ACTA CHEMICA SCANDINAVICA 3 (1949) 1—12

On the Change in Particle Size Distribution of Polymethyl Methacrylate during Polymerization

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In an investigation of some commercial samples of polymethyl methacrylate Kinell[1] has shown that the mass-frequency curves of these substances had at least three maxima. The samples had probably been prepared in bulk polymerization. Therefore it has been regarded of interest to study this type of polymerization especially with respect to the distribution of particle sizes and the change in this distribution during polymerization. The preliminary results of these measurements are presented in this paper.

The polymerization of vinyl compounds takes place in at least three steps. The first of these is the activation of the monomer. This can be done thermally, photo-chemically or by means of a suitable initiator. The monomeric molecule will in all these cases be transfered into an excited state. In this investigation benzoyl peroxide has been used as an initiator. It can be assumed that the benzoyl peroxide is split into two radicals according to one of the following two formulae:

$$(C_6H_5CO_2)_2 \rightarrow 2\ C_6H_5CO_2^* \qquad (1)$$

$$(C_6H_5CO_2)_2 \rightarrow 2\ C_6H_5^* + 2\ CO_2 \qquad (2)$$

These radicals react with the monomer to a new radical. In this way the reaction chain leading to the formation of polymeric molecules is started. Denoting the initiator molecule with R_2, the radicals formed in the dissociation of the initiator with R* and the monomer with M, we get the following reaction scheme:

$$R_2 \longrightarrow 2R^* \qquad (3)$$

$$R^* + M \longrightarrow M^* \qquad (4)$$

$$M^* + M \longrightarrow M_2^*$$

$$M_n^* + M \longrightarrow M_{n+1}^* \qquad (5)$$

The reaction should proceed indefinitely if the growing molecule could not in different ways undergo deactivation. Firstly this can occur if a hydrogen atom is transfered from either a monomer molecule, a polymer molecule, M_n, or a molecule from the solvent, T, etc. In this case a new radical will be formed. Secondly two growing molecules can react and form one stable molecule. Thirdly a transfer of a hydrogen atom can occur between two growing molecules. In this case two stable polymer molecules will be formed, one of which contains a carbon-carbon double bound. These posibilities are summerized in the following formulae:

$$M_n^* + M \longrightarrow M_n + M^* \quad (6)$$

$$M_n^* + M_m \longrightarrow M_n + M_m^* \quad (7)$$

$$M_n^* + T \longrightarrow M_n + T^* \quad (8)$$

$$M_n^* + M_m^* \longrightarrow M_{n+m} \quad (9)$$

$$M_n^* + M_m^* \longrightarrow M_n + M_m \quad (10)$$

Evidence that the initiator is split into free radicals according to reaction (3) can be obtained if the polymer is analyzed for these radicals. Each polymer molecule must contain at least one such radical if the assumption is correct. Measurements of this kind have been made on polystyrene [2] and on polymethyl methacrylate [3]. As regards reaction (8), this transfer of hydrogen atoms can be performed by means of intentionally added substances — chain transfer substances. This is often done in order to affect the mechanical properties of the polymer. The chain transfer reaction does not change the amount of free radicals and hence the overall rate of reaction is not affected, presupposing that the reactivity of the new radical is the same as that for a growing molecule. The molecular weight of the polymer will, however, be lower.

In the bulk polymerization of methyl methacrylate the rate of reaction increases very slowly in the beginning. At 10—20 per cent conversion the rate increases very rapidly and all the remaining monomer can be polymerized in a few minutes. The reaction is almost »explosive». Finally the reaction ceases because the monomer is used up. Due to the amount of heat liberated during the polymerization (for polymethyl methacrylate Tong and Kenyon [4] have determined the heat of polymerization to 13.0 ± 0.2 kcal/mol) a considerable rise in temperature can be expected within the reaction mixture during the explosive part of the reaction. An increase in temperature of about 100° C has been observed by Norrish and Brookman [5] and Schulz and Blaschke [6]

for bulk polymerization of methyl methacrylate. In kinetic measurements it is therefore of importance to find out the extent to which it is possible to maintain an isothermal state. No reliable results can be obtained unless this condition can be fulfilled.

It is of interest in this connection to give a short discussion of the reasons for the explosive course of the polymerization reaction. The following three possibilities can be quoted.

Firstly the polymer is a very bad heat conductor. Thus the liberated heat of polymerization has to be removed by means of convection currents in the reaction mixture. This will be the more difficult the higher the degree of conversion, because the viscosity of the mixture increases rapidly. As a consequence of this the temperature rises as does the rate of reaction.

Secondly, Schulz and Blaschke[7] have assumed that each growing molecule can in some way give rise to the formation of two or even more activated molecules. Thus the total amount of activated molecules is increased and the rate of reaction will be higher.

Thirdly, according to Norrish and Smith[8], the deactivation of the growing molecules will be rendered more difficult the higher the viscosity of the mixture. The amount of free radicals is increased and thus the rate of reaction is becoming higher.

The first of these possibilities is of course quite true. However, Norrish and Smith[8] have shown that the explosive course of the reaction does occur even at lower temperatures. In an experiment with methyl methacrylate at a temperature of 25° C they were able to keep the reaction temperature constant within a tenth of a degree, and yet the conversion versus time curve had the same shape as at higher temperatures. Thus the influence of temperature on the reaction rate is of second hand importance. According to the theory of Schulz and Blaschke their conclusion depends upon the result that the degree of polymerization does not change with time. This result is, however, in agreement neither with measurements of Norrish and Brookman nor with the results which will be reported in this paper. Later Schulz and Harborth[9] have published an investigation in which the results of Schulz and Blaschke concerning the constant degree of polymerization are explained as being due to the fact that the polymerisations were not performed isothermally.

The theory of Norrish and Smith is founded on the following observations. According to Norrish and Brookman the mean molecular weight increases approximately in proportion to the amount of polymer formed. Furthermore the increase in rate of reaction occurs at a higher degree of conversion if the polymerization is performed at a higher temperature or with a larger amount of initiator. In both these cases a polymer with a lower molecular weight is

formed and hence the viscosity of the reaction mixture is lower. Another piece of evidence for this theory has been given by Eriksson (unpublished). The dimensions of the reaction ampoules have been shown to have no influence on the reaction rate. This is true for ampoules with not too large dimensions. Thus the warming up of the reaction mixture is not of primary importance. The present investigation also will give some contributions to this question.

EXPERIMENTAL

The method used in the polymerization experiments was essentially the same as that used by Norrish and Brookman [5]. The reaction was performed at 80 °C and in small, evacuated tubes of glass containing 4—5 ml monomer, immersed in a thermostat. In order to be able to follow the change in conversion with time, about ten tubes were used for each amount of initiator. After given time intervals the tubes were taken up and cooled down in melting ice to zero degrees. The time elapsing between the moment when the tube was immersed in the thermostat and cooled down in the ice bath was used as the time of polymerization. The amount of polymer formed was determined by dissolving the reaction mixture in acetone. This solution was poured with stirring into methanol. The precipitated polymer was dried to constant weight in vacuum at room temperature. The different polymers were characterized by determining their intrinsic viscosity in an Ostwald viscosimeter.

The monomer was distilled four times at ordinary pressure. Each time about 10—15 per cent of the monomer was allowed to polymerize in the distillation bulb. This was done intentionally in order to remove from the monomer all inhibiting or foreign chain transfering substances, which could possibly have an influence upon the molecular weight of the polymer formed. As is seen in the following this monomer has a rather great tendency to polymerize without any initiator. Therefore one must not exclude the fact that it contains small amounts of peroxides formed with atmospheric oxygen.

The initiator used was recrystallized benzoyl peroxide marked »Benzoylsuperoxid reinst, für wissenschaftl. Zwecken. Dr. Theodor Schuchardt, Görlitz, Germany».

RESULTS

The results obtained in five polymerization experiments with different concentrations of initiator are summarized in Table 1. At a conversion of 15—20 per cent the reaction rate starts to increase rapidly. The intrinsic viscosity increases simultaneously to about twice its earlier value. Further information about the course of the polymerization can be obtained by using Schulz's [10] statement that the intrinsic viscosity of polymethyl methacrylate is proportional to the number average of the molecular weight. Dividing the amount (m) of polymer formed with the intrinsic viscosity $[\eta]$ a number is obtained which is proportional to the number of polymer molecules formed. In this way the curves in Fig. 1 have been constructed. The number of mole-

Table 1. Degree of conversion and intrinsic viscosity as a function of time in polymerization of methyl methacrylate in bulk with different amount of initiator. Temperature of reaction: 80° C. Initiator: benzoyl peroxide. Intrinsic viscosity measured in chloroform solution and calculated with 1 g polymer in 100 ml solution as a concentration unit; all values extrapolated to zero concentration.

Concentration of initiator zero		
Time of reaction in min	Degree of conversion in per cent	Intrinsic viscosity
73	4.1	—
167	11.5	4.50
313	23.8	7.18
Concentration of initiator $0.99 \cdot 10^{-6}$ g mol per ml		
30	5.7	3.14
60	11.1	3.29
75	14.4	—
85	16.8	3.58
100	20.2	—
115	26.0	4.00
130	30.4	—
145	43.1	4.80
160	77.7	6.52
200	91.7	6.95
Concentration of initiator $20.6 \cdot 10^{-6}$ g mol per ml		
11.5	8.8	1.02
20	16.9	1.10
31	26.2	1.16
96	96.3	2.20

Concentration of initiator $2.01 \cdot 10^{-6}$ g mol per ml		
Time of reaction in min	Degree of conversion in per cent	Intrinsic viscosity
30	7.7	2.68
64	16.4	2.87
75	19.6	—
94	27.2	3.12
115	52.0	4.48
126	85.0	6.30
132	89.4	—
152	92.3	—
180	91.8	—
210	93.3	—
240	93.0	—
287	93.4	6.55
Concentration of initiator $41.3 \cdot 10^{-6}$ g mol per ml		
10	10.5	0.75
15	17.5	0.82
30	40.1	1.08
90	96.2	1.43

cules formed is a linear function of time at all concentrations of initiator used. The linearity ceases as soon as the reaction starts to be explosive. In this part of the reaction the number of molecules formed per unit of time increases until most of the monomer is used up.

The dependence of the reaction rate on the amount of initiator can be seen from Fig. 2, where the quantity $m/[\eta] \cdot t$ for the first part of the reaction has

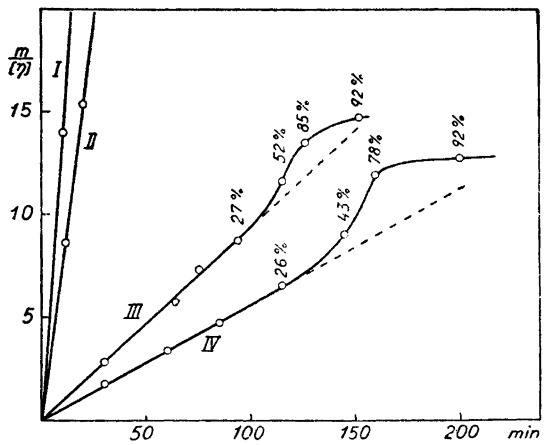

Fig. 1. Number of molecules formed as a function of time at different concentrations of initiator, I: 41.3 · 10^{-6} g mol/ml, II: 20,6 · 10^{-6} g mol/ml, III: 2.01 · 10^{-6} g mol/ml, IV: 0.99 · 10^{-6} g mol/ml. The numbers on curves III and IV represent the corresponding degrees of conversion.

been plotted against the original amount of initiator. The points do not correspond to a straight line. This can be understood by assuming that a chain transfer occurs between growing polymer and monomer (reaction (6)). For a stationary condition, *e. g.* activated molecules are formed at the same rate in reaction (3) as they are consumed in reactions (9) or (10) the following expression is obtained:

$$[M^*] = \text{const.} \sqrt{[R_2]} \tag{12}$$

where $[M^*]$ and $[R_2]$ are the concentrations of activated molecules and initiator molecules respectively. The rate of reaction is determined from:

$$\frac{d\,[N]}{dt} = k_6\,[M]\,[M^*] + k_9\,[M^*]^2 + k_{10}\,[M^*]^2 \tag{13}$$

if a cessation reaction of type (7) is disregarded in this early stage of the polymerization. [N] is the concentration of polymer molecules. Using equ. (12) one obtains:

$$\frac{d\,[N]}{dt} = a \cdot \sqrt{[R_2]} + b\,[R_2] \tag{14}$$

The curve drawn in Fig. 2 corresponds to an expression of this type, namely:

$$\frac{m}{[\eta] \cdot t} = 26.5\,\sqrt{C} + 30.3 \cdot 10^3\,C \tag{15}$$

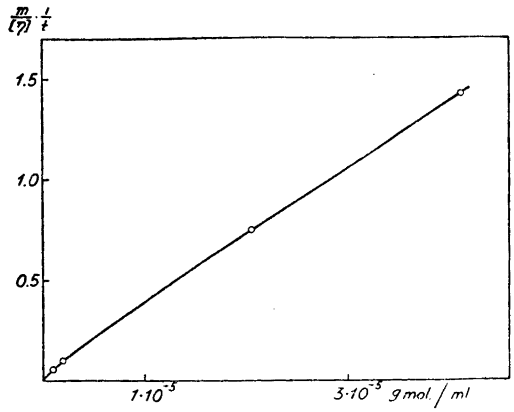

Fig. 2. Rate of reaction as a function of amount of initiator.

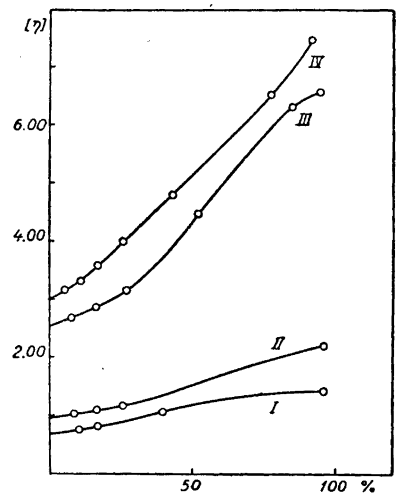

Fig. 3. Intrinsic viscosity as a function of degree of conversion. For further explanation cf. Fig. 1.

where C is the original concentration of the initiator. This shows that a chain transfer reaction of type (6) exists. In some investigations to determine the rate constants of the chain reaction Bamford and Dewar [11] have shown that such a chain transfer reaction is important in the thermal polymerization of styrene. During the later part of the polymerization reactions of the type (9) and (10) are suppressed due to the increasing viscosity of the reaction mixture. Thus the mean life of the activated molecules is longer and their chance to deactivate according to reaction (6) is greater. Each reaction chain will be comprised of a larger number of molecules. This gives a higher value of d [N]/dt. At a conversion of about 75 per cent the lack of monomer causes both the rate of polymerization and the rate of formation of polymer molecules to decrease. The polymerization ceases completely at a conversion of 92—96 per cent depending upon whether the amount of initiator is low or high.

It can be expected that the influence of the changes in the reaction mixture on the process of deactivation will cause the polymer formed to be very inhomogeneous. In order to obtain qualitative information about this, a study has been made of the relation between intrinsic viscosity [η], and degree of conversion, m. According to the results in Fig. 3 the intrinsic viscosity increases with the degree of conversion for all amounts of initiator used. The intrinsic viscosities, however, are mean values for all the polymer molecules

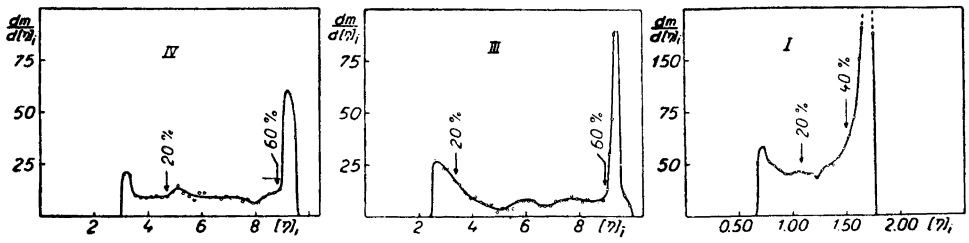

Fig. 4. Mass-frequency curves of polymethyl methacrylate formed at a concentration of initiator of 0.99 · 10⁻⁶ (IV), 2.01 · 10⁻⁶ (III) and 41.3 · 10⁻⁶ (I) mol/ml respectively.

formed during a certain time interval. A more suitable quantity for this discussion is the increment in intrinsic viscosity, $[\eta]_i$. This is defined as the mean viscosity of all the polymer molecules formed at a certain degree of conversion. Wall [12] has indicated a simple way to calculate this quantity from the relation between $[\eta]$ and m. If a polymer molecule is formed very rapidly, it can be referred to a definite degree of conversion. Assuming that this molecule cannot later take part in the reaction for instance through reaction (7), one obtains:

$$[\eta] = \frac{\int_0^m [\eta]_i \mathrm{d}m}{\int_0^m \mathrm{d}m}$$

and after differentiation:

$$[\eta]_i = [\eta] + \frac{\mathrm{d}[\eta]}{\mathrm{d}m} \cdot m$$

The derivative $\mathrm{d}[\eta]/\mathrm{d}m$ can be calculated graphically from Fig. 3. Plotting m against the obtained values of $[\eta]_i$ the mass-distribution curves of each polymer are obtained. The derivatives of these curves give the mass-frequency curves of the polymer in question. These curves are shown in Fig. 4. The arrows demarcate the parts which are formed during the stationary, the explosive and the final stage respectively. The per cent numbers give the degrees of conversion. These curves are of course rather inaccuarate as the experimental errors are quite large. This is especially true for curve I. Furthermore, it must be pointed out that they are not true frequency curves, as the viscosity increment is a mean value too. Some conclusions can, however, be drawn. All curves have the same general shape. The peak to the left is probably the beginning of a normal frequency curve, *e. g.* a curve which should be obtained if the polymerization could proceed during the entire time according to the conditions which prevail during the first stage. Such a curve can be

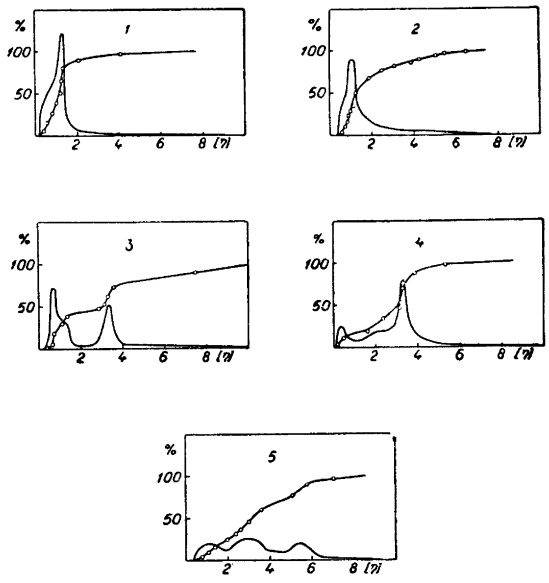

Fig. 5. Mass-distribution and mass-frequency curves of samples of polymethyl methacrylate taken at degrees of conversion of 11.2 (1), 28.4 (2), 53.2 (3), 83.2 (4), and 93.6 (5) per cent respectively.

calculated from the reaction scheme and has only one maximum. In this case, however, the explosive stage of the reaction complicates the frequency curves. The cessation reactions cannot proceed in a normal way and the molecules are getting larger. The large peak to the right can be explained, if a chain transfer reaction of type (6) predominates. This will put an upper limit on the growth of the activated molecules.

In order to get more reliable information about the change in particle size distribution during the polymerization, five samples of polymethyl methacrylates were prepared. All of them were made from the same monomer at a constant temperature of 80° C and with benzoyl peroxide as an initiator in a concentration of $5.49 \cdot 10^{-6}$mol/ml. The time of polymerization was choosen in such a way that samples corresponding to suitable degrees ot conversion were obtained: 11.2, 28.4, 53.2, 83.2, and 93.6 per cent respectively. The samples were divided up into a number of fractions by means of fractional precipitation with cyclohexane from benzene solutions. The intrinsic viscosities of the fractions were measured in benzene solution and the mass-distribution curves constructed according to Schulz. From these, the mass-frequency curves were calculated. All the curves are shown in Fig. 5. Samples 1 and 2 have frequency curves with only one maximum at $[\eta] = 1.3$ and 1.1 respectively. Both of the curves are skew. Sample 2 contains more high molecular material than sample 1. At higher degrees of conversion, frequency curves with two

maxima are obtained (samples 3 and 4). The maximum to the right is in both cases situated at $[\eta] = 3.3$. The maximum to the left corresponds to a rather high amount of substance with a rather low molecular weight (sample 3). For high degrees of conversion this maximum seems to be divided up in two maxima (sample 4). At still higher conversions all the maxima seem to flatten out (sample 5). The last frequency curve shows striking similarities with the curves in Fig. 4. This curve is, however, different from the curves obtained by Kinell but as the degree of conversion for his samples were not known, a comparison can hardly be made. The general shape of the curves is the same.

