* The X-ray photograph of this specimen contains interferences from the hexagonal B-8 structure only.

2. *Setregruben, Østfold.* A slight decrease in lattice constants is observed and the specimen is distinctly more ferromagnetic than No. 1.

3. *Åmdal, Froland.* In addition to the interferences caused by the hexagonal structure, the photograph also contains interferences from the low symmetric structure. The presence of a phase with this structure makes the mineral more magnetic than No. 2.

4. *Kongens Grube, Kongsberg.* A powdered crystal, of which we have no analysis, gave a photograph showing the presence of both the hexagonal phase and that of low symmetry.

5. *Herzog Friedrich Grube, Kongsberg.* The hexagonal phase has disappeared and only interferences from the monoclinic phase are visible on the photographs. This mineral has the highest sulfur content and has also the strongest ferromagnetic properties.

Weissenberg photographs have been taken of some small pyrrhotite crystals from Kongsberg, deposited on silver threads. The crystals were hexagonal and showed no superstructure interferences that could indicate a larger elementary cell than that found for the B-8 type.

The lattice constants found for the Norwegian pyrrhotites agree very well with those found for the synthetic preparations. By comparing the values in Tables 1 and 4 for the B-8 structure, the c-axis is, however, seen to vary less with the sulfur content for the minerals than for the synthetic samples. The homogeneity range of the hexagonal pyrrhotite minerals therefore seems to be narrower than found for the synthetic preparations. Disregarding the differences in formation between the natural and the synthetic pyrrhotites, this might be explained by the fact that the minerals are probably closer to thermodynamic equilibrium at "room temperature" than the synthetic preparations.

Table 4. Lattice constants in Å units of some Norwegian pyrrhotites.

Locality	a, $(b\sqrt{3})$	b	c	β
Bø, Valle Setesdal	(5.967_4)	3.445_3	5.735_9	(90°)
Setregruben, Østfold	(5.964_8)	3.443_8	5.733_9	(90°)
Åmdal, Froland	(5.963)	3.443	5.729	(90°)
	5.951	3.436	5.695	90.4°
Kongens Grube, Kongsberg	(5.963)	3.443	5.732	(90°)
	5.952	3.437	5.699	90.4°
Herzog F. Grube, Kongsberg	5.953	3.429	5.680	90.4°

Of the analyzed pyrrhotites the sample richest in sulfur has the monoclinic structure. The sample from Åmdal, Froland with somewhat less sulfur is a mixture of the phase with the monoclinic structure and the phase with the hexagonal structure, whereas the two samples poorest in sulfur are pyrrhotites with hexagonal structure. This investigation of Norwegian pyrrhotites thus also supports the conclusion drawn from studying synthetic preparations that the monoclinic phase has a greater sulfur content than the hexagonal phase.

Minerals which contain the superstructure phase — troilite — and the hexagonal pyrrhotite phase intermixed are not yet known. Their existence, however, seems probable and we have in progress an X-ray study of the iron rich, natural pyrrhotites.

D. Comparison with previous results

If we compare our results with those of Byström [6], the agreement between the lattice constant determinations is very good. The lattice constants found by him for Swedish pyrrhotites vary within these limits:

Hexagonal structure	$a = 3.438-3.445$ Å	
	$c = 5.724-5.740$ Å	
Monoclinic structure	$a = 5.945-5.954$ Å	$b = 3.432-3.438$ Å
	$c = 5.689-5.701$ Å	$\beta = 89.45°-89.66°$ ($90.34°-90.55°$)

As well from Byström's results as from ours it can be seen that there is a marked difference in the length of the c-axis of the hexagonal and the monoclinic structure.

The analyses in Byström's study of pyrrhotites gave no indications that the hexagonal and the monoclinic phase differ with respect to their sulfur content. From the high temperature study of synthetic pyrrhotites by Haraldsen [5] it was known that the c-axis of pyrrhotites contracts with increasing temperature, especially at the 325° C transformation (β-transformation). Byström made the assumption that the pyrrhotites should display monoclinic structure *above* the 325° C transformation temperature and sometimes have been cooled down to their present temperature without change into the hexagonal structure. He pointed out that this assumption was in accordance with the temperature variations of the length of the c-axis. In order to test his explanation the monoclinic pyrrhotite from Herzog Friedrich Grube, Kongsberg was annealed at 350° C for 7 days and quenched. The X-ray photograph of this preparation showed that the monoclinic deformation had disappeared and that the structure was of the ordinary B-8 type with lattice constants:

$a = 3.435$ Å $\qquad\qquad$ $c = 5.692$ Å

This result is in agreement with our X-ray high temperature investigation (see later) as we found no signs that a monoclinic deformation exists above the 325° C transformation. It therefore seems impossible to maintain the assumption made by Byström that the monoclinic pyrrhotite is a β-transformed, high temperature modification in the system iron sulfur.

Allen, Crenshaw, Johnston and Larsen [16] found that hydrothermal syntheses at 200° C gave orthorhombic pyrrhotites while syntheses at 80° C gave hexagonal pyrrhotites. Byström found very good agreement between the axial ratios of his monoclinic pyrrhotites, determined by means of X-rays, and those found goniometrically by Larsen for the hydrothermally prepared orthorhombic pyrrhotites. He therefore pointed out that the small angular deformation might have been overlooked by Larsen and that the orthorhombic crystals in reality were monoclinic. Applying this explanation to the apparently orthorhombic pyrrhotites found in nature we arrive at the conclusion that the pyrrhotites are either hexagonal or monoclinic, with or without superstructures, and that no orthorhombic pyrrhotites seem to exist.

Allen *et al.*[16] could give no proof that the hexagonal and the "orthorhombic" crystals prepared by them had the same composition and if we suppose that the "orthorhombic" (monoclinic) pyrrhotites which had been formed at 200° C had higher sulfur content than those formed at 80° C there seems to be no objection against the assumption that both the hexagonal and the low symmetric phase can exist as stable phases at room temperature.

The pyrrhotites which display the low symmetric (monoclinic) structure are richest in sulfur, having a composition close to Fe_7S_8. They also have ferromagnetic properties and have either been formed at low temperatures or have been cooled down slowly so that the transformation from the B-8 structure, stable at higher temperatures, to the low symmetric structure has taken place. The hexagonal pyrrhotites are poorer in sulfur and have paramagnetic or weak ferromagnetic properties. If their sulfur content equals that of the monoclinic pyrrhotites they must have been formed at comparatively high temperatures and have cooled down so rapidly that a transformation to the monoclinic structure did not take place.

Results obtained by Kiskyras [17] who investigated the magnetic properties of a large number of natural pyrrhotites fit into this picture. He concluded the existence of two types of pyrrhotites: one of hydrothermal origin which is ferromagnetic and rich in sulfur (52.8 atomic per cent S) and another from high temperature deposits (pegmatic or contact metamorphic) which is poorer in sulfur (52.5 atomic per cent S) and has weak magnetic properties. As Kiskyras reasons in terms of the results of Allen *et al.*[16], he incorrectly supposes

that the sulfur rich ferromagnetic pyrrhotites have the higher symmetry instead of the lower of the two types.*

HIGH TEMPERATURE X-RAY INVESTIGATION

X-ray studies of synthetic pyrrhotites at higher temperatures have been performed by Haraldsen [5] in order to investigate if the observed changes in magnetic properties were connected with special structures or structural changes. The present study is especially concerned with the ranges of homogeneity of the synthetic pyrrhotite phases at higher temperatures. It also seemed of interest to see if the orthorhombic pyrrhotite reported by Allen *et al.*[16] could be found at higher temperatures in the pyrrhotites synthesized by dry methods.

A series of X-ray photographs were taken at 185—190° C. This temperature was chosen in order to be in the middle of the stability range of the α-transformed pyrrhotites. The preparations were the same as those used for the room temperature investigation and had been annealed at 290° C for 40 days. In Table 5 are listed the composition of the preparations and the lattice constants found at 185—190° C. The temperature of the X-ray camera was maintained constant to $\pm$ 3° C.

Table 5. Lattice constants in Å units of synthetic pyrrhotites at 185—190° C.

Composition	°C	a, ($b\sqrt{3}$)	b	c	β	Hexagonal axial ratio
$Fe_{1.020}S$	190	(6.063_9)	3.501_0	5.807_7	(90°)	1.659
$Fe_{1.000}S$	190	(6.064_6)	3.501_4	5.807_2	(90°)	1.659
$Fe_{0.971}S$	185	(6.051_8)	3.494_0	5.788_0	(90°)	1.657
$Fe_{0.943}S$	185	(6.036_7)	3.485_3	5.769_4	(90°)	1.655
$Fe_{0.926}S$	190	(6.030_0)	3.481_4	5.752_6	(90°)	1.653
$Fe_{0.893}S$	190	(6.009_3)	3.469_5	5.720_5	(90°)	1.649
$Fe_{0.877}S$	190	5.994	3.460	5.697	90.4°	
$Fe_{0.833}S$	190	5.994	3.460	5.697	90.4°	

$Fe_{1.000}S$. Haraldsen [5] connected the appearance and disappearance of the superstructure interferences in FeS with the α-transformation at 138° C.

* After this paper was written, S. V. Lipin, Zapiski Vsesoyuz. *Mineral. Obshchestva (Mém. soc. russe minéral.)* **80** (1951) 258, *Ch. A.* **46** (1952) 3342 discusses the magnetic properties of pyrrhotite. Minerals heated to above 280° C show an approximate proportionality between magnetic susceptibility and sulfur content while this is not the case for unheated natural pyrrhotites. His results on the latter point are thus in disaccord with those of Kiskyras [17].

Confirming this, $Fe_{1.000}S$ exhibits no superstructure interferences at 190° C. The interferences can be indexed assuming an ordinary B-8 structure. Results of the film measurements and intensity calculations are listed in Table 6. The agreement between observed and calculated intensities is not as good as could be expected from a parameter free B-8 structure, and the deviations are most likely due to slight changes in the positions of the atoms.

Table 6. Powder photograph of FeS at 190° C. FeK radiation. Intensity refers to a doublets, a_2 and β interferences are omitted.

Θ	$\sin^2\Theta \cdot 10^4$		$h\,k\,i\,l$	Intensity	
	obs.	calc.		obs.	calc.
18.63	1021	1020	$a_1a_2 10\bar{1}0$	5	4.9
19.20	1082	—	?	2	—
19.50	1114	1113	$a_1a_2 0002$	1	0.5
21.11	1297	1299	$a_1a_2 10\bar{1}1$	5	3.6
27.51	2133	2133	$a_1a_2 10\bar{1}2$	20	16
33.58	3059	3057	$a_1 11\bar{2}0$	10	10
36.39	3519	3520	$a_1 10\bar{1}3$	3	1.8
39.69	4078	4076	$a_1 20\bar{2}0$	2	1.7
40.25	4175	4168	$a_1 11\bar{2}2$	1	1.1
41.31	4358	4354	$a_1 20\bar{2}1$	4	1.6
41.82	4446	4446	$a_1 00\bar{0}4$	3	2.6
46.06	5185	5187	$a_1 20\bar{2}2$	12	11
47.66	5464	5465	$a_1 10\bar{1}4$	3	3.3
54.16	6572	6577	$a_1 20\bar{2}3$	4	1.7
57.63	7134	7134	$a_1 21\bar{3}0$	1	4.1
59.43	7414	7412	$a_1 21\bar{3}1$	6	5.1
60.02	7503	7503	$a_1 11\bar{2}4$	20	25
63.18	7964	7966	$a_1 10\bar{1}5$	4	2.9
65.22	8244	8245	$a_1 21\bar{3}2$	30	41
67.40	8523	8522	$a_1 20\bar{2}4$	4	5.8
72.32	9078	—	?	6	—
73.28	9172	9172	$a_1 30\bar{3}0$	20	26
78.99	9635	9635	$a_1 21\bar{3}3$	20	21

$Fe_{0.971}S$ to $Fe_{0.926}S$. Between the composition $Fe_{1.000}S$ and $Fe_{0.971}S$ a pronounced shift in the interference positions is observed. This continues until the composition $Fe_{0.877}S$ is reached, indicating that an extended range of homogeneity exists. Contrary to what was observed on $Fe_{1.000}S$, weak additional interferences are found on our $Fe_{0.971}S$, $Fe_{0.943}S$ and $Fe_{0.926}S$ preparations. In Table 7 are listed the additional low angle interferences occuring on

a $Fe_{0.971}S$ photograph. The cell dimensions and atomic positions of the superstructure cell cannot be determined with any certainty by the powder method and we will therefore not discuss the various ways in which these additional interferences might be explained.

Table 7. Additional low angle interferences observed on $Fe_{0.971}S$ at 185° C. FeK Radiation

Int.	w	w	w	vw	vw	m	w—	vw	w—
$\sin^2 \Theta \cdot 10^4$	534	560	576	612	650	678	913	949	1589

$Fe_{0.893}S$. A decrease in cell dimensions from the composition $Fe_{0.926}S$ is observed. However, the weak additional interferences have disappeared.

$Fe_{0.877}S$ to $Fe_{0.833}S$. At the composition $Fe_{0.877}S$, a structural deformation is found. The assumptions used to derive the lattice constants at 190° C were the same as for the room temperature photograph. At the composition $Fe_{0.833}S$ interferences from the FeS_2-pyrite-phase are observed and there are no appreciable changes in lattice constants from $Fe_{0.877}S$ to $Fe_{0.833}S$.

In Fig. 2 the dimensions of the *b*-and *c*-axes are plotted as function of the composition. No break is observed at the transition from hexagonal to monoclinic structure. At 190° C therefore, a homogeneity range apparently exists all the way from $Fe_{1.000}S$ to $Fe_{0.877}S$.

Another series of high temperature photographs were taken at 360° C i.e. just above the β-transformation temperature. The derived lattice constants are listed in Table 8. Neither superstructure interferences nor interferences

Table 8. Lattice constants in Å units of synthetic pyrrhotites at 360° C.

Composition	*a*	*c*	*c/a*
$Fe_{1.020}S$	3.539	5.788	1.635
$Fe_{1.000}S$	3.538_6	5.786_5	1.635
$Fe_{0.971}S$	3.531_2	5.768_1	1.633
$Fe_{0.926}S$	3.520_9	5.741_5	1.631
$Fe_{0.893}S$	3.507_5	5.706_5	1.627
$Fe_{0.877}S$	3.503	5.702	1.628
$Fe_{0.833}S$	3.503_8	5.703_9	1.628

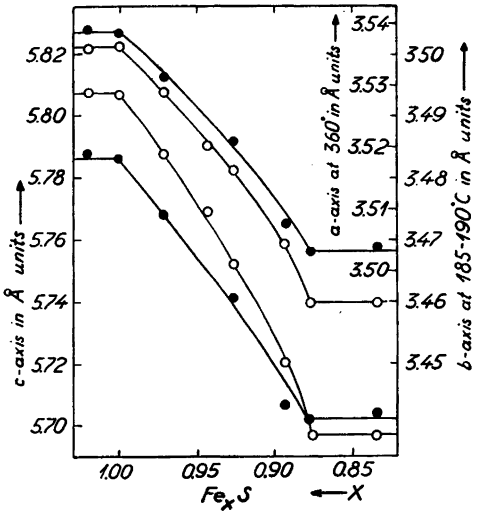


Fig. 2. Lattice constants of synthetic pyrrhotites at 185—190° C (— ○ —) and 360° C (— ● —).

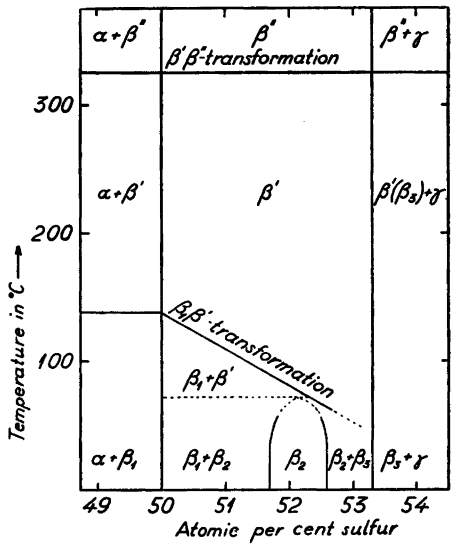


Fig. 3. Tentative phase diagram for synthetic pyrrhotites.

indicating lower symmetry are observed and the lattice constants decrease in an orderly manner. It appears from Fig. 2 that the pyrrhotite phase at this higher temperature has a range of homogeneity between the limits $Fe_{1.00}S$ and $Fe_{0.88}S$. It can be mentioned that Coughlin [18] in a study of the high temperature heat content of iron sulfides found $Fe_{1.030}S$ (49.26 atomic per cent S) to be a single phase preparation at higher temperatures. Although our results are not conclusive at this point, a slight extension of the range of homogeneity on the iron rich side seems to have taken place at 360° C.

In a few cases the sealing of the thin walled quartz capillaries was unsatisfactory, which caused a slight oxidation of the sample to take place. X-ray photographs then showed a decrease in cell dimensions of the pyrrhotite phase. Later on, pyrite and iron oxide interferences were observed. This has some connection with results found by Kiskyras [19]. He observed that the weathering process made pyrrhotite ores and rocks more ferromagnetic and connected that with an increase in sulfur content. The results of our X-ray study show that the pyrrhotite diminishes its cell dimensions during the oxidation process and this is most likely due to increased sulfur content.

When we took X-ray photographs of the synthetic pyrrhotites after they had been cooled down from 360° C to room temperature, no monoclinic defor-

mation could be observed on the $Fe_{0.877}S$ and the $Fe_{0.833}S$ preparations. Furthermore, the $Fe_{0.971}S$ sample was not found to split into the two phases which are stable at room temperature (see page 1455). This explains the results which were found both by Hägg and Sucksdorff [2] and by Haraldsen [4], namely that a single phase with hexagonal structure is the only existing pyrrhotite phase. Evidently the phase relations as given by their preparations correspond to equilibrium approach at higher temperature than those given by our preparations annealed at 290° C.

The sulfur rich limit for the homogeneity range of pyrrhotites at 20°, 185° and 360° C is found close to the composition Fe_7S_8. This is in perfect accord with the results of Roberts [2] and Haraldsen [4] whereas Juza and Biltz [15] report the somewhat lower value 52.8 atomic per cent sulfur as the sulfur rich limit of the homogeneity range of the pyrrhotite phase. Recently, Lukes, Prutton and Turnbull [20] studied the reaction between $FeCl_2$ and H_2S gas, and found that the amount of H_2 gas formed during the reaction corresponded to the formation of $Fe_{0.877}S$ (53.27 atomic per cent S). The experiments were carried out at various temperatures between 343.7° C and 455.6° C. At the highest temperature an increase in the H_2 gas formation took place, indicating a higher sulfur content, but the authors pointed out that this might be due to incidental errors.

Our exploratory X-ray photographs of pyrrhotites at 550° C did not reveal any extension of the homogeneity range beyond the composition $Fe_{0.87}S$. On the other hand Allen *et al.*[16] determined the sulfur rich limit at 565° C to be $Fe_{0.833}S$ (54.55 atomic per cent S). Later, Merwin and Lombard [21] found that pyrrhotite had a composition between $Fe_{0.846}S$ and $Fe_{0.835}S$ (54.17 and 54.50 atomic per cent S) in equilibrium with pyrite and sulfur vapor at 678° C (pS = 455 mmHg). The value $Fe_{0.80}S$ (55.5 atomic per cent S) found by Hägg and Sucksdorff [2] as the solubility limit for sulfur in pyrrhotite at 650° C seems to be somewhat high.

SOME CONSIDERATIONS REGARDING THE TRANSFORMATIONS

Figure 3 gives the phase relations of the pyrrhotites as they appear from the present investigation. Below the $\beta_1\beta'$-transformation temperature there exist three phases — β_1, β_2 and β_3 — separated by two phase regions.

The β_1 phase exists at the stoichiometric composition at room temperature and is stable up to 138° C. The atomic arrangement within its superstructure cell has not yet been worked out.

As the sulfur content is increased above the stoichiometric composition, a two phase region between the β_1 and the β_2 phase appears, and the $\beta_1\beta'$-trans-

formation temperature decreases. On further increase in sulfur, the homogeneity range of the β_2-phase follows and then a narrow two phase region between the β_2 and β_3 phases. The pyrrhotite phase richest in sulfur, the β_3 phase, has no appreciable range of homogeneity.

Some doubt arises as to the existence of the $\beta_1\beta'$-transformation in the sulfur rich preparations. The transformation was observed by Roberts [2] as well as by Haraldsen [3] at the composition $Fe_{0.909}S$ (52.40 atomic per cent S). On Roberts' transformation diagram, a point is also drawn at 40° C and the composition $Fe_{0.885}S$ (53.05 atomic per cent S) but this transformation point is not discussed in the text. Not enough information is present either to ascertain the presence of a transformation in the pure β_3 phase on change into the β' phase or the limits of the $\beta_1 + \beta_2$, and $\beta_2 + \beta_3$ regions.

In the temperature range between the $\beta_1\beta'$- and the $\beta'\beta''$-transformation, we have found no definite proof of two phase regions. The structural relationship in this range which we have designated by β', is, however, rather complicated. The following features are characteristic of the β' phase:

1. Superstructure interferences are found at the intermediate compositions.
2. A structural deformation is observed at the sulfur rich limit.
3. The axial ratio is above the ideal value 1.633.
4. The intensity ratios of the interferences differ from those at 360° C in some preparations.

Much additional work is required to assure whether or not the transition between the structures occuring in the β' region is continuous under equilibrium conditions.

At 360° C a single pyrrhotite phase exists between the limits $Fe_{1.00}S$ and $Fe_{0.88}S$. As the sulfur content increases from $Fe_{1.000}S$ a proportional part of the iron atoms is missing in the structure. If this loss of atoms only affects every second layer in the c-direction, a transition from a B-8 (NiAs) to a C-6 $Cd(OH)_2$ structure type can be said to occur. In the system $CoTe—CoTe_2$, Tengnér [22] supposed that a statistical deficiency existed only as long as the metal deficit is small. Since but a relatively small part of the iron can be missing in the pyrrhotite phase, the vacant lattice sites are probably distributed at random on the positions 000; $00\frac{1}{2}$. A preferential deficiency of iron atoms in say $00\frac{1}{2}$ would cause intensity changes and the occurrence of additional interferences. We have not been able to observe that, and furthermore, the close to ideal axial ratio in the pyrrhotites at 360° C may be an indication of a statistical missing of the iron atoms. The pyrrhotite phase on Fig. 3 designated with β'' is therefore supposed to have an ordinary B-8 structure with iron atoms missing at random when the sulfur content exceeds the stoichiometric ratio.

SUMMARY

1. X-ray studies of synthetic pyrrhotite samples annealed at 290° C and quenched, reveal the existence of three phases with different compositions. The β_1-phase exists at the stoichiometric composition $Fe_{1.000}S$ with a B-8 like superstructure and the lattice constants

$$a = 5.965_9 \text{ Å} \qquad c = 11.758_2 \text{ Å}$$

The β_2-phase with B-8 structure has a range of homogeneity between $Fe_{0.935}S$ and $Fe_{0.900}S$ (51.69 to 52.60 atomic per cent S). The lattice constants at the iron rich limit are

$$a = 3.448_2 \text{ Å} \qquad c = 5.760_4 \text{ Å}$$

and at the sulfur rich limit:

$$a = 3.440_9 \text{ Å} \qquad c = 5.720_2 \text{ Å}$$

The β_3-phase has a low symmetric structure and a composition close to $Fe_{0.877}S$ (53.27 atomic per cent S). These monoclinic lattice constants were computed:

$$a = 5.952 \text{ Å}, \quad b = 3.433 \text{ Å}, \quad c = 5.690 \text{ Å}, \quad \text{and } \beta = 90.4°$$

Preparations rapidly cooled from above the 325° C transformation temperature do not reveal the monoclinic deformation and the two phase regions.

2. The lattice constants found for pyrrhotite minerals agree very well with those found for the β_2 and β_3 phases. Chemical analyses confirm that the sulfur content of the low symmetric β_3 phase is higher than the sulfur content of the β_2 phase in the pyrrhotite minerals. Distinct ferromagnetic properties are connected with the low symmetric structure.

3. X-ray high temperature studies on synthetic pyrrhotites were performed at 185—190° C and at 360° C. At the former temperature probably a single phase, the β' phase, exists between the limits $Fe_{1.000}S$ and $Fe_{0.877}S$ (50 to 53.27 atomic per cent S). At the sulfur rich limit the monoclinic deformation still exists.

$Fe_{1.000}S$: $a = 3.501_4$ Å, $c = 5.807_2$ Å

$Fe_{0.877}S$: $a = 5.994$ Å, $b = 3.460$ Å, $c = 5.697$ Å and $\beta = 90.4°$

By raising the temperature to 360° C the monoclinic deformation disappears and the lattice constants vary within these limits:

$Fe_{1.000}S$: $a = 3.538_6$ Å, $c = 5.786_5$ Å

$Fe_{0.877}S$: $a = 3.503$ Å, $c = 5.702$ Å

No extension of the range of homogeneity is observed at 550° C.

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A Spectrophotometric Study in Infra-Red of the Hydrolysis of Dimethylamido-ethoxy-phosphoryl Cyanide (Tabun)

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Dimethylamido-ethoxy-phosphoryl cyanide, generally known as Tabun belongs to a class of organic phosphorus compounds which are biochemically characterized by their ability to inhibit the vital enzyme cholinesterase. Tabun is one of the most potent of these compounds.

Information concerning the hydrolysis of Tabun and similar derivatives is of great toxicological interest. In the first place every physiological action must occur in an aqueous phase, in which there exists the possibility of hydrolysis of the inhibitor. In the second place, according to a theory proposed by Burgen [1], it is probable that the enzyme inhibition is caused by a phosphorylation of some active group in the enzyme. If this is the case it is possible that the rate of hydrolysis runs parallel with the power to phosphorylate enzyme protein, *i.e.* that the rate of hydrolysis may be the measure of the inhibitory capacity of the compound as pointed out by Topley [2]. Several exceptions of this rule, however, will be found. The polarity of the molecule, for instance, will certainly influence the inhibition mechanism, and for reasons easily understood a very active phosphorylating compound, which reacts with water very quickly, will act as a weak inhibitor, as it is destroyed before meeting the enzyme.

The hydrolysis of the cyanide group of Tabun has previously been investigated by Holmstedt [3]. He compared these results with toxicity tests made concurrently in white mice. However, he found that the rapidly decreasing toxicity during the hydrolysis was not proportional to the splitting off of the cyanide group. This discrepancy may be explained by the dimethylamino group also being easily split off as pointed out by the present writer in a work recently carried out by Homstedt and Larsson [4].

The present investigation was undertaken to give a more clear view of the course of the hydrolysis of Tabun. By following the alterations of the absorption bands in the infra-red spectrum, the author has succeeded in elucidating the reaction in a simple and perspicuous manner. As far as the author knows, no examinations of this kind have been carried out before. In the work by Holmstedt and Larsson, quoted above, the absorption at 13.70—14.25 μ (730—702 cm $^{-1}$) was assigned to the vibrations of the P—N linkage in the combination P—N$\begin{smallmatrix}\diagup\mathrm{C}\\ \diagdown\mathrm{C}\end{smallmatrix}$ analogously to Meyrick and Thompson's [5] correlation of the absorption at 12.20—12.70 μ (820—788 cm $^{-1}$) with the vibrations of the P—O bond in the skeleton P—O—C. The latter correlation, however, has been met with some criticism by Daasch and Smith [6]. To prove that the absorption at 13.70 μ (730 cm $^{-1}$) in the spectrum of Tabun really originates from the P—N linkage, the author has followed the decreasing absorption at this wave-length during the hydrolysis. The amount of the dimethylamine formed has simultaneously been determined by a colorimetrical method according to Dowden [7]. Furthermore the infra-red spectra of two bis-(dimethylamido)-phosphoryl derivatives have been recorded and the absorption band of their dimethylamino groups has been discussed.

EXPERIMENTAL

As the measuring of the infra-red spectra was performed in a rock salt cell, it was necessary to transfer Tabun and its hydrolysis products to a suitable solvent, from which the water could be removed. The best result was obtained with dioxane.

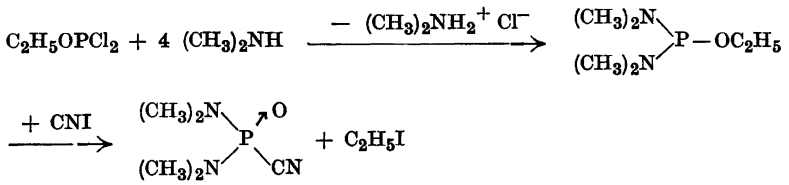
A series of samples consisting of 0.300 ml of Tabun in 0.150 ml of distilled water, the amounts measured by means of an AGLA micrometer syringe, were vigorously shaken and kept in a water bath at 25.0 $\pm$ 0.2° C. At intervals a sample was mixed with 4.00 ml of dioxane. The solution was dried with 1.0 g of anhydrous sodium sulphate for 30 minutes with shaking, after which the solution was sucked off through a glass filter. The infra-red spectrum of the filtrate was then recorded between 3.8 to 4.9 μ and between 12.0 to 14.0 μ with a Beckman IR 2 Infrared Spectrophotometer. A rock salt cell was used and the thickness of the sample was 0.10 mm.

In a similar series the splitting off of the dimethylamine was determined colorimetrically according to the method reported by Dowden [7]. The principle of this method is to convert the dimethylamine into dimethyl-dithiocarbamic acid with carbon disulphide in a benzene solution. The cupric salt of this acid, which is insoluble in water but rather soluble in benzene, has an amber tint. The color-intensity of the samples was measured in 10 mm cells with a Beckman DU Spectrophotometer at 4360 Å (absorption maximum). The amount of dimethylamine was calculated from the expression:

$$E = \varepsilon \cdot c \cdot d$$

where E = optical density, ε = extinction coefficient, c = concentration expressed in micromole dimethylamine per ml and d = cell thickness in cm. From a standard curve which obeys Lambert-Beer's law within the actual concentration range, the value of ε was calculated to 5.14 ± 0.07.

The infra-red absorption spectra of bis-(dimethylamido)-phosphoryl cyanide and the corresponding fluoride were recorded between 2.5 and 15.0 μ exactly in the same manner as reported in the work quoted above [4]. The former was prepared in the following way:



The dimethylamine, dissolved in ether, was slowly added to a solution of ethoxy-phosphine dichloride in ether with strong cooling and stirring and in an atmosphere of nitrogen. When the reaction was complete, the mixture was filtered and the ether was distilled off. The residue was fractionated three times *in vacuo*. To the bis-(dimethyl-amido)-ethyl phosphite obtained, dissolved in ether, a solution of cyanogen iodide in ether was added. The reaction mixture was kept between + 10° to + 20° C. After addition of CNI the ether was evaporated and the residue fractionated twice *in vacuo*. B.p. 91—94°/0.9 mm Hg. The latter reaction is a variation of the isomerization of Arbusow [8]. (Found: P 19.4; CN 15.4. $C_5H_{12}N_3OP$ (161.15) requires P 19.22; CN 16.15. $n_D^{20} = 1.4518$).

The syntheses of Tabun and of bis-(dimethylamido)-phosphoryl fluoride were performed by Holmstedt [3], the latter in the way described by Schrader [9].

RESULTS AND DISCUSSION

As shown from Fig. 1 the decrease in per cent of the absorption at 13.70 μ agrees well with the amount of dimethylamine liberated during the hydrolysis. This proves the proposition made before that the stated absorption band can be correlated with the vibrations in the P—N<C/C> group. The infra-red spectrophotometric method, however, is not quite suitable to quantitative determinations, because it is affected with some sources of error which cause too large scattering of the values. The sigmoid nature of the curve is due to the successively increasing acidity in the solution which catalyses the splitting off of the dimethylamino group [10].