No profound discussion of these curves can be given at this early stage of the present investigation. Some remarks will, however, be made as to the skewness of the frequency curves of samples 1 and 2. One may suppose that the skewness depends upon a competition between various reactions. Assuming a stationary state the discussion can be limited to reactions (5), (6), (9), and (10) if only the first period of the polymerization is taken into account. Denote by p_1, the probability that a growing molecule will add another monomer (reaction (5)), by p_2 the probability that the same molecule will undergo chain transfer (reaction (6)), by p_3 the probability that it will react with another growing molecule giving two deactivated molecules (reaction (10)) and finally by p_4 the probability, that it will react with another growing molecule giving only one deactivated molecule (reaction (9)). All these probabilities are independent of the degree of polymerization presupposing that the reactivity of the growing molecules is always the same. If the final fate of the growing molecule is limited only to one or more of these reactions we have:

$$p_1 + p_2 + p_3 + p_4 = 1$$

The probability that a growing molecule will form a polymer molecule with the degree of polymerization n is $p_1^{n-1}p_2$, $p_1^{n-1}p_3$ or $^1/_2\ p_1^{n-2}p_4\ (1-p_1)\ (n-1)$ for the cessation reactions (6), (10) or (9) respectively. The massfrequency curve is then given by the expression:

$$f(n) = \frac{(1-p_1)^2}{2(p_2+p_3)+p_4}\left[2(p_2+p_3)\,p_1^{n-1}n + p_4\,(1-p_1)\,p_1^{n-2}\,(n-1)n\right] \quad (16)$$

with

$$\sum_1^\infty f(n) = 1$$

This function, however, cannot have inflexion points in the same way as the frequency curves of samples 1 and 2. It is also difficult to find values on the

probabilities p_1, p_2, p_3, and p_4, which give the very rapid decrease in the experimental frequency curves at increasing molecular sizes. The discrepancies between the experimental and theoretical curves are probably due to changes in reactivity of the growing molecule with the degree of polymerization. With increasing weight the thermal motion of the molecules will be slower and hence the number of collisions between molecules will diminish. Furthermore a long, growing molecule may screen off its own reactive center. In both of these cases the possibility of further reaction is reduced.

The previous discussions can be summarized in the following conclusion. It is convenient to divide the polymerization reaction into three phases. During the first phase, the stationary stage, the polymerization and initiation rates are both constant. Deactivation of growing radicals occurs during this period both by chain transfer and by a bimolecular reaction between two growing chains. During the second phase, beginning at a conversion of 10—20 %, the reaction mixture is so viscous that the deactivation between growing radicals ceases to occur or at least is rendered more or less difficult. As a consequency during this stage of polymerization radicals are obtained with a longer average lifetime. Thus the same kinetic chain can give rise to a larger number of stable molecules than during the first stage. The rate of polymerization increases and the molecular weight of the polymer will be higher. The third phase is characterized by the gradual cessation of the reaction. The chain transfer here imposes an upper limit on the growth of the polymer radicals. The polymerization stopes entirely at a conversion of 92—96 % depending upon whether the initial concentration of initiator is low or high. The three maxima of the mass frequency curve of the final product seem to correspond to the three different stages of the polymerization.

SUMMARY

The change in degree of conversion and intrinsic viscosity with time have been studied for the polymerization of methyl methacrylate in bulk using benzoyl peroxide as an initiator. Distribution and frequency curves have been obtained both at various amounts of initiator and at various degrees of conversion. These curves show several maxima. The influence of different types of cessation reactions on the distribution of molecular sizes have been discussed.

This investigation is a part of some research work carried out at the request of AB. Bofors Nobelkrut, Bofors, and the Government Commission on Industry in Sweden.

The author wishes to acknowledge his indebtness to Professor The Svedberg for his very kind interest and for the many facilities which have been put at the author's disposal.

The author also wishes to thank Fil.lic. P.-O. Kinell for his interest in this work and for many valuable discussions.

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Received November 12, 1948.

ACTA CHEMICA SCANDINAVICA 3 (1949) 13—16

Tuberculostatic Derivatives of *p*-Aminobenzoic Acid

II. Derivatives of 2-Halogeno-4-aminobenzoic Acid

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According to Johnson *et al.* [1] and Wyss *et al.* [2] 2-chloro-4-aminobenzoic acid is bacteriostatically active; this effect is neutralized by *p*-aminobenzoic acid and therefore seems to be of the same nature as the effect of sulfanilamide. As is well known the effect of sulfanilamide is greatly enhanced by the introduction of heterocyclic substituents in the amide group, and consequently it seemed possible that heterocyclic substituted amides of 2-chloro-4-aminobenzoic acid would show a higher activity than the free acid.

To investigate this problem we have synthesized some heterocyclic amides of 2-chloro-, 2-bromo- and 2-iodo-4-aminobenzoic acid. The compounds were prepared along conventional lines by reaction of the 2-halogeno-4-nitro-benzoylchlorides with the appropriate amine in pyridine solution, followed by catalytic reduction of the nitroamides thus formed.

The bacteriostatic activity of these heterocyclic derivatives, of 2-chloro- and 2-iodo-4-aminobenzamide and of 2-iodo-4-amino-benzoic acid was tested on the following bacteria:

Staphylococcus aureus, *Staphylococcus albus*, *Diplococcus pneumoniae* (type I), *Enterococcus*, *Proteus vulgaris*, *Escherichia coli*, *Eberthella typhosa*, *Salmonella paratyphi B*, *Shigella paradysenteriae*, *Shigella sonnei*, *Klebsiella pneumoniae* and *Corynebacterium diphteriae (gravis)*.

The bacteriostatic effect was tested in the following manner: the bacteria were inoculated in streaks on blood agar; strips of filter paper moistened with solutions or suspensions of the compounds were placed across the streaks of bacteria and the cultures were incubated at 37° for 24 hours. In case of a bacteriostatic effect the growth of the bacteria stops at a shorter or longer distance from the filter paper strips.

This method has been found to be very convenient for qualitative testing of the effect of bacteriostatic substances. All the compounds here described were found to be completely inactive. Most of the compounds are very slightly soluble even by addition of a little hydrochloric acid (the solutions investigated generally contained 10 mg/100 ml). The unsubstituted amides are somewhat more soluble and the sodium salt of 2-iodo-4-aminobenzoic acid is highly soluble; these compounds were however found to be inactive too.

On the other hand the last mentioned substances have been found by Lehmann to possess a pronounced bacteriostatic effect against *Mycobacterium tuberculosis* (these results are to be published elsewhere). In this respect these compounds resemble *p*-aminosalicylic acid, which similarly is only slightly active against bacteria other than *M. tuberculosis*.

EXPERIMENTAL

2-Chloro-4-nitrotoluene was prepared by chlorination of *p*-nitrotoluene with antimony trichloride as a catalyst [3]. After destillation a recrystallization from ethanol was necessary to obtain a melting point of 64—65°.

2-Chloro-4-nitrobenzoic acid was prepared by the method of Ullmann and Wagner [4] with some slight modifications: 21 g of 2-chloro-4-nitrotoluene and 200 ml of 0.1 *N* sodium hydroxide were heated to boiling in a 3 l roundbottomed flask provided with reflux condenser and separatory funnel. A solution of 31 g of potassium permanganate in 1.9 l of water was added at the same rate as it was reduced by the toluene. When all was added and the colour of the permanganate had disappeared the solution was filtered, cooled, acidified and extracted with ether (the solution may also be concentrated to a small volume and acidified; the chloro-nitrobenzoic acid then separate). The ether solution gave by evaporation a raw product melting at ca 138°; a recrystallization from water (lost 15 %) raised the melting point to 141°. Yield of 2-chloro-4-nitrobenzoic acid 7.2—8.3 g (30—34 %). From the manganese dioxide 7.4—9.5 g of the starting material could be recovered unchanged.

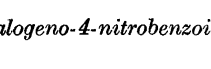
2-Bromo-4-nitrotoluene was prepared by bromination of *p*-nitrotoluene with iron as a catalyst.

2-Bromo-4-nitrobenzoic acid has previously been obtained by Frejka and Vitha [6] by oxidation of 2-bromo-4-nitrotoluene with nitric acid and potassium chlorate and mercury as catalysts. This method in our hands, however, gave only very small yields. Therefore we prepared the bromocompound in exactly the same way as the chloro compound and with about the same yield (32 %). Melting point of the raw product 161—164°, after recrystallization from water 166—167°.

2-Iodo-4-nitrobenzoic acid was prepared from 2-amino-4-nitrobenzoic acid by diazotation [7]. Yield 74 %, m. p. 144—145° after recrystallization from water.

These acids were converted into acid chlorides by the action of thionyl chloride. As a rule 4 g of the acid was heated with 8 ml of thionyl chloride until all had dissolved; after the excess of thionyl chloride had been removed *in vacuo*, the acid chloride was added, without further purification, to the equivalent amount of the heterocyclic amine dissolved in 10—20 ml of cold, anhydrous pyridine. After standing at room temperature

for some hours, the reaction mixture was poured into water and the amide which separated was filtered, dried, and recrystallized from acetic acid, pyridine or — in some cases — ethanol (most of the compounds are almost insoluble in ethanol). Yields of the crude products 75—85 % except for the derivative of 2-amino-4,6-dimethylpyrimidine, which could be obtained only in ca. 30 % yield. The compounds form white or pale yellow crystals which are only sparingly soluble in most solvents; melting points and analyses are presented in Table 1.

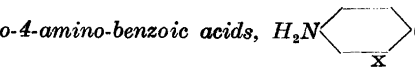
Table 1. Amides of 2-halogeno-4-nitrobenzoic acids, 

No.	X	R	Formula	M. p. °C	N % calc.	found
1	Cl	H	$C_7H_5O_2N_3Cl$	170—71	13.96	14.12
2	Cl	2-pyridyl	$C_{12}H_8O_3N_3Cl$	166—68	15.13	15.07
3	Cl	2-thiazolyl	$C_{10}H_6O_3N_3SCl$	262—64	14.81	14.61
4	Cl	2-(5-methyl)-thiadiazolyl	$C_{10}H_7O_3N_4SCl$	259—61	18.76	18.64
5	Cl	2-(4,6-dimethyl)-pyrimidyl	$C_{13}H_{11}O_3N_4Cl$	154—55	18.27	18.53
6	Br	2-pyridyl	$C_{12}H_8O_3N_3Br$	154—55	13.05	12.83
7	Br	2-thiazolyl	$C_{10}H_6O_3N_3SBr$	286—87	12.80	12.82
8	Br	2-(5-methyl)-thiadiazolyl	$C_{10}H_7O_3N_4SBr$	245—48	16.32	16.54
9	I	H	$C_7H_5O_3N_2I$	210—11	9.59	9.74
10	I	2-thiazolyl	$C_{10}H_6O_3N_3SI$	298—99 (d.)	11.20	11.41

Nos. 1, 5, 6, 8 and 9 were recrystallized from ethanol, nos. 2, 3 and 4 from acetic acid and nos. 7 and 10 from pyridine.

In addition to the heterocyclic amides the unsubstituted amides of 2-chloro- and 2-iodo-4-nitrobenzoic acid were prepared by addition of the acid chlorides to ice-cold, concentrated aqueous ammonia.

The amino derivatives were prepared from the nitro derivatives by catalytic hydrogenation; no splitting off of the halogen atoms was observed, even by prolonged action of hydrogen. The unsubstituted amides, the pyridine, and the pyrimidine derivatives were hydrogenated in alcohol solution (1—2 g of the nitro compound suspended in 25—50 ml of ethanol to which was added ca 0.1 g of PtO_2). The solid gradually went into solution; when the calculated amount of hydrogen had been absorbed (after 3—12 hours) the solution was filtered and concentrated *in vacuo;* upon the addition of water a precipitate separated which was filtered and recrystallized from concentrated or dilute alcohol. In case of the thiazole and thiadiazole derivatives the hydrogenation proceeded extremely slowly on account of the slight solubility of the nitro compounds in ethanol. When, however, the nitro compounds were dissolved in hot glacial acetic acid, the hydrogenation could be performed in the course of a few hours. After absorption of the calculated amount of hydrogen the solutions were diluted with water, neutralized, and filtered. These amino compounds were also recrystallized from ethanol. Yields 60—80 %. Melting points are

Table 2. Amides of 2-halogeno-4-amino-benzoic acids, 

X	R	Formula	M. p. °C	N % calc.	found
Cl	H	$C_7H_7O_2NCl$	162—64	16.35	16.29
Cl	2-pyridyl	$C_{12}H_{10}ON_3Cl$	171—72	16.96	16.79
Cl	2-thiazolyl	$C_{10}H_8ON_3SCl$	227—28	16.57	16.61
Cl	2-(5-methyl)-thiadiazolyl	$C_{10}H_9ON_4SCl$	261—62	20.85	20.82
Cl	2-(4,6-dimethyl)-pyrimidyl	$C_{13}H_{13}ON_4Cl$	201—202	20.25	20.34
Br	2-thiazolyl	$C_{10}H_8ON_3SBr$	183—85	14.09	14.19
Br	2-(5-methyl)-thiadiazolyl	$C_{10}H_9ON_4SBr$	215—17	17.88	18.04
I	H	$C_7H_7ON_2I$	174—75	10.70	10.82
I	2-thiazolyl	$C_{10}H_8ON_3SI$	200—01	12.17	12.19

presented in Table 2. The amino compounds are much more soluble in alcohol than the nitro derivatives. They can be dissolved in an excess of acid, separating again for the most part by diluting these solutions with water.

SUMMARY

Amides of 2-chloro , 2-bromo- and 2-iodo-4-aminobenzoic acid, unsubstituted and containing heterocyclic substituents in the amide group, were prepared. These compounds were found to be without any bacteriostatic effect on a great number of pathogenic bacteria, but some of them have some effect upon *Mycobacterium tuberculosis.*

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Received November 11, 1948.

ACTA CHEMICA SCANDINAVICA 3 (1949) 17—21

Formation of Biliverdin from Legcholeglobin, the Green Pigment in Leguminous Root Nodules

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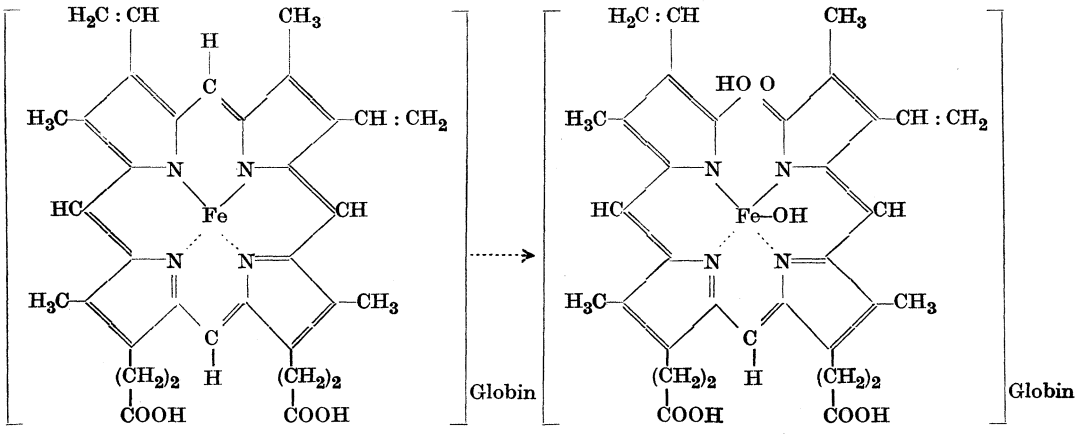
Virtanen and his collaborators [1–4] have shown that the leghemoglobin present in the leguminous root nodules changes to a »green pigment» when the symbiotic nitrogen fixation ceases. The pigment was isolated from the nodules, purified in a high degree by repeated precipitation with ammonium sulphate, and characterized as a precursor of bile pigments. This green pigment to which we preliminarily propose the name *legcholeglobin* until its constitution is fully explained, was noted to resemble in the first place the choleglobin of Lemberg [5] prepared from hemoglobin by oxidation with oxygen in the presence of ascorbic acid. The legcholeglobin was assumed to arise in the nodules from leghemoglobin through a similar oxidation either with hydrogen peroxide or oxygen. The initial and final products of the reaction were assumed to be the following:



Leghemoglobin Legmetcholeglobin

It has not yet been sufficiently explained whether the green pigment contains both bi- and trivalent iron or only either of them and at what stage the possible oxidation of iron to the ferric form takes place.

An important step in the investigation of the structure of legcholeglobin is the finding to be reported in this paper that a fully characterized bile pigment, biliverdin, is formed from the prosthetic group of legcholeglobin. Lemberg, Lockwood and Legge [6] have earlier shown that bile pigments, biliverdin and biliviolins, can be split from choleglobin by a weak acid (acetic acid). Liebecq [7] has shown that biliverdin and biliviolins can also be split from the pseudohemoglobin of Barkan and Schales [8] obtained from hemoglobin by oxidation with hydrogen peroxide in the presence of cyanide, provided that the pseudohemoglobin is not fully denatured during the procedure.

We have employed in our experiments pea plants grown in sterile cultures, inoculated with an effective *Rhizobium* strain. After six weeks growth the culture flasks with plants were transferred into the dark whereby the reddish nodules turned green in three days.

Samples of 4 g (fresh weight) were taken from the separated green nodules, crushed in a mortar with 8 ml glacial acetic acid to a homogeneous suspension, and centrifuged. 15 min after crushing 20 ml ether were added in a separatory funnel to the grass-green acetic acid solution. Legglobin and the main part of acetic acid were washed with small amounts of water from the ether phase, whereupon biliverdin was extracted with small amounts of 5 % hydrochloric acid. The HCl-extracts (2, 3 ml) were joined, filtered clear, made up to exactly 3 ml with water, and the absorption spectrum was determined with Beckman Quartz Spectrophotometer (1 cm cell). The spectrum obtained is given in Fig. 1, curve II. The maxima lie at about 665 mμ and at about 355 mμ. The curve is very similar to the spectrum of a pure biliverdin-hydrochloride measured in glacial acetic acid solution (Fig. 1, curve I). A somewhat differing location of the maxima in these biliverdin preparations may be due to some impurities, *e. g.*, small amounts of other bile pigments. For comparison, a typical spectrum is also given (Fig. 1, curve III) of 5 % HCl-extract from choleglobin prepared according to Lemberg *et al.* [6] at pH 7.4 with 3 h incubation from the cow oxyhemoglobin. It appears from the curve that this preparation contains abundantly biliviolins.

From the choleglobin preparations of Lemberg [6] and pseudohemoglobin preparations of Barkan [7] biliviolins are extractable with 10 and 20 % hydrochloric acid. In the corresponding extracts of legcholeglobin no biliviolins could be detected. Besides, the oxidation products obtained by Lemberg and Barkan from the hemoglobin of blood leave deeply brown pigments in the ether layer after HCl-extraction, whereas the ether solution obtained from

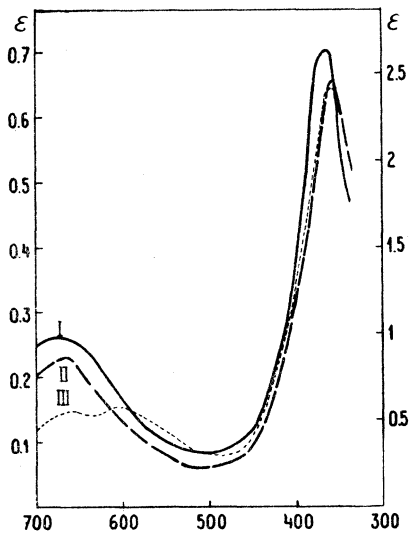


Fig. 1. Spectra of different biliverdin preparations. The ε values on the right refer to curve I, those on the left to curves II and III.

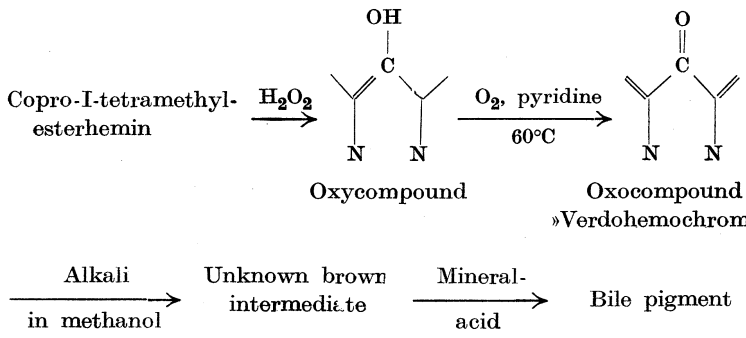
I. Pure biliverdin.
II. Biliverdin from legcholeglobin.
III. Biliverdin with high percentage of biliviolins from choleglobin preparation according to Lemberg.

legcholeglobin is only slightly yellowish after the removal of biliverdin. These observations indicate that legcholeglobin is an appreciably more homogeneous and purer substance than the artificial preparations of choleglobin and pseudohemoglobin.

In the soybean nodules the transfer of the plants into the dark does not catalyze the oxidation of leghemoglobin to green pigment as effectively as in pea [2]. The change of colour requires weeks. We have noted in the soybean grown out-of-doors that with the formation of green pigment the globin part is simultaneously denatured, whereby the prosthetic group and the denatured protein are linked together in some other way than in the native legcholeglobin. Then the prosthetic group can no longer be split by acid treatment. In the nodules of pea, where the change of colour takes rapidly place in the dark, the amount of the denatured green pigment is small compared with that of the undenatured one.

Regarding the structure of legcholeglobin the following facts must be considered. The absorption spectrum of legcholeglobin does not show any marked maxima between 500 and 600 mμ, so it does not seem probable, that the porphin ring is unbroken in the molecule. The easy formation of biliverdin from the legcholeglobin supports this view. Thus the opinion that the porphin ring has opened at the formation of legcholeglobin is well founded on the basis of the observations so far. Barkan and Schales, Lemberg and collaborators, and Liebecq consider the case to be such when pigments of pseudohemoglobin-choleglobin-type are formed.

Hans Fischer *et al.*[9] have found that the porphin ring has not been broken in the »green hemins»[10], another type of precursors of bile pigments, also called »verdohemochromogens» by Lemberg[11]. By oxidation of synthetic copro-I-tetramethylester-hemin they prepared the corresponding »oxo-compound» which has an oxo-group in one of the ms-positions. It has the properties of »verdohemochromogens» but can be reduced to the initial porphyrin.



Thus, the porphin ring of the »green hemins» must still be intact, contrary to the opinion of Lemberg[11]. In the light of the above findings, an intact porphin ring is, however, not probable in legcholeglobin.

The »green pigment», legcholeglobin, isolated in this laboratory in 1945 from the root nodules of pea, is as far as we know the first precursor of the bile pigments, formed in nature which has been isolated in high degree of purity. It is noteworthy, that this compound is found in the plant kingdom, in the root nodules of legumes — the only place where hemoglobins have hitherto been met in the vegetable kingdom. The research on the formation and breakdown of leghemoglobin in the nodules will possibly give further explanation also to the formation of the hemoglobin of blood and to its transformation into bile pigments.

SUMMARY

The green pigment which is formed in the leguminous root nodules as a transformation product of the red pigment, leghemoglobin, at the ceasing of nitrogen fixation is a precursor of the bile pigments. It yields biliverdin by the action of acids. The observations so far are in accordance with the idea that the porphin ring is open in the green pigment. The pigment is called legcholeglobin.

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Received December 16, 1948.

ACTA CHEMICA SCANDINAVICA 3 (1949) 22—28

Amperometric Titrations with Indicators

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During the last few years amperometric titrations have proved to have a very wide field of applicability. Compared with conductometric and potentiometric titrations they offer many advantages, yet they have also certain limitations. Thus it is not possible according to principles adopted hitherto to determine a reducible (or oxidizable) substance in the presence of large amounts of more easily reducible (oxidizable) substances. Generally speaking it is difficult to use polarographic methods in the analysis of metals with high negative half-wave potentials. Magnesium, for instance, can not be determined polarographically, and the determination of calcium is influenced by even small amounts of the alkali metals.

A new principle in amperometric titrations, the use of *indicator substances*, will be described in this paper *. This principle makes it possible to determine certain metals not analyzed polarographically before, and the disturbing influence of many metals with low half-wave potentials can be eliminated by its application. The diffusion current measured is due to a suitable indicator, which is added to the solution. If the indicator is reducible at a sufficiently low potential neither the polarographic behaviour of the original sample nor that of the reagent added by the titration has any significance. Some examples will elucidate this principle.

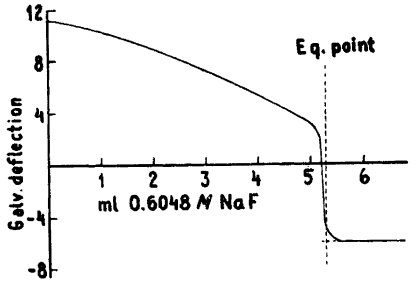
I. TITRATION OF ALUMINUM WITH FLUORIDE SOLUTION

Aluminum forms with fluorides the complex ion AlF_6^{3-}. It might therefore be expected that the amperometric determination of aluminum could be carried out with a sodium fluoride solution. The half-wave potential of alu-

* A preliminary report of the methods here described was given at the 6th Scandinavian Chemist Congress in August 1947.

Fig. 1. Titration of aluminum with sodium fluoride.

5 ml 0.1 M Al^{3+}
0.4 ml 0.1 M Fe^{3+}
25 ml alcohol
Excess of solid NaCl
Water to 50 ml



minum is, however, so high (—1.75 V) that the titration offers some difficulties, especially if large amounts of other salts are present. We have added a few drops of a ferric salt as indicator. Ferric iron is reduced by mercury without requiring the application of any potential to the dropping mercury electrode. If the saturated calomel electrode (SCE) is used as an external anode, the diffusion current of the ferric ion is obtained right at the start of the polarogram when the applied e. m. f. is zero. Both aluminum and iron form complex ions with fluorides, but the aluminum complex is more stable than that of iron. If, therefore, a sample containing both aluminum and iron is titrated with a sodium fluoride solution, the reaction between fluoride and aluminum will precede the reaction between fluoride and iron. In the presence of 50 % of alcohol the bulk of the iron will not react with fluoride until nearly all the aluminum has been converted into AlF_6^{3-} ions. The reduction of the ferric fluoride complex requires a potential of about — 1.4 V. If no potential is applied, the diffusion current, therefore, will change during the titration as shown in Fig. 1. The negative values observed after the end-point are due to the fact that the residual current corresponding to the potential $\pm$ 0 (SCE) is negative.

In order to make the equivalence point more distinct it is advantageous to saturate the solution with sodium chloride, which promotes the precipitation of Na_3AlF_6.

The optimum pH range is between about 2.5 and 3.5.

The apparatus employed was extremly simple. As no external potential is needed, the circuit consists of the sample solution, a calomel electrode, and the dropping electrode connected through a galvanometer (sensitivity = 5.7×10^{-8}A/unit of deflection).

Procedure. Add 0.5 ml 0.1 *M* ferric chloride, 25 ml alcohol, and an excess of solid sodium chloride to a 20—25 ml sample containing 10—40 mg aluminum. Remove dissolved oxygen by passing nitrogen or carbon dioxide through the sample, introduce

into the solution a calomel electrode and the mercury dropping electrode, and then titrate with 0.6 *M* sodium fluoride solution. The end-point of the titration is determined in the way indicated in Fig. 1. A volume of fluoride solution equivalent to the added ferric salt is subtracted from the total volume of fluoride solution consumed.

For the reagent a 10 ml semi-micro burette may be used.

The results of a few titrations are given in Table 1.

Table 1. Titration of aluminum with sodium fluoride. Volume = 50 ml.

ml 0.1 *M* Al-sol.	ml 0.635 *M* NaF		% error
	calc.	found	
5.00	4.72	4.70	— 0.4
8.00	7.56	7.56	± 0
8.00	7.56	7.57	+ 0.1
8.00	7.56	7.54	— 0.3
10.00	9.45	9.46	+ 0.1
10.00	9.45	9.40	— 0.5

The agreement is fairly satisfactory. The molarity of the sodium fluoride solution can be determined by titrating a solution of alum $KAl(SO_4)_2 \cdot 12\ H_2O$.

It may be mentioned that the reaction between aluminum and fluoride takes place in steps and is not complete at the equivalence point unless alcohol and sodium chloride are added. The equilibrium constants of aluminum fluoride in water solution have been determined by Brosset [1].

The error caused by interaction of the mercury on the bottom of the cell and the ferric ions is usually negligibly small. If necessary, the error can be minimized by collecting the falling drops in a narrow cavity in the bottom of the cell.

II. TITRATION OF MAGNESIUM WITH FLUORIDE SOLUTION

Magnesium also forms with fluoride ions a complex ion MgF_3^-, which is more stable than the FeF_6^{3-} ion. It might therefore be expected that the determination of magnesium should be possible by a method quite analogous to that described for aluminum.

Experiments conducted in order to investigate this possibility gave, however, no satisfactory results. The values were too low and varied considerably. It is likely that these observations are due to the fact that magnesium fluoride is temporarily precipitated during the titration and reacts but slowly with further amounts of fluoride ions to form complex MgF_3^- ions. Somewhat better results were obtained when the titration was carried out very slowly.

The difficulties mentioned can be avoided if the magnesium solution is added to an excess of sodium fluoride, the solution warmed and titrated back with a standard solution of an aluminum salt. The best results were obtained if also the aluminum solution was added in excess, and the final titration conducted with standard fluoride solution.

Procedure: Take a measured excess of 0.6 *M* sodium fluoride and add the sample solution containing magnesium. Heat the solution to boiling, cool, and add a measured excess of standard aluminum sulfate solution, 0.5 ml 0.1 *M* ferric chloride, and alcohol until it forms about 50 % of the solution. Add an excess of solid sodium chloride and titrate with sodium fluoride as described above.

The accuracy was not quite so good as in the titration of aluminum. The error was about $\pm$ 1 %.

III. TITRATION OF CALCIUM WITH FLUORIDE SOLUTION

Not only titrations involving complex formation but also precipitation titrations can be carried out by amperometric methods using indicator substances. As an example the titration of calcium with fluoride may be described.

Calcium fluoride is a rather slightly soluble salt — the solubility product in water is 4×10^{-11} — and consequently the possibility of using a ferric salt as an indicator seemed to exist. On adding sodium fluoride solution calcium fluoride will precipitate, and the diffusion current due to ferric ions will not reach its minimum until most of the calcium has been precipitated.

The titration is performed in a manner similar to that described above for the determination of aluminum. However, no sodium chloride should be added as the solubility of calcium fluoride is increased in the presence of large amounts of salts. As before, about 50 % alcohol is necessary to obtain a sharp end-point.

Procedure: Add to the sample solution 0.5 ml 0.1 *M* ferric chloride and alcohol to double the volume. Introduce into the solution a calomel electrode and the mercury drop electrode, and titrate with 0.6 *M* sodium fluoride after removing the air from the solution. The shape of the titration curve is seen in Fig. 2.

The weight ratio in the determination of calcium is not so advantageous as it is in the determination of aluminum. One millimole of fluoride corresponds to only 1/6 millimole of aluminum = 4.5 mg, but corresponds to ½ millimole of calcium = 20 mg. Hence the accuracy attainable in titrating very dilute calcium solutions is not very great. Highly concentrated solutions are not

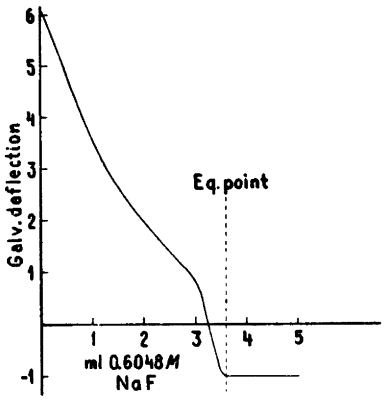


Fig. 2. Titration of calcium with sodium fluoride.

10 ml 0.1 M Ca^{2+}
0.4 ml 0.1 M Fe^{3+}
25 ml alcohol
Water to 50 ml

suitable either. In the latter case the precipitate is not quite pure, and the diffusion current may be influenced by very large precipitates. Thus the accuracy in the titration of calcium was less than in the titration of aluminum. By titrating amounts from 40—100 mg in a volume of about 50 ml the maximum error was about 1 %.

IV. TITRATION OF CALCIUM WITH SODIUM OXALATE SOLUTION

It is possible to titrate calcium amperometrically not only with a fluoride but also with an oxalate. The indicator must in this case be the salt of a metal forming a slightly soluble oxalate, although it must be more soluble than calcium oxalate. The half-wave potential of this metal must not be too high. Various experiments revealed that a cadmium salt was the most suitable for this purpose. In order to decrease the solubilities of the precipitated salts it was necessary to titrate in the presence of alcohol as in the methods presented above.

As the difference between the solubility of calcium oxalate and that of cadmium oxalate is small ($L_{CaC_2O_4} = 2 \times 10^{-9}$, $L_{CdC_2O_4} = 1.5 \times 10^{-8}$) a coprecipitation of the two oxalates can scarcely be avoided. It is advantageous to add a rather large amount of cadmium and to subtract from the result the amount of oxalate corresponding to the added cadmium. Fig. 3 shows the shape of the titration curve.

Procedure: Add to the calcium solution 2 ml of a 0.02 *M* cadmium chloride solution and an amount of alcohol corresponding to about 50 %. Remove the oxygen by passing nitrogen through the solution. Titrate in the usual way with 0.1 *M* sodium oxalate solution applying an emf. of — 0.8 V (SCE). The equivalence point is obtained from the titration curve as shown in Fig. 3.

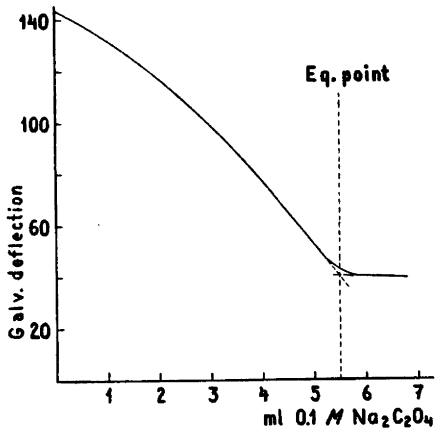


Fig. 3. Titration of calcium with sodium oxalate.

5 ml 0.1 M Ca^{2+}
2 ml 0.02 M Cd^{2+}
25 ml alcohol
Water to 50 ml
Potential = — 0.8 V

The results were about as accurate as when titrating with fluoride. A correction for the small amounts of cadmium remaining in the solution at the equivalence point can, if necessary, be applied.

It may finally be mentioned that the direct titration of aluminum, calcium and magnesium with a fluoride solution is also possible *visually*. Ferric thiocyanate formed by adding one drop of ferric chloride and an excess of ammoniumthiocyanate was used as indicator; the red colour disappears at the endpoint of the titration. The determination of aluminum has been described elsewhere by one of the authors [2], and the determination of calcium is reported in the following paper in this issue [3]. In the literature Treadwell and Bernasconi [4], and Uri [5] previously have described potentiometric methods in which fluoride has been used as reagent.

SUMMARY

A new principle in amperometric titrations — the use of indicator substances — is described. The diffusion current is due to an indicator, which reacts with the reagent at the equivalence point. The following titrations are considered:

1. Titration of aluminum with sodium fluoride using ferric chloride as indicator.
2. Titration of magnesium with sodium fluoride using ferric chloride as indicator.
3. Titration of calcium with sodium fluoride using ferric chloride as indicator.
4. Titration of calcium with sodium oxalate using cadmium chloride as indicator.

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Received December 15, 1948.

ACTA CHEMICA SCANDINAVICA 3 (1949) 29—31

A Titrimetric Method for the Determination of Calcium

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Recently one of the authors described a titrimetric method for the determination of aluminum with a sodium fluoride solution using ferric thiocyanate as indicator [1]. The method is based on the fact that aluminum and ferric iron form complex ions with fluorides; as the aluminum complex is more stable than the iron complex the fluoride reagent reacts first with aluminum, and the red colour of ferric thiocyanate disappears at the equivalence point. The results obtained were quite satisfactory, and therefore we conducted experiments to determine whether the same indicator could be used also in precipitating calcium with a sodium fluoride solution.

Although the determination of calcium is a very common analysis it seems that a satisfactory volumetric method permitting its direct titration has not yet been found. From this point of view investigations of new titrimetric principles seemed desirable.

The titration of calcium with a sodium fluoride solution has formerly been performed employing physicochemical methods for the detection of the endpoint. Uri [2] recently described a potentiometric method, and in this issue one of the authors reports on an amperometric method [3].

The solubility product of calcium fluoride is 4×10^{-11}. Hence the calcium concentration in the equivalence point is 2×10^{-4}, and the fluoride concentration 4×10^{-4}, a value which is too high to permit a sharp jump in pF at the equivalence point. By adding alcohol the solubility can be decreased, and as a consequence the colour change becomes sharper. One or a few drops of 0.1 *M* ferric chloride will be enough, but a rather large excess of ammonium thiocyanate is necessary. The concentrations were the same as those used in titrating aluminum.

Procedure. Add to the solution — 10—20 ml in volume and containing if possible 50—100 mg Ca — 0.1 ml 0.1 *M* ferric chloride, 4 ml 60 % ammonium thiocyanate and

alcohol to double the volume. Titrate with 0.5 *M* sodium fluoride until the red colour disappears.

A correction due to the consumption of fluoride by the indicator should be made. For the reagent a 10 ml semi-micro burette has been found suitable.

The molarity of the sodium fluoride solution can be determined by titrating a solution of alum $KAl(SO_4)_2 12\ H_2O$, which is easily available very pure. This titration is performed as above, but the alcoholic solution should be saturated with solid sodium chloride. The purpose of the sodium chloride is to precipitate the sodium cryolite formed; the reaction will then be more complete and the colour change consequently sharper. In his study of the potentiometric determination of calcium Uri [2] prescribed that also in the precipitation of calcium fluoride the solution should be saturated with sodium chloride. According to our experience the addition of sodium chloride is not to be recommended, as neutral salts increase the solubility and thus make the potential curve less steep. Due to the fact that potassium alum is slightly soluble in alcohol high potassium concentrations should be avoided in titrating aluminum. For this reason sodium fluoride and ammonium thiocyanate ought not to be replaced by the corresponding potassium salts.

The results of some calcium titrations are given in Table 1.

Table 1. Titration of calcium with sodium fluoride.

ml 0.1 *M* $CaCl_2$	ml 0.5 *M* NaF calc.	found	% error
10	4.00	4.02	+ 0.5
10	4.00	4.00	± 0
10	4.00	4.01	+ 0.25
10	4.00	4.04	+ 1.0
10	4.00	4.02	+ 0.5
20	8.00	8.00	± 0
20	8.00	7.93	— 0.9
20	8.00	8.03	+ 0.4

The equivalence ratio in titrating calcium is not quite so advantageous as in titrating aluminum, and hence the accuracy is perhaps somewhat lower. With 0.5 *M* sodium fluoride the colour change can, however, be determined within 1—2 drops, if the volume is about 50 ml.

The optimum pH range is between about 2.5 and 3.5. At pH values above 3.5 the colour intensity of ferric thiocyanate decreases, and below 2.5 the calcium fluoride begins to dissolve.

SUMMARY

A rapid method for the direct titration of calcium is described. Sodium fluoride is used as reagent, a little ferric chloride and an excess of ammonium thiocyanate serve as indicator, and the solution should contain about 50 % alcohol. At the end-point the colour of the solution changes from red to colourless. A pH value between 2.5 and 3.5 is the most suitable.

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Received December 15, 1948.

ACTA CHEMICA SCANDINAVICA 3 (1949) 32—35

Antihistamine Agents

II. 2-Imidazolinylmethyl Ethers of Carbocyclic Carbinols

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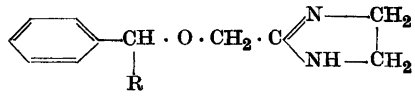
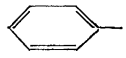
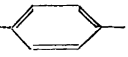
In a recent communication[1] the syntheses of 2-[(diphenylmethoxy)-methyl]-imidazoline (I) was described. As this compound exhibited promising properties as an antispasmodic and antihistamine agent, it seemed to be of interest to investigate some further compounds of this group.

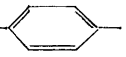
The present paper deals with the preparation of some modifications of the parent imidazoline (I), one phenyl group of which has been substituted or exchanged for another carbocyclic group (II—V). Some compounds containing substituents of a heterocyclic nature will be described later.