The absorption spectra of the two bis-(dimethylamido)-phosphoryl derivatives are illustrated in Fig. 2. and their absorption bands given in Table 1. In the spectra of these two compounds the absorption band characteristic of the P—N linkage is displaced to about 13.20 μ (758 cm^{-1}). The displacement

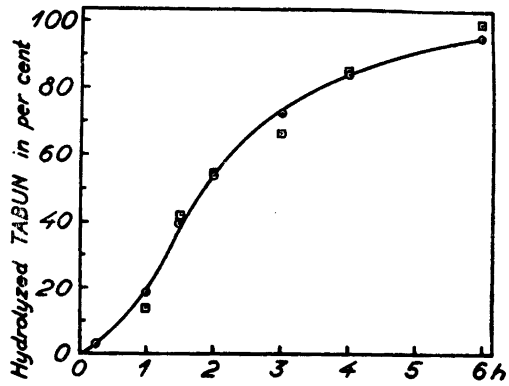


Fig. 1. Hydrolysis of Tabun in regard to the dimethylamino group.
⊙ *Colorimetrical determination.*
⊡ *Infra-red spectrophotometric determination.*

to higher frequencies indicates that this linkage in the bis-(dimethylamido) derivatives is stronger than in the monoderivatives which is also manifested by the much slower hydrolytic rate of the former. The bis-(dimethylamido) compounds are very weak inhibitors of cholinesterase *in vitro* which agrees with the hydrolytic stability. *In vivo* or in presence of liver slices, however, they become strong inhibitors which depends upon these compounds being somehow activated in the body [11]. In the spectrum of the bis-(dimethylamido)-phosphoryl fluoride an absorption band appears at 11.80 μ (847 cm^{-1}) which arises from the P—F bond. This band has been pointed out by Daasch and

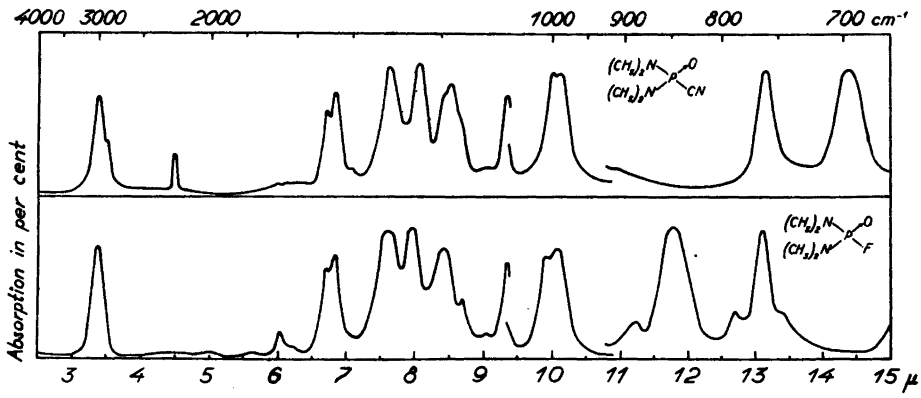


Fig. 2. Absorption curves of bis-(dimethylamido)-phosphoryl cyanide and the corresponding fluoride.

Smith [6] also. The absorption bands of the remaining groups agree well with these obtained in an earlier study of the spectra of phosphoryl derivatives [4].

In Fig. 3 is shown the absorption spectrum of Tabun hydrolyzed for four hours in comparison with the spectrum of unhydrolyzed Tabun. The hydrolysis was followed using the method mentioned above.

The very strong absorption band which appears at 3.97 μ (2 519 cm^{-1}) can probably be assigned to the vibrations of the bonded hydroxyl group according to Daasch and Smith [6].

It appears from the curves that the absorption at 4.48 μ (2 232 cm^{-1}) caused by the vibrations of the cyanide group decreases during the hydrolysis, but not so fast as the absorption of the dimethylamino group. The hydrocyanic acid simultaneously formed is expected to give an absorption band at 4.73 μ (2 114 cm^{-1}). This absorption is not arisen in the spectrum, because some of the hydrocyanic acid evaporates from the very concentrated reaction mixture. This has been established by analysis of the gasphase. The residue is probably adsorbed by the sodium sulphate. Furthermore this absorption band has been recorded in preliminary experiments using diluted aqueous solutions which have been measured in calcium fluoride cells directly. In the figure this band is marked with a dotted line. A quantitative determination of the splitting off of the cyanide group by following the decreasing absorption at 4.48 μ is even more difficult than that of the dimethylamino group. An

Table 1. Absorption bands of bis-(dimethylamido)-phosphoryl cyanide and the corresponding fluoride.

λ = *Wave-length in* μ.
ν = *Frequency in* cm^{-1}.
s = *strong*, *m* = *medium*, *w* = *weak*.

Bis-(dimethylamido)-phosphoryl cyanide				Bis-(dimethylamido)-phosphoryl fluoride			
λ	ν	λ	ν	λ	ν	λ	ν
3.38	2 958 s	8.43	1 186 s	3.36	2 976 s	9.36	1 068 s
3.51	2 849 m	8.54	1 171 s	6.03	1 658 w	9.94	1 006 s
4.48	2 232 m	9.05	1 105 w	6.70	1 493 m	10.09	991 s
6.72	1 488 m	9.36	1 068 s	6.83	1 464 s	11.26	888 w
6.85	1 460 s	10.03	997 s	7.62	1 312 s	11.80	847 s
7.08	1 412 w	10.14	986 s	7.97	1 255 s	12.71	787 m
7.64	1 309 s	13.18	759 s	8.43	1 186 s	13.13	762 s
8.07	1 239 s	14.41	694 s	8.70	1 149 m	13.40	746 m
				9.05	1 105 w		

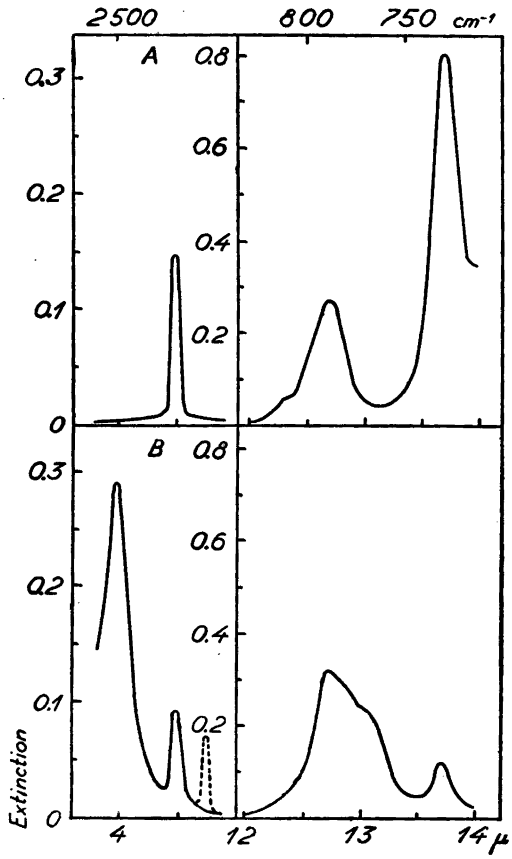


Fig. 3. Absorption curves of unhydrolyzed (A) and hydrolyzed (B) Tabun.

exact determination of the intensity of the cyanide absorption is complicated because of the overlapping absorption of the hydroxyl group.

As shown from the figure the intensity of the absorption band at 12.70 μ (788 cm^{-1}) is unchanged, but a little broadening of the band can be observed. This indicates that no splitting off of the ethoxy group will occur, on the assumption that this absorption corresponds to the P—O—C group as Meyrick and Thompson [5] have proposed. The alteration of the absorption band at 13.70 μ (730 cm^{-1}) has in detail been discussed above.

The spectrophotometric method is only a qualitative test of the hydrolysis, but it rapidly gives a perspicuous view of the hydrolytic course of a compound. A quantitative examination of the hydrolysis of Tabun to investigate its reaction mechanism in connection with its detoxification is now in progress.

SUMMARY

It has been shown that the decreasing absorption at 13.70 μ (730 cm^{-1}) in the spectrum of Tabun is proportional to the amount of dimethylamine liberated during the hydrolysis. This argument proves the hypothesis that the stated absorption band is caused by vibrations in the P—N<C/C skeleton.

The infra-red absorption spectra of bis-(dimethylamido)-phosphoryl cyanide and the corresponding fluoride are recorded and the reasons of the displacement of the absorption of the dimethylamino group to shorter wave-lengths are discussed. The synthesis of the former compound is described.

A qualitative infra-red spectrophotometric method for following the course of the hydrolysis of Tabun is described. In the acid environment the dimethylamino group is split off more rapidly than the cyanide group, but the P—O—C linkage seems to be stable.

The author is indebted to the Chief of the Research Institute of National Defence, Dept. 1, Professor Gustaf Ljunggren, for the kind interest he has shown in this work.

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On the Reversible Enzyme Inhibition by Tetraethyl Pyrophosphate (TEPP)

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Much attention has been brought to compounds with strong inhibiting action on cholinesterases. One type of these "anticholinesterases" are reversible inhibitors (*e. g.*, physostigmine, prostigmine). The second type of inhibitors are represented by, amongst others, the organophosphorus compounds which act as powerful inhibitors (see the review by Koelle and Gilman [1]). These latter compounds have been regarded as irreversible inhibitors. Neither dilution nor dialysis can restore the inactivated cholinesterase activity. However, two observations have been published recently [2,3] which demonstrate the *in vitro* reversibility of the tetraethyl pyrophosphate (TEPP) inhibition in some cases. Earlier, an observation of this kind was made by Grob and Harvey [4] who showed that the *in vivo* inhibition of erythrocyte acetylcholinesterase by TEPP was reversible for the first 24 hours. The reversible TEPP inhibition *in vitro* was demonstrated by Hobbiger [2] with the esterases of erythrocytes, brain, intestine, and salivary glands; human serum was exceptional. The observation of Wilson [3] was made with purified acetylcholinesterase from the electric tissue of *Electrophorus*. The enzyme, inhibited by TEPP, was reactivated by reactions of the enzyme-inhibitor complex with water or more rapidly with choline or hydroxylamine.

A similar demonstration of the reversibility of TEPP inhibition had been made in this laboratory when these two papers [2,3] came to our knowledge. The experiments reported here have been carried out with esterases of various sources; special attention has been given to the cobra venom acetylcholinesterase.

METHODS AND MATERIAL

The esterase activity was measured with the Warburg manometric technique previously described in detail [5]. The enzyme activity is expressed in b_{30} values, *i. e.*, the amount of CO_2 in μl evolved during 30 minutes minus the corresponding value for non-enzymic hydrolysis. All measurements were made at 25° C.

In most experiments reported in the following, the enzyme source was a dried and crystallised preparation of cobra venom *(Naja naja)*. The venom was dissolved in bicarbonate-Ringer's solution. In this solution the esterase is stable for months [6]. Human plasma and human erythrocytes and also *Helix* blood have been employed in some experiments.

Acetylcholine chloride was used as substrate; the final concentration in the reaction mixture was 3.30×10^{-3} *M*. Tetraethyl pyrophosphate (TEPP) was a pure sample *.

RESULTS

Various enzyme preparations were incubated with TEPP at pH 7.4. The concentration of the inhibitor was chosen to give about 90 % inhibition. Cholinesterases prepared from the following sources were tested: human plasma, human erythrocytes, cobra venom, and *Helix* blood.

In most of the experiments the TEPP treated enzyme solutions together with the controls were kept in the refrigerator (4.5° C). The esterase activity was measured after one hour and then after various lengths of time, continuing for 60 to 80 days. The results are illustrated in Table 1 and Figs. 1 and 2.

Table 1. Reactivation of cholinesterase from various sources after inhibition by TEPP. Values refer to per cent of control for each determination.

Days of incubation with TEPP	Human plasma	Human erythrocytes	Cobra venom	*Helix* blood
1/24	5.5	8	4	3.5
1	7	10	4	3.5
5	7.5	14	5	7
10	—	—	21	12
21	12	21	51	17
49	25	29	68	22.5
59	27	39	—	—
77	—	—	71	25

* TEPP was synthesised and kindly placed at our disposal by Dr. Bo Holmstedt, Research Institute of National Defence, Department I, Ulriksdal, Sweden.

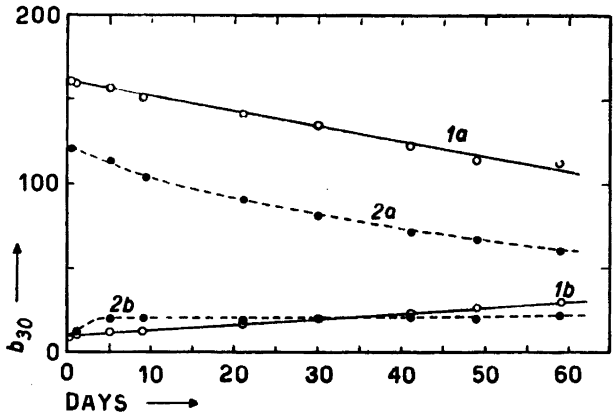


Fig. 1. Reactivation of human plasma cholinesterase (1) and human erythrocyte acetylcholinesterase (2) after inhibition by TEPP. Incubation at 4.5° C. a: controls; b: TEPP treated solutions.

Reactivation of esterase activity after inhibition by TEPP was observed in all cases studied. The recovery process was fastest with solutions of cobra venom, slowest with human plasma and *Helix* blood. For example, in case of cobra venom little reactivation occurred during the first 5 days, but after 3 weeks the activity was 50 %, after 11 weeks 70 % of control. Erythrocyte esterase activity was restored to about 20 % of control after 3 weeks.

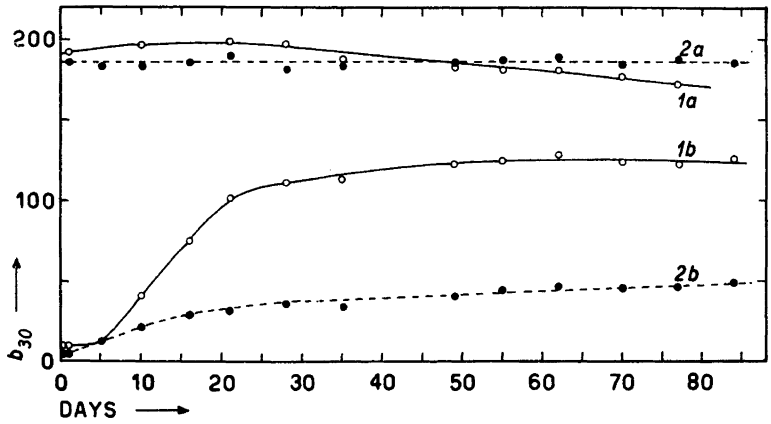


Fig. 2. Reactivation of acetylcholinesterase of cobra venom (1) and Helix blood (2) after inhibition by TEPP. Incubation at 4.5° C. a: controls; b: TEPP treated solutions.

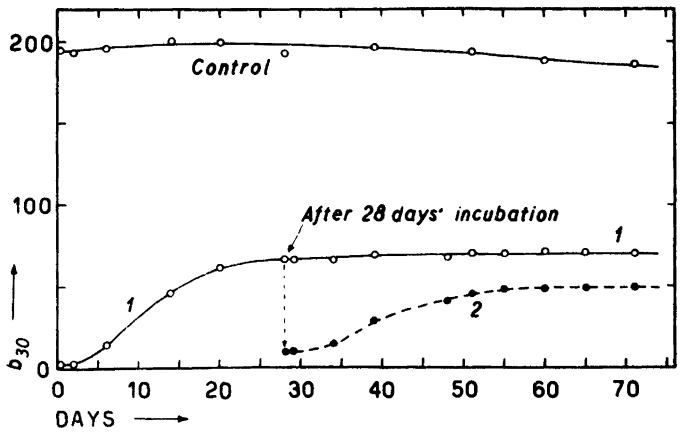


Fig. 3. Reactivation of cobra venom acetylcholinesterase after inhibition by TEPP. Incubation at 4.5° C. After a 28 day incubation period with TEPP this solution (1) was again treated with TEPP (2).

Fig. 3 also illustrates the reversibility of the TEPP inhibition of cobra venom acetylcholinesterase. After a 28 day incubation period, the TEPP treated solution, the activity of which was 33% of control, was divided into two parts. One part was treated with another portion of TEPP to reduce the activity to about 5%, the other part was diluted with bicarbonate solution and used as a second control. The esterase activity was again found to be restored in the solution which had been treated twice with TEPP. After another 30 days the activity was 72% of the second control, 26% of the first control.

The reactivation of cholinesterases after inhibition by TEPP may be due to dissociation of the enzyme-inhibitor complex or to a destruction of inhibitor. The complex, most probably, is a phosphorylated enzyme. In the reactivation processes described above it appears likely that water reacts with this phosphorylated enzyme in the same way as it does with the acetylated esterase in the acetylcholine hydrolysis. The former process is a slow one in comparison with the latter. The rate of reactivation after TEPP inhibition is measurable, for other organophosphorus compounds tested it is too slow to be detected. This is the case with DFP (di*iso*propyl fluorophosphate) and Tabun (dimethylethoxy-phosphoryl cyanide) [7].

It has been reported by Wilson *et al.* [3,8] that acetylated acetylcholinesterase (of electric tissue) reacts with hydroxylamine in a way similar to the reaction with water. It was demonstrated later that hydroxylamine also reacts with the phosphorylated enzyme. At that time the present authors had performed experiments with hydroxylamine which was shown to have a remark-

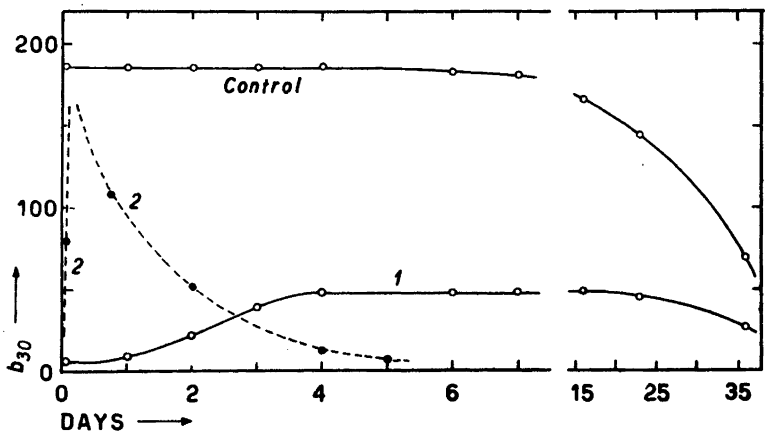


Fig. 4. Reactivation of cobra venom acetylcholinesterase after inhibition by TEPP. The effect of hydroxylamine on this process. The solutions were kept at 25° C. 1) No hydroxylamine; 2) Hydroxylamine added after 10 minutes' incubation, 0.05 M NH_2OH in the incubation mixture.

able effect on the reaction process after inhibition by TEPP. A typical experiment carried out with cobra venom acetylcholinesterase is shown in Fig. 4. The esterase was incubated for 10 minutes with TEPP, and hydroxylamine was then added to give 0.05 *M*. The reaction between TEPP treated enzyme and hydroxylamine of that concentration is too rapid to be measured accurately. In about two hours the activity was restored almost completely.

After restoration of activity another process begins. This is a comparatively slow reaction between the reactivated enzyme and phosphorylated hydroxylamine. In this process the esterase is again inhibited. The inhibition observed was 50% after about one hour, it was almost complete after 5 days. The difference in reaction rates between these two processes, dephosphorylation and subsequent inhibition, has made it possible to detect reactivation of TEPP inhibited enzyme.

Other compounds also have been tested for their influence on the reactivation process after inhibition by TEPP. Up to the present, cobra venom has been used in these experiments and pH of the incubation mixtures has been 7.4. Choline, arginine, and creatinine have been tested so far. Arginine at a concentration of 10^{-3} *M* has practically no influence on the rate of reactivation. Creatinine at a concentration of 7×10^{-3} *M* seems to react with phosphorylated enzyme and thereby the esterase activity is restored to a certain degree more rapidly than in the absence of this reagent.

Choline and all choline derivatives investigated are competitive and reversible inhibitors of cholinesterases [5]. Choline reacts with TEPP inhibited esterase demonstrated with 1.43×10^{-2} *M* choline chloride. The activity was restored to 25% of control after 2 days (for comparison with control, see Fig. 2). Higher choline concentrations would give better results, as was shown by Wilson [3] with the electric tissue esterase.

DISCUSSION

The inhibiting action of organophosphorus compounds on cholinesterases has been regarded an irreversible process until quite recently. *In vitro* experiments, however, have demonstrated conclusively that esterases inhibited by some of these compounds (see below) are reactivated although this process under ordinary conditions is comparatively slow. Thus, TEPP inhibited cholinesterases of various sources were reactivated; these sources were erythrocytes, brain, salivary glands [2], and electric tissue [3]. In the present paper reactivation was demonstrated also with cobra venom and *Helix* blood, both materials containing acetylcholinesterase. A small reactivation was also observed with the esterase of human plasma which was an exception in Hobbiger's series. TEPP has been the only phosphate investigated that renders the inhibited enzyme reversible; however, among the newly discovered "dialkylphosphostigmines" the lower homologoues are also reversible inhibitors [11] (see below). The inhibition by DFP *in vitro* is irreversible or its reversibility is too slow a process to be detected. The inhibition by Tabun also is not found to be reversible.

Various factors influence the degree of inhibition *in vitro* by these "irreversible anticholinesterases" (see review by Whittaker [9]). Most certainly the inhibition can be interpreted as a phosphorylation process, in which the enzyme is not destroyed. A direct proof for this has been produced by Jansen *et al.*[10] with α-chymotrypsin inhibition by ^{32}P labelled DFP. A similar process is most probably valid for cholinesterases. The phosphorylation is a comparatively slow reaction. This first reaction is supposed to be reversible and is dependent upon the various reactive groups around the phosphoryl group, $\rangle P = O \leftrightarrow \rangle P^+ \rightarrow O^-$. We do not know exactly the distribution of electric charges in this linkage, but it is certain that the phosphorus atom is relatively positive. The more positive this atom is, the greater reactivity is possessed by the compound. The phosphorus atom in DFP is more electrophilic than in TEPP which explains the difference in reactivation after enzyme inhibition. Wilson [3] has recently discussed these problems from a similar point of view.

In the reactivation process after inhibition by TEPP, described above, the inhibitor is not regenerated. Therefore, after the initial complex has been formed one part of the inhibitor is supposed to set free. This secondarily formed complex is now susceptible to hydrolytic dissociation or dephosphorylation. In case of dialkylphosphates, Burgen and Hobbiger [11] have recently found evidence for the theory that the duration of action of these compounds is determined by the dialkylphosphoryl residue.

No doubt, there is a great difference between the chemical structures of the classical "anticholinesterases", physostigmine and prostigmine, on one hand, and the "irreversible inhibitors", the organophosphorus compounds, on the other. The high affinity for cholinesterases, common to both these groups of inhibitors, may be due to similarity in electronic structures of the carbonyl group (in urethanes) and the phosphoryl group. As the phosphorus atom in the phosphoryl compounds, the carbon atom in the urethanes is also electrophilic. Both groups react with the active centre II [12] (the esteratic site, according to Wilson *et al.*). If the inhibitor molecule contains, in addition to one of these electrophilic groups, a positively charged atom, *e.g.*, a quaternary nitrogen atom as in prostigmine or the recently synthesised "dialkylphosphostigmines" [11], there is a two-point contact, now also with the active centre I (the anionic site). This latter combination is reversible. The inhibitor molecule of the complex formed is not altered as in the case of an one-point contact at the centre II. This probably explains the difference in kinetic behaviour of the various "anticholinesterases".

SUMMARY

Cholinesterases inhibited by tetraethyl pyrophosphate (TEPP) are reactivated slowly in vitro. The reversible inhibition by TEPP has been demonstrated with human plasma, human erythrocytes, cobra venom, and *Helix* blood. The rates of this recovery process are different for various enzyme preparations. The highest rate was obtained with cobra venom acetylcholinesterase, the lowest with human plasma and *Helix* blood.

The enzyme-inhibitor complex reacts with hydroxylamine to yield reactivated enzyme and hydroxylamine phosphate, which in its turn is a slowly reacting inhibitor of the enzyme.

The effects of choline, arginine, and creatinine respectively on the recovery process are shortly reported.

The mechanism of action of enzyme inhibition by organophosphorus compounds is discussed from a general point of view.

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Intermolecular Free Lengths in the Liquid State. I. Adiabatic and Isothermal Compressibilities

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It was pointed out in a preliminary communication that certain properties of the liquid state can be studied to advantage as a function of the free length between the molecules (Jacobson [1])*. These properties include compressibility, surface tension, viscosity and diffusion constants *i.e.*, those that are mainly dependent on the forces between the molecules.

The reason for choosing the intermolecular free length for these studies instead of the distances between centres such as are usually employed are as follows. The intermolecular forces, which in one way or another determine the said properties of liquids, consist of attractive forces and repulsive forces. These forces have opposite directions but are numerically equal under given external conditions. The attractive forces are dependent on the distance between what are called the centres of attraction of the molecules, whereas the repulsive forces are dependent on the distance between the surfaces of the molecules. The distance between centres of attraction is a property extremely difficult of definition, one reason being that this centre does not coincide with the geometrical centre of the molecules of the liquid (Hildebrand [2], Hudleston [3]). The distances between the surfaces of the molecules, on the other hand, have a clear physical significance, and thus lend themselves more easily to clear definition. For these reasons, we may anticipate the most simple relations if the intermolecular free length is used.

In some subsequent works surface tension, viscosity, thermal expansion and molecular association will be related to the intermolecular free length. The present work will deal with the adiabatic and isothermal compressibilities for liquids and liquid mixtures.

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* Owing to an unfortunate printer's error equation (1) was wrongly given. It should read:

$$j = kL^p$$

DEFINITIONS

The intermolecular free length in a liquid can be obtained if there is taken as basis a molecular model similar to that employed by Eyring [4] in his theory of the liquid state (*Cf.* also Brinkman [5]).

At absolute zero a liquid has the molar volume $V_0 = M/\varrho_0$, where M is the molecular weight and ϱ_0 is the density at $0°$K. The molecules have then their closest packing possible, which is shown in Figure 1. The total surface of all the molecules in one mole is designated by Y and will here be called the internal surface of the liquid. If the temperature is raised to $T°$K the liquid is expanded as a whole through molecular oscillations, though the molecules themselves are not expanded. If the molar volume is designated by V_T the increase in volume will be equal to the available volume $V_a = V_T - V_0$ (Kittel [6]). The free length L between the surfaces of the molecules, hereafter designated the free length, can be obtained when the surface Y is unaltered after a rise in temperature

$$L = \frac{2\,V_a}{Y} \tag{1}$$

This equation involves an approximation and gives values for the free length that are too large. Nevertheless, equation (1) will be regarded as a definition of the free length at temperatures considerably lower than the critical temperature, for the following reasons.

Any exact computation of the free length has to be based on a given type of packing and a given form of the molecules of the liquid. For hexagonal packing of spherical molecules, the length according to the Eyring theory is $L_{hex} = (2^{\frac{1}{2}}/N)^{\frac{1}{3}}\,(V_T^{\frac{1}{3}} - V_0^{\frac{1}{3}})$ where N is Avogadro's number. For organic liquids of various types at room temperature, L according to (1) is app. 20% greater than L_{hex}. For practical purposes, it is of lesser importance that one should regularly get too great a numerical value of the free length. It is more essential that L according to (1) should have a clear physical significance which always makes possible a simple computation from V_T and V_0, even in those cases where an exact treatment may be impossible, *i.e.*, in liquid mixtures and with molecular association. With rising temperature the error in L according to (1) increases and therefore the normal boiling point of the liquid concerned should be put as the upper temperature limit for the range of applicability of the definition.

One particular advantage of equation (1) is that it does not necessarily require spherical molecules. For spherical molecules the internal surface is $Y = (36\,\pi N V_0^2)^{\frac{1}{3}}$. For a molecule of another form the internal surface will be

$$Y = \mathrm{f}(36\pi N V_0^2)^{\frac{1}{3}} \tag{2}$$

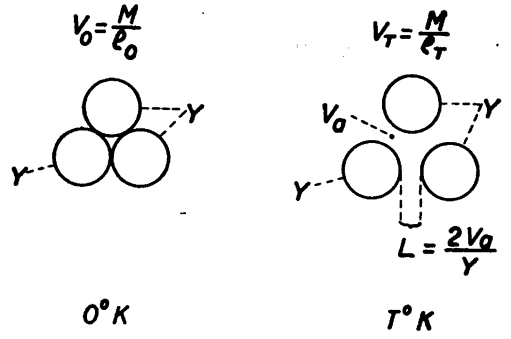


Fig. 1. The intermolecular free length (L) can be obtained from the available volume ($V_a = V_T - V_0$) and the internal surface (Y) of the liquid.

where f is a form factor giving the ratio between the surface of the molecule and the imagined spherical surface which encloses the same volume as the volume of the molecule. By the introduction of the form factor f it is possible to compute L for any form of the molecules. For all low molecular weight substances one may with good approximation put $f = 1$, and this has been done in all cases in the present work. For high molecular weight substances with highly asymmetrical molecules f is considerably greater than 1. The form factor has been introduced to make (1) applicable to solutions of such high molecular weight substances.

Equation (1) includes the zero volumes V_0. This can be obtained in two ways. V_0 can be theoretically computed according to Sugden [7] for any chemical compound of known composition and structure by the addition of atomic and structural constants. Greater accuracy of V_0 is obtained, however, if the critical temperature T_c is known and Sugden's formula can be used, which gives the variation of molar volume with the temperature

$$V_0 = V_T \left(1 - \frac{T}{T_c}\right)^{0.3} \tag{3}$$

ADIABATIC COMPRESSIBILITY OF NORMAL LIQUIDS AT 20° C

The adiabatic compressibility β_{ad} is easy to compute with great accuracy from measured values of the density ϱ of the liquids and the sound velocity u according to the formula $\beta_{ad} = 1/\varrho u^2$. Detailed information is furnished by the literature regarding the compressibility of organic liquids (Bergmann [8]; Lagemann *et al.*[9]; Weissler [10]; Schaaffs [14]). All non-associated organic liquids for which it has been possible to find accurate values for critical temperature and adiabatic compressibility have been tabulated in Table 1. The critical

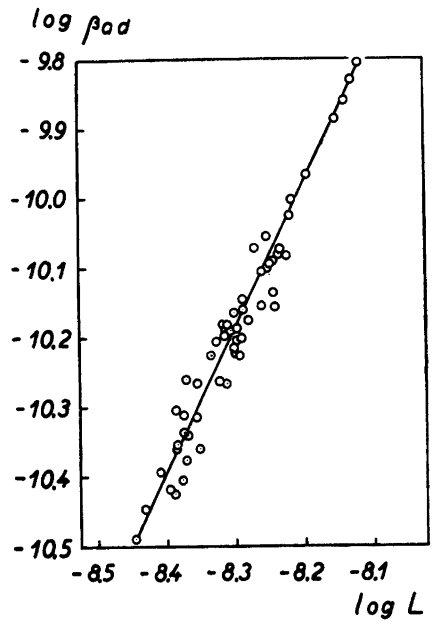


Fig. 2. Adiabatic compressibilities as a function of intermolecular free lengths in 54 organic non-associated liquids at 20° C.

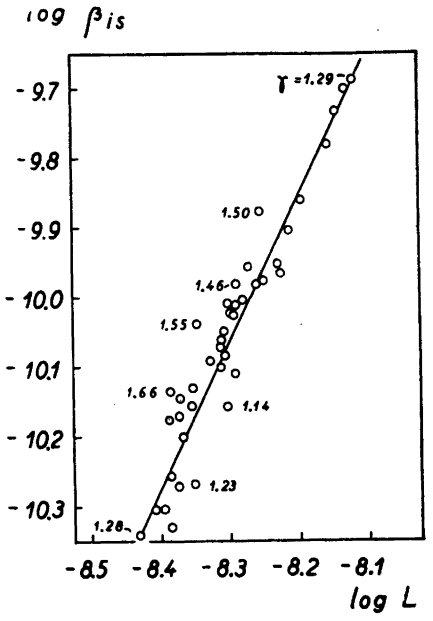


Fig. 3. Isothermal compressibilities as a function of intermolecular free lengths in 40 organic non-associated liquids at 20° C.

temperatures, taken from Landolt-Börnstein Physikalisch-Chemische Tabellen and International Critical Tables, are required for computation of the values for V_0 given in the table. The free length L has been computed according to (1) from molar volumes V_0 and V_T. In Figure 2 the adiabatic compressibilities for these 54 non-associated liquids have been plotted as a function of the free length both in logaritmic scale. As will be seen from the figure all points lie around a straight line corresponding to a relation of the type

$$\beta_{ad} = k \cdot L^p \tag{4}$$

where $p = 2.082$ and $\log k = 7.097$ for c.g.s. units at 20° C.