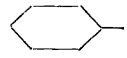
The new imidazolines were prepared by treating two moles of the sodium salt of the corresponding carbinol with one mole of 2 (chloromethyl)-imidazoline hydrochloride. The reaction product was isolated by precipitation with hydrogen chloride.

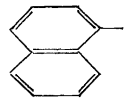


I. R = 

II. R = CH_3— 

III. R = CH_3O— 

IV. R = 

V. R = 

These imidazolines were rather sensitive especially to acids. Addition of strong acids to the aqueous solutions of their salts caused cleavage of the molecule at the ether linkage, the corresponding carbinol and salts of 2-(hydroxymethyl)-imidazoline being formed.

The bases could be precipitated from the aqueous solutions of their salts by sodium carbonate and ammonia but not by sodium bicarbonate. They could not be distilled without decomposition even at 10^{-3} mm Hg.

Results of preliminary tests* of the antihistaminic and antispasmodic potency of the hydrochlorides of the imidazolines are summarized in the following table. The tests were carried out on isolated guinea pig ileum. Activity is expressed in terms of β-dimethylaminoethyl benzhydryl ether hydrochloride (Benadryl) as the unit of activity.

Table 1. Effect of 2-imidazolinylmethyl ethers.

Compound	Effect in reducing the spasm produced by		
	Histamine	$BaCl_2$	Acetylcholine
I	0.5	0.9	0.7
II	0.6	0.8	0.3
III	0.2	0.3	0.1
IV	0.07	1.6	0.7
V	0.05	1.5	0.8
Benadryl	1	1	1

EXPERIMENTAL

2-[(*p*-Methyldiphenylmethoxy)-methyl]-imidazoline

p-Methylbenzohydrol was prepared in 94 % yield from *p*-methylbenzophenone by reduction with aluminium isopropoxide following the procedure given for the preparation of benzohydrol in *Organic reactions*[3]. *p*-Methylbenzohydrol (35.0 g) was dissolved in toluene (150 ml), powdered sodium (4.0 g) was added, and the mixture allowed to stand at room temperature overnight. Next day, 2-(chloromethyl)-imidazoline[3] (13.5 g) was added and the mixture warmed at 60° for 30 minutes with vigorous stirring. After cooling, the separated sodium chloride was filtered off, and the toluene solution precipitated with dry hydrogen chloride in ether. In order to remove sodium and ammonium chlorides the precipitate was dried in air and dissolved in water, whereupon the solution was made alkaline with sodium carbonate.

The resulting oil was extracted with ether, and dry hydrogen chloride in ether was added to the dried ether layer. The hydrocloride (8.1 g) was collected and washed with light petroleum. M. p. 180—184°. After two purifications by dissolving in acetone and precipitating with light petroleum, it melted at 188—189°.

* For these tests acknowledgment is made to Dr. S. Wiedling of our Department of Biology. Details will be reported elsewhere.

$C_{18}H_{20}N_2O \cdot HCl$ (318.8) Calc. N 8.9 Cl 11.2
Found » 9.0 » 11.3

The free base was obtained by dissolving the hydrochloride in water and precipitating with sodium carbonate solution. This yielded an oil, which soon crystallised. After recrystallisation from ether the free imidazoline melted at 98—99°.

$C_{18}H_{20}N_2O$ (280.4) Calc. N 10.0 Eq. wt. 280.4
Found » 10.1 » » 281.2 (titr. with 0.1 *N* H_2SO_4 with methyl red as indicator.)

With picric acid the base gave a picrate with m.p. 166—168°.

2-[(*p*-Methoxydiphenylmethoxy)-methyl]-imidazoline

p-Methoxybenzohydrol was prepared from the corresponding ketone by reduction with aluminium isopropoxide in the same way as the preceding carbinol. Yield 91 %. The reaction between *p*-methoxybenzohydrol (21.4 g), sodium (2.3 g) and 2-(chloromethyl)-imidazoline hydrocloride (7.75 g) was carried out as in the preceding experiment. The precipitated oily hydrochloride was boiled with acetone and insoluble salts were filtered off. On cooling and diluting with light petroleum, white crystals (6.5 g) with the m.p. 145—148° separated. After purification in the same way the hydrochloride melted at 150—151°.

$C_{18}H_{20}N_2O_2 \cdot HCl$ (332.8) Calc. Cl 10.65 N 8.4
Found » 10.7 » 8.4

It was impossible to obtain the base in crystalline form. For further characterisation of the base, its bioxalate was prepared by adding a solution of oxalic acid in ether to an ether solution of the base. After recrystallisation from water the bioxalate melted at 152—152.5°.

$C_{18}H_{20}N_2O_2 \cdot (COOH)_2$ (386.4) Calc. C 62.2 H 5.7
Found » 62.1 » 5.8

With picric acid a picrate melting at 143—145° was obtained.

2-[(Cyclohexylphenylmethoxy)-methyl]-imidazoline

This compound was prepared from cyclohexylphenylcarbinol[4] (28.9 g), powdered sodium (3.45 g), and 2-(chloromethyl)-imidazoline hydrochloride (11.6 g) in the usual way. The crude hydrochloride was boiled with acetone, and the acetone filtered and diluted with light petroleum. After cooling, white crystals (5.9 g) melting at 172—174° were collected. Repeated recrystallisations from acetone-light petroleum raised the m. p. to 180—180.5°.

$C_{17}H_{24}N_2O \cdot HCl$ (308.9) Calc. N 9.1 Cl 11.5
Found » 9.1 » 11.6

Addition of alkali to an aqueous solution of the hydrochloride gave the crystalline base, which after recrystallisation from light petroleum melted at 86—87°.

$C_{17}H_{24}N_2O$	(272.4)	Calc.	N	10.3	Eq.wt. 272.4
		Found	»	10.5	» 273.1

The base gave a bioxalate melting at 144—146°, recrystallised from ethyl acetate.

$C_{17}H_{24}N_2O \cdot (COOH)_2$	(362.4)	Calc.	C	63.0	H	7.2
		Found	»	63.1	»	7.1

The picrate melted at 131—132°.

2-[(α-Naphthylphenylmethoxy)-methyl]-imidazoline

This imidazoline was prepared in the usual way with some slight modifications. The reaction between α-naphthylphenylcarbinol[5] (29.0 g) and sodium (2.85 g) was rather slow and the mixture was therefore refluxed for four hours before the 2-(chloromethyl)-imidazoline hydrochloride (9.7 g) was added. The mixture was then stirred at 60° for 45 minutes. The precipitated, semi-solid hydrochloride was separated from inorganic salts by dissolving in acetone and diluting with light petroleum. The crude hydrochloride (5.8 g), m. p. 176—180°, obtained in this way was purified by repeated recrystallisations from ethanol-light petroleum. M. p. 204—205°.

$C_{21}H_{20}N_2O \cdot HCl$	(316.4)	Calc.	N	7.9	Cl	10.1
		Found	»	8.1	»	10.0

The free base was obtained as an oil, which did not crystallise. From the solution of the base in ether the bioxalate was prepared. M. p. 126—128° after recrystallisation from a mixture of ethyl acetate and light petroleum.

$C_{21}H_{20}N_2O \cdot (COOH)_2$	(406.4)	Calc.	C	68.0	H	5.45
		Found	»	67.8	»	5.35

SUMMARY

The preparation of four 2-imidazolinylmethyl ethers of carbocyclic carbinols is described. Results from preliminary pharmacological tests are reported.

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Received December 2, 1948.

ACTA CHEMICA SCANDINAVICA 3 (1949) 36—40

Purification du calcium

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Pour un certain nombre de réactions dans l'ammoniac liquide, nous avons eu besoin d'un calcium très pur et divisé; nous avons donc examiné et analysé du calcium produit et purifié d'après les méthodes connues.

Le calcium électrolytique, ultérieurement fondu, est contaminé par du silicium, de l'aluminium, du fer, du manganèse, du magnésium, du potassium, du sodium, par de petites quantités de métaux lourds et par du baryum et du strontium. Ce calcium contient en plus des quantités variables de chlorure, d'azoture, d'oxyde et de carbure de calcium. Ces impuretés s'élèvent en général à 5 pour cent avec prépondérance de l'azoture de calcium.

Si l'on a besoin d'un calcium d'une pureté plus grande que le calcium électrolytique, on doit soumettre ce calcium à une purification par distillation fractionnée dans le vide suivant la méthode employée primitivement par Guntz [1] et plus tard utilisée et perfectionnée par Biltz et Hüttig [2], Botolfsen [3], Antropoff et Falk [4] et autres. Cette méthode est employée actuellement pour la purification du calcium en production industrielle. On place le calcium impur dans un creuset en fer, que l'on chauffe dans un appareil en porcelaine ou en quartz opaque ou en fer. Les vapeurs du métal se condensent sur un tube en acier poli, intérieurement refroidi par un courant d'eau.

Le calcium obtenu d'après cette méthode est cristallisé, d'une couleur blanche argentée quand il est nouvellement distillé. Par un chauffage lent jusqu'à 850—900 °C, on obtient un produit très friable. Par un chauffage rapide à une température un peu plus élevée, environ 950—1000 °C, on obtient un produit compact. Plus la distillation est rapidement effectuée, plus les cristaux sont petits et plus le métal est homogène.

Par une distillation fractionnée dans le vide, le sodium et le potassium sont distillés les premiers et se déposent au sommet du condensateur et peuvent par conséquent être séparés du calcium, tandis que le magnésium se sépare difficilement. Les impuretés non volatilisables s'accumulent dans le résidu.

Il est difficile par une seule distillation à une température trop élevée de séparer le chlorure; le produit obtenu est par conséquent moins pur. L'hydrure de calcium ne se décompose que partiellement et ne peut être séparé que par des distillations répétées.

Les vapeurs de calcium son très réactives, le calcium distillé peut donc être facilement contaminé, par ex. par suite de combinaisons avec les matériaux de l'appareillage ou par suite d'un vide insuffisant. Le calcium électrolytique contient en plus des gaz occlus qui se séparent pendant la distillation; ces gaz réagissent avec les vapeurs du calcium et contaminent le calcium distillé. Ce n'est qu'avec une série de distillations que l'on arrive à se débarasser de ces impuretés.

Kroll [5] a montré que les combinaisons du calcium sont facilement réduites dans le vide par le silicium, le titane, l'aluminium et le béryllium. Actuellement, on peut se procurer par Dominion Magnésium Ltd. Canada le calcium produit par cette méthode de réduction de l'oxyde de calcium par l'aluminium [6]. En employant ce procédé, il faut être très prudent dans le choix des matériaux, afin que la quantité de magnésium contenue dans ces produits soit très faible.

Nous avons analysé plusieurs échantillons de calcium métallique produit et purifié d'après ces méthodes. Dans la table I sont indiqués les chiffres moyens donnés par quelques analyses pour les impuretés les plus importantes.

Table 1.

Ca I = calcium électrolytique et fondu ultérieurement.
Ca II = calcium électrolytique, distillé dans le vide, fabriqué en grandes quantités.
Ca III = calcium électrolytique, distillé dans le vide au laboratoire.
Ca IV = calcium produit d'après le procédé de Dominium Magnesium Ltd.

%	Si	Al	Fe	Mg	Na	$CaCl_2$	Ca_3N_2	CaC_2
Ca I	0,25	0,26	0,54	1,30	0,35	0,64	3,30	0,12
Ca II	0,01	0,22	0,55	1,26	pas trace	0,14	0,39	0,02
Ca III	0,01	0,04	0,09	1,60	pas trace	0,07	4,93	0,04
Ca IV	0,01	0,01	0,01	0,42	pas trace	0,16	2,68	0,07

Ces analyses montrent une pureté de 93 pour cent pour le calcium électrolytique et de 93—97 pour cent pour le calcium distillé dans le vide.

La pureté de ces échantillons n'était pas suffisante pour nos travaux. Après une nouvelle distillation dans le vide, très minutieuse afin d'éviter la contamination du calcium, et en ayant soin de manipuler et de conserver le

produit distillé dans de l'argon pur, on arrive à réduire la proportion d'impuretés, spécialement la quantité d'azoture de calcium.

La purification se fait donc d'après les principes énoncés précédemment. Mais l'appareil doit être constitué par un alliage d'acier en chrome, nickel et molybdène, ne contenant que très peu de carbone, ceci pour empêcher la formation de carbure de calcium. L'emploi d'un tel alliage d'acier est nécessaire pour que l'appareil résiste aux grandes variations de température qui ont lieu pendant l'opération, et pour éviter la contamination du calcium. Le calcium obtenu par cette méthode se présente, ainsi que nous l'avons constaté précédemment, en grands paquets cristallins, et doit être réduit en poudre dans une atmosphère d'argon.

Nous avons pour cette raison mis au point pour la purification ultérieure du calcium, un procédé d'un principe entièrement différent:

On sait que l'ammoniac liquide dissout les métaux alcalins et alcalino-terreux, en donnant des solutions colorées [7]. La couleur de ces solutions, lorsqu'elles sont étendues, est d'un bleu très beau, tandis que les solutions concentrées ont une couleur mordorée. Les métaux lourds, silicium et aluminium, sont insolubles dans l'ammoniac liquide; il en est de même avec les combinaisons du calcium qui pourraient se présenter, surtout l'oxyde, l'azoture, le carbure et le chlorure de calcium.

En traitant le calcium à purifier avec de l'ammoniac liquide et en prenant soin que la dissolution se fasse à une température basse, entre — 70 °C et — 35 °C, les impuretés ne seront pas dissoutes, à l'exception du potassium, du sodium et de petites quantités de magnésium. Le potassium et le sodium sont facilement solubles, tandis que le magnésium ne se dissout que difficilement dans l'ammoniac liquide. Les impuretés peuvent être retenues par filtration à basse température dans un appareil spécial, toutes les opérations se faisant dans une atmosphère d'ammoniac tout à fait pur. Si le filtrage a été fait avec un filtre très serré, on a dans la solution le calcium, avec comme seules impuretés le potassium, le sodium et le magnésium.

Puisque le potassium et le sodium peuvent être séparés par une distillation dans le vide comme précédemment décrite, on peut, en partant d'un calcium distillé dans le vide, obtenir une purification très poussée, en traitant ultérieurement le calcium avec l'ammoniac liquide.

Le liquide filtré est ensuite évaporé à la température d'ébullition de l'ammoniac. Lorsque l'évaporation de l'ammoniac est terminée, on a comme résidu de l'hexamine de calcium. Par un chauffage lent dans le vide, l'hexamine de calcium se décompose en ammoniac et en un calcium pur, très divisé et d'une couleur grise. Ce calcium est très actif et prend feu au contact de l'air.

On pourrait croire qu'avec ce procédé, on risquerait de provoquer la formation d'amidure, d'hydrure ou d'azoture de calcium. Botolfsen [3] a, en effet, montré que l'hexamine de calcium se décompose d'une manière explosive quand il est chauffé dans le vide et donne comme produits de réaction de l'azoture et de l'hydrure de calcium. Nous avons constaté que ces réactions n'ont pas lieu lorsque la dissolution et la filtration se font rapidement, de manière à ne laisser l'hexamine de calcium en contact avec les métaux lourds non dissous que pendant un temps très court. Ces métaux agissent en effet comme catalyseurs pour transformer entre autres choses l'hexamine en amidure, lequel par chauffage donne de l'azoture. La décomposition explosive de l'hexamine de calcium, qui se produit de temps en temps, est due probablement à ces catalyseurs.

Dans la table 2 se trouvent les analyses du calcium avant et après la purification par l'ammoniac liquide suivant notre méthode. Nous nous sommes servis pour la filtration d'un filtre en verre ayant des pores d'un diamètre de 40—90 μ. Les métaux ont été déterminés spectroscopiquement.

Table 2.

En %	Si	Al	Fe	Mg	Mn	Na, K	Ba	Sr	Pb	Cu, Cr	Ca Cl_2	Ca_2N_3	CaC_2
Avant purification	0,07	0,35	0,55	1,26	env. 0,01	pas trace	<0,01	<0,01	traces	traces	0,14	0,40	0,20
Après purification	0,007	0,13	pas trace	0,42	0,004	pas trace	<0,01	<0,003	pas trace	traces	0,02	rien	rien

Il ressort de cette table que les impuretés ont diminué dans la proportion de 3 à 0,6 pour cent environ.

L'appareillage employé était en verre d'Iena, et plus spécialement les filtres dont nous nous sommes servis. Les filtrations se font d'une manière automatique dans une atmosphère d'ammoniac pur. Il faut éviter de se servir de robinets en verre partout où la dissolution de calcium doit circuler. Ces robinets sont toujours bloqués. Nous nous sommes donc servis de tubes en polyvinyle munis de pinces à vis, l'expérience ayant montré que ces tubes ne sont pas attaqués et n'attaquent pas non plus la solution de calcium. Toutes les pièces rodées ont été enduites d'un mélange de glycérol, sucre et graphite. Ce mélange n'est pas attaqué par l'ammoniac.

Nous nous proposons de donner plus tard une description détaillée du procédé, de l'appareillage et des méthodes microanalytiques que nous avons utilisées.

RÉSUMÉ

Nous avons examiné et analysé du calcium produit et purifié d'aprés les methodes connues. Les analyses montrent une pureté de max. 97 pour cent. Les difficultés d'obtenir une plus grande pureté par distillation dans le vide sont discutées. Une nouvelle methode est publiée par laquelle on peut obtenir une purification très poussée: Après distillation dans le vide, le calcium est traité avec l'ammoniac liquide. Le calcium se dissout mais les impuretés sont retenues par filtration. Le filtrat est evaporé, l'hexamine de calcium decomposé et on obtient le calcium pur.

L'auteur saisit l'occasion pour remercier Mme Astri Tönsager et M. S. Rutlin qui ont bien voulu l'aider dans quelques analyses du calcium. Nous désirons aussi remercier M. le Professeur H. Haraldsen de nous avoir permis de nous servir de son laboratoire et la Fondation Nansen pour son aide financière.

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Manuscript reçu le 14 Septembre 1948.

ACTA CHEMICA SCANDINAVICA 3 (1949) 41—49

Investigations of Vicilin and Legumin

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In the seeds of different species of the family *Leguminosae* there occur two well-defined globulin components, legumin and vicilin, which were first isolated from seeds of peas, *Pisum sativum*, by Osborne [1]. He made many analyses with high accuracy on dried preparations of the two components. It is, however, impossible to decide if his preparations were monodisperse, and if the proteins had been denatured. However, with the ultracentrifuge one may investigate the homogeneity of protein solutions. Legumin has been investigated in the ultracentrifuge by Sjögren and Svedberg [2] and they determined the sedimentation constant of legumin to be $s_{20} = 13.1$ S (the value reduced to water basis) [3]. During the last year [4] it was shown that legumin and vicilin occur practically throughout the family *Leguminosae*. Methods of separation of the two globulin components have been developed which are based on the fact that legumin is precipitated at pH 4.7 and vicilin is not [4]. With this method it is possible to separate the components from each other completely, and molecular weight determinations have given the values $M = 186\,000$ for vicilin and $M = 331\,000$ for legumin [4]. Thus the molecular weight of legumin is about twice that of vicilin.

CHEMICAL DIFFERENCES BETWEEN LEGUMIN AND VICILIN

It is not yet clearly settled if vicilin and legumin are two different globulins. Vicilin could be a dissociation product of legumin, or legumin could be formed from vicilin. This question will be taken up in this paper. Osborne and Campbell [5] found small but distinct differences in the chemical composition, especially in the sulphur content, and the difference in solubility also seems to confirm the hypothesis that we are dealing with two substances which are not very closely related. In this paper some quantitative determinations of tyrosine and tryptophan will be reported. The method of determination was

that developed by Goodwin and Morton [6], and only a brief account of the method will be given here. Only a few amino acids, tyrosine, tryptophan and phenylalanine, show absorption in the region 250—320 mμ. The absorption of tyrosine and tryptophan is much stronger than that of phenylalanine, and using 0.1 *N* NaOH as solvent the maximum of the absorption of phenylalanine occurs near 258.5 mμ with a low molecular extinction. Goodwin and Morton have selected wave-lengths at which phenylalanine makes no contribution. The extinction curves of tyrosine and tryptophan intersect at 257.15 and 294.4 mμ. By using the intersecting point 294.4 mμ it is possible to determine tyrosine and tryptophan with a rather high accuracy even if phenylalanine is present.

Experimental

About 10 mg of substance was dissolved in 10.0 ml 0.1 *N* NaOH. The absorption of this solution was determined in a Beckmann photoelectric spectrophotometer at 280, 294.4, 340 and 370 mμ.

Method of calculation

The observed extinction coefficients at 280 and 294.4 mμ are used in the determinations of tyrosine and tryptophan. These values must, however, be corrected for the irrelevant absorption, and for this the extinction coefficients at 340 and 370 mμ are used. The molecular extinction coefficients for tyrosine are $E_{280} = 1576$ and $E_{294.4} = 2375$; for tryptophan $E_{280} = 5225$ and $E_{294.4} = 2375$. If one considers the irrevelant absorption as a straight line in the wave-length range 280—370 mμ, the correction factor for an observed extinction coefficient E^{obs} is

$$\varepsilon = E_{370} + \frac{E_{340} - E_{370}}{370 - 340} (370 - \lambda) \tag{1}$$

$\lambda = 280$ and 294.4 mμ. Then

$$E^{corr} = E^{obs} - \varepsilon \tag{2}$$

If the concentration of tyrosine is y g · mol/l and that of tryptophan $(x—y)$ g · mol/l, we have:

$$x = \frac{E^{corr}_{294.4}}{2375} \tag{3}$$

and

$$E^{corr}_{280} = y \cdot 1576 + (x—y)\ 5225 \tag{4}$$

(3) and (4) give

$$y = \frac{5225 \cdot E_{294.4}^{corr} - 2375 \cdot E_{280}^{corr}}{2375\ (5225 - 1576)} \qquad (5)$$

The molecular weights are for tryptophan 204.2 and for tyrosine 181.2. If A grams of protein is dissolved in 10.0 ml 0.1 *N* NaOH, we have $\frac{(x-y) \cdot 204.2}{A}$ % tryptophan and $\frac{y \cdot 181.2}{A}$ % tyrosine.

The results of the measurements are found in Table 1. The measurements were made on dried preparations of legumin and vicilin from peas. Some of the preparations were over one year old, and some of them were newly prepared.

Table 1. Determination of the tyrosine and tryptophan content of vicilin and legumin by measurement of absorption in the ultra-violet. The values of E are observed values.

Preparation	Substance mg	E_{280}	$E_{294.4}$	E_{340}	E_{370}	Tyrosine %	Tryptophan %
Vicilin							
10	10.0	0.432	0.560	0.020	0.013	3.88	0.29
11	10.7	0.432	0.570	0.022	0.016	3.74	0.24
22	10.1	0.424	0.550	0.011	0.007	3.81	0.30
27	10.3	0.430	0.560	0.019	0.013	3.79	0.27
29	9.8	0.409	0.538	0.012	0.007	3.85	0.26
					Average:	3.81	0.27
Legumin							
8	10.0	0.658	0.658	0.027	0.021	3.84	1.19
9	10.6	0.762	0.743	0.029	0.022	3.98	1.39
10	10.1	0.660	0.662	0.040	0.032	3.81	1.16
11	10.6	0.762	0.748	0.048	0.034	3.97	1.30
22	10.4	0.695	0.698	0.045	0.032	3.85	1.15
					Average:	3.89	1.24

It is seen from the values in Table 1 that vicilin and legumin contain about the same amount of tyrosine, 3.81 and 3.89 %, which values are considerably higher than those obtained by Osborne and coworkers [7,8]. With the method described here, the tyrosine content can be determined with satisfactory accuracy; the maximum deviation from the average is 1.8 % for vicilin and 2.3 % for legumin. In the tryptophan determinations the deviations are considerably higher, 11 % for vicilin and 12 % for legumin. According to these investigations vicilin contains 0.27 % tryptophan and legumin 1.24 %, which values show that vicilin and legumin from peas differ in

their chemical composition. Therefore vicilin and legumin cannot be very closely related. It must be stated that the analyses described here were made on fractions of vicilin and legumin which were shown to be homogeneous in the ultracentrifuge. It is interesting to compare these investigations with the results obtained by Osborne and Campbell [5]. They investigated legumin from different species (pea, lentil, horse bean, vetch) and found that legumin preparations from the different species had the same chemical composition. The same held for vicilin from the different species, but distinct differences in chemical composition between legumin and vicilin could be shown. The following table is taken from Osborne and Campbell.

Table 2. The composition of legumin and vicilin according to Osborne and Campbell.

	Legumin					Vicilin			
	Pea	Lentil	Horse bean	Vetch	Average	Pea	Lentil	Horse bean	Average
Carbon	51.74	51.73	51.72	51.69	51.72	52.36	52.13	52.38	52.29
Hydrogen	6.90	6.89	7.01	6.99	6.95	7.03	7.02	7.04	7.03
Nitrogen	18.04	18.06	18.06	18.02	18.04	17.40	17.38	17.52	17.43
Sulphur	0.42	0.40	0.39	0.43	0.41	0.18	0.17	0.15	0.17
Oxygen	22.90	22.92	22.82	22.87	22.88	23.03	23.30	22.91	23.08

From the experiments described above the following conclusions can be drawn. The differences in tyrosine and tryptophan content of vicilin and legumin from peas show that we are dealing with two well defined globulin components which are not very closely related. The results of Osborne and Campbell [5] show that legumin and vicilin from other species in the *Leguminosae* are identical with legumin and vicilin from peas, in respect to the chemical composition. Ultracentrifugal experiments [4] showed that all leguminosae plants investigated (about 30 species) contain vicilin and legumin.

THE ISOLATION OF LEGUMIN

The difference in tryptophan content in legumin and vicilin was assumed to be great enough for quantitative determinations of the two globulin components when they occurred in the same solution. This proved to be true under certain conditions. When pure preparations of legumin and vicilin were dissolved in 0.1 *N* NaOH in known concentrations, it was possible to determine the tryptophan content of such a solution, and with a simple mathematical calculation the amounts of legumin and vicilin could be determined from this value with satisfactory accuracy. When this method was tried on some pre-

parations in which legumin and vicilin had not yet been separated by isoelectric precipitation, very high tryptophan values were obtained. This must depend on a third substance with high tryptophan content. Experiments were carried out to isolate this third component. Therefore the tyrosine and tryptophan content was determined on preparations of pea globulins containing both vicilin and legumin, which had been precipitated by dialysis against water. In these investigations the determinations of the absorption were made in the following way. The wet precipitate was dissolved in 0.1 *N* NaOH and the solution investigated in the Beckmann photometer. The supernatants were similarly treated by adding NaOH to 0.1 *N*. In this way it was impossible to calculate the percentage content of tyrosine and tryptophan. With the use of equations (1)—(5) the fraction

$$F = \frac{y}{x—y}$$

can be obtained which is the molar ratio $C_{\text{tyrosine}}/C_{\text{tryptophan}}$ of the solution. This fraction is for vicilin $F = 16.1$ (average value from 15 determinations) and for legumin $F = 3.6$ (from 19 determinations). In solutions containing a mixture of these two globulin components, one should have

$$3.6 < F < 16.1$$

As was said above, preparations in which legumin and vicilin had not yet been separated abnormally high tryptophan values were obtained, *i. e.* $F < 3.6$. The separation of the two components was carried out by repeated dialysis against buffer solutions of pH 4.7 and water according to the scheme below. After each dialysis against water both the precipitate and supernatant were investigated in the Beckmann photometer in the manner described above. The values are given in Table 3. The preparation containing both legumin and vicilin (fraction 9 in the preparation scheme) had $F = 1.6$, which is very low.

As is seen from Table 3 most of the substance with high tryptophan content ($F < 1$) remained in solution after dialysis against water, but part of it was precipitated. The F values for vicilin and legumin increase after each dialysis, but it is impossible to get still higher values if the dialyses are repeated more times than in the preparation scheme described here, *i. e.* the component with high tryptophan content is completely removed after the second dialysis against water (fractions 17 and 23 in the preparation scheme).

Preparation scheme for the isolation of vicilin and legumin from peas.

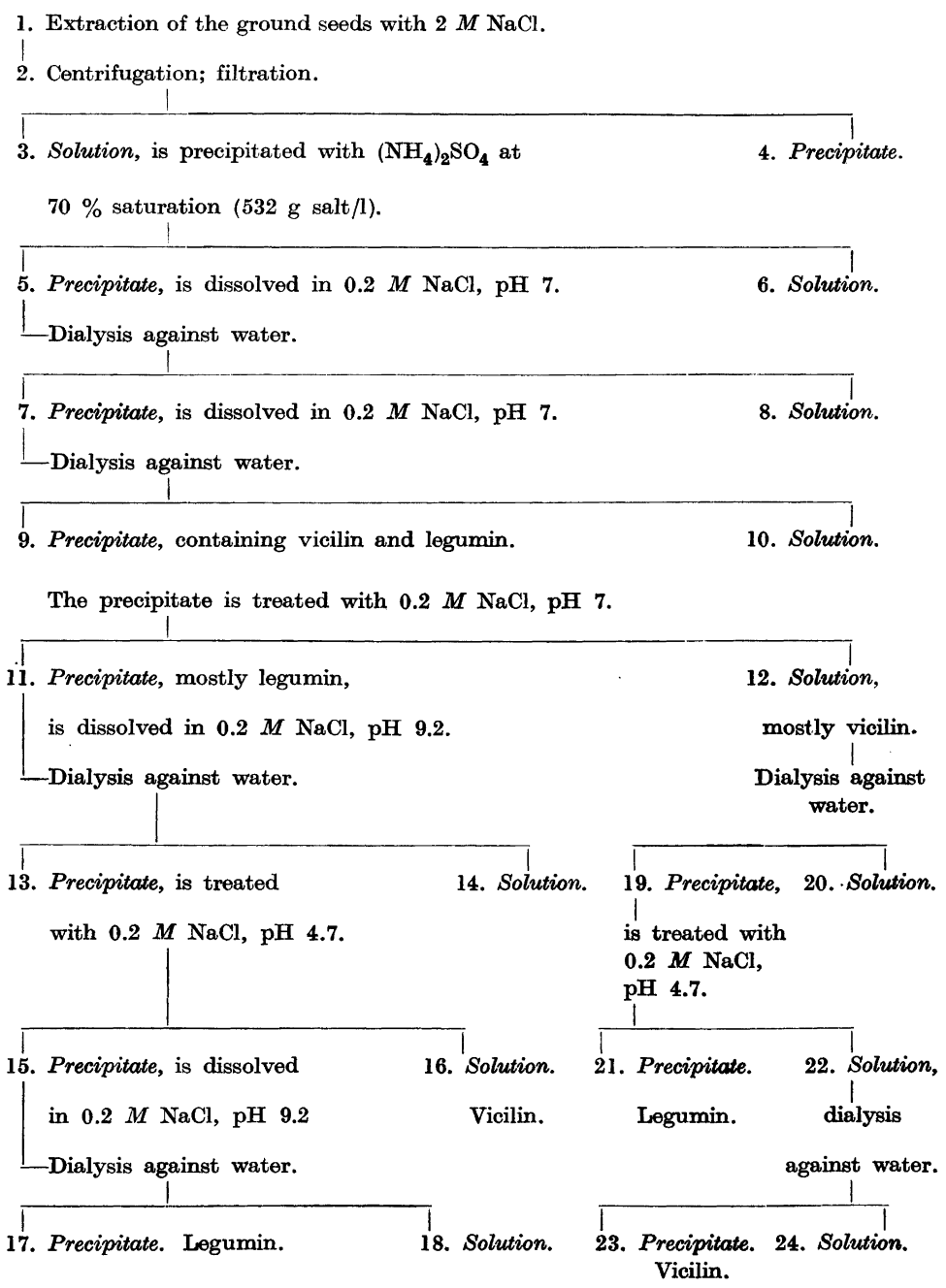

Table 3. Determination of $F = C_{tyrosine}/C_{tryptophan}$ *in precipitates and supernatants after dialysis of vicilin and legumin against water.*

	Fraction in the preparation scheme	Precipitate F	Solution (supernatant) F
Vicilin			
Before 1st dialysis	(12)	—	3.5
After 1st dialysis	(19) (20)	12	0.5
After 2nd dialysis	(23) (24)	16	—
Legumin			
Before 1st dialysis	(11)	1.2	—
After 1st dialysis	(13) (14)	3.6	0.3
After 2nd dialysis	(17) (18)	3.9	0.8

This component with high tryptophan content ($F < 1$) has been isolated in larger amounts in the form of a white amorphous powder by drying in vacuum the supernatants from dialyses against water. Ultracentrifugal analyses showed that it is a low-molecular substance, and part of it goes through the sac during dialysis. It is probably identical with legumelin, first obtained by Osborne [1], who defined legumelin as a substance, which is partly precipitated by dialysis and this fact has been confirmed in the experiments described above.

A SEROLOGICAL COMPARISON BETWEEN LEGUMIN AND VICILIN

In order to decide whether legumin and vicilin have similar serological properties in spite of differences in chemical constitution and molecular weight, some experiments with intravenous injections of rabbits have been carried out [9]. One rabbit was given intravenous injections with a solution of legumin and another rabbit was injected with a solution of vicilin. The two globulin components had been isolated from seeds of *Pisum sativum*. As far as could be seen from ultracentrifugal experiments the two components were entirely separated. When the immunization was finished, precipitin reactions were carried out on the immune sera obtained according to Table 4. Precipitin reactions on the above sera were also carried out with globulin solutions from seeds from other species in the family *Leguminosae*. These solutions contained both legumin and vicilin [4].

At the experiment described in this paper, each rabbit was injected every seven days, with 0.1 % solutions of legumin respectively vicilin. The total quantity injected in each rabbit was 0.015 g of the protein. In the precipitin

reactions 0.25 ml serum was mixed with 0.25 ml of 0.5 % protein solution in a test tube at 20° C. Experiments were also carried out at lower concentrations but the same results were obtained as those described here. Altogether six experiments have been made, but in no case any deviations from the results in Table 4 have been observed.

Table 4. Precipitin reactions of globulin solutions from various species in the Leguminosae. The sera used were obtained by injecting rabbits with solutions of vicilin and legumin from peas.

Protein and species	Legumin serum Reaction after 2 h	Legumin serum Reaction after 24 h	Vicilin serum Reaction after 2 h	Vicilin serum Reaction after 24 h
Legumin				
Pisum sativum	2 +	3 +	2 +	3 +
Vicilin				
Pisum sativum	—	2 +	—	2 +
Legumin + Vicilin				
Glycine Soja	—	+	—	+
Lathyrus Clymenum	+	2 +	+	2 +
Lathyrus odoratus	2 +	3 +	2 +	3 +
Lathyrus silvestris	+	2 +	+	2 +
Lupinus albus	—	2 +	—	2 +
Lupinus angustifolius	+	2 +	+	2 +
Medicago sativa	—	+	—	+
Vicia faba	—	2 +	—	2 +
Vicia sativa	+	2 +	+	2 +

As can be seen from Table 4 all the globulin solutions from different species of the *Leguminosae* gave precipitin reactions with both sera from pea globulins called legumin serum and vicilin serum in the table. This shows that the seed globulins from the different species in this family are immunologically very closely related. It may also be mentioned that α- and γ-globulin from barley and wheat [10, 11] gave no reactions with the sera used above. As is seen from Table 4 vicilin gave precipitin reaction with legumin serum, and legumin with vicilin serum. In spite of differences in chemical composition vicilin and legumin are immunologically related. The cross reactions of the pea globulins may of course depend upon inhomogeneity of the vicilin and legumin preparations, not detectable in the ultracentrifuge.

SUMMARY

1. The tyrosine and tryptophan content of the globulins vicilin and legumin from seeds of *Pisum sativum* has been determined by measurement of absorption in the ultra-violet.

2. Vicilin contains 3.81 % tyrosine and 0.27 % tryptophan, legumin contains 3.89 % tyrosine and 1.24 % tryptophan.

3. From the tryptophan content it is seen that vicilin and legumin differ in chemical composition, as was also shown by Osborne and Campbell by sulphur analyses. Thus vicilin and legumin are two well defined globulin components. It is not very probable that one of these components can be formed from the other by dissociation or association. It has previously been shown in the ultracentrifuge that vicilin and legumin occur in the seeds of most species investigated in the *Leguminosae*.

4. A low-molecular substance with high tryptophan content has been isolated. This substance may be legumelin.

5. A serological experiment with vicilin and legumin from seeds of 10 different species in the family *Leguminosae* showed that the globulin solutions from these species are immunologically very closely related.

The author wishes to express his thanks to his teacher Prof. The Svedberg for his great interest in these experiments. This work has been done with the aid of a grant from the *Rockefeller Foundation*.

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Received December 22, 1948.

ACTA CHEMICA SCANDINAVICA 3 (1949) 50—55

Über eine einfache thermodynamische Beziehung zwischen Phasengleichgewichten in 2-Komponentsystemen

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Den Gegenstand der vorliegenden Arbeit bildet eine zwischen verschiedenen Hydraten und wässrigen Lösungen herrschende Beziehung, die bisher — wohl mehr zufällig — in der thermodynamischen Literatur noch nicht behandelt worden zu sein scheint.

Wir betrachten einen kristallinischen Stoff (K), der imstande ist, mit ν Molen Wasser unter Bildung eines kristallinischen Hydrates (H) zu reagieren:

$$K + \nu\ H_2O \rightarrow H \qquad (1)$$

Bei gegebener Temperatur und gegebenem Druck ist von den folgenden drei Phasengleichgewichten mindestens eines thermodynamisch instabil, bezogen auf die anderen:

I. s, K
II. s, H
III. H, K

wobei s eine gesättigte Lösung darstellt. Es lässt sich nun zeigen, dass zwischen gewissen Eigenschaften dieser drei Phasengleichgewichte ein einfacher Zusammenhang besteht.

Wir wollen die beiden folgenden isothermen und isobaren Überführungsprozesse betrachten: a) Transport von einem Mol K von I nach II. b) Transport von ν Molen H_2O von III nach II (vgl. Fig. 1).

Die Affinität des aus den beiden Teilprozessen bestehenden Gesamtprozesses muss null sein. Von der Richtigkeit dieser Bedingung überzeugt man sich leicht, wenn man die vorgenannten Teilprozesse durch die beiden folgenden ersetzt: α) Transport von einem Mol K von I nach III. β) Transport von einem Mol H von III nach II. Da sowohl Anfangszustand wie Endzustand bei

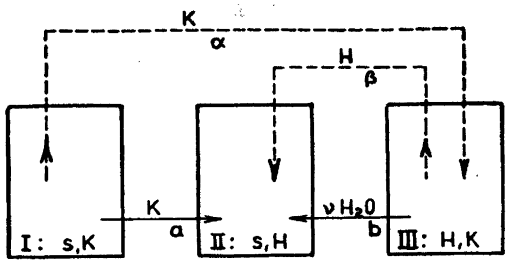

Fig. 1. Schematische Darstellung der betrachteten Stofftransporte.

diesem Totalprozess mit dem ersten identisch sind, sind die beiden Totalprozesse thermodynamisch äquivalent. Da jedoch sowohl α wie β Überführungsprozesse kristallinischer Substanzen im Gleichgewicht bei Konstanz von Temperatur und Druck darstellen, ist die Affinität jedes dieser Teilprozesse null. Folgende Gleichung hat daher exakte Gültigkeit:

$$A^* = \mu_{K(II)} - \mu_{K(I)} + \nu(\mu_{H_2O(II)} - \mu_{H_2O(III)}) = 0 \qquad (2)$$

(A^* = Affinität; μ = chemisches Potential.) Falls die idealen Gasgesetze auf den Gaszustand angewandt werden können, folgt aus (2):

$$\frac{a_{K(I)}}{a_{K(II)}} = \left(\frac{p_{H_2O(II)}}{p_{H_2O(III)}}\right)^{\nu} \qquad (3)$$

(a = Aktivität). Diese Beziehung kann zur Berechnung von Sättigungskonzentrationen in instabilen Lösungen, zur Berechnung von Zersetzungsdrucken von Hydraten, von Aktivitätskoeffizienten u. a. dienen. Im folgenden werden einige Beispiele für die Anwendung von (3) angeführt.