In order to test the utility of equation (4) for computation of the intermolecular free length L from compressibility data such a computation has been made and the result is given in Table 1. It will be seen that the agreement is good between L computed from molar volumes according to the definition (1) and L computed from compressibilities according to (4). The percentage of deviation in the values thus computed for L is on the average 3.3 %. This deviation is mainly due to the difficulty in determining V_0 exactly, and therefore

Table 1. Intermolecular free lengths of organic liquids calculated from adiabatic compressibilities (eq. (4)) and from molar volumes (eq. (1)).

Substance	Formula	Molar volume at absolute zero, V_0	Molar volume 20°C, V_T	Compressibility at 20°C, β_{ad} 10^{-12} cm²/dyne	Intermolecular free length, L 10^{-8} cm	
					Calc. from V_0 and V_T	Calc. from β_{ad}
2-Methylbutane	C_5H_{12}	86.1	116.4	156.2	0.76	0.760
Pentane	C_5H_{12}	86.7	116.2	147.7	0.74	0.740
Benzene	C_6H_6	71.3	89.0	64.9	0.50	0.499
Cyclohexane	C_6H_{12}	86.2	108.0	78.2	0.55	0.545
Hexane	C_6H_{14}	100.8	131.8	130	0.70	0.696
Toluene	C_7H_8	86.7	106.4	65.5	0.49	0.501
Methylcyclohexan	C_7H_{14}	102.6	128.5	84.2	0.58	0.565
Heptane	C_7H_{16}	115.7	146.5	108	0.64	0.637
o-Xylene	C_8H_{10}	101.1	121.9	62.1	0.47	0.488
m-Xylene	C_8H_{10}	101.5	123.0	64.5	0.49	0.497
p-Xylene	C_8H_{10}	101.8	123.4	65.8	0.49	0.502
Ethylbenzene	C_8H_{10}	100.4	122.3	64.4	0.49	0.497
Octane	C_8H_{18}	130.8	162.5	99.3	0.60	0.612
Pseudocumene	C_9H_{12}	113.4	137.2	61.0	0.50	0.484
Cumene	C_9H_{12}	114.9	138.3	63.3	0.49	0.492
Mesitylene	C_9H_{12}	115.0	139.3	62.5	0.50	0.489
Ethylbromide	C_2H_5Br	57.7	74.6	84.5	0.56	0.566
Amylbromide	$C_5H_{11}Br$	100.0	123.5	85	0.53	0.568
Bromobenzene	C_6H_5Br	88.3	104.9	49.5	0.41	0.438
Octylbromide	$C_8H_{17}Br$	144.7	172.7	61.4	0.50	0.485
Carbontetrachloride	CCl_4	77.4	96.5	71.4	0.51	0.522
Chloroform	$CHCl_3$	63.3	80.3	66.5	0.52	0.504
Methylenechloride	CH_2Cl_2	49.5	63.6	62.8	0.51	0.491
Acetylenetetrachloride	$C_2H_2Cl_4$	87.7	104.9	45.6	0.43	0.421
Ethylenedichloride	$C_2H_4Cl_2$	63.3	79.1	54.0	0.49	0.456
Propylchloride	C_3H_7Cl	67.9	88.3	94.4	0.60	0.597
Chlorobenzene	C_6H_5Cl	84.4	101.7	54.1	0.44	0.457
p-Chlorotoluene	C_7H_7Cl	100.1	118.7	54.8	0.42	0.460
n-Octylchloride	$C_8H_{17}Cl$	140.4	170.5	70.0	0.55	0.517
Methyleneiodide	CH_2I_2	68.4	80.6	32.4	0.36	0.357
Methyliodide	CH_3I	48.9	62.3	63.3	0.49	0.493
Ethyliodide	C_2H_5I	64.3	80.6	68.2	0.50	0.511
Iodobenzene	C_6H_5I	94.1	111.4	44.1	0.41	0.414
Fluorobenzene	C_6H_5F	75.1	93.8	69.0	0.51	0.514
Pyridine	C_5H_5N	66.4	80.5	48.8	0.42	0.435
Piperidine	$C_5H_{11}N$	79.7	99.0	59.3	0.51	0.477
Aniline	C_6H_7N	77.4	91.1	35.7	0.37	0.374
Methylaniline	C_7H_9N	92.6	108.9	40.4	0.39	0.397
o-Toluidine	C_7H_9N	90.5	107.4	37.5	0.41	0.383
Dimethylaniline	$C_8H_{11}N$	107.3	126.8	46.0	0.42	0.423
Nitromethane	CH_3NO_2	42.6	53.6	48.5	0.44	0.433
Nitrobenzene	$C_6H_5NO_2$	86.0	102.0	38.2	0.40	0.387
o-Nitrotoluene	$C_7H_7NO_2$	99.3	117.9	41.9	0.43	0.404
m-Nitrotoluene	$C_7H_7NO_2$	100.0	118.5	38.9	0.42	0.390
Methylacetate	$C_3H_6O_2$	61.6	79.8	73.5	0.57	0.529
Ethylacetate	$C_4H_8O_2$	76.4	98.1	79.0	0.59	0.548
Ethylether	$C_4H_{10}O$	77.2	103.8	138	0.72	0.716
Acetylacetone	$C_5H_8O_2$	84.5	103.2	53.9	0.48	0.456
Propylacetate	$C_5H_{10}O_2$	91.3	114.6	80.3	0.56	0.552
Butylacetate	$C_6H_{12}O_2$	106.1	133.4	82.6	0.60	0.560
Benzaldehyde	C_7H_6O	85.3	101.5	43.7	0.41	0.412
Amylacetate	$C_7H_{14}O_2$	120.0	148.8	83.0	0.58	0.561
Acetophenone	C_8H_8O	97.8	117.1	43.6	0.45	0.412
Carbondisulphide	CS_2	47.8	60.3	59.0	0.46	0.477

also L according to (1). The uncertainty regarding V_0 is due among other things to errors in the critical temperature values available. Nevertheless, V_0 cannot be computed with greater accuracy than 0.5 %, even in favourable cases with a correct value for the critical temperature. But even a small error in V_0 has relatively great effect on L, owing to the fact that the difference $V_T - V_0 = V_a$ is only 20—30 % of V_T. From this it follows that neither V_a nor L can be given with greater accuracy than about 2 %. This explains why only two decimal places have been included in Table 1 for L values computed according to equation (1).

As regards the accuracy possible in the free length computed according to (4) from compressibilities, it should be noted that the uncertainty respecting L is probably always of the order of 1 %. This is due to several contributing factors, one being that the internal compressibility of the molecules may be thought to have a disturbing effect. With macromolecular solutions correction should be made for the relatively high internal compressibility of these molecules (10^{-11} cm^2/dyne).

ISOTHERMAL COMPRESSIBILITY OF NORMAL LIQUIDS AT 20° C

It is interesting to see whether there exists for the isothermal compressibility a relation similiar to that demonstrated for the adiabatic. The isothermal compressibility β_{is} is related to the adiabatic β_{ad} through the expression $\beta_{is} = \gamma\beta_{ad}$, where $\gamma = c_p/c_v$ is the ratio of the specific heats of the liquid. As it is not possible to obtain γ directly, the isotermal compressibility has been computed for 40 organic liquids from the relation usually employed $\beta_{is} = \beta_{ad} + T\alpha^2/\varrho c_p$ where T is the absolute temperature, α the coefficient of cubic expansion, ϱ the density and c_p the heat capacity at constant pressure. Some examples of the values used and results obtained are assembled in Table 2. In Figure 3, β_{is} has been plotted as a function of L. It will be seen that for most of the substances there applies a relation of the type

$$\beta_{is} = k \cdot L^p \tag{5}$$

where $p = 2.058$ and $\log k = 7.012$ for c.g.s. units at 20° C. For many of the substances, however, the deviation from the straight line is greater than was the case with the adiabatic compressibility. On closer investigation of these deviating substances it appears to be common for them that the ratios of the specific heats differ considerably from $\gamma = 1.3$, which is about the value for most organic liquids. There does not exist any simple generally applicable relation between γ and L. Thus the investigation suggests that the isothermal compressibility does not follow (5) so well as the adiabatic follows equation (4).

Table 2. Examples of values used for calculating isothermal compressibilities and obtained intermolecular free lengths of some organic liquids at 20° C.

Substance	c_p 10^{-7}	a 10^{-3}	γ	Compressibility β_{is} 10^{-12} cm²/dyne	Intermolecular free length, L 10^{-8}cm	
					Calc. from V_0 and V_T	Calc. from β_{is}
Pentane	2.332	1.608	1.354	200.0	0.74	0.759
Hexane	2.249	1.350	1.279	166.3	0.70	0.694
Heptane	2.225	1.244	1.276	137.8	0.64	0.633
Octane	2.205	1.160	1.256	124.8	0.60	0.603
Benzene	1.737	1.215	1.438	93.3	0.50	0.524
Cyclohexane	2.118	1.20	1.33	103.8	0.55	0.552
Toluene	1.676	1.099	1.373	89.9	0.49	0.514
Ethylbenzene	1.712	0.957	1.281	82.5	0.49	0.493
Nitromethane	1.725	1.19	1.44	69.6	0.44	0.454
Nitrobenzene	1.503	0.83	1.29	49.3	0.40	0.384
Aniline	2.072	0.850	1.280	45.7	0.37	0.370
Pyridine	1.775	1.03	1.37	66.6	0.42	0.445
Carbontetrachloride	0.846	1.22	1.45	103.7	0.51	0.551
Chloroform	0.942	1.26	1.50	99.7	0.52	0.541
Ethylbromide	0.904	1.42	1.53	129.2	0.56	0.614
Carbondisulphide	0.996	1.18	1.55	91.4	0.46	0.519

In stating this, however, it should be remembered that the accuracy of the computed isothermal values is much lower than the accuracy of the adiabatic values. In what follows we shall deal chiefly with the adiabatic compressibility.

DEPENDENCE ON TEMPERATURE

Plentiful data are available for many liquids regarding the dependence of the adiabatic compressibility on temperature in the range 0—50° C (Freyer *et al.*[11]; Lagemann *et al.*[9]). This makes possible a study of the dependence of the constants p and k on temperature. The values computed are given in Table 3. The compressibility values at 0° C appear to be of low accuracy, so that the values for p and k at this temperature are comparatively uncertain. For purposes of comparison p and k are also given for the isothermal compressibility computed from liquids with values for γ in the neighbourhood of 1.3.

If another definition than (1) for the free length is desired, say L_{hex}, the constants p and k can be converted with the aid of equation (4). It has not

Table 3. Values of the constants p and k in c.g.s. units for adiabatic and isothermal compressibilities at various temperatures.

Temp. °C	Adiabatic compressibility		Isothermal compressibility	
	p	log k	p *	log k *
0	(2.010)	(6.543)	(2.003)	(6.605)
10	2.041	6.778	2.028	6.787
20	2.082	7.097	2.058	7.012
25	2.106	7.274	2.073	7.131
30	2.128	7.459	2.089	7.246
40	2.174	7.820	2.121	7.490
50	2.220	8.181	2.155	7.750

* Computed from liquids with a ratio of specific heats of about $\gamma = 1.3$.

been possible to find any definition for L which makes p and k independent of temperature.

LIQUID MIXTURES

To investigate whether equation (4) is applicable to liquid mixtures, determination of compressibilities has been made in a number of these by measuring sound velocities with an interferometer. This instrument has been described in an earlier work (Jacobson [12]). The compressibility was obtained to an accuracy of 0.1 %. Liquids with widely differing free lengths were taken for the different mixing experiments. The results are given in Tables 4—7. In order to make possible a computation of L from molar volumes, the definition (1) has been revised to make it useable for mixtures as follows

$$L = \frac{2[1/\varrho - \{(w_1 V_{01}/M_1) + (w_2 V_{02}/M_2)\}]}{(w_1 Y_1/M_1) + (w_2 Y_2/M_2)} \tag{6}$$

where ϱ is the density of the mixture, w, V_0 and Y are the weight fraction, the zero volume and the internal surface of the two liquids with the subscripts 1 and 2. As will be seen from Tables 4—7 the agreement is very good between the free lengths computed from the compressibility according to (4) and from molar volumes as per (6). The average error is 0.8 % for all 16 mixtures investigated. The greatest deviations were demonstrated for the mixtures of methylene iodide and ethyl ether. The compressibility for this series of mixtures has been plotted in Figure 4 as a function of the free length. The reason for the relatively large errors in these experiments is without doubt largely due to the evaporation of the ether in the course of the measurements.

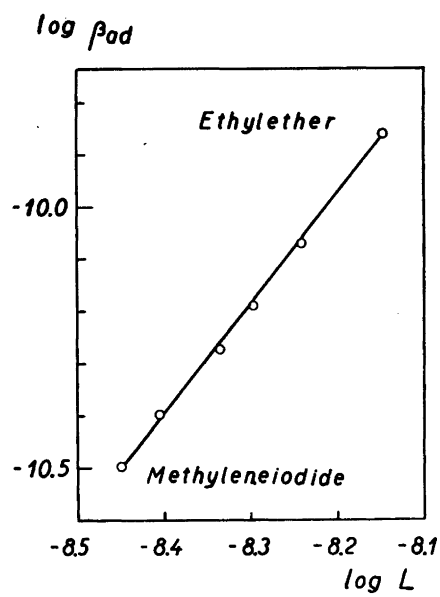


Fig. 4. Adiabatic compressibilities as a function of intermolecular free lengths in mixtures of methylene iodide and ethyl ether at 20° C.

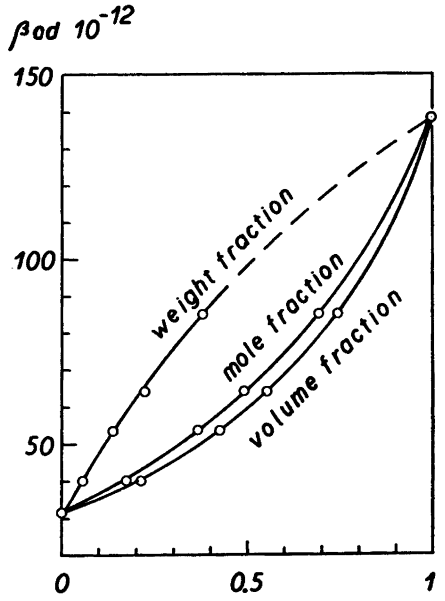


Fig. 5. Adiabatic compressibilities plotted as functions of different concentration units of mixtures of methylene iodide and ethyl ether at 20° C.

As regards the mathematical treatment of the measurement results with liquid mixtures the following should be stated. In Figure 1 the compressibilities of pure liquids have been plotted as a function of the free lengths. If two liquids are mixed then, provided equation (4) is valid, points for a series of mixtures should fall on the straight line between the points for the respective pure liquids. Owing to errors in V_0 and consequently in L, this line does not usually coincide with the line drawn in Figure 1 corresponding to $p = 2.082$ and $\log k = 7.097$. One must therefore for numerical treatment of the results compute new values for p and k for each series of mixtures of two liquids. These values are obtained by (4) from the values for the compressibilities and free lengths of the two pure liquids concerned. The values for the free lengths of the different mixtures can then be computed from their compressibilities (equation (4)). These will then be comparable with those computed according to the definition (6). In the experiment with the mixtures of methylene iodide and ethyl ether the values obtained were $p = 2.1103$ and $\log k = 7.3310$.

There is another and equally good manner in which to surmount this difficulty which arises because of errors in V_0 for the pure liquids. It is possible

Table 4. Experimental and calculated values for different fractions by weight of toluene in pentane at 20° C.

Fraction by weight	Density$_4^{20}$	Adiab. compress. β_{ad} 10^{-12}cm^2/dyne	Intermolecular free length, L 10^{-8}cm		Deviation %
			Calc. from eq. 6	Calc. from β_{ad}	
0.000	0.6278	147.4	0.706	0.706	—
0.288	0.6838	118.9	0.646	0.639	−1.1
0.509	0.7330	102.2	0.598	0.597	−0.2
0.651	0.7676	91.46	0.568	0.567	−0.2
0.827	0.8151	77.39	0.529	0.525	−0.8
1.000	0.8676	66.09	0.488	0.488	—

Table 5. Experimental and calculated values for different fractions by weight of methylene iodide in ethyl ether at 20° C.

Fraction by weight	Density$_4^{20}$	Adiab. compress. β_{ad} 10^{-12}cm^2/dyne	Intermolecular free length, L 10^{-8}cm.		Deviation %
			Calc. from eq. 6	Calc. from β_{ad}	
0.000	0.7154	138.0	0.714	0.714	—
0.618	1.407	85.02	0.575	0.568	− 1.2
0.787	1.896	64.03	0.505	0.496	− 1.8
0.861	2.237	53.26	0.462	0.455	− 1.5
0.945	2.804	40.05	0.393	0.397	+ 1.0
1.000	3.325	31.81	0.356	0.356	—

Table 7. Experimental and calculated values for different fractions by weight of diphenylmethane in hexane at 30° C.

Fraction by weight	Density$_4^{20}$	Adiab. compress. β_{ad} 10^{-12}cm^2/dyne	Intermolecular free length, L 10^{-8}cm.		Deviation %
			Calc. from eq. 6	Calc. from β_{ad}	
0.000	0.6657	132.0	0.648	0.648	—
0.258	0.7316	105.3	0.596	0.596	0.0
0.514	0.8091	81.82	0.542	0.542	0.0
0.690	0.8706	66.68	0.504	0.502	− 0.4
0.877	0.9489	52.41	0.458	0.459	+ 0.2
1.000	1.002	43.94	0.430	0.430	—

Table 6. Experimental and calculated values for different fractions by weight of pyridine in hexane at 20° C.

Fraction by weight	Density$_4^{20}$	Adiab. compress. β_{ad} 10^{-12}cm^2/dyne	Intermolecular free length, L 10^{-8}cm.		Deviation %
			Calc. from eq. 6	Calc. from β_{ad}	
0.000	0.6701	120.4	0.629	0.629	—
0.277	0.7361	97.29	0.575	0.573	− 0.3
0.494	0.7936	82.94	0.541	0.534	− 1.3
0.688	0.8566	69.50	0.499	0.495	− 0.8
0.856	0.9224	57.53	0.453	0.456	+ 0.7
1.000	0.9816	49.16	0.424	0.424	—

to compute according to (4) and (1) from the compressibilities of these liquids such values for V_0 that when plotted in Figure 1 they fall on the line $p = 2.082$ and $\log k = 7.097$.

The very good agreement between L computed from compressibilities and L computed from molar volumes for mixtures constitutes the strongest evidence of the general applicability and utility of equation (4) for computation of intermolecular free lengths.

ASSOCIATED LIQUIDS

The possibility demonstrated of computing from compressibility data the free lengths between molecules in liquids is of special interest for associated liquids. If the compressibility is plotted in the ordinary way as a function of the free length for associated liquids, without taking into account molecular association, it is found that the points for these always fall to the left of the mean line corresponding to normal liquids in Figure 1. This means that the free length in the liquid, according to the computations (equation (1)) is too small for the empirical relation (4) to hold good. The conditions with the lower fatty acids are shown in Figure 6. If the free lengths are computed by using monomeric molecular weights, then all will fall to the left of the line for normal liquids. But according to current opinion these acids occur at 20° C in associated state as dimers. If this is taken into account and the double molecular weight is used for the computation of L from (1) then all the acids will fall in the neighbourhood of the line for normal liquids. This supports the utility of the method stated here for computation of free lengths from

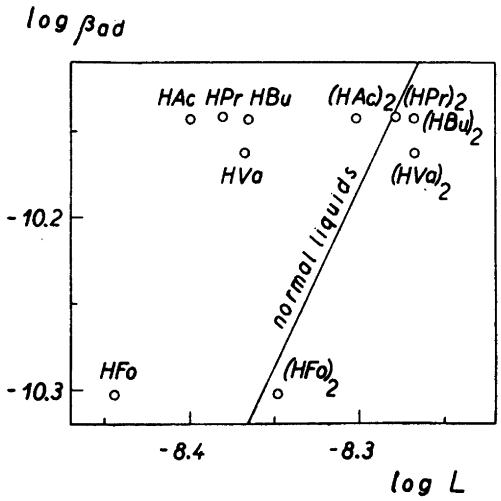


Fig. 6. Adiabatic compressibilities plotted as functions of intermolecular free lengths in fatty acids calculated as monomers and dimers. HFo = formic acid, HAc = acetic acid, HPr = propionic acid, HBu = butyric acid and HVa = valeric acid.

compressibilities and also, of course, the generally accepted opinion of organic acids as dimers.

It is in fact possible to compute in a simple manner association factors if the compressibility and zero volume of the compound are known. It is also possible to study easily association in liquid mixtures. A report on this will be given in a later work, when comparison will be made between association factors computed from different properties and in different ways.

GENERAL DISCUSSION

Compressibilities and ultra sonic velocities have been closely studied by a large number of investigators (Bergmann [8]). Efforts have been made to utilise values obtained to draw conclusions regarding the chemical constitution of the liquids (Parthasarathy [13]; Schaaffs [14]; Rao [15]). The relations obtained, however, have been very complicated or have proved not to be of general application. Thus it has been demonstrated by several authors (Lagemann *et al.*[9]; Weissler [10]; Natta *et al.*[16]) that Rao's molar sound increments are not constant. This is understandable as it has been shown that the compressibility and thus to a large extent sound velocities are dependent on the free length between the molecules. The free length in turn varies, of course, with chemical constitution, but probably not in any simple manner. There seems to be small possibility of finding such a general correlation between free length and chemical constitution.

Detailed studies have also been made of compressibility and sound velocity in liquid mixtures. Attempts have been made to find relations between these properties and the concentration of the components comprised in the mixtures. Various concentration units, such as mole fraction, weight fraction and volume fraction have been tried with the object of finding linear relations with the compressibility (or the sound velocity). It has, however, been possible only in special cases to find such linear relations and these are rather to be regarded as accidental. The shape of the curve obtained when the compressibility (sound velocity) is plotted as a function of the concentration is entirely dependent on the choice of concentration units. This may be seen from Figure 6, in which the compressibility has been plotted as a function of different concentration units in mixtures of methylene iodide and ethyl ether. If the same is done with the sound velocity the curves will be still more complicated. Sometimes such curves are used for drawing conclusions regarding associations in solutions (Parshad[17]). This, according to what is stated above, is not possible. Information regarding association in liquid mixtures can, however, as may be seen from the above, be obtained from compressibility by relation to the intermolecular free length.

SUMMARY

An empirical relation has been demonstrated between the intermolecular free length L in the liquid state and the adiabatic compressibility $\beta_{ad} = kL^p$ where k and p are constants dependent to some extent on temperature. At 20° C $p = 2.082$ and $\log k = 7.097$ for c.g.s. units. The free length is defined as $L = 2\ V_a/Y$ where the available volume $V_a = V_T - V_0$ is the difference between the molar volumes at T°K and O°K. Y is the surface of all the molecules per mole. The relation has been tested for 54 organic non-associated liquids and for 16 liquid mixtures. It has been found that the intermolecular free length can be computed from the relation stated to an accuracy of some few per cent. Association factors in liquids and liquid mixtures can be obtained from compressibility data. The isothermal compressibility follows a relation similar to that stated for the adiabatic but probably with poorer accuracy.

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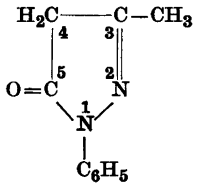
Studies on Pyrazolones

I. Light Absorption and Constitution of Certain 4-Halo-5-pyrazolones

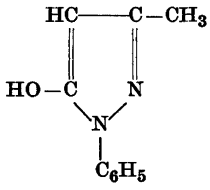
GUNNEL WESTÖÖ

Institute of Chemistry, University, Lund, Sweden

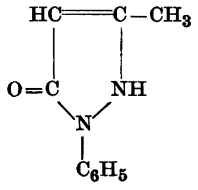
By studying the light absorption of 1-phenyl-3-methyl-5-pyrazolone and its N- and C-alkyl derivatives, Biquard and Grammaticakis[1] showed in 1941 that 1-phenyl-3-methyl-5-pyrazolone exists in three forms (I—III) in a state



I

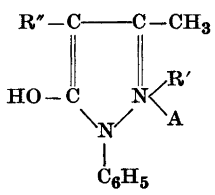


II

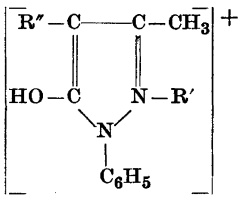


III

of equilibrium. These forms had already been proposed by Knorr [2] in 1896. In 1940 and 1941, Valyashko and Bliznyukov [3] published investigations on the light absorption of various pyrazolones and pyrazoles, and, on the basis of the absorption curves, deduced the structures of the substances. They confirm the salt formula (IV) proposed by Knorr [2] (the formula is written in a modern



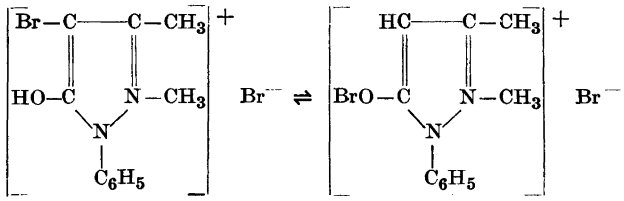
IV



V

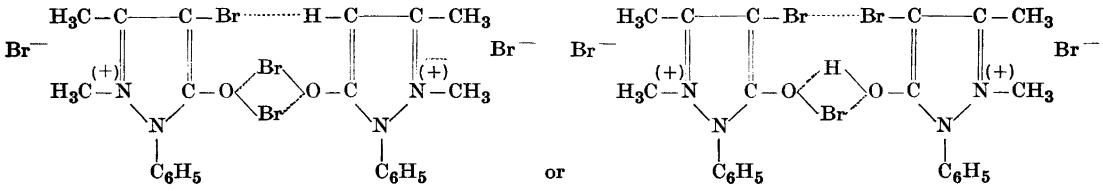
a) R′ = H R″=CH_3
b) R′ = H R″=Br
c) R′ = CH_3 R″=Br
d) R′ = H or CH_3
R″ = H

way in V). For monobromoantipyrine hydrobromide, Kitamura and Sunagawa[4]



VI VII

suggest oscillation between the two forms VI and VII, and also analogous



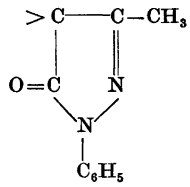
VIII

formulas for antipyrine perbromide (VIII). In the present paper the light absorption curves of some 4-halo-5-pyrazolones * and 5-pyrazolone salts under various conditions are reported and the structures of the substances are discussed.

The stimulus to this investigation were some observations made during the preparation of halopyrazolones. When 1-phenyl-3,4-dimethyl-5-pyrazolone **, $PyHCH_3$, is dissolved in glacial acetic acid and an equimolar amount of bromine in the same solvent is added, the solution turns red. This is because with concentrated solutions in such anhydrous solvents as glacial acetic acid and chloroform (but not ethanol) only half of the pyrazolone is brominated in accordance with the formula

$$2\ PyHCH_3 + Br_2 \rightarrow Py(CH_3)Br + PyHCH_3 \cdot HBr$$

* The iodo-compounds were so sensitive to light that their absorptions could not be measured.

** Throughout this paper Py will be used as a substitute for the radical 

In dilute solutions the greater dissociation of the salt into acid and pyrazolone allows the reaction to continue further than halfway. In the salt Py*H*$CH_3 \cdot HBr$, however, the italicized hydrogen atom, which is easily substituted in the corresponding free pyrazolone, does not react with bromine. If water is added, the salt is decomposed and the remaining bromine is rapidly absorbed.

$$PyHCH_3 \cdot HBr \xrightarrow{H_2O} PyHCH_3 + HBr; \quad PyHCH_3 + Br_2 \rightarrow PyBrCH_3 + HBr$$

The same effect is observed in the preparation of 1-phenyl-3-methyl-4,4-dibromo-5-pyrazolone. The reaction in concentrated glacial acetic acid solution between one mole of 1-phenyl-3-methyl-5-pyrazolone and two moles of bromine stops when half the bromine has reacted.

$$PyH_2 + Br_2 \rightarrow PyHBr \cdot HBr$$

When water is added, a second bromine atom is introduced.

$$PyHBr \cdot HBr \xrightarrow{H_2O} PyHBr + HBr; \quad PyHBr + Br_2 \rightarrow PyBr_2 + HBr.$$

These facts show that whereas PyHCH$_3$ and PyHBr possess a hydrogen atom easily substituted by bromine, the corresponding HBr-salts do not.

On the other hand, repeated attempts to synthesize 1,3-diphenyl-4-bromo-5-pyrazolone hydrobromide from equimolar amounts of bromine and pyrazolone in glacial acetic acid have failed. Part of the diphenyl-compound combined with two bromine molecules, forming the yellow 1,3-diphenyl-4,4-dibromo-5-pyrazolone, even when a large excess of the pyrazolone was present. The different behaviour towards bromine of 1,3-diphenyl-4-bromo-5-pyrazolone hydrobromide and the hydrobromides of $PyHCH_3$ and PyHBr is certainly not caused by different structures, but is a consequence of the weaker basic strength of the diphenyl-compound, which allows dissociation of the salt into free base and acid even when the acid is present in moderate excess.

When antipyrine reacts with bromine in chloroform or glacial acetic acid, 4-bromoantipyrine hydrobromide (1-phenyl-2,3-dimethyl-4-bromo-5-pyrazolone hydrobromide) is formed [4]. (This compound was first prepared by Knorr [5], who regarded it as 3,4-dibromoantipyrine.) If bromine is added in excess, antipyrine perbromide [4, 6] is obtained. The affinity of the bromoantipyrine salt for the halogen can not be caused by dissociation in this case, as 4-bromoantipyrine has no reactive hydrogen. *Thus the existence of a reactive hydrogen atom in 4-bromoantipyrine hydrobromide is certain.* Consequently a difference in structure between this salt and the hydrobromides of PyHBr and $PyHCH_3$ is very likely. This opinion is supported by the different colours of

the salts. The hydrobromide of 4-bromoantipyrine is distinctly yellow. Other pyrazolone salts are colourless unless the anions are coloured. Kitamura and Sunagawa [4] attribute the colour to a perbromide impurity. However, the substance still retains its colour after purification by precipitation from absolute alcohol solution by the addition of dry ether. But if it is dissolved in 95 per cent alcohol and precipitated with ether, a colourless product having the composition 1-phenyl-2,3-dimethyl-4-bromo-5-pyrazolone hydrobromide monohydrate is obtained. Here no perbromide can be present, for this compound is precipitated by ether in orange-yellow crystals from 95 per cent as well as from absolute alcohol. The monohydrate turns yellow when it is dried under a pressure of 0.1 mm Hg at room temperature over phosphorus pentoxide. Moreover, it the hydrate is dissolved in glacial acetic acid, a yellow solution is obtained that is decolorized by the addition of water. From the yellow solution the yellow, anhydrous 4-bromoantipyrine hydrobromide may be precipitated by dry ether. The same colour-changes as described above are found if 4-bromoantipyrine hydrobromide is prepared from pure 4-bromoantipyrine and bromine-free hydrobromic acid. Thus the yellow colour is not caused by an impurity but is characteristic of 4-bromoantipyrine hydrobromide.

As the difference in reactivity of the pyrazolone salts is accompanied by a difference in light absorption, it was of interest to characterize the compounds by absorption curves. The salts are insoluble in hydrocarbons but readily soluble in alcohol, where, however, they suffer extensive dissociation into pyrazolone and acid. Hence the absorption was measured in ethanol containing sulphuric acid.

Diagram 1 shows the light absorption of $PyHCH_3$, PyHBr and monobromoantipyrine in ethanolic solution with varying amounts of acid, a considerable excess of which is necessary to suppress the free base. The absorption curves of the salts differ distinctly from those of the free pyrazolones and are in accordance with the structure V. This is evident from a comparison with the light absorption curves of 1-phenyl-3-methyl-5-chloropyrazole and 1-phenyl-3-methyl-5-methoxypyrazole, investigated by Valyashko and Bliznyukov [3]. Thus in ethanol solution, salts of $PyHCH_3$ are represented by V a, of PyHBr by V b and of 4-bromoantipyrine by V c, no difference in structure existing between the various salts.

The light absorptions of PyH_2 and antipyrine in neutral and hydrochloric acid alcohol solutions measured by Valyashko and Bliznyukov [3] show essentially the same variations as the above. On account of the similarity between the light absorption curves of PyH_2 and antipyrine in concentrated, alcoholic hydrogen chloride and the corresponding curves of 1-phenyl-3-methyl-5-

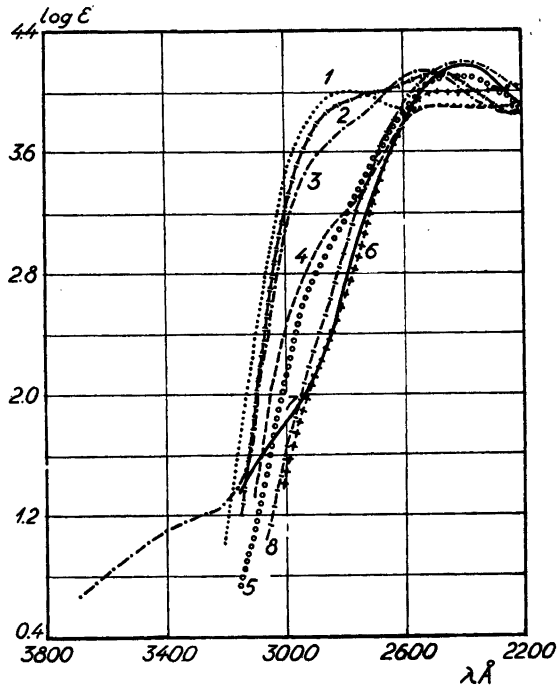
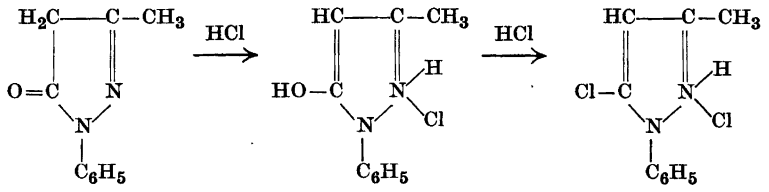
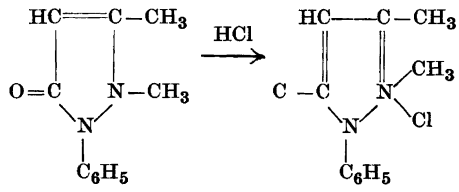


Diagram 1. 1) 4-Bromoantipyrine in ethanol ($3.6 \cdot 10^{-3}$, $3.6 \cdot 10^{-4}$ and $3.6 \cdot 10^{-5}$ M solutions) 2) 1-Phenyl-3,4-dimethyl-5-pyrazolone in ethanol ($4.5 \cdot 10^{-3}$, $4.5 \cdot 10^{-4}$ and $4.5 \cdot 10^{-5}$ M solutions) 3) 1-Phenyl-3-methyl-4-bromo-5-pyrazolone in ethanol (4.10^{-3}, 4.10^{-4} and 4.10^{-5} M solutions) 4) 4-Bromoantipyrine in ethanol with 50 ml of concentrated sulphuric acid per liter (4.10^{-3}, 4.10^{-4} and 4.10^{-5} M solutions) 5) 1-Phenyl-3-methyl-4-bromo-5-pyrazolone in ethanol with 50 ml of concentrated sulphuric acid per liter (4.10^{-3}, 4.10^{-4} and 4.10^{-5} M solutions) 6) 4-Bromoantipyrine in ethanol with 150 ml of concentrated sulphuric acid per liter (4.10^{-3} 4.10^{-4} and 4.10^{-5} M solutions) 7) 1-Phenyl-3,4-dimethyl-5-pyrazolone in ethanol with 150 ml of concentrated sulphuric acid per liter (4.10^{-3}, 4.10^{-4} and 4.10^{-5} M solutions) 8) 1-Phenyl-3-methyl-4-bromo-5-pyrazolone in ethanol with 150 ml of concentrated sulphuric acid per liter (4.10^{-3}, 4.10^{-4} and 4.10^{-5} M solutions).

chloropyrazole and 1-phenyl-3-methyl-5-chloropyrazole-2-chloromethylate, the Russian authors conclude that PyH_2 has changed into 1-phenyl-3-methyl-5-chloropyrazole (IX) and antipyrine into 1-phenyl-3-methyl-5-chloropyrazole-2-chloromethylate (X) via the salts. It seems preferable to ascribe the great similarity of the curves solely to the stable pyrazole structure of both the salts (V d) and the 5-chloro-pyrazole (XI a). This is not only because sulphuric acid has the same effect on the pyrazolone spectra as hydrogen chloride, but also because a hydroxyl group attached to a pyrazole nucleus should not be substi-

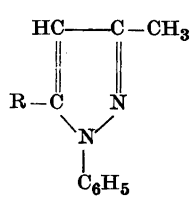


IX



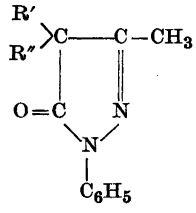
X

tuted by halogen on mere treatment with hydrogen halide, and because dilution alone suffices to regain the unchanged pyrazolone from the solution in strong hydrochloric acid (*cf.* Michaelis and Pasternack [7]).



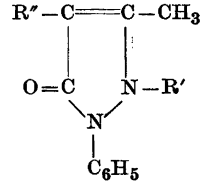
XI

a) R=Cl



XII

a) R′=CH_3 R″=Br
b) R′=H R″=Br



XIII

a) R′=CH_3 R″=Br

In contrast to $PyHCH_3$ and PyH_2, PyHBr exhibits quite different light absorption curves in neutral alcohol and in solvents with smaller dielectric constants, *e.g.* chloroform (Diagram 2). This indicates that different molecular structures are present in alcohol and chloroform solution. 1-Phenyl-3,4-dimethyl-4-bromo-5-pyrazolone, $Py(CH_3)Br$, which can only exist in one form (XII a), is characterized by the same light absorption curve in ethanol, strongly acidified ethanol and chloroform (Diagram 2). This curve resembles the curve of PyHBr in chloroform very much, which shows that PyHBr has chiefly the structure XII b in that solvent. The absorption, especially above 3 200 Å, proves that in alcoholic solution PyHBr is present in the form XII b only

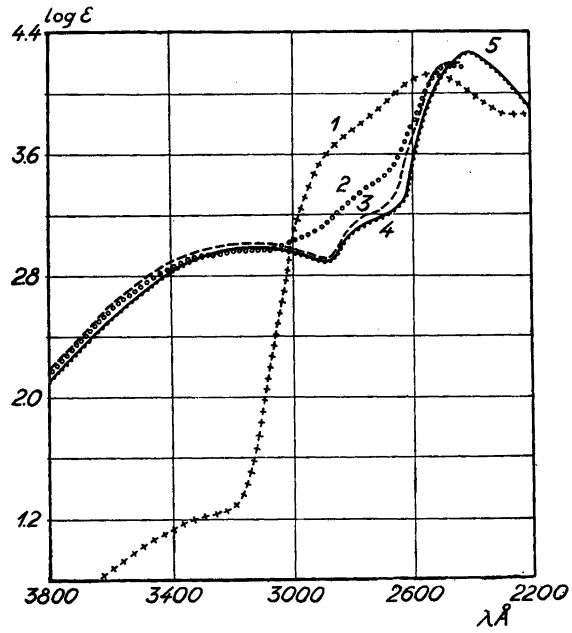


Diagram 2. 1) 1-Phenyl-3-methyl-4-bromo-5-pyrazolone in ethanol (4.10^{-3}, 4.10^{-4} and 4.10^{-5} M solutions) 2) Ditto in chloroform (4.10^{-3}, 4.10^{-4} and 4.10^{-5} M solutions) 3) 1-Phenyl-3,4-dimethyl-4-bromo-5-pyrazolone in chloroform (4.10^{-3}, 4.10^{-4} and 4.10^{-5} M solutions) 4) Ditto in ethanol (4.10^{-3}, 4.10^{-4} and 4.10^{-5} M solutions) 5) Ditto in ethanol with 150 ml of concentrated sulphuric acid per liter (4.10^{-3}, 4.10^{-4} and 4.10^{-5} M solutions).

to a very small extent. If Beer's law is valid, not more than 2 % of the substance possesses that structure.

It is interesting to compare the light absorption curves of $Py(CH_3)Br$, $Py(CH_3)Cl$, $Py(CH_3)OH$* and $Py(CH_3) \cdot PyCH_3$ (Diagram 3). They are all derivatives of the structure I of 1-phenyl-3-methyl-5-pyrazolone. The introduction of halogen or hydroxyl into the 4-position markedly increases the light absorption at wave-lengths longer than 2 900 Å. From the curves is evident that the greater absorption in the visible range is caused by a long-wave displacement and broadening of an already existing band, the maximum extinction coefficient of which is, however, decreased. The halogens and the hydroxyl group thus lower the difference in energy between the excited and ground states of the molecule. (Red shifts caused by halogens have been described before, *e.g.* by Mohler and Polya [8]). A similar displacement on intro-

* The compound $Py(CH_3)OH$ was prepared in cooperation with Prof. S. Veibel and will be described in another paper.

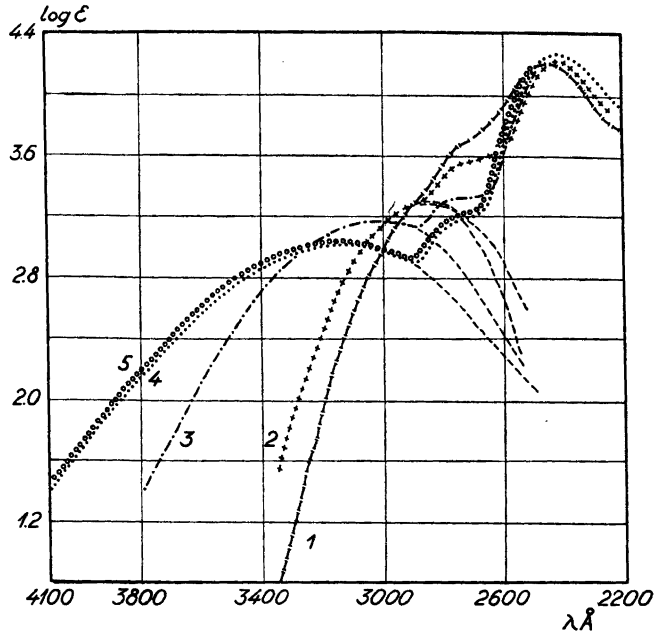


Diagram 3. 1) 1,1-Diphenyl-3,3',4,4'-tetramethyl-[4,4'-bi-2-pyrazoline]-5,5'-dione in ethanol ($5.0 \cdot 10^{-3}$, $5.0 \cdot 10^{-4}$ and $5.0 \cdot 10^{-5}$ M solutions). 2) 1-Phenyl-3,4-dimethyl-4-hydroxy-5-pyrazolone in ethanol ($5.8 \cdot 10^{-3}$, $5.8 \cdot 10^{-4}$ and $5.8 \cdot 10^{-5}$ M solutions) 3) 1-Phenyl-3,4-dimethyl-4-chloro-5-pyrazolone in chloroform ($4.3 \cdot 10^{-3}$, $4.3 \cdot 10^{-4}$ and $4.3 \cdot 10^{-5}$ M solutions) 4) 1-Phenyl-3,4-dimethyl-4-bromo-5-pyrazolone in ethanol (4.10^{-3}, 4.10^{-4} and 4.10^{-5} M solutions) 5) Ditto in chloroform (4.10^{-3}, 4.10^{-4} and 4.10^{-5} M solutions).

duction of halogen into the 4-position of 1-phenyl-3-methyl-5-pyrazolone or 1,3-diphenyl-5-pyrazolone is shown in Diagram 4.

Substances with antipyrine or pyrazole structures like XIII, V and XI do not give rise to the above band, as is evident from the absorption curves of monobromoantipyrine (XIII a), the acid sulphate of PyHBr (V b) (Diagram 1) and of 1-phenyl-3-methyl-5-chloropyrazole [3] (XI a). This band is thus associated with the structure XII and its two chromophores C = O and C = N. In the halogen-compounds, the polarizable halogen atoms, linked with the same C-atom (4) as the chromophores, evidently furnish electrons capable of interaction with the π-electrons of the double bonds and thus cause the red shift. This involvement of electrons from the C-Hal-group in the pyrazolone nucleus must diminish the electron density around this group, which explains the electrophilic reactions of the halogen atoms of $Py(CH_3)Hal$, PyHHal and $PyHal_2$. All these compounds, with the exception of PyHCl, oxidize hydrogen

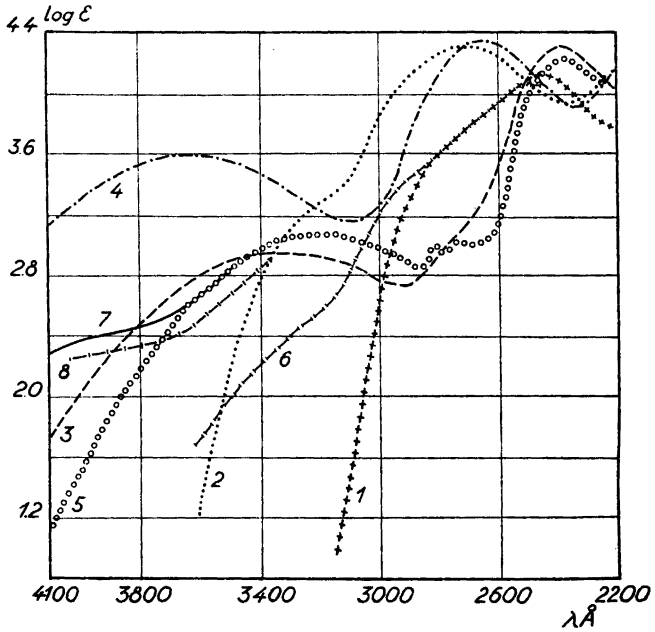


Diagram 4. 1) 1-Phenyl-3-methyl-5-pyrazolone in ethanol ($7.3 \cdot 10^{-3}$, $7.3 \cdot 10^{-4}$ and $7.3 \cdot 10^{-5}$ M solutions) 2) 1.3-Diphenyl-5-pyrazolone in ethanol ($4.8 \cdot 10^{-3}$, $4.8 \cdot 10^{-4}$ and $4.8 \cdot 10^{-5}$ M solutions) 3) 1-Phenyl-3-methyl-4,4-dibromo-5-pyrazolone in ethanol (4.10^{-3}, 4.10^{-4} and 4.10^{-5} M solutions) 4) 1,3-Diphenyl-4,4-dibromo-5-pyrazolone in ethanol ($3.3 \cdot 10^{-3}$, $3.3 \cdot 10^{-4}$ and $3.3 \cdot 10^{-5}$ M solutions) 5) 1-Phenyl-3-methyl-4,4-dichloro-5-pyrazolone in ethanol ($6.7 \cdot 10^{-3}$, $6.7 \cdot 10^{-4}$ and $6.5 \cdot 10^{-5}$ M solutions) 6) 1-Phenyl-3-methyl-4-chloro-5-pyrazolone in chloroform ($6.2 \cdot 10^{-3}$, $6.2 \cdot 10^{-4}$ and $6.2 \cdot 10^{-5}$ M solutions) 7) Antipyrine perbromide in chloroform ($2.8 \cdot 10^{-3}$ M solution) 8) Ditto ($1.6 \cdot 10^{-3}$ M solution).

iodide to iodine in alcoholic solution at room temperature (*e.g.* $PyCl_2 + 2\ HI \rightarrow PyHCl + I_2 + HCl$ or $PyHI + HI \rightarrow PyH_2 + I_2$), the reaction velocity increasing from the chloro to the iodo compounds. Furthermore they can attack an inert metal such as mercury at room temperature.

The investigation of the light absorption of the salts in chloroform and acetic acid must be done with rather dilute solutions, where the dissociation is consequently important. The higher the concentration, the more is the equilibrium of the dissociation reaction, salt $\rightleftharpoons$ pyrazolone + acid, displaced towards the left, and the more should the light absorption curve be displaced towards the curve of the undissociated salt. If the structure of the salts is the same in chloroform and acetic acid as in ethanol, the absorption at longer wave-lengths should be suppressed. Diagrams 5 and 6 show that this is the

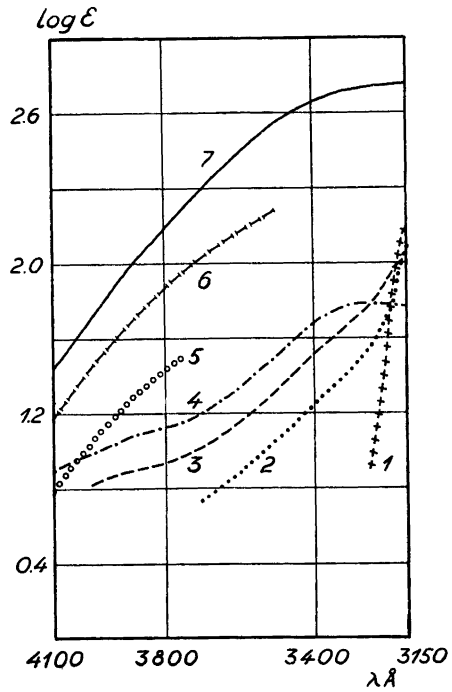


Diagram 5. 1) Monobromoantipyrine in chloroform (10^{-2} M solution) 2) Monobromoantipyrine hydrobromide in chloroform (10^{-2} M solution) 3) Ditto ($1.7 \cdot 10^{-2}$ M solution) 4) Ditto ($3.4 \cdot 10^{-2}$ M solution) 5) 1-Phenyl-3-methyl-4-bromo-5-pyrazolone hydrobromide in chloroform ($6.7 \cdot 10^{-2}$ M solution) 6) Ditto (10^{-2} M solution) 7) 1-Phenyl-3-methyl-4-bromo-5-pyrazolone in chloroform ($4 \cdot 10^{-3}$ M solution) (Log ε decreases only slightly when the concentration of PyHBr is increased).

case with solutions of PyHBr · HBr. Thus, the salts of PyHBr have the same constitution in chloroform and glacial acetic acid as in ethanol (V b) and, consequently, a hydrogen atom attached to the oxygen in position 5 of the pyrazolone molecule V is not substituted by bromine at room temperature (pp. 1500—1501).

The light absorption of the hydrobromide of monobromoantipyrine in chloroform and acetic acid solutions above 3 300 Å (Diagrams 5 and 6), however, increases with the concentration quite differently. It follows that the hydrobromide of monobromoantipyrine possesses not only the constitution V c. There must be an equilibrium between at least two forms. The alternative VII proposed by Kitamura and Sunagawa [4], which well explains the ability of the substance to act as a brominating agent, assumes that the bromine atom can easily shift from position 4 to the oxygen atom in position 5 and back

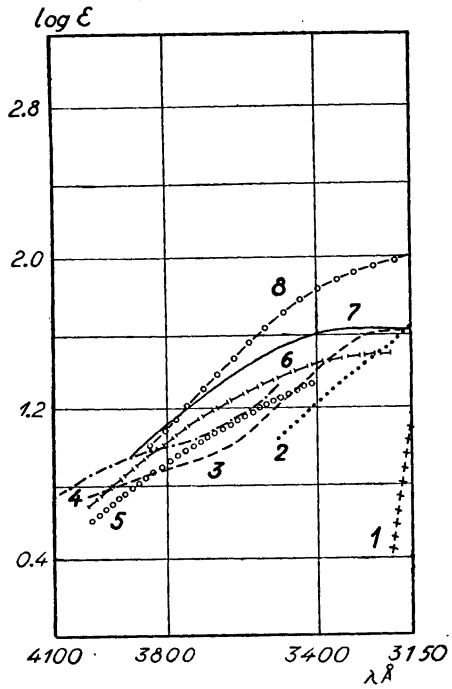
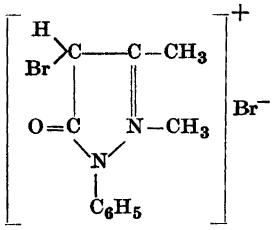


Diagram 6. 1) Monobromoantipyrine in glacial acetic acid (10^{-2} M solution) 2) Monobromoantipyrine hydrobromide in glacial acetic acid (10^{-2} M solution) 3) Ditto ($5 \cdot 10^{-2}$ M solution) 4) Ditto (10^{-1} M solution) 5) 1-Phenyl-3-methyl-4-bromo-5-pyrazolone hydrobromide in glacial acetic acid (10^{-1} M solution) 6) Ditto ($5 \cdot 10^{-2}$ M solution) 7) Ditto (10^{-2} M solution) 8) 1-Phenyl-3-methyl-4-bromo-5-pyrazolone in glacial acetic acid (10^{-2} M solution).

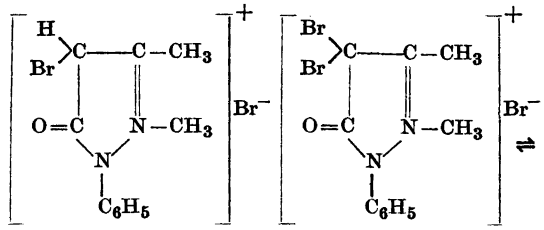
again. There is little precedence for such shifts. Moreover, it was shown above that the hydrogen of the 5-hydroxy-group of the closely related PyHBr · HBr is not easily substituted by bromine. Accordingly, an equilibrium between the forms V c and XIV seems preferable.

A substance with the structure XIV is likely to absorb light in the visible range like compounds of the structure XII (R″ = halogen) (Diagram 2). This explains the colour of monobromoantipyrine hydrobromide and the light absorption curves in Diagrams 5 and 6.

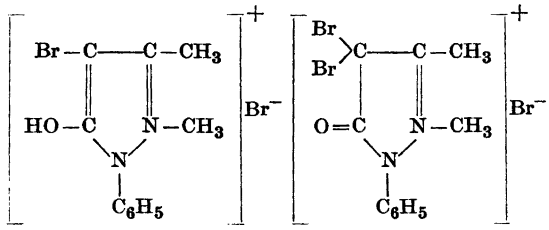
In a subsequent paper, "Studies on Pyrazolones VII", it will be shown that $PyBr_2$ and $Py(CH_3)Br$ can act as brominating agents. Accordingly the formation of bromoacetone from acetone and monobromoantipyrine hydrobromide [4] is no argument against the formula XIV.



XIV



XV



XV

The structure XIV is also in accordance with the existence of an antipyrine perbromide (Kitamura and Sunagawa [4]), for the hydrogen atom in the 4 position of XIV ought to be easily substituted by bromine. The double salt formula XV, which is consistent both with the light absorption (strongly dependent on the concentration) of the substance (Diagram 4) and with its brominating and oxidizing properties, seems a resonable formula for the perbromide.

EXPERIMENTAL

I. 1-Phenyl-3,4-dimethyl-4-halo-5-pyrazolones

1-Phenyl-3,4-dimethyl-4-chloro-5-pyrazolone. 1-Phenyl-3,4-dimethyl-5-pyrazolone (5 g) was dissolved in glacial acetic acid (40 ml), and water (300 ml) was added. Chlorine was introduced with vigorous stirring of the solution. The colourless reaction product was collected by filtration and washed with water. The yield of crude chloropyrazolone was 98%. It was purified by recrystallization from ethanol (cooling in a freezing mixture). M.p .68° C. (Found: C 59.2; H 5.1; Cl 15.97; N 12.67. Calc. for $C_{11}H_{11}ClON_2$ (222.7): C 59.3; H 5.0; Cl 15.92; N 12.58.) When the reaction: $PyHCH_3 + Cl_2 \rightarrow Py(CH_3)Cl + HCl$ takes place in dilute acetic acid, $Py(CH_3)Cl$ precipitates as soon as formed and is thus prevented from being further attacked by the chlorine.

1-Phenyl-3,4-dimethyl-4-bromo-5-pyrazolone. $PyHCH_3$ (20 g) and bromine (17 g) were dissolved separately in glacial acetic acid. A few pieces of ice were placed in the pyrazolone solution and the bromine solution was poured into it with stirring. The bromine combined rapidly and completely with the pyrazolone. First ice and then water were added in portions with stirring to precipitate the yellow $Py(CH_3)Br$. The yield was

quantitative. M.p. 83° C. (Found: C 49.5; H 4.1; Br 29.88; N 10.46. $C_{11}H_{11}BrON_2$ (267.3) requires C 49.4; H 4.1; Br 29.92; N 10.49.)

The bromination of $PyHCH_3$ in glacial acetic acid was studies in the following way. When one mole of bromine was added per mole of $PyHCH_3$, a solution strongly coloured by bromine was obtained. The colour appeared when slightly more than half of the bromine had been added. The acetic acid and the bromine that had not reacted were removed by evaporation under reduced pressure. Colourless crystals and a yellow solution were obtained by extraction of the residue with dry ethyl ether. $Py(CH_3)Br$ crystallized from the ether solution on evaporation. The yield was somewhat more than half a mole of $Py(CH_3)Br$ per mole of $PyHCH_3$ added. The colourless product, which was insoluble in ether, contained 0.371 equivalents of bromide ions per 100 grams, the same amount of strong acid (HBr) and twice as much total acid, all determined by potentiometric titrations. In a buffered alcoholic solution half of the hydrogen atoms react with the bromine atoms in $Py(CH_3)Br$ with formation of HBr (the rest of the molecules gives $Py(CH_3) \cdot PyCH_3$) in quite the same way as $PyHCH_3$ (*cf.* Smith [9]). These facts show that the salt $PyHCH_3 \cdot HBr$ has been formed. The substance may be purified by dissolving it in anhydrous alcohol and precipitating it with anhydrous ether, or alternatively by dissolving it in 95 per cent alcohol and precipitating it with ether. In the latter case the molecule takes up two molecules of water and is transformed into $PyHCH_3 \cdot HBr \cdot 2H_2O$, identical with the salt that is obtained on treating $PyHCH_3$ with concentrated hydrobromic acid. (Found: C 43.2; H 5.6; Br 26.13. Calc. for $C_{11}H_{12}ON_2 \cdot HBr \cdot 2H_2O$ (305.4): C 43.3; H 5.6; Br 26.09.)

1-Phenyl-3,4-dimethyl-4-iodo-5-pyrazolone. An equivalent amount of dilute sodium hydroxide was added to a saturated solution of 1-phenyl-3,4-dimethyl-5-pyrazolone in ethanol. Much crushed ice and ice water were added, followed by the addition (with efficient stirring) of an equivalent amount of iodine in aqueous potassium iodide. Yellow crystals of 1-phenyl-3,4-dimethyl-4-iodo-5-pyrazolone separated immediately. The yield of crude product was 98%. For purification the iodopyrazolone was dissolved in glacial acetic acid at room temperature, cooled in an ice-bath, and precipitated with a small amount of crushed ice. M.p. 70° C. (Found: C 42.0; H 3.5; I 40.4; N 8.92. $C_{11}H_{11}ION_2$ (314.1) requires C 42.1; H 3.5; I 40.4; N 8.92.)

$PyCH_3I$ is sensitive to light and heat. On heating, the reaction: $2\ Py(CH_3)I \rightarrow Py(CH_3)PyCH_3 + I_2$ takes place.

All the 1-phenyl-3,4-dimethyl-4-halo-5-pyrazolones are readily soluble in alcohol, ether, chloroform, ethyl acetate and glacial acetic acid, but are insoluble in water. Alkali splits off the halogen atoms quantitatively as ions, and the reaction is suitable for analytical purposes (*cf.* p. 1514). There is no simple exchange of halogen by hydroxyl, but the rest of the pyrazolone is attacked too.

Examples. $Py(CH_3)Br$ (0.3777 g) was dissolved in ethanol (30 ml), and 2.5 *N* sodium hydroxide solution (15 ml) was added. The solution became orange-red. After one hour it was acidified with 5 *N* sulphuric acid (yellow colour) and titrated potentiometrically with 0.1006 *N* $AgNO_3$-solution. 14.05 ml were consumed, which is equivalent to 29.88% of bromine (calc. 29.92). In the case of $Py(CH_3)Cl$, more concentrated alkali, 25 ml of 2.5 *N* sodium hydroxide to 25 ml of ethanol, and a longer time of reaction, two hours, were used.

Neither Volhard titration nor weighing of the silver halide is suitable because the product of hydrolysis has strong reducing properties and transforms the excess of silver nitrate to metallic silver. Therefore potentiometric titration is necessary.

II. 1-Phenyl-3-methyl-4,4-dihalo-5-pyrazolones

1-Phenyl-3-methyl-4,4-dichloro-5-pyrazolone. This compound has been prepared by Knorr [5] by treating 1-phenyl-3-methyl-5-pyrazolone either with chlorine or with phosphorus pentachloride. The former method gives a poor yield and the latter is somewhat laborious. The compound is prepared more simply in the following way.

PyH_2 was dissolved in about six parts of glacial acetic acid and chlorine was introduced with stirring. An excess must be avoided because $PyCl_2$ reacts with chlorine. The solution was cooled, and $PyCl_2$ was precipitated with crushed ice and ice-cold water. Alternatively, water may be added before the introduction of the halogen. The advantage of this variation was that $PyCl_2$ precipitated as soon as formed and so was protected from oxidation by the chlorine. In this case the product was green, but it was easily purified by crystallization from ethanol. In either case the yield was 95—98%. M.p. 65° C. (Found: C 49.5; H 3.3; Cl 29.15. Calc. for $C_{10}H_8Cl_2ON_2$ (243.1): C 49.4; H 3.3; Cl 29.17.)

1-Phenyl-3-methyl-4,4-dibromo-5-pyrazolone. The following procedure differs from the methods described by Knorr [5] and Smith [9] mainly in avoiding both heating and long reaction time. — PyH_2 (10 g) and bromine (18.4 g) were dissolved separately in glacial acetic acid. A few pieces of ice (about 10 g) were added to the pyrazolone solution and the bromine was introduced with stirring. The solution acquired a clear yellow colour. Crushed ice was added with stirring, causing yellow crystals of $PyBr_2$ precipitate. The precipitation was completed by addition of water. The yield was quantitative.

As in the preparation of 1-phenyl-3,4-dimethyl-4-bromo-5-pyrazolone, the bromination in concentrated glacial acetic acid solution stops when half the bromine has reacted (p. 1501). Addition of water makes the reaction continue. The intermediate product, PyHBr · HBr, can be precipitated by addition of anhydrous ether to the glacial acetic acid solution. It may be purified by dissolution in a small amount of anhydrous ethyl alcohol and precipitation with anhydrous ether. That the precipitated substance is the salt PyHBr · HBr, may be inferred from the following data:

1. $C_{10}H_9BrON_2 \cdot HBr$ (334.1) requires C 35.9; H 3.0. Found: C 35.7; H 3.05.
2. Each molecule contains one ionizable and one non-ionizable bromine atom. On potentiometric titration with silver nitrate solution 0.304 equivalents of bromide per 100 g of substance were found (calc. for PyHBr · HBr: 0.299 equivalents per 100 g).
3. The product reacts in buffered alcoholic solution in the same way as PyHBr, *e. g.* formation of hydrogen bromide and pyrazole blue (*cf.* Knorr [2]): 2 PyHBr → 2 HBr + Py = Py.
4. On titration with barium hydroxide solution (phenolphthalein as indicator) the equivalent weight 165 was found. (Calc. for PyHBr · HBr 167.)

1-Phenyl-3-methyl-4,4-diiodo-5-pyrazolone. 1-Phenyl-3-methyl-5-pyrazolone (4 g) in ethyl alcohol (15 ml) was neutralized by 2.3 *N* sodium hydroxide (19.5 ml, equivalent to both hydrogen atoms in the pyrazolone). Ice-water (1.5 l) was added and then, with stirring, an equivalent amount of iodine (180 ml of a 0.5 *N* solution in aqueous potassium iodide). 1-Phenyl-3-methyl-4,4-diiodo-5-pyrazolone was obtained as a yellow precipitate. PyI_2 adsorbs sodium and potassium iodide and must be washed carefully with water. It is very unstable and sensitive to light and is difficult to purify by crystallization. (Found: C 28.3; H 1.9; N 6.50; 0.97 % sulphate ash. $C_{10}H_8I_2ON_2$ (426.0) requires C 28.2; H 1.9; N 6.58.)

The $PyHal_2$ compounds are easily reduced, PyI_2 even by cold methanol or ethanol. As has already been shown by Knorr in the case of $PyBr_2$ the dihalogenated pyrazolones are attacked by alkali, halide ions being split off. The reaction is not complete enough to be suitable for analysis. For analytical purposes one can use sodium to cleave off the halogen (Rauscher [10]).