Falls man Temperaturen unterhalb des Schmelzpunktes des Hydrates betrachtet, dann enthält (3) eine Grösse ($a_{K(I)}$), die sich auf ein instabiles System bezieht, wogegen die anderen Grössen Eigenschaften stabiler Systeme darstellen. Als Beispiel für die Berechnung der Wasserlöslichkeit eines Anhydrids in einem solchen instabilen System sei die Löslichkeit der wasserfreien Traubensäure bei 20° C angeführt. Diese wurde in einer vorhergehenden Arbeit [1] mit Hilfe von (3) zu $x = 0{,}0260$ berechnet, während die Löslichkeit des bei dieser Temperatur stabilen Hydrates 0,0209 beträgt (x = Molenbruch). Die Richtigkeit des berechneten Wertes konnte bestätigt werden durch seine Anwendung bei der Berechnung der Razemisierungsaffinität der Weinsäure,

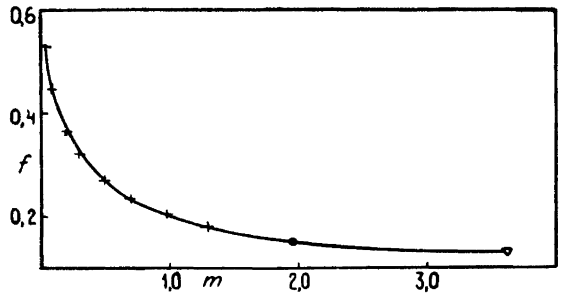

Fig. 2. Aktivitätskoeffizienten von Natriumsulfat in wässriger Lösung.

+ : Literaturwerte
○ : extrapoliert
△ : berechnet nach (4).

wobei Übereinstimmung mit der nach anderen Methoden ermittelten Affinität erzielt wurde.

Falls alle Werte für die in (3) enthaltenen Dampfdrucke und Konzentrationen vorliegen, kann (3) für die Ermittelung der Aktivitätskoeffizienten von K angewandt werden. Als Beispiel hierfür wollen wir das System Na_2SO_4—H_2O betrachten, in welchem das Dekahydrat auftritt. Hierfür liegen in der Literatur folgende Werte vor:

Löslichkeiten:

$$m_{Na_2SO_4(I)} = 3{,}63\ ^{2};\quad m_{Na_2SO_4(II)} = 1{,}96\ ^{3}$$

(m = Konzentration in Mol per 1000 g H_2O.)

Dampfdrucke (mm Hg):

$$p_{H_2O(II)} = 21{,}95\ ^{4}\quad p_{H_2O(III)} = 19{,}16\ ^{5}$$

(alle Werte beziehen sich auf die Temperatur 25° C).

Auf Grund der Gleichung

$$\left(\frac{m_{Na_2SO_4(I)}}{m_{Na_2SO_4(II)}} \cdot \frac{f_{Na_2SO_4(I)}}{f_{Na_2SO_4(II)}}\right)^3 = \left(\frac{p_{H_2O(II)}}{p_{H_2O(III)}}\right)^{10} \tag{4}$$

ergibt sich für das Verhältnis der Aktivitätskoeffizienten der Wert 0,85. Die Aktivitätskoeffizienten von Na_2SO_4 in wässriger Lösung wurden von Harned und Hecker [6] mit Hilfe von EMK-Messungen bis zu einer Molarität

von 1,3 bestimmt. Diese Werte sind in Fig. 2 dargestellt. Obwohl (4) nicht die Absolutwerte der Aktivitätskoeffizienten zu berechnen ermöglicht, lässt sich auf Grund der vorliegenden Werte durch eine Extrapolation der Kurve bis $m = 1{,}96$ doch eine recht gute Schätzung dieser Grössen vornehmen. Die aus der Fig. 2 ersichtlichen Werte der Aktivitätskoeffizienten betragen demnach 0,15 für die mit dem Dekahydrat, bezw. 0,13 für die mit dem Anhydrid gesättigte Lösung. Die Kurve zeigt die gleiche Form wie die entsprechende, bis zu hohen Konzentrationen gemessene Kurve für Li_2SO_4[7]. Ein ausgeprägtes Minimum scheint bis zu einer Molarität von 3,6 nicht aufzutreten.

Eine Kontrolle der dem vorliegenden Resultat zugrundeliegenden experimentellen Werte ist in gewissem Grade möglich durch Berechnung des Dampfdruckes der mit dem Anhydrid gesättigten Lösung, für den in der Literatur der experimentelle Wert 20,4 mm Hg vorliegt[4]. Durch Anwendung der Gibbs-Duhem'schen Gleichung

$$m \frac{\mathrm{d} \log f_{Na_2SO_4}}{\mathrm{d}m} + 55{,}5 \frac{\mathrm{d} \log f_{H_2O}}{\mathrm{d}m} = 0$$

auf die Kurve (Fig. 2) erhält man für das Verhältnis der Aktivitätskoeffizienten des Wassers in den beiden gesättigten Lösungen:

$$\frac{f_{H_2O(I)}}{f_{H_2O(II)}} = 1{,}01$$

Auf Grund der Gleichung

$$\frac{x_{H_2O(I)}}{x_{H_2O(II)}} \cdot 1{,}01 = \frac{p_{H_2O(I)}}{p_{H_2O(II)}}$$

erhält man:

$$p_{H_2O(I)} = 20{,}2 \text{ mm Hg}$$

in befriedigender Übereinstimmung mit dem obenerwähnten experimentellen Wert.

Die Berechnung von Zersetzungsdrucken kann in solchen Fällen von Bedeutung sein, in denen die Gleichgewichtseinstellung infolge von Reaktionsträgheit der kristallinischen Phasen, Ausbildung instabiler Phasen oder mangelhaft ausgebildeter Kristallgitter zu langsam vor sich geht. Als Beispiel für eine solche Berechnung wollen wir das Kobaltojodat wählen, von dem ein Tetrahydrat und ein Dihydrat bekannt ist. Für die Löslichkeiten dieser

Hydrate sowie des Anhydrids liegen Bestimmungen in einem verhältnismässig grossen Temperaturbereich vor [8]. Die im folgenden aufgeführten Werte der Sättigungskonzentrationen gelten für 18° C.

Bodenkörper	Sättigungskonzentration (Mol/l)
Tetrahydrat	0,0203
Dihydrat	0,0110
Anhydrid	0,0252

Infolge der Schwerlöslichkeit der drei Stoffe kann der Dampfdruck über den gesättigten Lösungen mit dem des reinen Wassers (15,5 mm Hg) identifiziert werden. Die Aktivitätskoeffizienten des Salzes in wässriger Lösung sind nicht bekannt. Jedoch weicht in diesem Konzentrationsbereich das Verhältnis der Aktivitätskoeffizienten für zwei gegebene Konzentrationen bei Salzen von gleichem Ladungstyp nur wenig voneinander ab. Deshalb wurden in der vorliegenden Berechnung für die betreffenden Verhältnisse der Aktivitätskoeffizienten die Durchschnittswerte von zehn Salzen des gleichen Ladungstyps aus vorliegenden Bestimmungen benutzt. Daraus ergab sich:

$$\frac{f_{(0,0252)}}{f_{(0,0203)}} = 0{,}96 \text{ und } \frac{f_{(0,0252)}}{f_{(0,0110)}} = 0{,}85$$

Durch Einsetzen der genannten Werte ergibt sich für das Gleichgewicht Tetrahydrat, Anhydrid (4,0):

$$\left(\frac{0{,}0252 \cdot 0{,}96}{0{,}0203}\right)^3 = \left(\frac{15{,}5}{p_{(4,0)}}\right)^4$$

und folglich $p_{(4,0)} = 13{,}6$ mm Hg.
Analog erhält man für das Gleichgewicht Dihydrat, Anhydrid (2,0):

$$\left(\frac{0{,}0252 \cdot 0{,}85}{0{,}0110}\right)^3 = \left(\frac{15{,}5}{p_{(2,0)}}\right)^2$$

und folglich $p_{(2,0)} = 5{,}7$ mm Hg.
Der entsprechende Gleichgewichtsdruck für das System (4,2) ergibt sich durch die bekannte Beziehung:

$$p_{(4,2)} = \frac{p^2_{(4,0)}}{p_{(2,0)}} = 32{,}4 \text{ mm Hg}$$

Bemerkenswert — in Anbetracht der Existenzfähigkeit des Tetrahydrates — ist der hohe Gleichgewichtsdruck des letztgenannten Systems, der mehr als doppelt so hoch ist wie der des reinen Wassers bei der gleichen Temperatur. Der Dampfdruck des Systems (s, 4) ist jedoch von dem des stabilen Systems (s, 2) kaum verschieden, und das gesamte (instabile) Existenzgebiet des Tetrahydrates liegt zwischen den Drucken 13,6 mm Hg und 32,4 mm Hg. Ferner ist die Umwandlungsgeschwindigkeit der kristallinischen Phasen anscheinend sehr gering. So soll in dem System (s, 4) die Umwandlung des Tetrahydrates zum Dihydrat erst nach wochenlangem Schütteln vollendet sein. Eine ähnliche relative Beständigkeit weist das Anhydrid auf. Unter diesen Umständen erscheinen die Bedingungen für die Messung der Gleichgewichtsdrucke zweier kristallinischer Phasen sehr ungünstig.

Falls das betrachtete System nicht ein Anhydrid und ein Hydrat, sondern zwei Hydrate verschiedener Zusammensetzung enthält, lässt sich eine mit (2) analoge Gleichung anwenden, die darüber hinaus noch ein Glied enthält, das der Überführung von Wasser von I nach II Rechnung trägt. In diesem Fall muss folglich auch der Dampfdruck über I bekannt sein.

ZUSAMMENFASSUNG

Eine thermodynamische Beziehung zwischen stabilen und instabilen Phasengleichgewichten in 2-Komponentsystemen wurde abgeleitet.

Die Anwendung dieser Beziehung für die Berechnung von Sättigungskonzentrationen in instabilen Systemen, von Aktivitätskoeffizienten und von Zersetzungsdrucken von Hydraten wurde an Hand von Beispielen erläutert.

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Eingegangen am 29. November 1948.

ACTA CHEMICA SCANDINAVICA 3 (1949) 56—60

Studies on Liver Arginase

I. On its Crystallization by Bach

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The procedure of purification of liver arginase published by Bach[1], in which he claims the crystallization of the enzyme, was repeated several times in this laboratory. The purpose of this communication is to report that the findings of Bach in regards to the purity of the preparation, the assumed crystalline structure, and the role of manganese are completely in error.

The purification method is relatively simple, though it requires the consumption of large quantities of ammonium sulfate for the repeated dialysis which is somewhat tedious.

EXAMINATION OF THE FINAL PRECIPITATE

The final product containing the round structures (assumed hexagonal crystals) contains considerable amounts of amorphous proteins. Thus, assigning arginase activity to a particular constituent in the mixture is impossible. Tests for the presence of catalase, phosphatase and esterase were carried out showing only the presence of the latter in rather large concentration.

The heterogeneous nature of the preparation was further ascertained by means of electrophoresis **. This was carried out in phosphate buffers of ionic strength 0.1 at pH values of 7.0 and 5.9. The solution containing 0.8 per cent protein was first dialyzed against running distilled water for 48 hours to remove the ammonium sulfate, followed by dialysis for 24 hours against the buffer which was to be used in the electrode compartments of the Tiselius aparatus.

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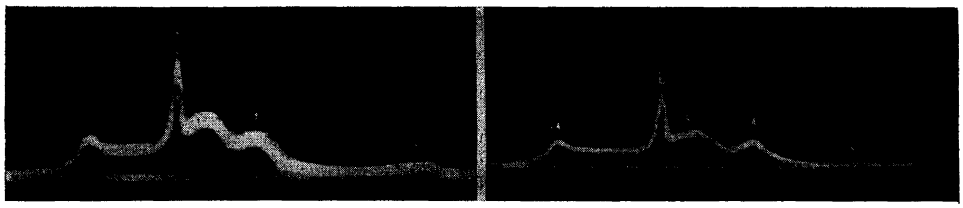
* On leave from Farouk Ist University, Alexandria, Egypt.

** Thanks are due to Dr. E. Hultin of this institute for running the tests and for his continuous help and cooperation.



a *b*

Fig. 1. Electrophoresis of Bach's purified arginase. a: pH 7.0 (ascending limb). b: pH 5.9 (ascending limb).

The diagrams reproduced (Fig. 1) show the presence of five proteins in the preparation. They all move towards the anode at both pH 7.0 and 5.9 except the slowest component a which moves towards the cathode at the latter pH. Table 1 gives the mobilities of the components at the two pH values.

Table 1. Calculated mobilities of the components present in Bach's purified arginase.

Boundary	Mobility ($\times 10^5$ cm^2 volt^{-1} sec^{-1})	
	pH 7.0	pH 5.9
a	0.35	— 0.66
b	0.92	0.25
c	1.45	0.46
d	1.75	0.80
e	2.95	2.00

Electrophoresis of arginase partially purified according to Mohamed and Greenberg [2] showed the presence of the three components having mobilities corresponding to boundaries b, c, and e (Fig. 2).

Fig. 2. Electrophoresis of purified arginase (Mohamed and Greenberg). pH 7.0 (descending limb).

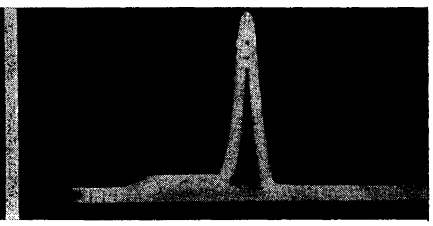


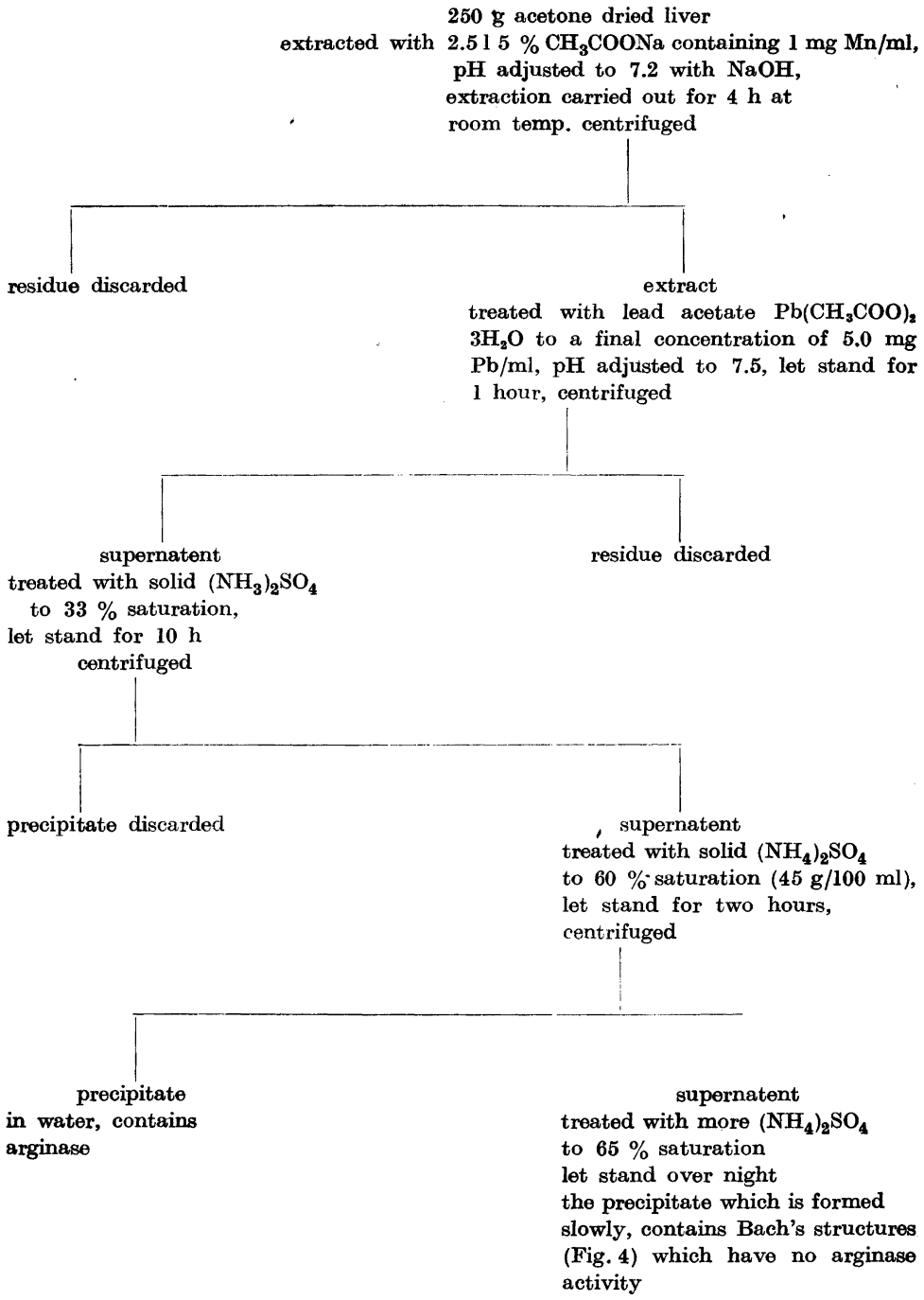
Fig. 3. Electrophoresis of crystalline liver esterase. pH 7.0 (ascending limb).

Fig. 4. Round structures of Bach.

The crystallization of a protein from liver possessing esterase activity was achieved [3]. Electrophoresis experiments were carried out at pH 7.0 (Fig. 3), and pH 5.8 showing the presence of a single homogeneous component with a mobility of 2.96 ($x10^5 cm^2 volt^{-1} sec^{-1}$) at pH 7.0 and 1.98 at pH 5.8. These values correspond very closely to those of component e in Bach's preparation.

A close study under the microscope of the round structures, claimed to be arginase crystals, revealed that each is composed of a network of filaments with part of the mother liquor and amorphous proteins entrapped inside along with one or more air bubbles. The presence of air bubbles could be observed by putting a slide with a part of the suspension in a vacuum desiccator. The bubbles could clearly be seen to expand in volume, then collapse on exposure to the air. The whole structure is then partly or completely destroyed. This also occurs when the preparation is left to dry on the slide giving the illusion af resolution.

The next step was to find out if these structures actually constituted the enzyme arginase. A procedure was finally adopted by which they were separated in a fraction which proved to be completely devoid of arginase activity. The enzyme was separated in a different fraction. The procedure is outlined as follows:


250 g acetone dried liver
extracted with 2.5 l 5 % CH_3COONa containing 1 mg Mn/ml, pH adjusted to 7.2 with NaOH, extraction carried out for 4 h at room temp. centrifuged
residue discarded
extract
treated with lead acetate $Pb(CH_3COO)_2$ $3H_2O$ to a final concentration of 5.0 mg Pb/ml, pH adjusted to 7.5, let stand for 1 hour, centrifuged
supernatent
treated with solid $(NH_3)_2SO_4$ to 33 % saturation, let stand for 10 h centrifuged
residue discarded
precipitate discarded
supernatent
treated with solid $(NH_4)_2SO_4$ to 60 % saturation (45 g/100 ml), let stand for two hours, centrifuged
precipitate
in water, contains arginase
supernatent
treated with more $(NH_4)_2SO_4$ to 65 % saturation
let stand over night
the precipitate which is formed slowly, contains Bach's structures (Fig. 4) which have no arginase activity

Moreover, if the crude liver extract is incubated with trypsin in presence of Mn ions before employing Bach's method the round structures fail to appear in the final product yet it still possesses powerful arginase activity.

ACTIVATION BY MANGANESE

Contrary to Bach's observation it was clearly found that manganous ions powerfully activate the enzyme in all stages of the purification. The purest stage in Bach's method (47 per cent saturated ammonium sulfate precipitate) is strongly activated by both cobalt and manganese salts. Incubation of the extract with 2.5×10^{-3} *M* of either Mn^{++} or Co^{++} for one hour at 40° C and pH 7.0 resulted in 2—3 fold activation.

SUMMARY

Electrophoretic studies of arginase purified according to Bach showed the heterogeneous nature of the preparation.

The round structures are neither crystalline nor do they contain arginase activity.

Manganese and cobalt salts are powerful activators of the enzyme in all stages of purification so far attained.

The author expresses his gratitude to Prof. K. Myrbäck, head of the institute for inviting him to work in his laboratories and for the support and keen interest he has been showing throughout the work. The continuous help and interest of Dr. G. Neumüller are deeply appreciated.

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Received October 11, 1948.

ACTA CHEMICA SCANDINAVICA 3 (1948) 61—64

On Relations between Activation Energies and Frequency Exponents in Chemical Kinetics

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It is well known from the literature [1–3] that a relation exists between the two parameters of the Arrhenius equation [4]. The relation is often approximately linear. As shown by Polanyi and Evans [3] similar relations also occur in the case of equilibrium constants, *e. g.* in case of solubilities.

An explanation of such relations has been given in a special case by Schwab[2]. Schwab considers the case of catalysts of different catalytic activities, and he has shown by rather conclusive experiments that there is a correlation between the preparation temperature of the catalyst and the activation energy and the frequency exponent (the logarithm of the frequency factor) of the same reaction catalysed by catalysts of different activities.