III. 1-Phenyl-3-methyl-4-halo-5-pyrazolones

1-Phenyl-3-methyl-4-chloro-5-pyrazolone. This substance has been prepared by Alphen [11] from $C_6H_5N = NC(CH_3) = CHCOOC_2H_5$ and HCl. In the present investigation it has been obtained from 1-phenyl-3-methyl-5-pyrazolone and chlorine and also by reduction of 1-phenyl-3-methyl-4,4-dichloro-5-pyrazolone.

It was shown above (p. 1512) that bromine reacts with PyH_2 in glacial acetic acid according to the equation: $PyH_2 + Br_2 \rightarrow PyHBr \cdot HBr$, and that further bromination does not occur until the acetic acid has been diluted with water. On the other hand, when chlorine was used as halogenating agent, even with less than 1 mole of chlorine per mole of pyrazolone, $PyCl_2$ was formed to a certain extent according to the equation: $PyH_2 + 2Cl_2 \rightarrow PyCl_2 + 2HCl$. Thus some of the unsubstituted pyrazolone remained in the form of $PyH_2 \cdot HCl$, (this salt, which easily absorbed one mole of water, could be precipitated by ether. Found: C 52.5; H 5.86; Cl 15.45. The hydrate, $C_{10}H_{13}ClO_2N_2$, (228.7) requires C 52.5; H 5.73; Cl 15.50.) On addition of ice the dichloro-compound precipitated first. If the precipitate was removed by filtration and the mother liquor allowed to stand a few hours, PyHCl separated from the solution.

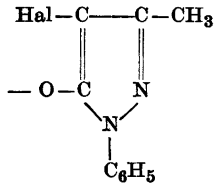
A better yield of PyHCl was obtained by reduction of $PyCl_2$ with hydrogen iodide at room temperature in the dark. $PyCl_2$ (15 g) was dissolved in ethyl alcohol (270 ml), and constant boiling hydroiodic acid (25 ml) was added. Then the reaction: $PyCl_2 + 2HI \rightarrow PyHCl + HCl + I_2$ took place. The liberated iodine was reduced as formed by adding a sodium thiosulphate solution dropwise (concentrated solution at the beginning in order not to precipitate $PyCl_2$ by the water!). After a short while PyHCl started to separate. When the reaction was finished as indicated by the iodine formation having ceased, the precipitation was completed by water. The yield of colourless product was 11.3 g (88 %). The small amounts of impurities present were removed by extraction with chloroform (boiling half a minute) or by recrystallization from ethanol. M.p. 153° C with decomposition. (Found: C 57.6; H 4.35; Cl 17.00; N 13.46; equ.wt 208.7 on titration with sodium hydroxide against phenolphthalein. Calc. for $C_{10}H_9ClON_2$ (208.7): C 57.5; H 4.35; Cl 16.99; N 13.43; equ.wt 208.7)

1-Phenyl-3-methyl-4-bromo-5-pyrazolone. This compound was prepared by a modification of the method of Knorr [5] (Compare Smith [9]). 1-Phenyl-3-methyl-5-pyrazolone was dissolved in glacial acetic acid, and an equivalent amount of bromine in the same solvent was added. After stirring a short while, the salt PyHBr · HBr usually separated. If seeding was necessary, crystals were prepared by adding ether to a small part of the solution. The colourless crystals were filtered by suction and washed successively with glacial acetic acid and ether. When impure PyH_2 was used, the salt was coloured yellow and was then purified by precipitation with dry ether from a solution in absolute alcohol. The pure salt was suspended in five parts of glacial acetic acid, and crushed ice was added with efficient stirring. The salt was decomposed by the water into hydrogen bromide and PyHBr, and at the same time it dissolved. When the acetic acid was sufficiently diluted, PyHBr separated as colourless crystals. The product was pure enough for most purposes,

but could be further purified by recrystallization from chloroform. Yield 95%. As PyHBr · HBr is more stable than PyHBr, the substance is best stored as the salt. (Found: Br 31.55; equ.wt 253.2 on titration with sodium hydroxide against phenolphthalein. Calc. for $C_{10}H_9BrON_2$ (253.2): Br 31.57; equ.wt 253.2.)

1-Phenyl-3-methyl-4-iodo-5-pyrazolone. 1-Phenyl-3-methyl-5-pyrazolone was dissolved in a little alcohol and an equimolecular quantity of sodium hydroxide solution was added. Much crushed ice and an equivalent quantity of iodine solution (in aqueous potassium iodide) were introduced with stirring. Yellow crystals of 1-phenyl-3-methyl-4-iodo-5-pyrazolone precipitated. (Found: C 40.2; H 3.0; I 42.3; N 9.39. Calc. for $C_{10}H_9ION_2$ (300.1): C 40.0; H 3.0; I 42.3; N 9.34.) PyHI is very sensitive to light and heat.

In contrast to the halogen atom of $Py(CH_3)Hal$ that of PyHHal cannot be hydrolyzed even by boiling with alkali hydroxide solutions. The diminished reactivity of the halogen atom in the alkali salts of PyHHal is to be expected, as it is attached to an unsaturated C-atom belonging to a stable pyrazole nucleus (XVI).



XVI

IV. *1-Phenyl-2,3-dimethyl-4-bromo-5-pyrazolone hydrobromide monohydrate.* Solutions of equimolar amounts of 1-phenyl-2,3-dimethyl-5-pyrazolone and bromine in glacial acetic acid were mixed, and the resulting 1-phenyl-2,3-dimethyl-4-bromo-5-pyrazolone hydrobromide was precipitated with dry ether. (Found: equ.wt 348 on potentiometric titration with silver nitrate. Calc. for $C_{11}H_{12}Br_2ON_2$ (348.1): equ.wt 348.1.) Solution of the yellow product in 95% alcohol and precipitation with ether gave colourless crystals of the monohydrate. (Found: C 36.2; H 3.85; equ.wt 366 on potentiometric titration with silver nitrate. $C_{11}H_{12}Br_2ON_2 \cdot H_2O$ (366.1) requires C 36.1; H 3.85; equ.wt 366.1.)

Antipyrine perbromide. Bromine (4.4 g) in acetic acid (15 ml) was mixed with a solution of antipyrine (2.3 g) in acetic acid (35 ml). On addition of ether antipyrine perbromide separated as orange-yellow crystals. Yield: 3.9 g. M.p. 171—172° C. (Found: C 33.9; H 3.0; Br 51.3. $C_{11}H_{11}Br_3ON_2$, $C_{11}H_{12}Br_2ON_2$ (775.2) requires C 34.1; H 3.0; Br 51.6.)

V. *Bromination of 1,3-diphenyl-5-pyrazolone.* A glacial acetic acid solution of bromine (7.0 g) was poured into a solution of 1,3-diphenyl-5-pyrazolone (10.3 g) in the same solvent. On precipitation with ether or toluene, only a sticky product was obtained. By means of ethyl acetate it was possible to isolate 1,3-diphenyl-5-pyrazolone hydrobromide (5.6 g) from the ether precipitate. (Found: equ.wt 159.2 on potentiometric titration with sodium hydroxide; *M* 318 on potentiometric titration with sodium hydroxide and silver nitrate. $C_{15}H_{13}BrON_2$ (317.3) requires equ.wt 158.6; *M* 317.3.) The ether-acetic acid solution contained 1,3-diphenyl-4,4-dibromo-5-pyrazolone, even when the amount of pyrazolone added was increased.

Light absorption measurements

All light absorption measurements were performed with a Beckmann spectrophotometer model DU in silica cells. Systems identical with the measured solutions except that they did not contain any pyrazolone were used as comparison solutions.

In the diagrams $\log \varepsilon = \log \log \frac{I_0}{I} - \log c \cdot l$ is plotted against the wave length λ (in Ångström units). I_0 and I are the intensities of the light before and after the passage of the cell, c is the concentration of the pyrazolone in moles per liter with one exeption, the bis-pyrazolone in Diagram 3, where c is expre sed in pyrazolone units per liter, and l is the length of the cell in cm.

SUMMARY

The 1-phenyl-3-methyl-4-halo-5-pyrazolones, 1-phenyl-3,4-dimethyl-4-halo-5-pyrazolones and 1-phenyl-3-methyl-4,4-dihalo-5-pyrazolones have been prepared, and the light absorption of these substances (except the iodo-compounds), of antipyrine perbromide, the acid sulphate of 1-phenyl-3,4-dimethyl-5-pyrazolone and the bromides and acid sulphates of 1-phenyl-3-methyl-4-bromo-5-pyrazolone and 1-phenyl-2,3-dimethyl-4-bromo-5-pyrazolone has been studied. The significance of the absorption curves with respect to the structures of the substances is discussed.

I wish to express my thanks to Professor S. Bergström for the use of a Beckmann spectrophotometer and to Dr. B. Mc Kusick who kindly revised the English text. I am indebted to *Kungliga Fysiografiska Sällskapet* for a grant.

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The Properties of Various Crystalline Horse Erythrocyte Catalase Preparations

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It has been recently pointed out that various crystalline erythrocyte catalases do not show constant activity [1,2]. A series of molecules of varying reactivities appear to comprise the usual material. In further investigations of such variability a number of horse erythrocyte catalases were studied in terms of activity, iron and hemin contents and absorption coefficients. Since the possibility of molecular kinetic variation appeared likely, sedimentation study of four samples of rather widely different activity and properties was also carried out. From these and partial specific volume and diffusion constant measurements, a molecular weight was calculated and compared with those obtained from the iron and heme contents. The most striking differences noted among the preparations studied were the variations in activity and crystallizability.

EXPERIMENTAL

The catalases were prepared essentially by the method of Bonnichsen [3] as described previously [1]. Particular care was taken to make the period of the Tsuchihashi [4] treatment as short as possible. In this respect sufficient ethanol-chloroform mixture was always added to satisfactorily precipitate (denature) the hemoglobin at room temperature within 30—60 minutes after the completion of the addition of this reagent. Two preparations (nos. 5 and 8) were unusual in that most of the material readily crystallized from water after the acetone precipitation and pH 4 acetic acid buffer treatments. The crystals were readily soluble on addition of a trace of ammonia and would begin to recrystallize after only several hours dialysis against distilled water. Some of the aqueous supernatants to recrystallized catalase preparation number 8 were examined to determine the extent of solubility. This was found to be as low as 0.003 g/100 ml at 3—5° C and pH 5.9—6.0, the hydrogen ion concentration of the supernatant to the exhaustively dialyzed crystals. Dilution of a 11.4 % solution of this material (no. 8) with 250 volumes of water caused

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it to show a marked turbidity within 10 minutes at room temperature. This was followed by the formation of well-defined crystals within 30 minutes. The above solubility properties of catalase preparations numbers 5 and 8 contrast strongly with catalase from horse erythrocytes which have been described in the literature. In these cases crystallization from water occurs only after prolonged standing in the cold of dialyzed and concentrated solutions and only relatively small amounts of the protein crystallizes. Numerous other of our enzyme preparations could not be crystallized under aqueous conditions analogous to those used for preparations nos. 5 and 8 although they showed the usual crystallizability from strong ammonium sulfate solution at room temperature as well as from concentrated aqueous solution after prolonged standing in the cold. This solubility difference is further indication of the lack of uniformity of various horse erythrocyte catalases in addition to those previously noted [2].

All activity measurements were made by the method of Bonnichsen, Chance and Theorell [5] after it was demonstrated to our satisfaction that it was equivalent to the method of von Euler and Josephson [6]. Iron was determined as the sulfosalicylate in ammoniacal solution and heme as the alkaline pyridine hemochromogen. Absorption measurements were made with a Beckman spectrophotometer.

Sedimentation and diffusion analyses were carried out in the Institute of Physical Chemistry of the University of Uppsala under the direction of Dr. K. O. Pedersen. The partial specific volume measurement was carried out by Dr. Carl Drucker of the same laboratory.

RESULTS

The various analytical data for the enzyme preparations studied are given in Table 1.

Table 1. Properties of Various Catalase Preparations.

Preparation	Kat. F.	Per cent Fe	Per cent Hemin	Absortion Coefficient [1] $\times 10^{-7}$ (β)		s_{20w}
				405 mμ	280 mμ	
2[4]	117 000	0.097	1.04	89.4	68.8	
4A	97 000	0.098	1.04	92.7	71.4	11.61 [2]
5Aq[5]	110 000	0.098	1.04	96.0	68.0	11.61 [2]
5S	76 000	0.094	1.06	94.9	72.5	11.61 [2]
6A	69 000	0.097	1.04	91.6	69.7	
8Aq	125 000	0.094	1.06	90.9	68.0	11.64 [3]
Average		0.096	1.04			

[1] Calculated for a molecular weight of 225 000.

[2] Sedimentation constant for single runs at 0.8% protein.

[3] Sedimentation constant for single run at 0.6% protein.

[4] The properties of this preparation were presented in a previous report [1].

[5] This preparation was also assayed for activity by Dr. Britton Chance in Philadelphia and found to possess a Kat. F, of 107 000.

Activity: A relatively wide range of activity is noted. All of the samples studied also invariably showed decreases in activity during the assay by the rapid method [5] for determining the Kat. F. and the firstorder reaction constants were extrapolated to zero time as was done previously [1]. The values reported in Table 1 were obtained at temperatures between 18—20° C. Bonnichsen, Chance and Theorell [5] have reported a value for this constant of 3.5×10^7 liter $mole^{-1}$ $sec.^{-1}$ at 22.5° for a catalase of Kat. F. of 61 000. We have used this figure to calculate the Kat. F. values in this work and have not corrected for the differences in temperature at which the assays were conducted. However, the low temperature coefficient of catalase would raise these figures only slightly.

The catalase samples usually tended to show activity decreases upon standing in the cold. This was usually synchronous with the formation of small amounts of a greyish precipitate. Samples stored in the frozen state also usually gave small amounts of insoluble material on thawing. Preparation no. 8 with an initial Kat. F. of 125 000 showed a value of only 90 000 after storage in 12 % solution at 1—3° C for a two-week period. The activity then remained constant over a further three-week period. A similar decrease for a high activity catalase has been previously reported [1]. Such findings are further indication that at least a portion of the molecules of such highly active eryhthrocyte catalases are more labile than has been generally assumed for this enzyme. Of particular interest is the ability to consistently obtain material of higher activity than the generally accepted Kat. F. 60 000 material.

Yields: The amount of material recovered from horse erythrocytes varied greatly and usually amounted to 10—60 % of the yields experienced by Bonnichsen [3]. In about one out of four fractionations suitable crystalline material, that is crystals not contaminated by amorphous material, could not be obtained. The most successful preparation was no. 8 in which experiment 2.5 g of three times aqueous crystallized enzyme was recovered from 35 liters of washed erythrocytes. In the case of preparation no. 5 only half of the total recovered catalase crystallized on aqueous dialysis (5 Aq). The remainder (5 S) could, however, be crystallized from ammonium sulfate. As shown by the data of Table 1, the latter fraction possessed a considerable lower activity than 5 Aq.

Absorption spectra: These were determined by means of a Beckman spectrophotometer. Dry weight determinations (constant weight at 105° C) on aliquots of exhaustively dialyzed samples were used to standardize the solutions employed. The same maxima as reported by other workers were found but differences in extinction coefficients were noted. Average values for the molar extinction coefficient (β) at 405 and 280 mμ were 92.6 and 69.7 cm^2 $mole^{-1}$

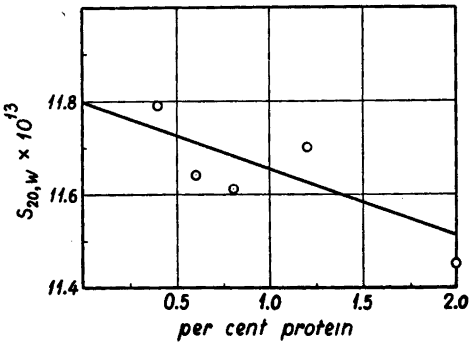


Fig. 1. Sedimentation constant of preparation 5 Aq at various protein concentrations.

respectively. The absorption coefficients (β) were calculated on the basis of a molecular weight of 225 000 since this is the value usually employed by other workers and hence will allow for direct comparison of results. Values for β may be readily calculated to the molecular weights reported in this paper. The results at 405 mμ in particular is considerably higher than that found by Bonnichsen [3] but is not reflected by a higher hemin content. Preparation 5 Aq while showing the same 280 mμ β reported by this worker gave a value at 405 mμ of 96 in contrast to the latter workers value of 86×10^7 cm^2 mole^{-1}. All of the preparations show a ratio of the 405 to the 280 mμ extinction of 1.30 or greater. A high value for this ratio should be synonomous with increased catalase purity in the absence of other proteins showing a higher absorption at 405 mμ than catalase. The low hemin content would appear to obviate the latter possibility.

Hemin and Iron Content: The hemin values determined for the various preparations were relatively constant. The average of 1.04 % is slightly lower than the value of 1.07—1.09 % reported by Bonnichsen [3], for horse erythrocyte catalse. A value for the molecular weight of 250 000 is obtained on the basis of the hemin content and the assumption of four hemins per mole of protein.

Some variation in the iron content was found for various samples, an average of 0.096 % obtaining. This is slightly higher than the value of 0.091—0.095 found by Bonnichsen [3]. From the iron content a molecular weight of 233 000 may be calculated.

Molecular Kinetic Studies: Three preparations, 4 A, 5 Aq and 5 S, which showed considerable variation in Kat. F. and absorption coefficients were first studied in the ultracentrifuge at a concentration of 0.8 %. A single symmetrical boundary was seen for preparations 4 A and 5 S. Sample 5 Aq showed a small amount of material of sedimentation constant of approximately 3 *S*. Identical average values of $s_{20w} = 11.61$ *S* were obtained in each case.

The spread in interval values during each experiment was sufficiently large to indicate that the remarkable close agreement of the individual experiments must be regarded as fortuitous. Nevertheless it indicates a close similarity in molecular mass for the above preparations of widely different activity. Further single sedimentation experiments were also carried out on preparations 8 Aq at 0.6 % protein, and 5 Aq at 0.4, 1.2 and 3.0 % protein. The value for the latter sample at the 3.0 % protein concentration was very low (10.45 S) and was not used for the extrapolation of the sedimentation constant to infinite dilution. The data for these experiments which are plotted to give Fig. 1 indicate that the sedimentation constant for horse erythrocyte catalase is close to 11.8×10^{-13} cm per sec. Our value for the sedimentation constant of a solution near 1 % protein is higher than the 11.0—11.3 S previously found for the main components of horse liver [7,8], and human erythrocyte and bacterial catalases [9].

Various diffusion experiments were carried out. The most technical perfect experiments performed on sample 8 Aq gave the following results.

Concentration	D_A	D_m
g/100 ml		
0.6	4.14	4.11×10^{-7} cm²/sec.
0.43	4.08	4.28×10^{-7} » »

The close agreement of the diffusion constant when calculated by both the height-area and moments method indicates that the enzyme was molecularly homogeneous.

After the diffusion experiments this material (8 Aq) was studied in the ultracentrifuge at a concentration of approximately 0.6 %. A single peak of sedimentation constant 11.64 S was observed. This result is again in remarkable agreement with the sedimentation results obtained for the other three catalase preparations studied in this concentration range.

Table 2. Physical constants of horse erythrocyte catalase.

Molecular weight:	Fe content	233 000
	Hemin content	250 000
	Molecular kinetic	269 000
V_{sp}		0.74
D_A		4.11×10^{-7} cm²/sec.
D_m		4.19×10^{-7} » »
s_{20w}		11.8×10^{-13} cm/sec.
f/f_0		1.17

The value of the partial specific volume was 0.74 which is near the 0.73 reported by Sumner and Gralén [10] for beef liver catalase, but considerably higher than the value of 0.715 found by Agner [7] for a horse liver catalase. From the above values of the sedimentation and diffusion constants and partial specific volume a molecular weight of 269 000 is calculated. This is considerably higher than the commonly accepted value of 225 000 for erythrocyte catalase and likewise higher than the values calculated from the hemin and iron contents. From the above data a dissymmetry factor (f/f_0) of 1.17 and an axial ratio near 4 is obtained if one assumes the molecule to be an elongated ellipsoid. The various physical data are summarized in Table 2.

SUMMARY

Variations in the analytical data for six crystalline horse erythrocyte catalase preparations indicate that this enzyme is not a uniformly pure protein as is ordinarily assumed. Support for this has already been presented in the form of denaturation studies on several preparations of this enzyme [2]. The variations in activity and conditions for crystallization are not reflected in any discernable molecular mass difference. It would appear that the present methods of preparation result in only slight modification of the catalase molecule but that these are synchronous with rather large changes in activity and in ease of crystallization.

Acknowledgement: The author is indebted to Dr. K. O. Pedersen for so graciously carrying out the diffusion and sedimentation experiments in his laboratory. We also wish to thank Dr. Carl Drucker for making the partial specific volume measurement. The kind interest of Professor Hugo Theorell during the course of the work is greatly appreciated.

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The Magnetic Properties of Crystalline Horse Erythrocyte Catalase and Some of its Derivatives

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The magnetic susceptibility as an adjunct to spectrophotometric technique is a powerful tool for the study of the nature of the heme-protein bonds in iron porphyrin proteins, as first shown by Pauling and his collaborators [1]. Previous work of the nature from this laboratory have been concerned with cytochrome c [2], horseradish peroxidase [3], horse liver catalase [4], and myoglobin [5]. The more recent investigations have utilized the apparatus described by Theorell and Ehrenberg [6]. This instrument provides a high degree of sensitivity and makes possible the accurate measurement of the magnetic susceptibility of small samples.

In connection with the systematic investigations of the magnetic properties of iron porphyrin proteins, it seemed opportune to investigate horse erythrocyte catalase. The study of this protein is somewhat more elucidative than the horse liver catalase studied previously by Theorell and Agner [4] since all the iron is present as hemin iron, and one is thus not faced with the problems introduced by the presence of biliverdin iron. The work reported here is concerned with the magnetic susceptibility of a highly active horse erythrocyte catalase as well as a number of its derivatives.

EXPERIMENTAL

A single sample of the enzyme was employed throughout the work. It represented the catalase preparation no. 8 Aq, described in the preceding article [7]. The enzyme had been crystallized from water three times and showed an initial Kat.F. of 125 000. Near the end of the investigations (approximately one month), the activity had fallen to 90 000.

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This is somewhat characteristic of the high activity catalases previously reported [7,8]. The fall in activity did not appear to affect the magnetic susceptibility of the enzyme as measured during this interval.

The recrystallized enzyme was dialyzed exhaustively against distilled water and the crystals dissolved by addition of a trace of ammonia to give a 12.125% solution. The iron content of the protein was found to be 0.0937%, which if one assumes four atoms of iron per molecule of catalase corresponds to a molecular weight of 238 000.

Solutions for magnetic susceptibility measurements were made by mixing 0.3 ml of the above solution with 0.1 ml of buffer or a particular reagent while the sample was being stirred magnetically. All solutions measured thus contained 9.094% protein. The ionic strength was 0.25 except in the case of the studies on the fluoride derivative. In these experiments a fluoride concentration of 0.5 *M* and a total ionic strength of 0.65 was employed. In studies of the effect of pH on magnetic susceptibility, the salt consisted chiefly of sodium chloride solutions containing either dilute sodium hydroxide or acetic acid. All pH measurements were made by means of a glass electrode immediately after the removal of the sample from the measuring tube. The glass electrode had been previously carefully calibrated against a hydrogen electrode. The actual magnetic measurements and calculations of susceptibilities were carried out as described by Theorell and Ehrenberg [5,6].

The preparation of catalase derivatives was carried out in the usual manner as described in previous work.

RESULTS

The magnetic susceptibility data for catalase and the derivatives which were measured here are shown in Table 1. These values have been corrected for the diamagnetism of catalase. The ferriprotoporphyrin of catalase cannot be removed without denaturation of the protein. Neither can a diamagnetic compound of catalase such as a ferro-carbon monoxide derivative be prepared easily. However, the diamagnetic gram susceptibilities of myoglobin, peroxidase and cytochrome c did not vary much [5,3,2]. The mean value of their diamagnetism was therefore taken for our calculations.

Table 1. Magnetic susceptibility of catalase and derivatives.

Compound	$\chi_{Fe} \times 10^6$ cgs	μ (Bohr magnetons)	Number of unpaired electrons	μ theoretical (Bohr magnetons)
Catalase (pH 4.8—10.4)	13 390	5.60	5	5.92
Fluoride-Catalase	13 350	5.60	5	
Cyanide-Catalase	2 240	2.29	1	1.73
Hydrogen-Sulfide-Catalase	2 700	2.51	1	
Azide-Catalase	12 310	5.36	5	

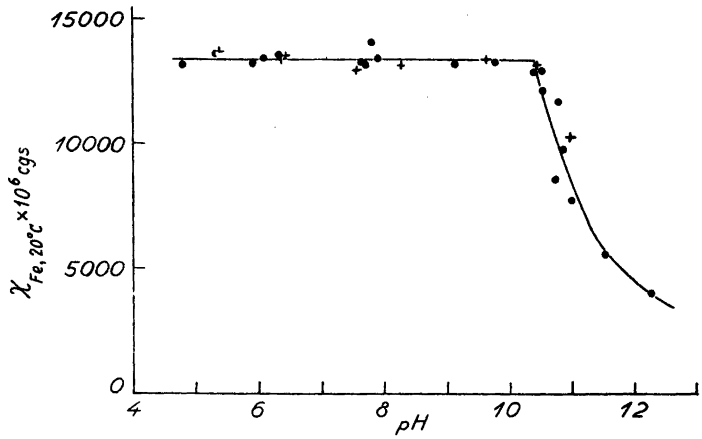


Fig. 1. Effect of pH on the paramagnetic susceptibility of the iron of catalase (•) and catalase fluoride (+).

Theorell and Agner's [4] diamagnetic correction, which contributes 30 % to the final χ_{Fe} of neutral catalase and fluoride catalase was probably somewhat too high. Our calculated diamagnetic contribution amounts to 23.6 % which accounts for practically the whole difference between the two sets of results for the strongly paramagnetic compounds of catalase. Our susceptibility values for the weakly paramagnetic cyanide and hydrogen sulfide compounds of about $2\,500 \times 10^{-6}$ cgs are to be expected from their spectral type. This is strongly in favour of the lower correction figure. A 30 % correction would give values of about $3\,700 \times 10^{-6}$ which are probably too high. It is interesting to note that if the susceptibilities obtained by Theorell and Agner [4] for the derivatives of horse liver catalase are corrected, first for the assumption that the fourth iron atom in the molecule is unaffected by the reagents, as described in their paper, and second for the too high diamagnetic correction, then their measurements are found to agree quite closely with our results.

No change in the magnetic susceptibility of catalase was found from pH 4.8 to approximately 10.4. At the latter point a sudden change is seen. Above this pH, horse erythrocyte catalase undergoes denaturative changes characterized by a marked increase in viscosity and loss of solubility at neutral pH. Almost identical results were obtained in the presence of 0.5 *M* fluoride over this pH range. This remarkably abrupt change in stability is very similar to that found by Theorell and Ehrenberg [5] in the case of myoglobin. At pH below 5.5, where acetate buffer was used, about 30 % of the catalase is combined with the acetate as was shown by Agner and Theorell [9]. Spectrophoto-

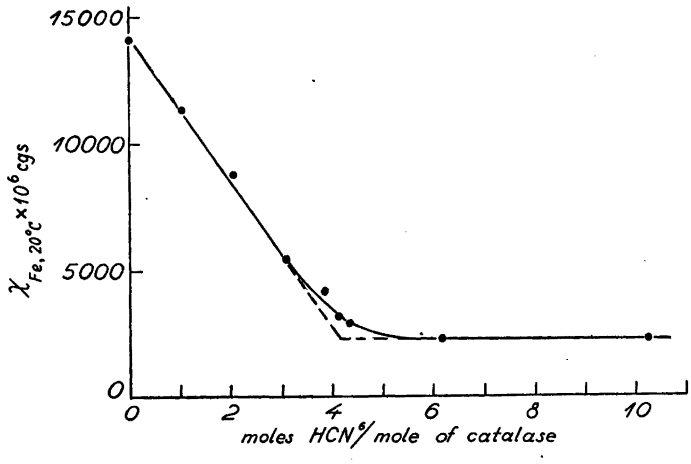


Fig. 2. Magnetometric titration of horse erythrocyte catalase with HCN at pH 7.75.

metric titration experiments with fluoride at pH values between 5.8 and 7.8 indicated that there is a pure competition between fluoride and hydroxyl ions. With fluoride ion concentration of 0.5 *M* there is present about 30 % catalase fluoride compound at pH 7.6 and about 80 % at pH 6.4. The two experiments at the latter pH were made with phosphate buffer so that the acetate could not interfere. Thus both the acetate and the fluoride compounds have about the same paramagnetic susceptibility as neutral catalase. The results for these experiments are plotted to give Figure 1. The mean value for the molar susceptibility (χ_{Fe}) of the iron in catalase for eleven observations between pH 4.8—10.4 is 13 390 $\times$ 10^{-6} ($\pm$ 100) cgs. (The iron of the denaturation product that has a typical hemichrome spectrum is found to have a molar susceptibility of 2 500 $\times$ 10^{-6} cgs as determined from extrapolation of the data of Figure 1.) The mean value of six observations of χ_{Fe} for catalase fluoride in the same pH range was 13 350 $\times$ 10^{-6} ($\pm$ 120) cgs.

In magnetometric studies of ferrimyoglobin fluoride, Theorell and Ehrenberg [5] noted three p*K* values in the region pH 6, 8, and 10, only two of which were spectrophotometrically operable. The magnetometric studies of catalase fluoride as shown in Figure 1 did not suggest any heme-linked groups to be dissociated over the pH range 4.8—10.4.

The magnetic susceptibility of the cyanide catalase derivative was determined for various ratios of cyanide to catalase at pH 7.75. The results for these experiments are plotted to give Figure 2. All of the iron atoms are titrated to the point of saturation when slightly over four (4.18) equivalents of cyanide per

molecule of catalase have been added. Assuming the dissociation constant of HCN to be 2×10^{-9} a $K_I = \frac{[HCN][FeOH]}{[FeCN]} = 18 \cdot 10^{-6}$ can be calculated from the data. This is only slightly higher than the values determined by Chance [10] in kinetic, spectrophotometric, and inhibition experiments. The susceptibility of the cyanide compound is determined to $2\,240 \times 10^{-6}$ cgs.

The hydrogen sulfide derivative of catalase gave a molar susceptibility of $2\,700 \times 10^{-6}$ cgs. This corresponds most closely to one unpaired electron for the iron atoms. The slightly higher value for hydrogen-sulfide-catalase as compared with the value found for cyanide catalase has been previously noted by Theorell and Agner [4].

The χ_{Fe} for azide catalase is close to that of catalasefluoride and free catalase.

From Table 1 it is apparent that the number of unpaired electrons for the iron of catalase and its fluoride and azide derivatives must be five. The measured susceptibility is somewhat lower than the theoretical value for five unpaired electrons, just as in ferri Hb[1], ferri Mb[5] and horse radish peroxidase[3].

The cyanide and sulfide derivatives show susceptibilities slightly higher than would be expected for one unpaired electron. However, when one considers that the orbital contributions may add about 0.5 Bohr magnetons to these values, the difference from 1.75 is not too great. The higher values may indicate some heme-heme interaction or a greater than usual orbital contribution.

The magnetic susceptibility data for horse erythrocyte catalase and derivatives as obtained in these studies support the results obtained earlier by Theorell and Agner [4] on horse liver catalase and proves the correctness of their assumption that biliverdin iron as present in liver catalase is held by essentially ionic bonds and does not take part in the formation of the covalent complexes so far studied.

SUMMARY

The magnetic susceptibilities of a sample of highly active crystalline horse erythrocyte catalase and some of its derivatives have been studied. The results obtained support most previous work on the electronic nature of the iron in this type of enzyme.

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Investigations in the Retene Field

V. The Structure of 4,9-Dinitro-3-hydroxyretene and of Some of its Derivatives

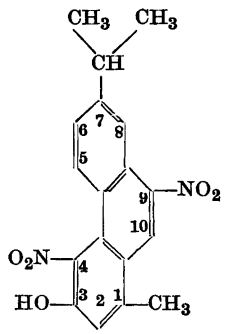
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Karrman [1] investigated the nitration of 3-hydroxyretene and some of its derivatives and prepared both mono- and dinitro-derivatives of the compounds. The structures of the nitro-derivatives were not completely elucidated although it was shown that the dinitro-derivatives could be obtained by further nitration of the mononitro-derivatives, and consequently, one nitro-group in the former series of compounds occupies the same position as in the corresponding mononitro-derivatives. Further, diazonium salts couple with mononitro-3-hydroxyretene but not with dinitro-3-hydroxyretene and this should indicate that one of the nitro-groups in the latter compound occupies the 4-position. Attempts to oxidize the nitro-derivatives to quinones were reported to fail. The formation of a quinone would have proved if a nitro-group is situated in the 9- or 10-position.

Sihlbom [2] proves that the mononitro-derivatives obtained by nitration of 3-acetoxyretene, 3-benzoyloxyretene, and 3-ethoxyretene were 9-nitro-derivatives. The present paper gives an account of the elucidation of the structures of the other mono- and dinitro-derivatives of 3-hydroxyretene and its derivatives described by Karrman [1].

On nitration even under vigorous conditions, 3-acetoxyretene gives solely 9-nitro-3-acetoxyretene [1]. On the other hand, mild nitration of 3-hydroxyretene yields solely a dinitro-derivative [1], in which one of the nitro-groups occupies the 9-position [2]. It may be assumed that the other nitro-group occupies the 2- or the 4-position, this also being valid for the dinitro-derivatives prepared from 3-methoxyretene and 3-ethoxyretene. Therefore, it was conceivable that amination of dinitro-3-hydroxyretene should give 2,9-dinitro-3-aminoretene or, more probably, 4,9-dinitro-3-aminoretene, a compound previously prepared (Karrman and Sihlbom [3], Sihlbom [4]).



4.9-Dinitro-3-hydroxyretene

Amination experiments with urea using the procedure described by Kym [5] gave only small yields of the dinitro-amine (about 1%) but appreciably higher yields (about 18%) were obtained when the amination was carried out by heating at 140° C for 10 hours in a solution of 35% ammonia (Merz and Ris [6]). By chromatographic purification of the crude product some unchanged starting material could be isolated. Prolongation of the reaction time or elevation of the temperature causes all the dinitro-3-hydroxyretene to react but owing to decomposition higher yields of the dinitro-amine were not obtained. The dinitro-amine was shown to be identical with 4,9-dinitro-3-aminoretene [3] by a mixed m.p. determination. Similarly, the acetyl-derivative was shown to be identical with 4,9-dinitro-3-acetylaminoretene [3]. As 4,9-dinitro-3-aminoretene can be more easily obtained in another way [3], the amination reaction was not investigated more closely, but this amination reaction indicates that 3-hydroxyretene, on nitration, yields 4,9-dinitro-3-hydroxyretene.

Karrman [1] also prepared dinitro-derivatives by nitration of 3-methoxyretene and 3-ethoxyretene. It has now been shown that these derivatives can be obtained by methylation, or ethylation, respectively, of 4,9-dinitro-3-hydroxyretene and thus their structure are proved. The methylation can be carried out with dimethyl sulphate in an alkaline ethanolic solution, but methylation with diazomethane is preferable, as the methylether is obtained directly in an almost pure state, in high yield. The ethylation was carried out with diethyl sulphate in alkaline ethanolic solution.

According to Karrman [1], nitration of 3-methoxyretene under mild conditions yields a mixture of two mononitro-3-methoxyretenes which on further nitration gives one dinitro-3-methoxyretene, shown above to be 4,9-dinitro-3-methoxyretene. It is therefore probable that the mixture contains 4-nitro-3-methoxyretene and 9-nitro-3-methoxyretene. Chromatographic separation (alumina, petroleum ether) showed that two mononitro-3-methoxyretenes were present in the ratio 4 : 5. For purposes of comparison the two anticipated compounds were prepared in the following way. 4-Nitro-3-acetoxy-9-aminoretene, prepared by partial reduction of 4,9-dinitro-3-acetoxyretene, was deaminated (diazotisation and reduction of the diazonium salt with hypophosphorous acid) and the reaction product, 4-nitro-3-acetoxyretene, was hydrolyzed. The 4-nitro-3-hydroxyretene, thus obtained, yielded 4-nitro-3-methoxyretene on methylation. 9-Nitro-3-methoxyretene was obtained by methylation of 9-nitro-3-hydroxyretene [1,2]. Mixed m.p. determinations

showed that the above-mentioned isomers, isolated chromatographically, were identical with 4-nitro-3-methoxyretene and 9-nitro-3-methoxyretene, respectively, the latter being present in the larger amount.

It may be of interest to compare the tendency of some of the compounds investigated to form molecular compounds with acetic acid. It has been reported previously (Karrman [1]) that 4,9-dinitro-3-hydroxyretene can crystallize with acetic acid (2 moles), a property not exhibited by 4,9-dinitro-3-acetoxyretene. Obviously, the acetoxy-group reduces the tendency of the compound to form such molecular compound. After elimination of the nitro-group in the 9-position, we again have a compound, 4-nitro-3-acetoxyretene that can crystallize with acetic acid (1 mole). Nevertheless, 4-nitro-3-hydroxyretene, and 9-nitro-3-hydroxyretene crystallize without acetic acid.

The compounds discussed in this paper are listed in Table 1.

Table 1. Compounds treated in the present paper.

Name	M. p. °C *
4-Nitro-3-hydroxyretene	148—149 (decomp.)
4-Nitro-3-acetoxyretene	137—138
4-Nitro-3-methoxyretene	169—170
9-Nitro-3-methoxyretene	140—141
4,9-Dinitro-3-hydroxyretene **	166—167
4,9-Dinitro-3-methoxyretene **	173—174
4,9-Dinitro-3-ethoxyretene **	227—228
4-Nitro-3-acetoxy-9-aminoretene	ca. 190 (decomp.)
4-Nitro-3-acetoxy-9-acetylaminoretene	» 220 »

* All melting points are approximately corrected.

** Compound previously described.

EXPERIMENTAL

Amination of 4,9-dinitro-3-hydroxyretene. Finely powdered 4,9-dinitro-3-hydroxyretene (2.0 g) suspended in 35% ammonia (40 ml) in a sealed glass tube was heated at 140 °C for 10 hours. The reaction product consisting of reddish-brown crystals and friable dark pieces was filtered off and washed with warm ammonia until the washings were colourless. After drying, the reaction product was dissolved in acetone and adsorbed on a column of alumina (10 cm^2 × 20 cm). Development with acetone caused the separation of many zones. The yellow, most quickly moving zone, which consisted chiefly of 4,9-dinitro-3-aminoretene was eluted, and the dark crystalline residue (0.5 g), obtained after evaporation of the solvent, was dissolved in benzene and again adsorbed on a column of

alumina (10 cm^2 × 20 cm). On development with benzene, an orange-coloured zone appeared and from this pure 4,9-dinitro-3-aminoretene was isolated (0.35 g, 18%); m.p. 210—211 °C, undepressed on admixture with authentic material prepared via nitration of 3-acetylaminoretene [3]. [Calc. for $C_{18}H_{17}O_4N_3$ (339.3): C 63.7; H 5.05. Found: C 63.8; H 4.97].

4,9-Dinitro-3-acetylaminoretene. A sample (0.1 g) of the amine obtained above was dissolved in a mixture of benzene (2 ml) and acetic anhydride (2 ml) and the mixture was set aside overnight at 60 °C. After evaporation of the benzene, water was added to decompose the excess of acetic anhydride and precipitate the reaction product, which, after two crystallizations from glacial acetic acid, was obtained as yellow needles; m.p. 233—234 °C, undepressed on admixture with 4,9-dinitro-3-acetylaminoretene prepared by nitration of 3-acetylaminoretene [3]. M. p., previously reported (Karrman and Sihlbom [3]), 235—236 °C. [Calc. for $C_{20}H_{19}O_5N_3$ (381.4): C 63.0; H 5.02. Found: C 62.8; H 5.11.]

4,9-Dinitro-3-methoxyretene. An ether solution containing excess of diazomethane was added to a solution of 4,9-dinitro-3-hydroxyretene (0.1 g) in ether (10 ml) and the reaction mixture was set aside overnight at room temperature. After evaporation of the ether, the residue (m.p. 166—168 °C) was purified by two crystallizations from glacial acetic acid; m.p. 173—174 °C, undepressed on admixture with dinitro-3-methoxyretene prepared by nitration of 3-methoxyretene [1]. Karrman [1] reports m.p. 172.2—172.5 °C. [Calc. for $C_{19}H_{18}O_5N_2$ (354.4): C 64.4; H 5.12. Found: C 64.6; H 5.20.]

4,9-Dinitro-3-ethoxyretene. Potassium hydroxide (1 g) dissolved in water (5 ml) was added to a solution of 4,9-dinitro-3-hydroxyretene (0.5 g) in ethanol (15 ml). Three portions of 1 ml of diethyl sulphate (each) were added to the stirred solution maintained at 30—40 °C during the course of 3 hours. After a further 2 hours, the yellow reaction product (0.25 g, 46 %) was filtered off, washed with ethanol, and crystallized from glacial acetic acid; m.p. 227—228 °C, undepressed on admixture with dinitro-3-ethoxyretene prepared by nitration of 3-ethoxyretene. [Calc. for $C_{20}H_{20}O_5N_2$ (368.4): C 65.2; H 5.47. Found: C 64.8; H 5.40.]

4-Nitro-3-acetoxy-9-aminoretene. Finely powdered 4,9-dinitro-3-acetoxyretene (5.0 g) was suspended in glacial acetic acid (50 ml) saturated with hydrogen chloride. A solution of $SnCl_2 \cdot 2H_2O$ (10 g) in glacial acetic acid (40 ml) saturated with hydrogen chloride was added with stirring at 20—25 °C during 2 hours. The amine formed by the reduction, precipitated as a sparingly soluble tin complex (6.6 g) which was filtered off and dried over potassium hydroxide in a vacuum. This material was dissolved in ethanol (90 ml), and pyridine (5 ml) and water (300 ml) were added. The precipitate formed on the addition of water, was filtered off, dried and extracted with boiling *n*-propanol (90 ml), in which solvent 4-nitro-3-acetoxy-9-aminoretene alone dissolved. On cooling, the amine crystallized as flat yellowish-brown prisms, m.p. ca. 190 °C (decomp.). Yield 2.6 g, 57 %. [Calc. for $C_{20}H_{20}O_4N_2$ (352.4): C 68.2; H 5.72. Found: C 68.4; H 5.76.]

The hydrochloride of the amine obtained above separated as colourless minute needles when hydrochloric acid (15 ml, ca. 4 *N*) was added to a solution of 4-nitro-3-acetoxy-9-aminoretene (0.1 g) in glacial acetic acid (10 ml); m.p. ca. 195 °C (decomp.). [Calc. for $C_{20}H_{21}O_4N_2Cl$ (388.9): C 61.8; H 5.44. Found: C 61.7; H 5.52.]

The picrate separated as yellow needles on cooling a hot ethanolic solution (35 ml) containing 4-nitro-3-acetoxy-9-aminoretene (0.1 g) and picric acid (0.3 g); m.p. ca. 185 °C (decomp.). [Calc. for $C_{26}H_{23}O_{11}N_5$ (581.5): C 53.7; H 3.99. Found: C 53.6; H 3.99.]

4-Nitro-3-acetoxy-9-acetylaminoretene. Acetic anhydride (1 ml) was added to a solution of 4-nitro-3-acetoxy-9-aminoretene (0.2 g) in benzene (15 ml) at room temperature.

After 4 hours ligroin (75 ml) was added slowly with stirring and the 4-nitro-3-acetoxy-9-acetylaminoretene separated as short light-yellow needles, m.p. ca. 220 °C (decomp.). [Calc. for $C_{22}H_{22}O_5N_2$ (394.4): C 67.0; H 5.62. Found: C 66.7; H 5.71.]

4-Nitro-3-acetoxyretene. 4-Nitro-3-acetoxy-9-aminoretene (2.0 g) was dissolved in warm glacial acetic acid (70 ml). On cooling to room temperature, a colourless precipitate formed. With stirring, a mixture of glacial acetic acid (5 ml) and conc. sulphuric acid (2 ml) was added, followed by a solution of sodium nitrite (0.4 g) in water (2 ml) added dropwise at 10—15 °C. The clear, reddish-brown diazonium salt solution was mixed with 50 % hypophosphorous acid (20 ml) and kept at 5 °C for 10 hours. The reaction product, 4-nitro-3-acetoxyretene, crystallized as long, yellow needles containing 1 mole of acetic acid (1.8 g, 80 %). Crystallization from ethanol gave plates containing no acetic acid, m.p. 137—138 °C. [Calc. for $C_{20}H_{19}O_4N$ (337.4): C 71.2; H 5.68. Found: C 70.8; H 5.75].

Crystallization from glacial acetic acid gives needle-shaped crystals containing 1 mole of acetic acid which can be removed by heating at 110 °C. [Calc. for $C_{22}H_{23}O_6N$ (397.4): C 66.5; H 5.83. Found: C 66.7; H 5.86.]

4-Nitro-3-hydroxyretene. A sample of 4-nitro-3-acetoxyretene (1.2 g) obtained above was suspended in ethanol (8 ml) containing conc. hydrochloric acid (0.3 ml) and the mixture was refluxed for 80 minutes by which time a clear yellowish-brown solution was obtained. Evaporation of the solvent and drying of the residue at 100 °C in a vacuum yielded rather pure 4-nitro-3-hydroxyretene (1.0 g) as a yellow crystalline mass. It crystallizes from glacial acetic acid as yellow needles containing no acetic acid; m.p. 148—149 °C (decomp.). [Calc. for $C_{18}H_{17}O_3N$ (295.3): C 73.2; H 5.81. Found: C 72.6; H 5.70.]

4-Nitro-3-methoxyretene. A solution of 4-nitro-3-hydroxyretene (0.1 g) in ether (10 ml) was mixed with ether containing excess of diazomethane and kept at room temperature for 10 hours. The residue obtained by evaporation of the ether, was crystallized twice from glacial acetic acid, giving 4-nitro-3-methoxyretene as yellow needles, m.p. 169—170 °C. [Calc. for $C_{19}H_{19}O_3N$ (309.4): C 73.8; H 6.19. Found: C 73.5; H 6.29.]

9-Nitro-3-methoxyretene. 9-Nitro-3-hydroxyretene was methylated in the same way as described above for the methylation of 4-nitro-3-hydroxyretene. Yellow needles, m.p. 140—141 °C. [Calc. for $C_{19}H_{19}O_3N$ (309.4): C 73.8; H 6.19. Found: C 74.2; H 6.24.]

Chromatographic separation of 4-nitro-3-methoxyretene and 9-nitro-3-methoxyretene. A mixture of these compounds was prepared by mild nitration of 3-methoxyretene [1]. A sample (0.25 g) of this mixture, dissolved in a mixture of benzene (10 ml) and petroleum ether (10 ml, b.p. 40—50 °C), was adsorbed on a column of alumina (10 cm^2 × 35 cm). On development with petroleum ether two light-yellow zones appeared. The lower zone yielded a yellow compound (0.10 g, m.p. 136—137 °C) which on crystallization from glacial acetic acid gave yellow needles; m.p. 140—141 °C, undepressed on admixture with 9-nitro-3-methoxyretene. [Calc. for $C_{19}H_{19}O_3N$ (309.4): C 73.8; H 6.19. Found: C 73.8; H 6.25.]

The yellow compound (0.08 g, m.p. 165—167 °C) isolated from the other zone separated as needle-shaped crystals from glacial acetic acid; m.p. 169—170 °C, undepressed on admixture with 4-nitro-3-methoxyretene. [Calc. for $C_{19}H_{19}O_3N$ (309.4): C 73.8; H 6.19. Found: C 73.8; H 6.30.]

SUMMARY

Previously prepared dinitro-derivatives of 3-hydroxyretene, 3-methoxyretene, and 3-ethoxyretene have been shown to have the nitro-groups in 4-

and 9-positions. From the mixture of mononitro-3-methoxyretenes, obtained by mild nitration of 3-methoxyretene, two components have been separated chromatographically and identified as 4-nitro-3-methoxyretene and 9-nitro-3-methoxyretene.

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Polarographic Investigation of Dilute Solutions of Cadmium Cyanide Complexes

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A polarographic study of very dilute solutions of zinc perchlorate in the presence of increasing amounts of potassium cyanide has previously been made by the authors [1]. We found that, although the ion $Zn(CN)_4^{--}$ evidently predominates, quite a series of complex zinc cyanide ions are formed, from $Zn(CN)_3^-$ ions to $Zn(CN)_6^{----}$ ions. For the latter Pines [2] in his polarographic studies got no reduction; but we found that it was polarographically reduced before the reduction potential of K^+ is reached, whereas Pines assumes that it does not decompose at lower potentials than —2 volts.

Pines [3] further assumes, in opposition to the results of v. Euler [4], and of later investigators, that the complex ion $Cd(CN)_3^-$ and not the ion $Cd(CN)_4^{--}$ is predominant in dilute solutions of cadmium cyanide complexes.

The question, whether the cyanide complexes of zinc or those of cadmium have the greater instability constant, has also been a matter of investigation and discussion. It has been found that they are of the same order of magnitude [4,5]. If, however, as supposed by Pines [2], the $Zn(CN)_4^{--}$ ion predominates in dilute potassium zinc cyanide solutions, and the $Cd(CN)_3^-$ ion in potassium cadmium cyanide solutions, the constants found are not directly comparable. It seems therefore that a further investigation as regards these phenomena is indicated.

Considering the above mentioned inconsistencies we found it of interest to make a polarographic study of cadmium perchlorate in the presence of increasing amounts of potassium cyanide under practically the same experimental conditions as formerly used for the zinc perchlorate — potassium cyanide solutions.

EXPERIMENTAL PROCEDURE

A polarograph from Radiometer with a photoelectric amplifier, an automatic registrator and a counter current arrangement was used as previously.

We took good precautions against oxygen waves. Hydrogen and nitrogen purified over red hot copper and bubbled through chromous chloride solutions were used. In order to avoid intrusion of air during the passage plastic tubing was substituted for rubber tubing. The solutions in the electrolytic vessel were the whole time kept under one of the above mentioned gasses.

Cadmium perchlorate solutions were prepared by precipitation of cadmium sulphate solutions with equivalent amounts of barium perchlorate solutions.

The molar concentration of the cadmium ions was of the order of magnitude of 10^{-3}. The concentration of potassium cyanide was increased from mole ratio $Cd^{++} : CN^{-} = 1 : 0$ to mole ratio $Cd^{++} : CN^{-} = 1 : 320$.

Inasmuch as some mercurous cyanide undoubtedly is formed during the electrolysis, the stationary pool of mercury on the bottom of the electrolytic cell will give a mixed potential. We therefore found it convenient as a check on our measurements to take a series of readings with a large saturated calomel half-cell as a non-polarizable reference electrode.

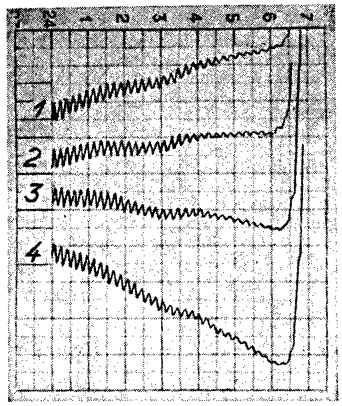


Fig. 1. Electro-reduction of cadmium perchlorate in aqueous solutions.

No	Volts		Counter current	l/sens.	Mol. conc.	
	E.M.F. applied	Starting point			$NaClO_4$	$Cd(ClO_4)_2$
1	2	0	2	1 × 1	0.1	0.0003
2	2	0	3	1 × 1	0.1	0.0003
3	2	0	4	1 × 1	0.1	0.0003
4	2	0	5	1 × 1	0.1	0.0003

EXPERIMENTAL RESULTS

During a previous investigation [6] we found that the polarographic reduction of Cd^{++} ions under certain circumstances can occur successively, the discharge wave being preceded by a smaller one. This pre-wave has been found also by reduction of some other metal ions under similar conditions. At that time an apparatus with counter current was not available, but with our present equipment we make use of a counter current, thereby getting more distinct waves. As will be seen from Fig. 1, which gives the results in pure cadmium perchlorate solutions, the value of the half wave potential for the pre-wave is found to be -0.35 volts against the mercury anode. The increase in current induced by the cadmium starts about -0.65 volts. On addition of cyanide the pre-wave disappears, inasmuch as the half-wave potential for the cadmium ion shifts towards less negative values.

The said value for the half-wave potential, $E_{\frac{1}{2}}$, is the same as formerly found. This is consistent with the assumption that in the presence of a relatively large excess of a stated supporting electrolyte the half-wave potential is fixed as long as the ionic strength is kept constant.

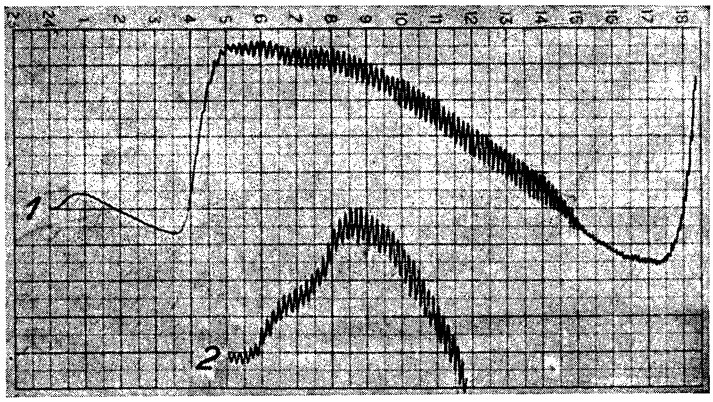


Fig. 2. Electro-reduction of solutions of cadmium perchlorate and potassium cyanide with the molar proportion Cd : CN = 1 : ½.

No	Volts		Counter current	1/sens.	Mol. conc.		
	E.M.F. applied	Starting point			$NaClO_4$	$Cd(ClO_4)_2$	KCN
1	2	0	1	3 × 10	0.1	0.001	0.0005
2	2	−0.5	4	5 × 1	0.1	0.001	0.0005

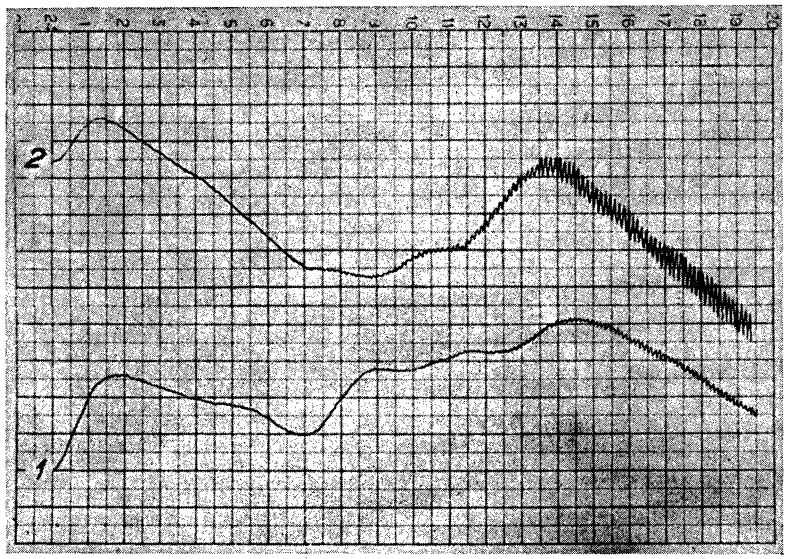


Fig. 3. Electro-reduction of solutions of cadmium perchlorate and potassium cyanide with the molar proportions Cd : CN = 1 : 2 and 1 : 4.

No	Volts		Counter current	1/sens.	Mol. conc.		
	E.M.F. applied	Starting point			$NaClO_4$	$Cd(ClO_4)_2$	KCN
1	1	0	4	1.5 × 10	0.1	0.001	0.002
2	1	0	5	1.5 × 10	0.1	0.001	0.004

On the addition of cyanide we found a shift towards less negative values for the Cd^{++} ion, although an increase of concentration usually entails a shift towards more negative values. The same has been found earlier for zinc.

As the concentration of cyanide increases, the Cd^{++} wave decreases, and when the mole ratio $Cd^{++} : CN^- = 1 : 5$ is reached the wave for the free metallic ion has grown rather ambiguous. At the ratio 1 : 6 it is no more distinguishable.

Simultaneously with the decrease of the discharge wave of the simple ion two continuously increasing complex waves appear. When a molar ratio $Cd^{++} : CN^- = 1 : 6$ has been reached the first complex wave disappears and a third complex wave becomes visible. In its turn the second wave decreases as the third wave increases. At a molar ratio $Cd^{++} : CN^- = 1 : 80$ the second and third waves can be said to be connected. At higher concentrations of cyanide the third complex wave only is present and is gradually reaching a constant height.

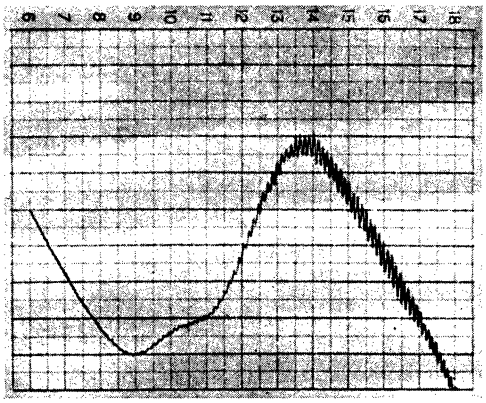


Fig. 4. Electro-reduction of solution of cadmium perchlorate and potassium cyanide with the molar proportion Cd : CN = 1 : 5.

Volts		Counter current	1/sens.	Mol. conc.		
E.M.F. applied	Starting point			$NaClO_4$	$Cd(ClO_4)_2$	KCN
1	−0.3	10	1 × 10	0.1	0.001	0.005

The general trend is the same whether we use a stationary mercury anode or a saturated calomel electrode as anode. With the latter anode we get the usual shift of the half wave potentials to an increased negative value of about 0.25 volts.

Fig. 2 shows that complex formation takes place even before the cyanide has reached the same molar concentration as the cadmium.

The curve taken with the greater sensitivity (2) shows two distinct waves at about −0.6 and −0.8 volts, suggesting that two different complexes have already been formed.

When the concentration of cyanide has grown to be equivalent to that of cadmium, the heights of the complex waves turn out to be of the same order of magnitude as that of cadmium. When the proportion 1 : 4 has been reached the cadmium wave has practically disappeared in relation to the complex waves (Fig. 3).

Even the relatively small increase from the proportion 1 : 4 to the proportion 1 : 5 gives an alteration of the shape of the curve, the cadmium wave having disappeared completely, the first complex wave having become very small in relation to the second wave, which has increased perceptibly. No third complex wave has as yet turned up (Fig. 4).

Even at the relatively small increase of cyanide given by the proportion 1 : 8 the first complex wave has completely disappeared, and the second complex wave has reached its maximal height. At the same time a third complex wave appears. This wave increases very slowly with increasing concentration of cyanide, although the second complex wave

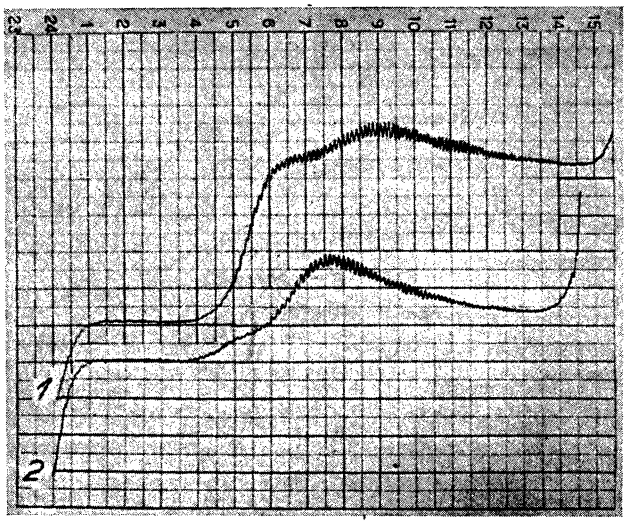


Fig. 5. Electro-reduction of solutions of cadmium perchlorate and potassium cyanide with the molar proportions 1 : 8 and 1 : 40.

No	Volts		Counter current	1/sens.	Mol. conc		
	E.M.F. applied	Starting point			$NaClO_4$	$Cd(ClO_4)_2$	KCN
1	2	0	0	3 × 10	0.1	0.001	0.008
2	2	0	0	3 × 10	0.1	0.001	0.040

Table 1. Height of Waves. Saturated calomel half cell as anode.

$[Cd^{++}]$ / $[CN^-]$	C_1			C_2			C_3		
	H_{obs}	F	H_{calc}	H_{obs}	F	H_{calc}	H_{obs}	F	H_{calc}
1 : 2	10	3×10	30	8	3×10	24			
1 : 5	—	—	—	23	7×10	161	Not measurable		
1 : 8	—	—	—	80	1.5×10	120	11	1.5×10	17
1 : 14	—	—	—	40	2×10	80	22	1.5×10	33
1 : 20	—	—	—	7	7×10	49	37	1.5×10	55
1 : 40	—	—	—	10	1.5×10	15	39	1.5×10	59
1 : 80	—	—	—	—	—	—	The waves are connected		
1 : 160	—	—	—	—	—	—	80	7×1	56
1 : 320	—	—	—	—	—	—	80	7×1	56

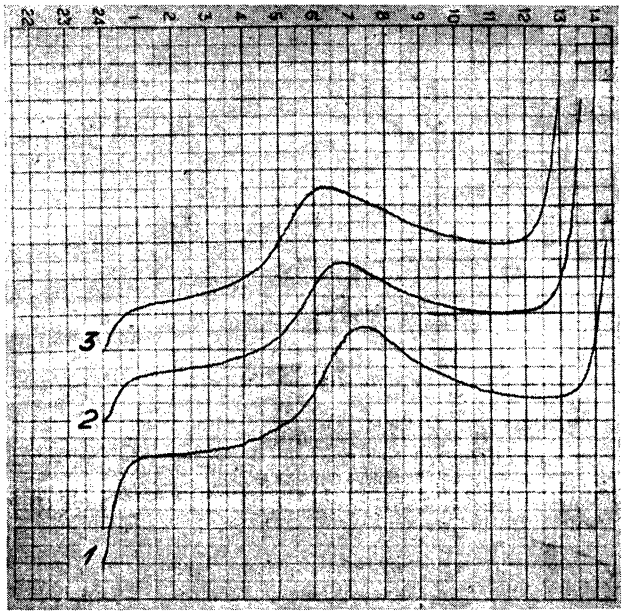


Fig. 6. Electro-reduction of solutions of cadmium perchlorate and potassium cyanide with the molar proportions 1 : 80, 1 : 160 and 1 : 320.

No	Volts		Counter current	1/sens.	Mol. conc.		
	E.M.F. applied	Starting point			$NaClO_4$	$Cd(ClO_4)_2$	KCN
1	2	0	0	3 × 10	0.1	0.001	0.08
2	2	0	0	3 × 10	0.1	0.001	0.16
3	2	0	0	3 × 10	0.1	0.001	0.32

decreases rapidly. When we have reached the proportion 1 : 40 the second complex wave has almost disappeared while the third complex wave is very pronounced (Fig. 5).

At the proportion 1 : 80 the second and the third complex waves are linked together and can not be distinguished from each other. With a very large excess of cyanide one well defined wave only is obtained. This must be ascribed to the third cyanide complex. At the ratios 1 : 160 and 1 : 320 we thus find practically the same curves (Fig. 6).

The figures 7 and 8 give some of the curves resulting from the use of a saturated calomel electrode as anode. The agreement between the two series of curves is good, inasmuch as we find corresponding waves. The form of the curves, however, differ somewhat as we have not used the counter current for the last series.

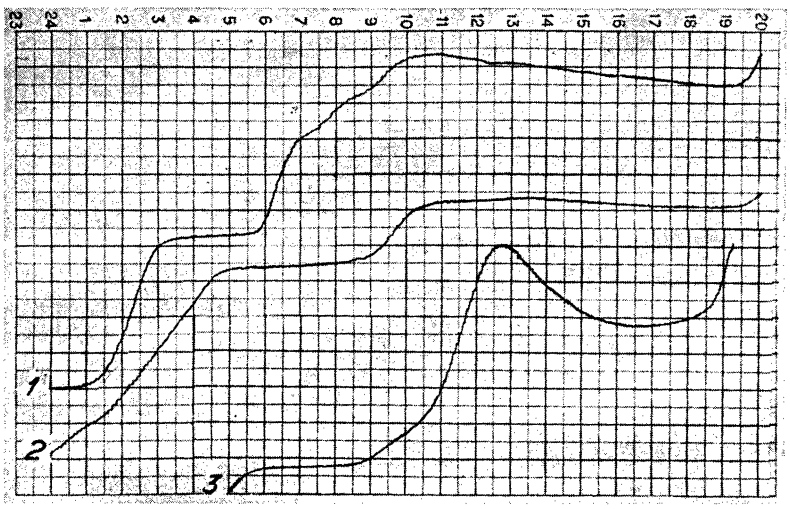


Fig. 7. Electro-reduction of solutions of cadmium perchlorate and potassium cyanide with the molar proportions 1 : 2, 1 : 8 and 1 : 40. Saturated calomel electrode as anode.