Of course, such assumptions are excluded in the case of reactions in homogeneous systems. For such systems rather satisfactory explanations have been given by Moelwyn-Hughes [5] and by la Mer and co-workers [6].

It seems, however, that in principle the matter may be treated in a simpler and perhaps more general way. The idea is that in the expression for the logarithm of the velocity constant, the factor over T or RT must represent the free energy of activation (comp.Christiansen [7]), but what we measure is on account of the restricted temperature range always the energy of activation that is, what we know is only the tangent of the $(\ln k,\ T^{-1})$ curve, not the curve itself.

We may remark in passing that strictly speaking we should in the above replace the word energy by enthalpy as in the case of solutions we always measure velocities at constant pressure.

Let

$$\ln k = -\frac{G}{T} + B \qquad (1)$$

where B is independent of temperature.

From this we get

$$H = T^2 \frac{\partial}{\partial T} \ln k = G - T \frac{\partial G}{\partial T} \tag{2}$$

If we have no theory for the evaluation of G as a function of T, we cannot do better than empirically to determine a value H_0 around a certain temperature T_0. In other words, we replace the true curve by its tangent, the position and direction of which may be derived from experiments with a fair accuracy. Let the empirical equation of the tangent be

$$\ln k = -\frac{H_0}{T} + I_0 \tag{3}$$

Now (1) and (3) are not identical, but at $T = T_0$ the values of $\ln k$ according to the two equations must coincide. We therefore get

$$G_0 - H_0 = T_0(B - I_0) \tag{4}$$

or, when applying (2):

$$\left(\frac{\partial G}{\partial T}\right)_0 = B - I_0 \tag{5}$$

Now there are cases where some theory shows that G depends significantly on T. The meaning of this somewhat loose expression may be illustrated by means of two examples.

1. A linear dependence on T cannot be significant as any member in G of the form aT after division by T must disappear in the constant B of equation (1).

2. A member in G of the form $bT \ln T$ cannot be significant if the pure number b is not excessively great, as in the expression for k we get in that case a factor of the form T^b before the exponential term against which the form of the exponential curve is very insensitive. The same thing appears from the fact that in expressions of the form

$$-\frac{Q}{RT} + b \ln T$$

$b \ln T$ can with fair approximation be considered a constant even if T at ordinary or higher temperature is varied between rather wide limits.

There are, however, cases where some theory shows that G is at least approximately of the form $A + KD^{-1}$, where A and K are constants while D is strongly

dependent on T. For instance in the case of ionic reactions in solutions D may represent the dielectric constant of the solvent [8,9]. It is known [5] that D is strongly variable with temperature as it may be represented approximatively by $D = Ce^{-\alpha T}$.

Consequently we get

$$\frac{\partial G}{\partial T} = KD^{-1}\alpha \tag{6}$$

or from (5)

$$B - I_0 = KD_0^{-1}\alpha \tag{7}$$

where D_0 is the value of D at T_0.

Furthermore from (2)

$$H = A + KD^{-1}\ (1 - \alpha T) \tag{8}$$

or

$$H_0 = A + KD_0^{-1}\ (1 - \alpha T_0) \tag{9}$$

By insertion of (7) we get

$$H_0 = A + (B - I_0)\ (\frac{1}{\alpha} - T_0) \tag{10}$$

that is if we compare different reactions with equal values of A and B and in the same solvent (same α), a linear relation between the different values of H_0 and I_0 will exist if the same T_0 is chosen. If the solvents are different (different α's), the relation may not be linear, but an interdependency will still exist, and if the different T_0's are chosen so as to make $\frac{1}{\alpha} - T_0$ constant the relation becomes linear.

By a completely analogous reasoning it can be proved that a similar relation prevails in the case of equilibrium constants.

It should be added that Waring and Becher have recently shown [10] that for the fluidity of liquids similar relations exist, and furthermore that the thermodynamic properties of the fluidity function seem to reappear in the functions expressing the velocity constants for reactions in the same liquids. This seems to mean that the fluidity (or the diffusion coefficient) enters into the expression for the free enthalpy of activation, G.

SUMMARY

A derivation from well known assumptions of the relationship between activation-energy and frequency-exponent is given.

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Received December 23, 1948.

ACTA CHEMICA SCANDINAVICA 3 (1949) 65—71

Relations Between Vapour Pressures and Solubilities of Hydrates

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Let S denote a non-volatile substance which forms two crystalline hydrates S_m and S_n, respectively with m and n molecules of water per molecule of S (m is different from n, one of them may be zero). p^0 denotes the vapour pressure of pure water, $p_{m,n}$ that of a mixture of S_m and S_n. p_m and p_n indicate the vapour pressures of the saturated solutions of, respectively, S_m and S_n. The activities of S in the same solutions are denoted by a_m and a_n.

When $(n-m)\,dx$ moles of water vapour are transferred, reversibly and isothermally, from pure water to the mixture of crystalline S_m and S_n, dx moles of S_n are produced at the expense of dx moles of S_m. The work gained is

$$dA = (n-m)\ RT \ln \frac{p^0}{p_{m,n}}\, dx \qquad (1)$$

The same transformation may be performed in the following way. dx moles of crystalline S_m are dissolved in a saturated solution of S_m and transferred, reversibly and isothermally, from this solution to a saturated solution of S_n. At the same time, $(n-m)\,dx$ moles of water are transferred from pure water to the saturated solution of S_n. By crystallization, dx moles of S_n are obtained. The work gained may be written as follows

$$dA = RT\left[\ln \frac{a_m}{a_n} + m \ln \frac{p_m}{p_n} + (n-m) \ln \frac{p^0}{p_n}\right] dx \qquad (2)$$

Combination of this equation with equation 1 leads to

$$(n-m)\ln p_{m,n} = \ln \frac{a_n}{a_m} + n \ln p_n - m \ln p_m \tag{3}$$

If S is a salt, a_n and a_m are the thermodynamic solubility products of S_n and S_m, that is, the solubility products containing the activities of the ions instead of the concentrations. If c_m and c_n denote the molar concentrations of the two saturated solutions, ν the number of ions produced by the dissociation of one molecule of S, and f_m and f_n the mean activity coefficients of the ions in the two solutions, equation 3 may be written as follows

$$(n-m)\log p_{m,n} = \nu \log \frac{c_n f_n}{c_m f_m} + n \log p_n - m \log p_m \tag{4}$$

If the solubilities are sufficiently small one may set $p_n = p_m = p^0$, and obtain

$$(n-m)\log \frac{p_{m,n}}{p^0} = \nu \log \frac{c_n f_n}{c_m f_m} \tag{5}$$

When we apply the Gibbs-Duhem relation to an aqueous solution of S, we obtain

$$x\,\mathrm{dln}\,a + (1-x)\,\mathrm{dln}\,a_{H_2O} = 0$$

where x denotes the mole fraction and a the activity of S while a_{H_2O} indicates the activity of the water. Owing to the proportionality between a_{H_2O} and the vapour pressure p one obtains from this relation

$$x\,\mathrm{dln}\,a + (1-x)\,\mathrm{dln}\,p = 0$$

and by integration

$$\ln \frac{a_n}{a_m} = \ln \frac{p_n}{p_m} - \int_{x_m}^{x_n} \frac{\mathrm{dln}\,p}{x} \tag{6}$$

On introducing this expression into equation 3, one obtains

$$(n-m)\ln p_{m,n} = (n+1)\ln p_n - (m+1)\ln p_m - \int_{x_m}^{x_n} \frac{\mathrm{dln}\,p}{x} \tag{7}$$

An exact solution of the integration in this formula cannot be given since p, in general, is not known as a function of x. If, however, the vapour pressures

of the unstable solutions in the concentration interval from x_m to x_n have been measured, a numerical value of the integral may be computed.

An approximate formula may be deduced from equation 7 when we assume that p, in the interval from x_m to x_n, is a linear function of x

$$p = P(1 - kx) \tag{8}$$

where the constants P and k are given by the expressions

$$P = \frac{x_n p_m - x_m p_n}{x_n - x_m}$$

and

$$k = \frac{p_m - p_n}{x_n p_m - x_m p_n} \tag{9}$$

By means of equation 8, one obtains

$$-\int_{x_m}^{x_n} \frac{\mathrm{d}\ln p}{x} = k\int_{x_m}^{x_n} \frac{\mathrm{d}x}{x(1 - kx)} = k \ln \frac{x_n}{x_m} - k \ln \frac{p_n}{p_m} \tag{10}$$

On introducing this expression into equation 7, one obtains

$$(n - m) \log p_{m,n} = (n + 1 - k) \log p_n - (m + 1 - k) \log p_m + k \log \frac{x_n}{x_m} \tag{11}$$

By means of this formula, one may calculate an approximate value of the vapour pressure $p_{m,n}$ of the mixture of the crystals S_m and S_n when the solubilities of the two hydrates and the vapour pressures of their saturated solutions have been measured.

Let S_m be the stable hydrate. If x_m, p_m, $p_{m,n}$, and, in addition, the vapour pressures and concentrations of a series of unsaturated solutions ($x < x_m$) have been measured, but the measurements have not been extended to solutions supersaturated with S_m, approximate values of x_n and of p_n may be found in the following way. The tangent to the experimental vapour pressure curve in the point x_m, p_m is drawn. It is assumed as an approximation that the slope ($-\alpha$) of this tangent is equal to the slope ($-kP$) of the straight line represented by equation 8. Hence, one has

$$P = p_m + \alpha x_m \tag{12}$$

$$k = \frac{\alpha}{p_m + \alpha x_m} \tag{13}$$

On introducing

$$p_n = P(1 - kx_n) \tag{14}$$

into equation 11, and rearranging the terms, one finds

$$(n + 1 - k) \log (1 - kx_n) + k \log x_n = (n - m) \log p_{m,n} + (m + 1 - k) \log p_m + k \log x_m - (n + 1 - k) \log P \tag{15}$$

By means of this formula, an approximate value of the solubility x_n of the unstable hydrate may be computed. The corresponding vapour pressure may be estimated by means of equation 14.

If only x_m, p_m, and $p_{m,n}$ (but not the vapour pressures of the unsaturated solutions) have been measured, one may, by means of equations 15 and 14, find rather rough approximations to x_n and p_n when one sets

$$\alpha = \frac{p^0 - p_m}{x_m}$$

and, consequently, according to equations 12 and 13

$$P = p^0 \text{ and } k = \frac{p^0 - p_m}{p^0 x_m}$$

We shall now examine the case where one of the hydrates, say the m-hydrate, exists in two crystalline forms, S_m and S_m' of which S_m is supposed to be the stable (or more stable) modification. In the following, the symbols referring to the form S_m' are dashed. When equation 3 is applied to S_m' instead of S_m, one obtains

$$(n - m) \ln p_{m,n}' = \ln \frac{a_n}{a_m'} + n \ln p_n - m \ln p_m'$$

From this equation and equation 3 by subtraction

$$(n - m) \ln \frac{p_{m,n}}{p_{m,n}'} = \ln \frac{a_m'}{a_m} - m \ln \frac{p_m}{p_m'} \tag{16}$$

By the same procedure one obtains from equations 5 and 7

$$(n - m) \log \frac{p_{m,n}}{p_{m,n}'} = \nu \log \frac{c_m' f_m'}{c_m f_m} \tag{17}$$

and

$$(n - m) \ln \frac{p_{m,n}}{p_{m,n}'} = - (m + 1) \ln \frac{p_m}{p_m'} - \int_{x_m}^{x_m'} \frac{\mathrm{d} \ln p}{x} \tag{18}$$

If we assume, as an approximation, that the linear relation 8 is valid in the interval between x_m and x_m', we obtain, in analogy with equation 11,

$$(n - m) \log \frac{p_{m,n}}{p_{m,n}'} = k \log \frac{x_m'}{x_m} - (m + 1 - k) \log \frac{p_m}{p_m'} \tag{19}$$

The constant k may be computed from the expression

$$k = \frac{p_m - p_m'}{x_m' p_m - x_m p_m'} \tag{20}$$

If only the vapour pressures of solutions where $x \leqq x_m$ have been measured, approximate values of the constants P and k in equation 9 may be found by means of equations 12 and 13. On introducing

$$p_m' = P (1 - k x_m') \tag{21}$$

into equation 19 and rearranging the terms, one obtains

$$(m + 1 - k) \log (1 - k x_m') + k \log x_m'$$
$$= (n - m) \log \frac{p_{m,n}}{p_{m,n}'} + k \log x_m - (m + 1 - k) \log \frac{p}{p_m} \tag{22}$$

By means of formulae 21 and 22 one may compute approximate values of the vapour pressure and concentration of the solution saturated with the unstable modification S_m' when $p_{m,n}$, $p_{m,n}'$, and the vapour pressure curve for $x \leqq x_m$ have been determined.

If only $p_{m,n}$, $p_{m,n}'$, x_m, and p_m have been determined, we may find very rough approximations to x_m' and p_m' by means of equations 22 and 21 when we set $P = p^0$ and $k = (p^0 - p_m)/p^0 x_m$.

The following examples will illustrate the application of some of the formulae given above.

1. Calculation of the decomposition pressure $p_{1,2}$ of the unstable dihydrate of calcium oxalate. The solubilities of the mono- and di-hydrate of calcium oxalate in water at 25° C have been measured by the author of this paper [1] who found $c_1 = 4.84 \cdot 10^{-5}$ and $c_2 = 7.65 \cdot 10^{-5}$. By means of the Debye-Hückel limiting law, $-\log f = 0.5 \cdot 2^2 \cdot \sqrt{4c}$, we find $-\log f_1 = 0.028$ and $-\log f_2 = 0.035$. Hence, by equation 5, $p_{1,2} = 2.42\ p^0$. Although the decomposition pressure is 2.42 times the vapour pressure of water the dry dihydrate does not decompose at room temperature. A 9 years old preparation, which had been stored in an ordinary powder glass, was still almost pure dihydrate.

2. Calculation of the decomposition pressure $p_{0,2}$ of potassium fluoride dihydrate. Lannung [2] has measured the concentrations and vapour pressures of aqueous solutions saturated with KF and $KF,2H_2O$ at 18° C. He found $x_0 = 0.272$, $p_0 = 2.8$ mm Hg, $x_2 = 0.201$, $p_2 = 5.2$ mm Hg. By means of equations 9 and 11, one computes: $k = 2.75$, $\log p_{0,2} = 0.292$, and $p_{0,2} = 2.0$ mm Hg. Lannung found by direct measurement $p_{0,2} = 2.1$ mm Hg.

3. Calculation of the solubility of anhydrous sodium bromide. $NaBr,2H_2O$ is stable in contact with its saturated solution below 51° C, above that temperature the stable form is NaBr. Lannung [2] has measured the vapour pressure $p_{0,2}$ of a mixture of the two hydrates and the vapour pressures of solutions of sodium bromide at 18° C. He found $p_{0,2} = 5.15$ mm Hg, and, for the solutions,

x	0.0501	0.0591	0.0727	0.0888	0.1086	0.1353 ($=x_2$)
p (mm Hg)	13.8	13.3	12.7	11.9	10.9	9.35

The slope of the tangent to the vapour pressure curve in the point x_2, p_2 is $\alpha = -58.2$ from which we find, by means of equations 12 and 13, $P = 17.22$ and $k = 3.38$. x_0 may now be computed from formula 15 ($m = 2$, $n = 0$). We find $x_0 = 0.160$. The corresponding vapour pressure is, according to formula 14, $p_0 = 7.9$ mm Hg. The solubility of anhydrous sodium bromide above the transition point 51° C has been determined by several investigators [3]. By extrapolation of the data one finds that the solubility of NaBr at 18° C is 53.0 g NaBr in 100 g saturated solution, or $x_0 = 0.165$.

SUMMARY

The paper gives the deduction of a series of exact or approximate formulae relating the vapour pressure of a mixture of two hydrates of the same substance to the solubilities of the hydrates in water and certain vapour pressure

data for the solutions. Examples illustrating the application of some of the formulae are given.

The formulae given in this paper were deduced on the suggestion of Mr. A. Lannung, the leader of the pharmaceutical department of The Royal Veterinary and Agricultural College. My thanks are due both to Professor N. Bjerrum and to Mr. A. Lannung for discussion of some of the problems.

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Received November 16, 1948.

ACTA CHEMICA SCANDINAVICA 3 (1949) 72—81

Beitrag zur Kenntnis der Natriummetaphosphate. III

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Es ist in den beiden vorhergehenden Aufsätzen [1] gezeigt worden, dass man mit dem Ionenaustauschverfahren eine experimentell einfache und schnelle Methode zur Verfügung hat zur Darstellung der Metaphosphorsäuren aus ihren Salzen. Diese Methode ermöglicht es, das chemische Verhalten dieser an sich instabilen Verbindungen an reinen Präparaten zu studieren. Im Anschluss an Untersuchungen der den Grahamschen Salzen entsprechenden Polymetaphosphorsäuren erschien es von Interesse, auf ähnliche Weise die primären Hydratationsprodukte des Phosphor-V-oxyds zu studieren, da hierüber bisher ebenfalls nur widersprechende Angaben vorliegen.

Nach Travers und Yu [2] soll beim Auflösen von Phosphor-V-oxyd in Wasser Dimetaphosphorsäure, $H_2P_2O_6$, entstehen, während Pascal [3] für den durch Erhitzen von Phosphor-V-oxyd mit Äthyläther dargestellten Metaphosphorsäure-Äthylester durch Schmelzpunktserniedrigung in Naphthalin ein Molekulargewicht von 657 fand, welches also einer sechsfachen Aggregation entsprechen würde. Von anderen Autoren wurde durch Messung der Siedepunktserhöhung in Chloroform ein Wert erhalten, welcher auf die dimere Form dieses Esters hindeutet.

Um festzustellen, ob es überhaupt möglich ist, bei der Auflösung von Phosphor-V-oxyd in Wasser zu einem einheitlich zusammengesetzten Produkt zu gelangen, wurde versucht, die bei der Hydratation unter verschiedenen Versuchsbedingungen gebildeten Phosphorsäuren analytisch zu erfassen. Die Analysen wurden nach dem von Wurtzschmidt und Schuhknecht [4] beschriebenem Verfahren durchgeführt, nach welchem in Bezug auf Ortho- und Pyrophosphat zuverlässige Werte zu erhalten sind.

Nach diesem Verfahren wird Orthophosphat durch Extraktion der mit Molybdat gebildeten und in organischen Lösungsmitteln löslichen Phosphor-Molybdänsäure mit Essigester abgetrennt, während Pyrophosphat durch Titration der bei der Fällung von saurem Natriumpyrophosphat mit Zinkjodid-Lösung freigemachten Jodwasserstoffsäure

mit Lauge bestimmt wird. Die Differenz zu dem gesamten in der Analysenlösung vorhandenen Phosphor wurde als Metaphosphat angenommen, was sich im Verlauf der Untersuchung auch als richtig erwies.

Für die Reaktion $P_2O_5 + aq \rightarrow$ Säure wurden zwei extreme Fälle gewählt, um Einblick zu gewinnen, welchen Einfluss die Versuchsbedingungen auf die Zusammensetzung der entstehenden Produkte haben. Als Ausgangsprodukt wurde im Sauerstoffstrom bei ca 280° C sublimiertes Phosphor-V-oxyd verwendet.

Versuch 1. Phosphor-V-oxyd wurde der Einwirkung feuchter Luft ausgesetzt, bis sich eine nahezu klare, sirupöse Masse gebildet hatte, welche sodann in dest. Wasser gelöst wurde.

Versuch 2. In einen grossen Überschuss eisgekühlten dest. Wassers wurde unter Umrühren Phosphor-V-oxyd portionsweise eingetragen.

Tabelle 1 gibt die in den beiden Lösungen analytisch bestimmten Werte für Ortho- und Pyrophosphat auf den totalen Phosphorgehalt der Lösung bezogen wieder. Der Rest wurde als Metaphosphat unbekannter Anionengrösse berechnet.

Tabelle 1. Zusammensetzung der nach Versuch 1 und 2 erhaltenen Phosphorsäuregemische.

Versuch Nr.	$(PO_4)^{3-}$ %	$(P_2O_7)^{4-}$ %	$(PO_3)_n^-$ %
1	85	10	5
2	5	5	92

Die in Tabelle 1 wiedergegebenen Werte sind auf ganze Zahlen abgerundet, da das angewendete Analysenverfahren eine genauere Bestimmung kaum zulässt. Aus den Analysenwerten ergibt sich, dass es ganz von den Versuchsbedingungen abhängig ist, bis zu welcher Stufe die Hydratation fortschreitet.