No	Volts		Counter current	1/sens.	Mol. conc.		
	E.M.F. applied	Starting point			$NaClO_4$	$Cd(ClO_4)_2$	KCN
1	2	0	0	3 × 10	0.1	0.001	0.002
2	2	0	0	7 × 10	0.1	0.001	0.008
3	2	−0.6	0	1.5 × 10	0.1	0.001	0.040

DISCUSSION

It might be of interest to give a survey of the change of height of the different complex waves in relation to the increasing concentration of cyanide.

A diagram of the results when a mercury anode is used will be found in Fig. 9. The heights of the different curves are given as ordinates and the relationship CN^- : Cd^{++} as abscissae. The corresponding data with a saturated calomel half cell as anode are given in Table 1 p. 1539.

The graphs show plainly that the cadmium wave decreases very rapidly as the complex waves appear. The first complex wave apparently disappears

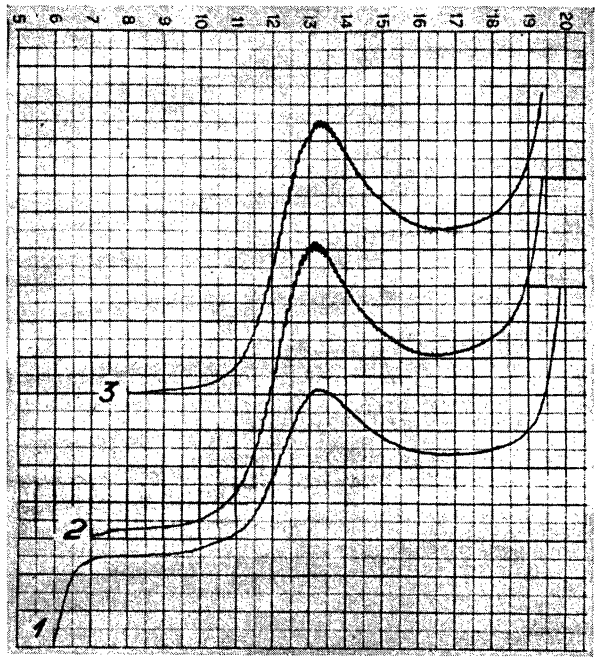


Fig. 8. Electro-reduction of solutions of cadmium perchlorate and potassium cyanide with the molar proportions 1 : 80, 1 : 160 and 1 : 320. Saturated calomel electrode as anode.

No	Volts		Counter current	1/sens.	Mol. conc.		
	E.M.F. applied	Starting point			$NaClO_4$	$Cd(ClO_4)_2$	KCN
1	2	−0.6	0	1.5 × 10	0.1	0.001	0.08
2	2	−0.7	0	7 × 1	0.1	0.001	0.16
3	2	−0.8	0	7 × 1	0.1	0.001	0.32

very abruptly, but the shapes of the polarographic curves suggest that the first and the second complex waves draw together in such a way that they are not to be distinguished from each other. That is also in keeping with the very rapid increase in height of the second complex wave, which reaches a maximum and diminishes rapidly as a third complex wave asserts itself.

It is evident from the diagram that as long as the excess of potassium cyanide does not exceed $Cd^{++} : CN^{-} = 1 : 20$ the complex corresponding to C_1

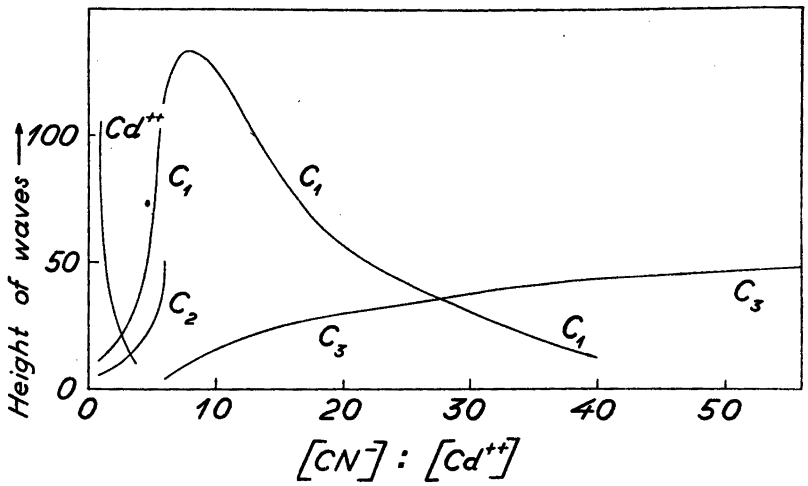


Fig. 9. Diagram of heights of cadmium waves and of cadmium complex waves in relationshipto the proportion $[CN^-]/[Cd^{++}]$.

is predominant. As the excess of cyanide surpasses $Cd^{++} : CN^- = 1:30$ the complex corresponding to C_3 prevails. It seems as if those two complexes may lay claim to special interest. The highest complex (curve C_3) appears when the excess of CN^- over Cd^{++} passes 6 : 1. When the excess grows very great, no increase in the height of the wave is observed and the C_1 curves disappear at $Cd^{++} : CN > 1 : 40$. It therefore seems reasonable to ascribe the C_3 curve to the complex ion $Cd(CN)_6^{----}$, which is the highest described cadmium cyanide complex ion.

The curve marked C_2 must indicate a lower cyanide complex than $Cd(CN)_6^{----}$. According to earlier research workers such as Britton and Dodd [5], who measured changes in pH with increase of cyanide, and Glasstone [7], who made an electrode potential titration curve for cadmium sulphate with sodium cyanide and a cadmium electrode, the $Cd(CN)_4^{--}$ ions are chiefly present in solution. Glasstone maintains that a $Cd(CN)_3^-$ ion is also indicated, whilst Britton and Dodd confine themselves to asserting that an anion having a lower cyanide content than that corresponding to $Cd(CN)_4^{--}$ must exist in solution.

Our curves then seem to show that beside the $Cd(CN)_4^{--}$ ion, which is predominant between the above mentioned concentrations (C_1), a lower complex ion, which we assume to be $Cd(CN)_3^-$, (C_2) exists in solutions having an excess of cyanide less than $Cd^{++} : CN = 1 : 6$. On further increase of cyanide this ion is not polarographically traceable.

SUMMARY

1. The polarographic behaviour of the system $Cd(ClO_4)_2$-KCN-H_2O has been investigated.

2. Even at quite small additions of cyanide ions complex waves appear, showing that cadmium cyanide complexes have been formed.

3. At an excess of cyanide greater than $Cd^{++} : CN = 1 : 4$ the cadmium wave has disappeared, and the complex waves dominate.

4. At the interval between $Cd^{++} : CN = 1 : 4$ and $Cd^{++} : CN = 1 : 20$ the complex ion $Cd(CN)_4^{--}$ dominates.

5. At a greater excess than $Cd^{++} : CN^- = 1 : 30$ the complex ion $Cd(CN)_6^{----}$ prevails. When the proportion $Cd^{++} : CN^-$ grows greater than 1 : 40 no other cadmium cyanide complex can be polarographically detected.

6. In solutions with a relatively small cyanide content still another complex is evident. We suggest that ion to be $Cd(CN)_3^-$.

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Desoxyribonucleic Acids

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The action of desoxyribonuclease (DRNase) from OX-pancreas on desoxyribonucleic acid (DRNA) isolated from calf thymus gland, can be differentiated into two stages[1,2]. An initial depolymerisation (disaggregation) is followed by splitting of the disaggregated nucleic acid into acid-soluble oligonucleotides of varying size down to dinucleotides[1,3,4,5] together with a non-diffusible residue or "core"[1,3,4]. Zamenhof and Chargaff[4] have shown that the quantity of this non-dialysable residue appears to decrease asymptotically with increasing duration of enzyme digestion and dialysis. The dialysis residue is characterised by an increased ratio of purines to pyrimidines and by a greater resistance to further enzymic degradation than the parent nucleic acid, and it was suggested by Chargaff[6] that possibly this finding could be explained by a dissymmetry of the nucleotide sequence in the nucleic acid moelcule.

As part of an extensive programme of investigation of the properties of DRNA's isolated from a variety of sources[7-11] and of their degradation by physical[12], chemical and enzymic[1,13] methods, which our Group has undertaken, some aspects of the enzymic method have been studied. In particular the first stage (disaggregation) of the enzyme action on DRNA from various animal, plant and microbial sources, has been analysed, and also the rate of formation of acid-soluble polynucleotides has been examined. The results obtained are described in this paper.

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MATERIALS AND METHODS

Stage 1. Initial disaggregation. — DRNA's isolated from the following sources were used in this investigation: calf thymus gland [14], herring testis [8], wheat germ [10], mouse sarcoma [8], virulent and avirulent *H. pertussis* [11] and mouse leukaemic tissue [15]. The methods of isolation employed were as described in the references quoted.

The DRNase was prepared from OX-pancreas according to the procedure of McCarty [16] as modified by Overend and Webb [1]. (For the freeze-dried preparation, Found: N 15.8; P 2.0 %.) Previous investigation has shown that enzyme samples prepared in this way behave essentially the same as the crystalline DRNase described by Kunitz [2].

The depolymerisation of the DRNA by DRNase was investigated viscosimetrically in Ostwald viscometers (flow times for water (4.5 ml.) of between 80 and 90 seconds), at 34.5 ± 0.25°. Approximately 2 per cent aqueous solutions of the nucleic acid specimens (dried in the frozen state) were analysed for total phosphorus content (method of Allen [17] as modified ny Jones, Lee and Peacocke [18]) and were then suitably diluted to make a final concentration of 0.116 mg of nucleic acid phosphorus per ml.

The buffer solutions used in the following experiments were made 0.01 *M* with respect to Mg^{++} and were used in such proportion that the final enzyme-substrate-buffer mixture contained sufficient Mg^{++} for optimum enzyme activity.

A mixture of the DRNA solution (3 ml) and 0.01 *M*-magnesium sulphate in 0.1 *M*-veronal buffer (pH 7.1) (1.0 ml) was introduced into the viscometer at 34.5° and the temperature allowed to equilibriate. Thereafter the viscosity was determined. The experiment was carried out with each of the samples of DRNA listed above. The relative viscosities of these specimens were as follows:

Source of DRNA	Thymus gland	Herring testis	Wheat germ	Mouse sarcoma	Virulent *H. pertussis*
ηr.	3.04	1.83	1.53	4.60	3.26

Continued:	Avirulent *H. pertussis*	Mouse Leukaemic Tissue
	2.31	4.61

In each case, after determination of the initial relative viscosity, a freshly prepared solution of DRNase (0.002%, 0.5 ml) preheated to 34.5° was added to the sample. Viscosity measurements were made at frequent intervals until the viscosity became constant. Representative results are shown in Fig. 1.

Stage 2. (a) *Formation of acid-soluble polynucleotides* — A solution of DRNase (0.01%, 1 ml), preheated to 37°, was added to each of a series of centrifuge tubes containing the substrate solution (2 ml) and 0.01 *M*-magnesium sulphate in 0.1 *M*-veronal buffer (pH 7.1) (1 ml) at 37°. Tubes were removed at intervals, cooled to 0° and 5 *N*-hydrochloric acid (0.1 ml) added to precipitate the acid-insoluble polynucleotides. The precipitates were removed at the centrifuge and aliquots (2 ml) of the clear supernatent solutions analysed for total phosphorus. Results are shown in Fig. 3. Control experiments showed no measurable liberation of "acid-soluble" phosphorus occurred when either the enzyme solution or the nucleic acid solutions were incubated alone at pH 7.1 for 4 hours at 37°.

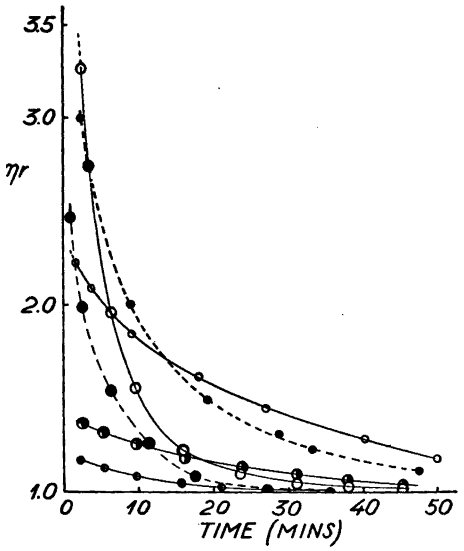


Fig. 1. The depolymerisation of the DRNA's from calf thymus gland (○——○), mouse sarcoma tissue (○——○), virulent H. pertussis (●---●), herring testis (◑——◑), wheat germ (◐——◐) and mouse leukaemic tissue (●---●) by DRNase.

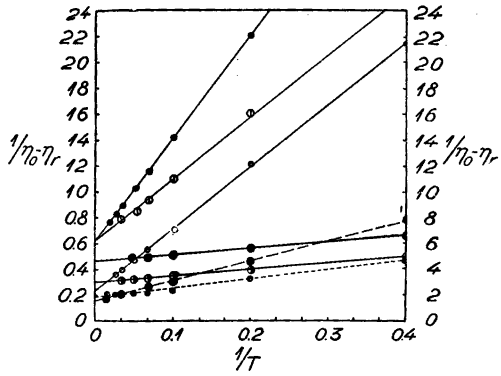


Fig. 2. Application of the modified Langmuir Isotherm $\frac{1}{\eta_0-\eta_r}=\frac{B}{A}\cdot\frac{1}{t}+\frac{1}{A}$ *to the viscosimetric data obtained from the enzymic depolymerisation of the DRNA's from wheat germ (◍——◍), herring testis (○——○) (Right hand axis), avirulent H. pertussis (●——●), virulent H. pertussis (◑——◑), calf thymus gland (●——●), mouse sarcoma tissue (◐---◐) and mouse leukaemic tissue (●---●) (Left hand axis).*

(b) *Dialysis of enzyme degradation products* — A mixture of DRNA solution (2X ml, where X = 15—25 ml), 0.1 *M* veronal buffer containing 0.01 *M* Mg^{++} at pH 7.1 (X ml) and 0.01% DRNase (X ml) was incubated at 37° for 15 hours. At the end of this period the addition of 5 *N*-hydrochloric acid (0.05 ml) to an aliquot (2 ml) of this solution (pH 5.7—6.0) gave no precipitation. The solution was then dialysed in cellophane against distilled water (10 vols.) at 4°. After 7, 24, 48, 72, 110 and 144 hours the dialysis sac was transferred to fresh distilled water and the diffusate was concentrated under diminished pressure to small volume. Thereafter it was transferred to a graduated flask and diluted to volume. Aliquots of this solution were analysed for total phosphorus content. Results are recorded in Fig. 4.

RESULTS

The initial measurements of the relative viscosities of the buffered nucleic acid solutions (*i.e.* before the addition of enzyme) were taken as an approximate indication of the degree of polymerisation of the nucleic acids. Representative results of changes in viscosities after addition of the enzyme are shown in Fig. 1. (Time intervals are recorded as the periods elapsing between the addition of the enzyme (zero time) and the mid-point of the flow-time in each

determination.) In an attempted interpretation of these results, the curves (Fig. 1) were extrapolated to t = 0 to obtain rough approximations of η_0 ($\eta_r = \eta_0$ at $t = 0$). From the values so derived, linear plots of $\frac{1}{\eta_0 - \eta_r}$ against 1/t were obtained, indicating that the results followed the modified Langmuir isotherm ($\eta_r = \eta_0 - \frac{At}{B+t}$ or $\frac{1}{\eta_0 - \eta_r} = \frac{B}{A}\frac{1}{t} + \frac{1}{A}$). Mean values of the constants A and B were calculated for each set of results from experimental values of η_r. and t. From these figures true values of η_0 were derived and using the latter the linear relationships in Fig. 2 were obtained. Values of A were derived from the intercepts of the graphs on the ordinate at $1/t = 0$ (when $\frac{1}{\eta_0 - \eta_r} = \frac{1}{A}$) and the constants B were calculated from the slope of the lines. Results so obtained are summarized in Table 1. From the previous equation it follows that at infinite time (t_∞), $A = \eta_0 - \eta_r$. Since on prolonged incubation the relative viscosity (η_r) of the enzyme-substrate mixture tends towards unity, it follows that $A = \eta_0 - 1$. That the values calculated for $\eta_0 - 1$ are in reasonable agreement with the values of A derived from Fig. 2 is apparent in Table 1.

On the assumption that at any given time during the enzymic depolymerisation, the relative viscosity of the enzyme-substrate mixture was proportional to the concentration of the unattacked DRNA, Maver and Greco [19] found that the hydrolysis of thymus DRNA by the DRNases of certain cathepsin preparations, follows the equation of a first order reaction. It may be pointed out, however, that for the enzyme-substrate systems now studied, Fig. 1 does not fit the equation $K = \frac{1}{t} \log \eta_0/\eta_t$

The second stage of the enzymic hydrolysis of the DRNA's, namely the splitting into acid-soluble polynucleotides was followed by the methods

Table 1.

Source of nucleic acid	η_0	$\eta_0 - 1$	A	B/A	B
Avirulent *H. pertussis*	2.99	1.99	2.10	0.46	0.97
Virulent *H. pertussis*	4.02	3.02	3.21	0.47	1.50
Thymus gland	2.47	1.47	1.59	7.90	12.56
Mouse sarcoma	5.58	4.58	5.26	0.73	3.84
Mouse leukaemic tissue	7.40	6.40	6.67	1.55	10.34
Herring testis	1.44	0.44	0.42	48.0	20.16
Wheat germ	1.14	0.14	0.16	48.0	7.68

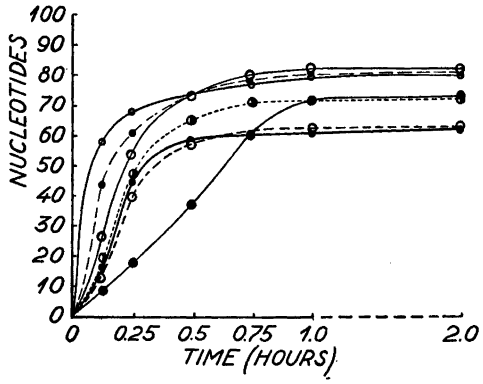


Fig. 3. The hydrolysis of the DRNA's from calf thymus gland (○——○), mouse leukaemic tissue (●---●), mouse sarcoma tissue (○—○), wheat germ (◑---◑), avirulent H. pertussis (●—●), virulent H. pertussis (○·····○) and herring testis (●—●) to acid- soluble nucleotides by DRNase.

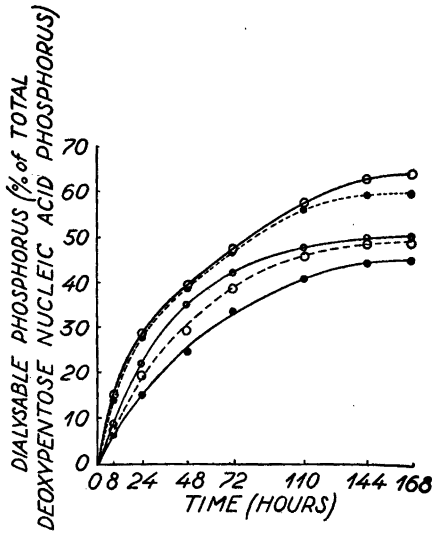


Fig. 4. Dialysis of the enzymic degradation products of the DRNA's from calf thymus gland (○——○), wheat germ (○---○), mouse sarcoma tissue (○—○), virulent H. pertussis (●·····●) and herring testis (●—●).

described in the Experimental section. The results shown in Fig. 3 are expressed to illustrate the relationship between the percentage hydrolysis of the substrate and the time of incubation with the enzyme. The curves (Fig. 3) show in each case an initial lag in the formation of acid-soluble polynucleotides which corresponds to the period of disaggregation of the nucleic acid. The curves showing the hydrolysis of the nucleic acid specimens from virulent and avirulent strains of *H. pertussis* so closely parallel one another that it seems likely that the two specimens are similar (see also Fig. 2). It appears that the DRNA from herring testis is more resistant than the other DRNA's to both enzymic hydrolysis (Fig. 3) and disaggregation (Figs. 1 and 2, and Table 1).

Under the conditions of the present experiments, hydrolysis ceases when about 60—80 percent of the nucleic acid has been rendered acid-soluble.

Preliminary quantitative measurements were made on the amounts of enzymic degradation products which underwent dialysis and the amounts of non-dialysable »cores». Fig. 4 shows that the quantity (based on phosphorus estimations) of non-dialysable nucleotide material varies from 45—65 per cent

according to the sample of DRNA used. The amounts of these non-dialysable fragments are greater than those reported by Chargaff and Zamenhof [4]. The differences are due probably to changes in the dialysis procedure rather than to large differences in the sizes of the polynucleotide fragments obtained in our experiments and those of Chargaff and Zamenhof. In the present experiments dialysis was carried out against repeated changes of distilled water and not against buffer solution as reported by Chargaff and Zamenhof [4]. This procedure served to remove effectively from the system metallic ions in the presence of which polynucleotides are known to dialyse to a greater extent [21]. It follows that dialysis against distilled water results in a larger amount of the nucleic acid remaining in the dialysis sac.

From the above results it is clear that for all the samples of DRNA studied, the mode of disaggregation and hydrolysis by pancreatic DRNase follows closely the pattern previously observed with DRNA from calf thymus gland. Furthermore, it seems that for individual samples of DRNA, the rates of disaggregation and hydrolysis do not run parallel.

The authors wish to thank Professor M. Stacey, F.R.S. and Dr. H. Fell, F.R.S. for their interest in this work.

SUMMARY

The enzymic degradation (by pancreatic DRNase) of DRNA's isolated from various sources has been studied. The initial stage of disaggregation has been followed viscosimetrically and a preliminary analysis made of the results obtained. These have been shown to follow the modified Langmuir Isotherm. It was illustrated that the rates of disaggregation and subsequent hydrolysis of the DRNA's by the enzyme do not run parallel.

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Short Communications

Separation of Bile Acids by Paper Chromatography. Bile Acids and Steroids. 3.

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The methods used for the separation of steroids by paper chromatography [1–7] are not suitable for bile acids. The separation of bile acids by reversed phase partition chromatography on columns has recently been developed by Bergström and Sjövall [9,10] with aqueous methanol and chloroform-heptane as phases, whereas Ahrens and Craig [8] have used acetic acid and isopropyl ether-heptane in a counter current extraction procedure.

We have now developed a method with similar solvent systems based on the observation that acetic acid is so firmly bound to filter paper that it can serve as stationary phase. Fig. 1 shows the separation of taurocholic, glycocholic, cholic, hyodesoxycholic and desoxycholic acid. Chenodesoxycholic acid cannot be clearly separated from desoxycholic acid with this method. The specificity of the spraying reagents is shown in Table 1.

In a paper that just appeared Kritchevsky and Kirk [17] report the separation of bile acids by paper chromatography in alkaline media.

Experimental. Solvents: Stationary phase: 70 % acetic acid; moving phase: isopropyl ether: heptane, 7 : 3. The phases are saturated with each other before use.

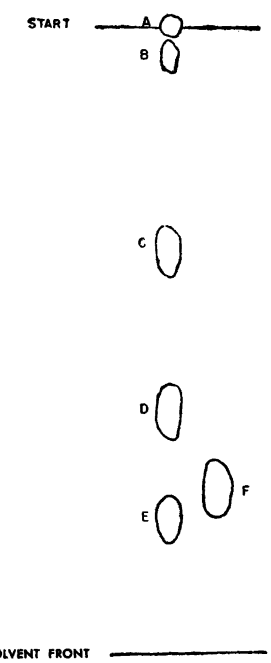


Fig. 1. Separation of taurocholic (A), glycocholic (B), cholic (C), hyodesoxycholic (F) and desoxycholic acid (E). Chenodesoxycholic acid (F) is run separately.
Paper impregnated with 70 per cent acetic acid. Moving phase: isopropyl ether-heptane 7 : 3 saturated with 70 per cent acetic acid.

The filter paper to be used for the chromatography (Whatman No. 1, cut in strips 9 × 40 cm) is wetted with the stationary phase. It is then dried at 90° C for 5 minutes. 25—50 μg of each acid is put on the starting line on an area with a maximum diameter of 0.5 cm. The paper is left

Table 1. Spraying reagents used for the detection of the bile acids.

Acid	Phosphomolybdic acid [11]	Rhodamine B [12]	Phosphoric acid [13]	m-dinitrobenzene [14]	Chlorine+KI+starch [16]	KI+I [15]
Cholic acid	+ + +	+	+ +	−	−	+ +
3, 7-dihydroxy-12-ketocholanic acid	+	+	−	−	−	−
3-hydroxy-7.12-diketocholanic »	−	+	−	−	−	−
3, 7, 12-triketocholanic »	−	+	−	+ +	−	−
Desoxycholic acid	+ +	+	−	−	−	−
3-hydroxy-12-ketocholanic acid	−	+	−	−	−	−
3, 12-diketocholanic »	−	+	−	+ +	−	−
Chenodesoxycholic acid	+ + +	+	−	−	−	−
3-hydroxy-7-ketocholanic acid	−	+	−	−	−	−
3, 7-diketocholanic »	−	+	−	+ +	−	−
Hyodesoxycholic acid	+ +	+	−	−	−	−
Lithocholic acid	−	+	−	−	−	−
3-ketocholanic acid	−	+	−	+ +	−	−
Taurocholic acid	+ + +	−	+ +	−	+ +	−
Glycocholic acid	+ + +	−	+ +	−	+ +	−

in the chromatographic chamber for 2 hours in an atmosphere saturated with vapors from both phases. The vapor equilibrium must be complete before the paper is brought in contact with the moving phase if tailing is to be avoided. Descending chromatography is used. When the chromatogram has been run it is dried at 90° C and the spots developed by some of the methods listed in Table 1.

Spraying reagents *cf.* Table 1.

Reported at the Second International Congress of Biochemistry in Paris 21—27 July 1952.

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Biochemical Aspects of the Plant Injury Caused by Ionizing Radiations

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Experiments have been performed in order to elucidate the effects of different factors on the lethalizing and growth inhibiting action of X-rays (175 kV, unfiltered radiation) and fast neutrons (about 3 MeV, obtained from a Be(dn)B reaction, the deuterons being accelerated to about 7 MeV in the 80 cm cyclotron of the *Nobel Institute for Physics*, Stockholm). The neutron doses were measured by the chemical dosimetry using the oxidation of ferrous iron [1]. Dry dormant as well as pre-soaked germinating seeds of barley have been subject to investigation. Experimental data will be published elsewhere [2].

When dormant seeds are irradiated with X-rays in the presence of sulfhydryl compounds (hydrogen sulphide or mercaptoacetic acid), or at the temperature of liquid air, a protection against the radiation damage is obtained. This indicates that the damage is partly due to indirect radiation effects, which owing to the low water content in the embryo (about 10 per cent) are probably different from those normally occurring in waterous tissue (embryos of germinating seeds contain about 70 per cent of water).

Germinating seeds are 6—7 times more sensitive than dormant ones to X-rays. This difference is not simply due to the difference in water content mentioned, since the protective action of anesthetics, *e.g.* magnesium chloride and propene, is independent of the water content. In dormant seeds, too, anesthetics exert some protection. Ether given in high, anesthetizing concentrations protects against radiation, whereas low concentrations, which stimulate germination or growth, at least under certain conditions, increase the radiation damage. Temperatures deviating from those giving optimal growth (20—30° C.) decrease the radiation sensitivity. These facts indicate an influence of the physiological state (chiefly factors associated with metabolic rate and irritability) on the sensitivity of the seeds to X-rays.

In germinating seeds an increase of the oxygen pressure within the range of 0—760 mm strongly affects the X-ray sensiti-

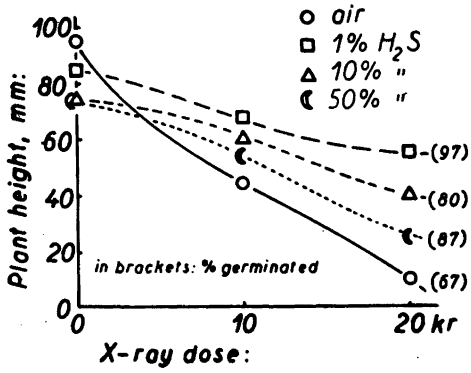


Fig. 1.

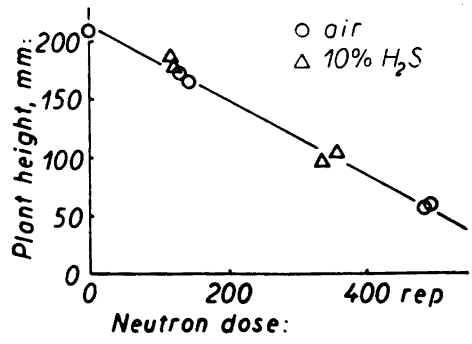


Fig. 2.

vity. The effect of oxygen may be due to: (a) a participation of O_2 in X-ray induced chemical reactions (if so, indicating an indirect effect of the radiation) or (b) an alteration of the metabolic rate *per se*. Probably both effects must be considered. Dormant seeds are much less sensitive to a change in the oxygen pressure, probably due to the very small and slow alteration of the metabolic rate. Other possibilities which have to be considered are however: a slow penetration of oxygen into or out of the dry seeds, or the absence of participation of oxygen in the chemical reactions induced in dormant seeds.

Seeds are 20—30 times more sensitive to fast neutrons than to X-rays, when equal doses are given. The difference between the radiation sensitivities to neutrons of dormant and germinating seeds is 2—3 times less than the corresponding difference in the case of X-rays. Hydrogen sulphide does not seem to protect against radiation damage caused by fast neutrons (compare Figs. 1 and 2 which show the effects of X-rays and neutrons, respectively, on the growth rate of hydrogen sulphide treated plants). Since, further, the oxygen pressure exerts a much smaller, if any, influence on the neutron sensitivity of germinating seeds, a fundamentally different mechanism of action of neutrons compared to that of X-rays has to be supposed, *i.e.* in the case of neutron irradiation the direct action of the radiation dominates. Such direct effects cannot be influenced by chemical and physiological factors in the same way as part of the X-ray effects. The difference in behaviour is probably related to the ionization densities produced in tissue (neutrons and X-rays give about 700 and 100 ion pairs per μ respectively). The field appearance of the damage caused by the two kinds of radiation is different, too: in the case of X-rays, plants not killed at a very early stage of development, survive as a rule, eliminating cells with severely damaged nuclei (chromosomes). In the case of neutrons, on the other hand, plants often germinate and grow normally for the first few days but then suddenly die. The elimination of chromosome damaged cells is much less marked. This difference leads to a much higher sterilizing and mutagenic action of the neutrons, determined in an analysis of the mature plants.

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2. Nybom, N., Gustafsson, Å., and Ehrenberg, L. *Botan. Notiser* **1952**, 343.

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Attempted Successive Applications of the Edman Degradation to Insulin

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The Fraenkel-Conrats [1] converted insulin to its phenylthiocarbamide derivative, and effected the removal of the N-terminal amino acids as the hydantoins by heating in 0.6 to 1.2 *N* hydrochloric acid at 75°. This adaptation of the Edman degradation [2] yielded the hydantoins of glycine, phenylalanine and alanine. Subsequent reapplications of the procedure were reported to release unincreased quantities of thiohydantoins, indicating that probably the vicinal peptide links only had been broken. A further adaptation of the procedure by Ottesen and Wollenberger [3], in which the acidity was reduced to 0.1 *N*, permitted the systematic degradation of hexapeptide, with, however, one extra peptide link being split during the second round. The modified procedure has now been applied to insulin. As a result of the first application, the number of free amino

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Degr. No.	Dissociat. Groups Reacted M/12000 g	Hydantoin Extracted M/12000 g	Amino Acids Expected	Amino Acids Found			
				Much	Moderate	Small	Trace
1	4.85	3.9	Glycine, Phenylalanine	Glycine	Phenylalanine		
2	14*	(6.2) **	Isoleucine, Valine	Isoleucine or Leucine, Valine	Glycine		
3	9.0	6.2	Valine, Aspartic acid	Valine, Aspartic acid		Glycine, Alanine	Leucine or Isoleucine
4	7.3	6.1	Glutamic acid	Glutamic acid		Glycine, Alanine, Leucine or Isoleucine	Aspartic acid, Valine
5	9.4	7.3	Glutamic acid	Glutamic acid, Alanine	Glycine, Valine, Leucine or Isoleucine	Aspartic acid	

* Including 6 moles of ammonia.
** Analysis insuccessful. This value taken from a parallel experiment.

groups was approximately doubled, and several amide links were split. Glycine and phenylalanine, but not alanine, were obtained in the first degradation; the expected products appeared through 4 more degradations, but extra amino acids gradually became prominent and numerous. Further diminution of the acidity to pH 2 resulted in excessively slow degradation.

The procedure was applied to solutions of insulin in guanidine chloride solution [1] and followed essentially according to Ottesen and Wollenberger [3]. The extent of reaction with phenyl isothiocyanate at pH 8 was taken as the sum of the hydrogen ions released during the reaction plus the loss of groups dissociating between pH 5.6 and 8.0, as shown by titration before and after the reaction. Extractions of hydantoin by ether were carried out at about pH 3; during degradations 3 and 5, the extractions were repeated at pH 8.3. Inappreciable amounts of additional hydantoin were obtained at the higher pH. Pure histidine yielded its hydantoin at pH 8. The observed extra degradation products suggested that the extra cleavages involved some of the amino groups of hydroxyamino acids, but the products could not be simply explained.

The results are summarized in the Table.

Except for the first 2 degradations, each stage was marked by a regular alternation in the position of the titration curve of the protein material indicating the orderly progress of the degradation procedure. The conditions of the cleavage cannot be considered satisfactory yet, however, for larger peptides.

The author wishes to thank Professor K. Linderstrøm-Lang for suggesting this study, and for his hospitality and counsel.

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Utilization of S^{35}-Labelled Sodium Sulphate in the Synthesis of Chondroitin Sulphuric Acid, Taurine, Methionine and Cystine

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The high incorporation of S^{35}-labelled sodium sulphate in sulpho-mucopolysaccharides of mammalian tissues has been demonstrated by different workers [1–4]. In several autoradiographic studies [5–8] on the sulphate fixation in different mammalian organs, it was shown that the main uptake of sulphate took place in those tissues, such as cartilage, the aorta, sclera, cornea and intestinal mucosa, which are known to contain large amounts of sulpho-mucopolysaccharides. On the other hand, it is known that S^{35} fed to rats as sulphate does not appear in cystine [9]. These facts thus speak in favour of the opinion that S^{35} administered as sulphate is mainly incorporated as ester sulphate in mucopolysaccharides.

In the present investigation a comparative study was made of the utilization of sodium sulphate in the synthesis of chondroitin sulphuric acid, taurine, methionine and cystine.

Four groups of adult white rats *, each group comprising 20 rats weighing 250–300 g, were given intraperitoneal injections of 1.5 mg of S^{35}-labelled Na_2SO_4 ** in 0.5 ml of distilled water (3.5×10^6 counts per minute per cm^2 measured as $BaSO_4$ at infinite thickness). The different groups of animals were sacrificed 2, 4, 16, and 24 hours, respectively, after the injection. The livers of all the animals in a single group were pooled and likewise the ribs with the intercostal muscles. The material thus obtained was handled in the following way.

* The same animals as those used for previous experiments [3].

** Obtained from *A.E.R.E.*, Harwell, England.

Chondroitin sulphuric acid was prepared from the cartilage of the ribs by applying the method of Jorpes [10] as modified by Strandberg [11]. The details of the method used and the analytical figures of the preparations obtained are given in a previous paper [3].

Isolation of taurine. The livers from each group of animals were minced into alcohol and the pooled livers ground in a Turmix blender (Swiss), filtered, and dried with more alcohol. The material was then extracted for two hours with boiling 3 : 1 alcohol : ether. The alcohol and alcohol-ether extracts were pooled and evaporated to dryness at reduced pressure. The residue was dissolved in a minimum of chloroform; this was poured into a large volume of acetone, and the precipitate filtered off. The material was twice more dissolved in chloroform and precipitated with acetone. The precipitate was then hydrolyzed in boiling 6 *N* HCl for 24 hours. After cooling, the solidified fatty acids were filtered off. The filtrate was evaporated to dryness, and the excess hydrochloric acid removed by repeated evaporations *in vacuo*. The final residue was dissolved in *N* HCl and applied to a column of the cation exchange resin Dowex 50 in the H^+ form. The length of the column was 20 cm and the diameter 4 cm. The separation was carried out with *N* HCl as the eluting solvent. The taurine emerges as a sharp peak very early in the chromatogram. This peak occurs between the solvent front and the peak of the first amino acid (aspartic acid). The fractions containing taurine were pooled and evaporated to dryness. Excess acid was removed by repeated evaporations *in vacuo*. The taurine was then chromatographed on starch, using the solvent system 2 : 1 propanol : 0.5 *N* HCl to check the identity and purity [12]. The taurine was then put through a small Dowex 50 column (20 cm × 0.9 cm) from which it was eluted in *N* HCl in a highly pure form. The identity and purity were further verified by paper chromatography.

Isolation of methionine and cystine. The liver material remaining after extracting the lipid components was extracted twice with 5% trichloroacetic acid (TCA) at 90° C for 15 minutes according to Schneider [13]. The residue was dried with alcohol and ether. About 5 g of this TCA-protein was hydrolyzed by boiling for 24 hours in a solution 6 *N* with respect to HCl and 50 per cent with respect to formic acid. The solution was freed from excess acid by repeated evaporations *in vacuo* and applied to Dowex 50 for the separation of the amino acids [14]. The column was 60 cm × 5 cm. To obtain a better resolution of methionine and

Substance	Radioactivity in counts/min./cm² at different times after injection			
	2 h	8 h	24 h	48 h
Chondroitin sulphuric acid isolated from the costal cartilage	1392	2136	2953	2933
Taurine isolated from the liver	32	68	53	35
Methionine isolated from the liver	—	—	3	—
Cystine isolated from the liver	—	—	4	—

isoleucine, the column was run at 70° C, which resulted in a complete separation of these two amino acids. The fractions corresponding to methionine and cystine, respectively, were pooled and evaporated to dryness. Both were then re-run on small Dowex 50 columns (20 cm × 0.9 cm) from which they were eluted in a few milliliters of 2.5 *N* HCl. No further purification of methionine and cystine was undertaken. As in the case of taurine, the identity and purity were checked by paper chromatography.

The *radioactivity* in the different samples was measured in the following manner. 80 mg of each of the preparations of chondroitin sulphuric acid were hydrolyzed with 6 *N* HCl for 4 hours on a boiling water bath and the sulphate precipitated as $BaSO_4$. In the case of taurine, methionine and cystine, $BaSO_4$ was precipitated after oxidation of about 40 mg of the substance with sodium peroxide according to Bailey. The different samples of $BaSO_4$ were then plated as described elsewhere[3] and measured by means of a Geiger-Müller counter with a 2.1 mg per cm² mica end-window tube. The radioactivity of the samples was expressed as counts/min./cm² at infinite thickness.

The results are given in the table. In agreement with earlier investigations [1,3] the incorporation of S^{35} in the sulphate group of chondroitin sulphuric acid of cartilage was very high after a single injection of labelled sulphate. A very low although significant uptake of S^{35} was demonstrated in taurine, isolated from the liver. The maximum uptake in chondroitin sulphuric acid and taurine was reached in 24 and 8 hours, respectively. As also shown by Tarver and Smith [9], S^{35} administered as sulphate was not utilized in the synthesis of cystine, nor did any incorporation of S^{35} take place in methionine.

The present investigation seems to afford further support to the opinion that the main uptake of administered sulphates occurs in mucopolysaccharides containing ester sulphates.

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Exchange of the Acetyl Group of Chondroitin Sulphuric Acid in Slices of Cartilage

HARRY BOSTRÖM and BENGT MÅNSSON

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In an earlier paper[1], we have reported on the exchange of the sulphate group of chondroitin sulphuric acid in slices of cartilage. The object of the present study was to investigate the exchange of the acetyl group by means of the previously described slicing technique, after addition of sodium acetate labelled with C^{14} in the methyl group. A relatively low but definite uptake of C^{14} was demonstrated for both the methyl and the carboxyl groups of the acetic acid.

Fresh costal cartilage from 1 to 3-day-old calves was freed from muscles, connective tissue and perichondrium, and sliced to a thickness of approximately 0.2 mm. The slices were incubated in portions of 10 g with shaking in an oxygen-carbon dioxide atmosphere (93.5 % O_2 plus 6.5% CO_2) at 37° C in Erlenmeyer flasks, each containing 50 ml of Krebs' solution [3], sodium acetate labelled with C^{14} in the methyl group *, and 50 000 I.U. of penicillin. The slices were taken at different times and boiled for five minutes. Sodium salts of chondroitin sulphuric acid were then prepared from all the samples, according to a method described elsewhere [2]. The yield was 200 to 300 mg from each portion of 10 g. The figures obtained on analyzing the different preparations are given in the table.

150 mg of the preparations obtained were hydrolyzed under reflux with 10% by volume of sulphuric acid for three hours at 100° C. The acetic acid was then isolated by distillation. 0.05 *N* NaOH was added to pH 7 (glass electrode control), and the solution evaporated to dryness. The sodium acetate was then degraded according to the method of Phares [4]. After plating of the barium carbonate obtained, employing a filtering technique, the radioactivity of each of the carboxyl and methyl carbons was measured in a Geiger-Müller counter with a 2.1 mg per cm^2 mica end-window tube.

Three different experiments, A, B, and C, were carried out in which 1.6 mg, 0.5 mg, and 1.0 mg, respectively, of sodium acetate labelled with C^{14} in the methyl group and with an activity of 2.5×10^6 c.p.m. per cm^2 (measured as $BaCO_3$ at infinite thickness) were added. The rate of uptake of C^{14} in the methyl and carboxyl carbon of the acetyl group of the chondroitin sulphuric acid was determined. The periods of incubation varied from 0 to 30 hours. In two control experiments the incubation was performed with cartilage slices which had been boiled for five minutes prior to incubation with 1.0 mg of labelled acetate.

The results of the experiments are shown in the figure. Curves A, B, and C represent the uptake of C^{14} in the methyl group of the acetyl group in the chondroitin sulphuric acid as a function of time. Curves a, b, and c denote the corresponding uptake in the carboxyl group. The points D mark the retention in the methyl carbon in the acetyl group of the chondroitin sulphuric acid prepared from slices that had been boiled prior to incubation with the isotope.

It is seen from curves A, B, C, and a, b, c that in slices of calves' cartilage some methyl-labelled sodium acetate is built into the acetyl group of the chondroitin sulphuric acid, the labelled carbon being found both in the methyl group and, although to a lesser extent, in the carboxyl group. If the slices were boiled prior to addition of the isotope, there was no uptake (experiment D).

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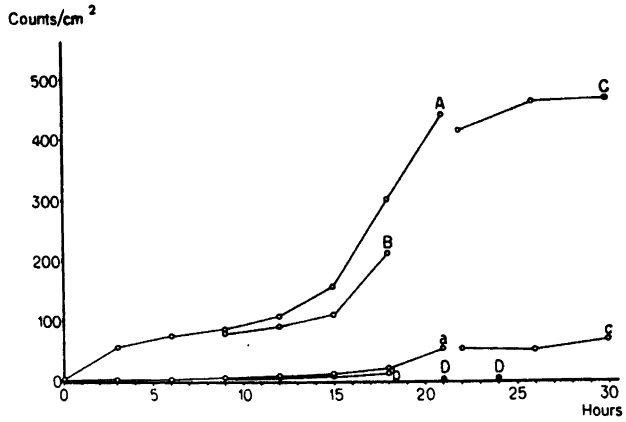
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The fact that the sodium acetate added was labelled in the methyl group only, but that the carboxyl group of the acetic acid from chondroitin sulphuric acid nevertheless also showed a low but definite uptake, suggests that some change in the precursor has occurred in the system examined. On the other hand, the methyl group showed a rate of uptake that was about ten times as high as that of the carboxyl group. This fact seems to support the supposition that an exchange, including an introduction of acetate, has occurred in the chondroitin sulphuric acid.

The possibility that the observed incorporation of C^{14} in the chondroitin sulphuric acid should be due to contamination with free labelled acetate, is considered to be ruled out on the following grounds. In the two samples in which the cartilage had been boiled prior to incubation with acetate (D), and in the sample that was boiled immediately after addition of the isotope (the time O on curve A), there was no significant uptake. Moreover, the applied method of preparation of chondroitin sulphuric acid includes extraction with NaOH followed by neutralization with glacial acetic acid and dialysis against running distilled water. With this process the precursor is diluted about 2 000 times with inactive acetate. Finally, the chondroitin sulphuric acid preparations obtained were allowed to pass through an anion exchange column (Dowex 2, 20 to 40 mesh, 30 cm × 1 cm) without any observable change in the content of isotope.

The possible contamination with proteins also appears to lack importance as a source of error. Firstly, the low content of nitrogen in the preparations (Table) rules out the presence of any large amounts of proteins. Secondly, the possibility that any C^{14}-labelled complexes would follow the acetate during the isolation and the subsequent degradation is very slight.

It is of some interest to compare the concentration of isotope in the acetyl group of the chondroitin sulphuric acid obtained in this experiment with the in-

Nitrogen % of dry substance		Sulphur % of dry substance		Acetic acid % of dry substance	
Found	Calculated	Found	Calculated	Found	Calculated
2.88 (2.61, 3.22)	2.78	5.69 (5.58, 5.78)	6.37	10.1 (9.6, 11.4)	11.9

corporation in the sulphate group of the chondroitin sulphuric acid obtained, when labelled sodium sulphate is added to slices of cartilage in about equivalent concentrations and under otherwise identical conditions. It was found that in the latter case the content of isotope was many times higher, as has been shown elsewhere [2]. At present, it is impossible to state whether these findings reflect a real difference in the speed of exchange of the acetyl groups and of the sulphate groups or whether the difference in the content of isotope found is merely due to different sizes of the sulphate and acetyl pools.

In addition, the course of the curves reproduced in the figure, indicating a considerably higher rate of uptake at the end of the experiment, differs from that of the curves representing the exchange of sulphate in the chondroitin sulphuric acid, which is almost linear during the first 20 hours. The cause of this discrepancy has not been investigated. The possibility that the increase in the C^{14} incorporation found in the later part of the former curve may be due to bacterial influence cannot be completely ruled out.

The authors wish to express their thanks to Professor Erik Jorpes for valuable discussions and stimulating advice during the work.

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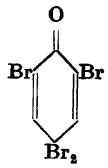
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Studies on Quinones and Hydroquinones. IV *. A Note on the Structure of Tribromophenol Bromide

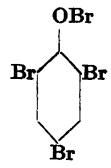
KARL ERIK ALMIN and BENGT LINDBERG

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It is possible to propose alternative structures, containing either a carbonyl group or with the hypobromite configuration, for the substances obtained by the bromination of certain quinones, with bromine methanol [1]. A simple substance for comparison is tribromophenol bromide for which two structures, the one (I a) quinonoid and the other (I b) a phenyl hypobromite, have been suggested. Elston, Peters and Rowe [2] investigated analogous polyhalo derivatives and found that the



I a



I b

ultraviolet absorption spectra supported the quinonoid structure. Recently Yasnikov and Shilov [3] studied the reaction between tribromophenol and bromine water containing radioactive Br, and concluded that tribromophenol bromide should have the quinonoid structure (I a). We have now determined the infrared absorption spectrum of tribromophenol bromide (Fig. 1). The two bands at 5.97 μ and 6.31 μ are due to the C = O group and the C = C

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corporation in the sulphate group of the chondroitin sulphuric acid obtained, when labelled sodium sulphate is added to slices of cartilage in about equivalent concentrations and under otherwise identical conditions. It was found that in the latter case the content of isotope was many times higher, as has been shown elsewhere [2]. At present, it is impossible to state whether these findings reflect a real difference in the speed of exchange of the acetyl groups and of the sulphate groups or whether the difference in the content of isotope found is merely due to different sizes of the sulphate and acetyl pools.

In addition, the course of the curves reproduced in the figure, indicating a considerably higher rate of uptake at the end of the experiment, differs from that of the curves representing the exchange of sulphate in the chondroitin sulphuric acid, which is almost linear during the first 20 hours. The cause of this discrepancy has not been investigated. The possibility that the increase in the C^{14} incorporation found in the later part of the former curve may be due to bacterial influence cannot be completely ruled out.

The authors wish to express their thanks to Professor Erik Jorpes for valuable discussions and stimulating advice during the work.

This investigation has been aided by grants from the *Rockefeller Foundation*, the *Swedish Medical Research Council*, the *Knut and Alice Wallenberg Foundation*, and the *Vitrum Company*, Stockholm.

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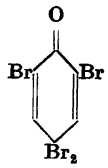
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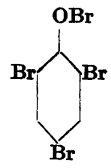
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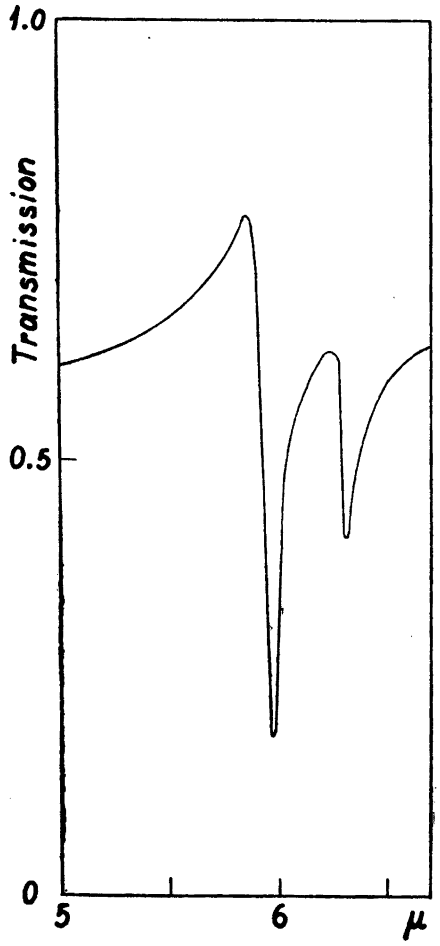


Fig. 1. Infrared spectrum of tribromophenol bromide.

bonds, respectively, and are very close to the corresponding bands in the spectra of quinone and 2,6-dimethylpyrone, in which the C = O bands are found at 6.01 μ and 5.99 μ and C = C bands at 6.28 μ and 6.21, 6.27 μ [4]. Thus the infrared spectrum of tribromophenol bromide furnishes conclusive evidence for the quinonoid structure (I a).

Experimental. The tribromophenol bromide was prepared in the usual manner and purified by crystallization from chloroform. The infrared absorption spectrum was determined, using freshly crystallized material in paraffin oil paste, with a Perkin Elmer infrared spectrometer, Model 12 C, with 67° calcium fluoride prism.

One of us (B. L.) wishes to thank *Statens Naturvetenskapliga Forskningsråd* for financial support.

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An Observation on the Parameter a of the Debye-Hückel Formula

AARNE ELLILÄ

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The writer has investigated the dissociation of acetic acid in water solutions of strong 1—1 electrolytes. In this report part of the results obtained is presented.

The investigation was carried out by a method developed by Kilpi [1]. The method is based on potentiometric titration of a weak acid with a strong hydroxide, and from the buffer capacity determined at one of the inflexion points of the titration curve the corresponding dissociation constant can be calculated. Eleven different salts were used as electrolytes. The concentrations used are expressed in moles of salt per liter of solution.

From the observations it was found that the negative logarithm of the dissociation constant, pK of acetic acid is an additive

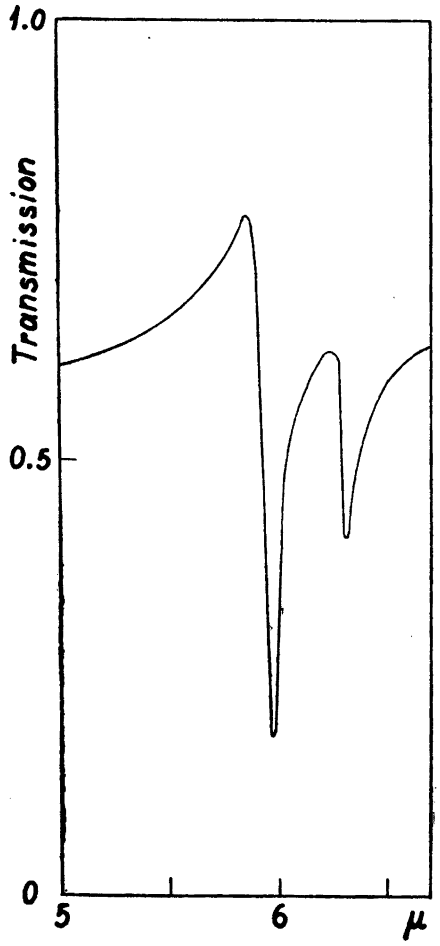


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From the observations it was found that the negative logarithm of the dissociation constant, pK of acetic acid is an additive

quantity. This was deduced from the invariabilities of

$$pK_{KCl} - pK_{NaCl} = pK_{KNO_3} - pK_{NaNO_3}$$

and

$$pK_{NaClO_4} - pK_{NaBr} = pK_{LiClO_4} - pK_{LiBr}$$

which were found to be true in all cases investigated and being dependent separatively on the anions and cations of the co-electrolyte at constant molarity. In the equations the indices refer to the salt, in the solution of which the measurement was performed.

This observation is in good agreement with the specific interionic attraction theory of Brönsted [2]. Recently Olson and Simonson [3] have reported on works which have led them to the conclusion, that only the oppositely charged ions influence each other. Combining these two observations, that of Olson and Simonson and of mine, it was possible to alter the orthodox semi-empirical Debye-Hückel formula applied to weak acids into the following form

$$pK = pK_0 - \left(\frac{0.5062\sqrt{I}}{1 + a_{H(-)}\sqrt{I}} + \frac{0.5062\sqrt{I}}{1 + a_{Ac(+)}\sqrt{I}} \right) + BI \qquad (1)$$

where $a_{H(-)}$ means the parameter, which characterizes the coulumbic influence of the anions of the co-electrolyte upon the H-ions and $a_{Ac(+)}$ the parameter, which characterizes the coulombic iufluence of the cations of the co-electrolyte upon the acetate ions. The following results were obtained at 25° C.

Electrolyte	Ionic strength I	pK	B
KCl	0.999	4.5605	0.2008
KCl	2.693	4.8285	
LiCl	0.999	4.4430	0.1400
LiCl	2.693	4.5835	

Using these values and that for $pK_0 = 4.7570$, determined by Harned and Ehlers [4], the term B in the fourth column was calculated assuming in the orthodox manner that $a_{H(-)} = a_{Ac(+)}$.

I	pK	
	usual	Ellilä
0.010	4.6715	4.6715
0.100	4.5620	4.5625
0.450	4.5135	4.5145
1.000	4.5605	4.5605
2.000	4.7100	4.7100
3.000	4.8835	4.8835

Introducing these results into the equation (1) four equations were obtained. Subtracting from each other those having the same value for I, the result was two equations with two variables. When solved, the following values were obtained: $a_{Ac(K)} = 1.755$; $a_{Ac(Li)} = 1.104$ and further $a_{H(Cl)} = 1.377$. When calculated in the usual manner ($a_{H(-)} = a_{Ac(+)}$), one gets the values $a_{KCl} = 1.550$, and $a_{LiCl} = 1.230$.

The table above includes pK-values at several ionic strengths in KCl-solution calculated by the usual manner and in the way presented in this paper. The table indicates, that the method of calculation does not influence the results.

A more detailed report on the work and on the results obtained will be published in the near future in the form of an academic dissertation.

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Determination of Xanthogenates by Titration with Perchloric Acid

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During recent years, the titration of weak acids and bases in non-aqueous medium has been greatly developed. Whilst working on the titration of weak bases with perchloric acid, we observed that xanthogenates can be rapidly and quantitatively titrated in this way. Hirschkind [1] determined potassium ethyl xanthogenate by titration with hydrochloric acid according to the equation:

$$C_2H_5OCSSK + HCl = C_2H_5OH + CS_2 + KCl$$

and determined the excess hydrochloric acid by titration with sodium hydroxide. Linck [2] found that this method was not generally applicable to other xanthogenates.

In the determination of the equivalent weight of an unknown alcohol, the potassium xanthogenate is prepared and titrated with iodine as described by Whitmore and Lieber [3]. This method has not been satisfactory in all cases, and Linck has suggested the addition of barium chloride to the solution of the xanthogenate to remove by-products and decomposition products, as some of these consume iodine during the titration.

In our method we dissolve the xanthogenate in glacial acetic acid and titrate directly with a solution of perchloric acid in the same solvent. The titration can be carried out both visually and potentiometrically. The process can be represented as follows:

$$HClO_4 + CH_3COOH = CH_3COOH_2^+ + ClO_4^-$$

$$ROCSS^- + CH_3COOH_2^+ = ROCSSH + CH_3COOH$$

This is probably not the only reaction which occurs. According to Cranendonk's experiments [4] the xanthogenates decompose rapidly in acid medium and the decomposition takes place *via* the free xanthogenic acid:

$$ROCSSK + CH_3COOH = ROCSSH + CH_3COOK$$

$$ROCSSH = ROH + CS_2$$

We have found that a solution of potassium ethyl xanthogenate in glacial acetic acid (1 %) decomposes very rapidly. Approximately 50 % was decomposed after 4 minutes and approximately 95 % after 6 hours. The course of the decomposition was followed by pipetting at regular intervals a certain quantity of the solution into water and immediately titrating with 0.1 *N* iodine.

We have attempted to recrystallize the potassium ethyl xanthogenate by dissolving in a little glacial acetic acid and subsequent addition of ether. Precipitation occurred immediately but the substance did not contain sulphur and was analyzed as a compound containing potassium acetate and acetic acid. At the same time we observed the smell of carbon disulphide. This also indicates that the decomposition proceeds as mentioned above.

Furthermore, our experiments have shown that the potentiometric titration curve for potassium ethyl xanthogenate is identical with the titration curve for potassium acetate.

An *equivalent* quantity of potassium acetate is produced when the xanthogenate is decomposed, and it is chiefly this compound which is titrated with perchloric acid:

$$CH_3COO^- + CH_3COOH_2^+ = CH_3COOH + CH_3COOH$$

Table 1. Analysis of Xanthogenates.

ROCSSK	Amount g	ml $HClO_4$ 0.1000 *N*	Mol. weight		Relative error %
			calc.	found	
methyl	0.3292	22.42	146.3	146.8	0.3
ethyl	0.2005	12.48	160.3	160.7	0.3
n-propyl	0.2037	11.68	174.3	174.4	–
*iso*propyl	0.2332	13.30	174.3	175.3	0.6
n-butyl	0.3148	16.68	188.4	188.7	0.2
*iso*butyl	0.2086	11.03	188.4	189.1	0.4
*sec.*butyl	0.2096	11.06	188.4	189.5	0.6
*iso*amyl	0.3146	15.58	202.4	201.9	0.3
allyl	0.2566	14.98	172.3	171.3	0.6
cyclo-hexyl	0.1958	9.10	214.4	215.2	0.4
benzyl	0.2288	10.22	222.4	223.9	0.7
β-methoxy-ethyl	0.3136	16.53	190.3	189.7	0.3

It is therefore possible to titrate the xanthogenate quantitatively even though it is partially decomposed in acid medium. Concurrent with the iodometrical determination of the course of the decomposition, samples were withdrawn at intervals and titrated with perchloric acid as described below. The observed consumption of perchloric acid was constant, regardless of the decomposition of the xanthogenate.

EXPERIMENTAL: Preparation and purification of the xanthogenates. The xanthogenates are prepared according to Whitmore and Lieber, with a few modifications as follows: About 1 g of the alcohol is weighed into a test tube and 0.5–0.6 g potassium hydroxide (approx. 0.01 mole) is added. The mixture is gently heated with constant shaking until the potassium hydroxide is dissolved. This often takes several minutes. In a few cases it may be necessary to add, at the most, 2 drops of water. The mixture is cooled whereby it sometimes becomes viscous or solid. After cooling about 1 ml carbon disulphide (approx. 0.017 mole) is added dropwise. If the liquid becomes warm it is cooled. The mixture is stirred for some minutes with a glass rod and 20 ml of ether are added. After vigorous shaking the xanthogenate produced is filtered through a Buchner funnel and washed with a few ml of ether. This is the normal routine method employed by our students when identifying alcohols.

The xanthogenate is purified by dissolving in the least possible amount of hot

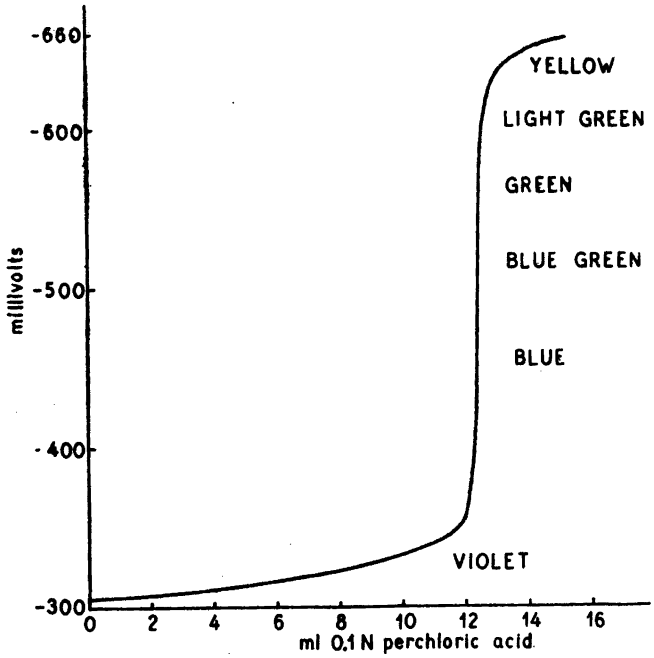


Fig. 1. Potentiometric titration curve of potassium butyl xanthogenate and colour changes of the indicator (crystal violet) during the titration.

acetone. The solution is filtered, and after cooling the xanthogenate is precipitated by addition of 20 ml anhydrous ether. The derivative is filtered off and washed with a few ml of anhydrous ether. The ether is allowed to evaporate spontaneously and the final drying is effected by means of infrared radiation.

The xanthogenates prepared in this manner are stable for several months.

Titration of the xanthogenates. Reagents used: Glacial acetic acid, not less than 99.7 % CH_3COOH. 0.1 *N* perchloric acid in glacial acetic acid. Crystal violet indicator, 0.5 g crystal violet dissolved in 100 ml glacial acetic acid. The 0.1 *N* perchloric acid is prepared and standardized according to Markunas and Riddick [5].

Procedure: About 0.200 g of the purified xanthogenate is dissolved in 15–20 ml glacial acetic acid. 2 drops of crystal violet indicator are added and the solution is titrated with 0.1 *N* perchloric acid to a distinct blue colour. The subsequent colours of the solution will be blue-green and then green, and only one or two drops (0.02–0.04 ml) 0.1 *N* perchloric acid are required to produce the colour changes from blue to blue-green and from blue-green to green. The results are given in Table 1.

We have determined the true end point potentiometrically (Fig. 1) using a calomel electrode, fibre type, as reference and a glass electrode, connected to a pH meter (Radiometer, pH meter 22). The visual end point was determined by observing the colour change of the indicator at the true end point.

As shown by Conant and Werner [6], and later by Seaman and Allen [7], the colour of crystal violet in acetic acid solution at the end point depends to a great extent on the ionic strength of the solution,

However, in the titration of the potassium xanthogenates the ionic strength at the equivalent point will be nearly constant since the slightly soluble potassium perchlorate is precipitated during the titration, and therefore the visual end point is unaffected by the amount of xanthogenate.

SUMMARY

It is shown that the potassium xanthogenates, prepared from 12 common alcohols, can easily be titrated with perchloric acid in glacial acetic acid solution, although the xanthogenates are rapidly decomposed in this medium. The titration can be carried out potentiometrically or visually, using crystal violet as indicator. Detailed instructions for the preparation, purification and the titration are given. The method described is recommended for the determination of the equivalent weight of unknown alcohols.

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