Zur Reindarstellung der nach Versuch 2 hauptsächlich entstehenden Metaphosphorsäure wurde, um die Bildung von Ortho- und Pyrophosphorsäure nach Möglichkeit auszuschalten, folgender massen verfahren: Phosphor-V-oxyd wurde unter kräftigem Umrühren portionsweise in 0,1 *N* Natronlauge unter Kühlung eingetragen bis zur neutralen Reaktion gegen Phenolphthalein. Es ergab sich hierbei eine anfänglich trübe Lösung, welche sich nach wenigen Minuten klärte und aus welcher das gebildete Salz durch Zugabe von Äthylalkohol gefällt werden konnte. Es wurde hierbei ein kristalliner, bisweilen seidenglänzender, fiberartiger Niederschlag erhalten, welcher abfiltriert, mit Alkohol und Äther gewaschen und schliesslich bei Raumtemperatur im Vacuum getrocknet wurde.

Die Analyse dieses Salzes ergab das für Metaphosphate charakteristische Atomverhältnis Na : P : O = 1 : 1 : 3. Der Phosphorgehalt auf P_2O_5 betrechnet ergab einen Wert von 56,5%, was auf einen Kristallwassergehalt entsprechend der Formel $NaPO_3 \cdot 4/3\ H_2O$ schliessen liess und mit dem Glühverlust übereinstimmte.

IONENGEWICHTSBESTIMMUNGEN DURCH DIALYSE

Sowohl an der nach Versuch 2 dargestellten wässrigen Lösung von Phosphor-V-oxyd wie auch an 0,1 *N* Lösungen des Natriumsalzes und der durch Ionenaustausch daraus dargestellten Säure wurde das Anionengewicht durch Dialyse bestimmt, wie dies schon früher [1] beschrieben worden ist. Die hierbei erhaltenen Werte sind in Tabelle 2 zusammengefasst. Für das Anion der Lösung von Phosphor-V-oxyd in Wasser wurde ein etwas niedrigerer Wert gefunden, als einer sechsfachen Aggregation entspricht, was sicher auf Spuren von Ortho- resp. Pyrophosphat zurückzuführen ist. Die Werte für das Natriumsalz und für die aus diesem durch Ionenaustausch dargestellte Säure weisen jedoch eine gute Übereinstimmung mit dem theoretischen Wert auf, welcher bei sechsfacher Aggregation für das Natriumsalz 612 beträgt. Für das Natriumsalz wurden hierbei auch bei verschiedener Dialysendauer nahezu konstante Dialysenkoeffizienten gefunden, woraus hervorgeht, dass nur *eine* Art von Metaphosphationen in der Lösung enthalten war.

Tabelle 2. Anionen- und Molekulargewicht dieser Metaphosphorsäure.

Präparat	Anionengewicht	Molekulargewicht
P_2O_5-Lösung (Vers. 2)	465	471
$[NaPO_3]_n$	480	618
$[HPO_3]_n$	475	481

DIE ÄQUIVALENTLEITFÄHIGKEIT DES NATRIUMSALZES

Der Verlauf der Äquivalentleitfähigkeit dieses Salzes mit steigender Verdünnung liegt, wie aus Fig. 1 hervorgeht, nahe den für Natriumtrimetaphosphat gefundenen Werten, jedoch ist der Kurvenverlauf mehr denen der Polymetaphosphate ähnlich. In Tabelle 3 und Fig. 1 sind die bei 20° C erhaltenen Messresultate wiedergegeben.

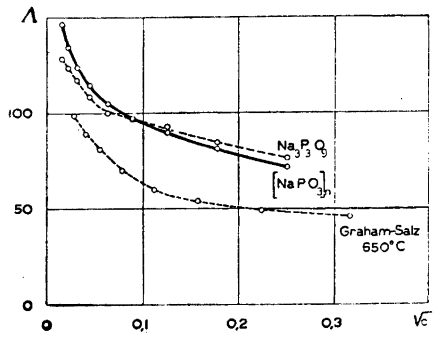


Fig. 1. Äquivalentleitfähigkeit bei 20° C.

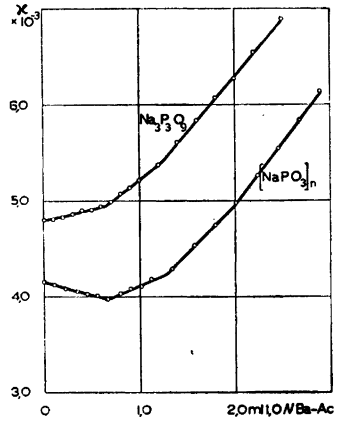


Fig. 2. Konduktometrische Titration von 20 ml 0,1 N. Natriummetaphosphat mit 1,0 N Bariumacetat.

Tabelle 3. Äquivalentleitfähigkeit von $[NaPO_3]_n$.

Verdünnung $\frac{1}{c}$	Λ 20° $[NaPO_3]_n$
16	71,8
32	81,5
64	89,8
128	97,6
256	105,0
512	114,7
1024	124,0
2048	134,5
4096	146,5

KOMPLEXBILDUNG MIT ERDALKALI-IONEN

Das Komplexbindungs vermögen des Natriumsalzes dieser Metaphosphorsäure wurde durch konduktometrische Titration der wässrigen Lösung mit Bariumacetat studiert. Aus der hierbei erhaltenen Titrationskurve, welche in Fig. 2 wiedergegeben ist, geht hervor, dass dieses Salz zur Bildung eines stabilen Komplexes der Zusammensetzung $Na_4[Ba(PO_3)_6]$ befähigt ist. Bei der Zugabe von Bariumacetat sinkt die Leitfähigkeit zunächst geradlinig ab bis zu einem Punkt bei $Na^+ : Ba^{2+}/2 = 3 : 1$, und die Leitfähigkeitskurve durchläuft hier einen scharfen Wendepunkt. Im weiteren Verlauf der Kurve werden zwei Knickpunkte schwach angedeutet, nämlich der eine bei $Na^+ : Ba^{2+}/2 =$

3 : 2 und der andere schliesslich im Äquivalenzpunkt. Die erhaltene Titrationskurve unterscheidet sich also deutlich von denen, welche bei der konduktometrischen Titration der Grahamschen Salze erhalten wurden und ist in ihrem Verlauf jenseits des Wendepunktes eher derjenigen des Natriumtrimetaphosphats ähnlich. Die sekundäre Dissociation des komplexen Anions $[Ba(PO_3)_6]^{4-}$ ist auch bei höherer Temperatur so niedrig, dass beispielsweise durch Zugabe von Sulfat-Ionen das komplex gebundene Barium nicht gefällt werden kann.

DAS THERMISCHE VERHALTEN DES NATRIUMSALZES

Das thermische Verhalten dieses Natriummetaphosphats wurde mit Hilfe von Le Chatelier's Methode untersucht. Diese Methode ermöglicht eine relative Bestimmung der Wärmemengen, welche bei den während der Erhitzung auftretenden thermischen Effekten aufgenommen oder abgegeben werden. Diese sind durch Aufnehmen von Abkühlungskurven nicht zu erfassen, da es sich hierbei um praktisch irreversible Reaktionen handelt, z. B. Kristallwasserabgabe, Kristallumwandlung und schliesslich Polymerisation.

Zur Feststellung des Schmelzpunktes und anderer thermischer Effekte wurde dieses Salz in einem Stahlblock gemäss Fig. 3 mit wasserfreiem Aluminiumoxyd als Vergleichssubstanz unter nahezu konstanter Temperatursteigerung von 13° C/min bis über die Schmelztemperatur erhitzt. Zur Messung der Temperatur des Stahlblockes sowie für das verwendete Differentialthermoelement wurden Hoskinsdrähte verwendet. Der Stahlblock wurde in einem elektrischen Ofen erhitzt.

Fig. 4 zeigt die Thermokurven für dieses Natriummetaphosphat sowie für Natriumdimetaphosphat, $Na_2P_2O_6 \cdot 2\,H_2O$ und Natriumtrimetaphosphat $Na_3P_3O_9$. Im Temperaturbereich zwischen Raumtemperatur und dem Schmelzpunkt konnten drei deutlich ausgeprägte, endotherme Effekte festgestellt werden, nämlich bei ca. 150, 330 und 540° C. Bei 150° C wird die Hälfte des Kristallwassers, $4\,H_2O$ auf $Na_6P_6O_{18}$ berechnet, abgegeben. Bei 330° C ist wahrscheinlich eine bei ca. 250° C beginnende Kristallumwandlung beendet, denn es konnte bei dieser Temperatur kein weiterer Gewichtsverlust festgestellt werden. Erst bei ca. 540° C, also nahe der Schmelztemperatur, wird unter starker Kontraktion der anfänglich voluminösen Kristallmasse das restliche Kristallwasser abgegeben. Bei 595° C beginnt dieses Natriummetaphosphat unter Polymerisation zu Grahamschen Salz zu schmelzen zum Unterschied von Natriumtrimetaphosphat, dessen Schmelztemperatur bei 630—640° C liegt. Der Übergang zu der hochpolymeren Verbindung scheint durch eine kaum merkbare Schmelzwärme gekennzeichnet zu sein.

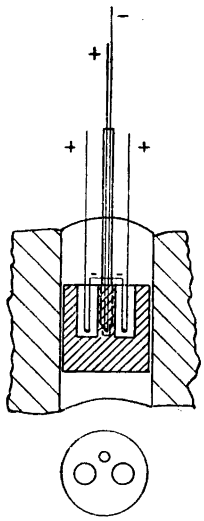


Fig. 3. Messapparatur zur thermischen Analyse.

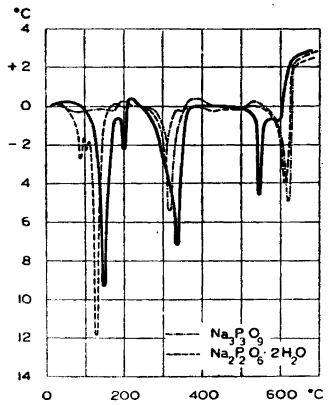


Fig. 4. Thermokurven der Natriummetaphosphate.

POTENTIOMETRISCHE TITRATION UND ZERFALLSGESCHWINDIGKEIT DIESER METAPHOSPHORSÄURE

Durch Ionenaustausch wurde aus dem Natriumsalz die diesem Salz entsprechende freie Metaphosphorsäure dargestellt und unmittelbar danach sowie bei verschiedenen Zeitpunkten nach der Darstellung potentiometrisch mit Kalilauge titriert.

Es ergab sich auch hier wiederum für die frisch bereitete Säure eine Titrationskurve, welche charakteristisch ist für eine starke Mineralsäure in Bezug auf sämtliche Wasserstoff-Ionen. In Analogie zu den früher untersuchten Metaphosphorsäuren bildet sich allmählich ein zweiter Potentialsprung aus, welcher sich langsam verschiebt. Fig. 5 zeigt die Titrationskurve der frisch bereiteten Säure und einige der für die gleiche Säure bei verschiedenen Alterungsgraden erhaltenen Potentialkurven. Der pH-Wert einer unzersetzten 0,1 *N* Metaphosphorsäure liegt bei 1,10—1,13. Mit zunehmender Alterung steigt dieser Wert und erreicht nach 720 h 1,40.

Die Ausbildung und Verschiebung des zweiten Potantialsprunges, welcher bei pH $\approx$ 10, also etwas niedriger als bei den Kurven der hydratisierten Trimeta- und Polymetaphosphorsäuren, liegt, gibt die Möglichkeit zur Bestimmung der Zerfallsgeschwindigkeit, wie dies schon früher dargelegt worden ist[1]. In Fig. 6 ist log $c\,[HPO_3]_n$ gegen die Zeit aufgetragen. Man erhält auch für diese Metaphosphorsäure eine nahezu konstante Halbwertszeit,

und es handelt sich also bei dem Zerfall dieser Säure um eine Reaktion erster Ordnung. Die graphisch ermittelte Halbwertszeit beträgt bei Raumtemperatur ca. 280 h, hieraus ergibt sich die Zerfallskonstante:

$$k = \frac{-2{,}303 \log \frac{1}{2}}{t\,\frac{1}{2}}$$

$$k = \frac{0{,}693}{280} = 0{,}0025\ \mathrm{h}^{-1}$$

Vergleicht man diesen Wert mit den für Trimetaphosphorsäure und für die Polymetaphosphorsäuren gefundenen Zerfallskonstanten, so zeigt sich, dass diese Metaphosphorsäure wesentlich stabiler ist, die Halbwertszeit beträgt mehr als das Fünffache. Auch betreffs der bei der Hydratation gebildeten Spaltprodukte zeigt sich bei dieser Metaphosphorsäure ein Unterschied zu den früher untersuchten Säuren darin, dass der zweite Potentialsprung um ca. 0,3 pH-Einheiten tiefer liegt, was seinen Grund in einem procentual höheren Anteil an Orthophosphat-Ionen hat.

Parallel mit der potentiometrischen Untersuchung dieser Säure wurde der Spaltungsverlauf analytisch nach dem eingangs beschriebenen Verfahren verfolgt. In Tabelle 4 sind die Analysenwerte der durch Hydratation und Spaltung dieser Säure bei Raumtemperatur gebildeten Ortho- und Pyrophosphat-Ionen in Abhängigkeit von der Zeit in Procent auf den totalen Phosphorgehalt der Ausgangslösung berechnet zusammengefasst. Zum Vergleich sind die mit Hilfe der potentiometrisch bestimmten Zerfallskonstante berechneten Werte für unzersetzte Metaphosphorsäure aufgeführt. Die Übereinstimmung der analytisch gefundenen und aus k berechneten Werte lässt den Schluss zu, dass der Zerfall dieser Säure nicht über irgendwelche Zwischenstufen vorsichgeht.

Tabelle 4. Die Spaltung dieser Metaphosphorsäure in Ortho- und Pyrophosphorsäure bei Raumtemperatur.

Alter der Metaphosphorsäure h	0,1 *M* H_3PO_4 %	0,05 *M* $H_4P_2O_7$ %	0,1 *N* $[HPO_3]_n$ %	
			gef.	ber.
24	4,5	2	93,5	94,1
96	13	6	81	78,6
240	28	14	58	54,8

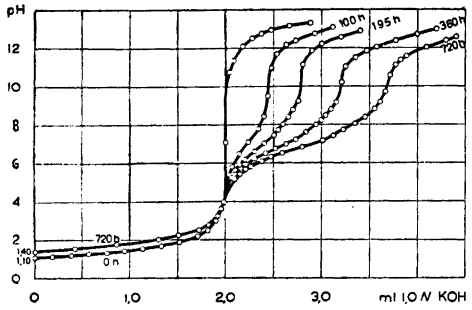


Fig. 5. Potentiometrische Titration von 20 ml 0,1 N Metaphosphorsäure bei verschiedenen Alterungsgraden.

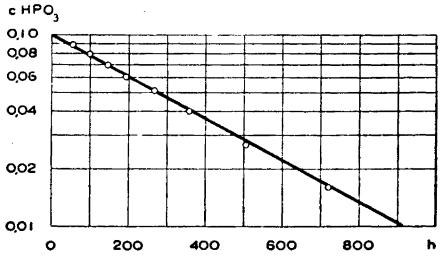


Fig. 6. Zerfallsgeschwindigkeit dieser Metaphosphorsäure bei Raumtemperatur.

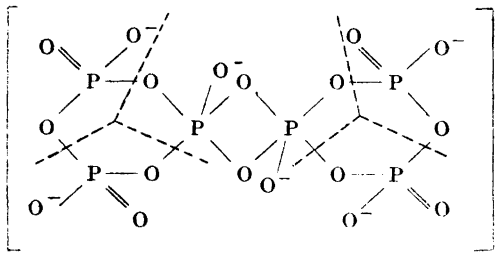
Es zeigt sich auch hier wiederum, dass die Hydratationsprodukte in einem bestimmten Mengenverhältnis zu einander stehen. Potentiometrische Vergleichstitrationen von hydratisierter Metasäure und Modellösungen, deren Zusammensetzung aus der Zerfallskonstanten berechnet wurde unter Zugrundelegung des analytisch ermittelten Verhältnisses Orthophosphat zu Pyrophosphat = 2 : 1, ergaben auch hier, wie dies schon früher bei Trimetaphosphorsäure und den Polymetaphosphorsäuren gezeigt worden ist, mit einander identische Titrationskurven.

Unter der Annahme, dass es sich bei dieser Säure tatsächlich um eine *Hexametaphosphorsäure* handelt, kann die Hydratation und Spaltung durch folgende Gleichung zum Ausdruck gebracht werden:

$$H_6P_6O_{18} + 5\ H_2O \rightarrow 4\ H_3PO_4 + H_4P_2O_7$$

DIE KONSTITUTION DIESES METAPHOSPHAT-ANIONS

Es erscheint schwierig, an Hand dieser Versuchsergebnisse mit einiger Sicherheit Aussagen über die Struktur dieses Metaphosphat-Anions machen zu können. Jedoch deutet der Verlauf der konduktometrischen Titrationskurve bei der Titration des Natriumsalzes mit Bariumacetat auf eine gewisse Ähnlichkeit mit dem Trimetaphosphat-Anion hin. Die Fähigkeit dieses Anions zur Komplexbildung mit Erdalkali-Ionen, ferner die Spaltung bei der Hydratation in 4 $(PO_4)^{3-}$ und 1 $(P_2O_7)^{4-}$ kann durch folgende Strukturformel zum Ausdruck gebracht werden.



Wie schon früher dargelegt worden ist [1], sind bei diesem Anion zwei der sechs Valenzen befähigt, ein zweiwertiges Metall-Ion komplex zu binden, während die übrigen vier Valenzen sich bezüglich der Salzbildung ähnlich verhalten sollten wie das Trimetaphosphat-Anion. Auch die Bildung eines kristallisierende Natriumsalzes mit 8 H_2O ist mit dieser Struktur in Einklang zu bringen, und zwar werden bei diesem Salz analog zu Natriumtrimetaphosphat je zwei Kristallwassermoleküle an die äusseren PO_3-Gruppen angelagert, wodurch auch diese Phosphoratome koordinativ sechswertig werden. Ein Hexametaphosphat nach der oben wiedergegebenen Strukturformel würde also den ersten Schritt der Polymerisation des Trimetaphosphats darstellen. Zum Unterschied von Grahamschen Salz ist dieses Metaphosphat jedoch noch zur Bildung kristallisierenden Salze befähigt.

SCHLUSSFOLGERUNGEN

Nach den im Vorstehenden beschriebenen Versuchsergebnissen zu urteilen entsteht bei der Hydratation des Phosphor-V-oxyds als primäres Reaktionsprodukt eine Metaphosphorsäure mit sechsfacher Aggregation. Die Salze dieser Säure sind also tatsächlich als *Hexametaphosphate* zu bezeichnen, eine Benennung, die immer noch fälschlicherweise für das Grahamsche Salz gebraucht wird. Die sechsfache Aggregation dieses Metaphosphats lässt vermuten, dass in dem Aufbau des Ausgangsstoffes eine Verkettung von sechs Phosphoratomen oder ein ganzzahliges Vielfaches davon in einer ähnlichen Konfiguration enthalten sein muss.

Für die Gasphase des Phosphor-V-oxyds haben Hampson und Stosick [5] auf Grund von Elektronenrefraktionsdiagrammen eine dem Hexamethylen-Tetramin ähnliche Struktur gefunden, welche vier Phosphoratome im Molekül enthält. Maxwell, Hendricks und Deming [6] sind jedoch auf Grund gleichartiger Untersuchungen zu dem Schluss gekommen, dass dem Phosphor-V-oxyd im gasförmigen Zustand eine andersartige Struktur zukommen muss. Die niedrige Sublimationstemperatur und der direkte Übergang von fester in gasförmige Phase lässt die Annahme zu, dass bei der Kondensation eine

so durchgreifende Umlagerung der Molekülstruktur kaum statthaben kann. Mit Hilfe von Hampsons Strukturformel ist jedoch eine sechsfache Aggregation des Phosphor-V-oxyds im festen Zustand nicht zu erklären.

ZUSAMMENFASSUNG

Untersuchungen der Hydratationsprodukte des Phosphor-V-oxyds lassen den Schluss zu, dass bei der Reaktion

$$P_2O_5 + aq \rightarrow \text{Säure}$$

primär eine Hexametaphosphorsäure gebildet wird. Durch Ionengewichtsbestimmungen wurde eine sechsfache Aggregation sowohl für die gebildete Metaphosphorsäure als auch für das dieser Säure entsprechende Natriumsalz gefunden. Die Äquivalentleitfähigkeit und das Komplexbildungsvermögen des Natriumsalzes in wässriger Lösung deutet auf eine Zwischenstellung in struktureller Hinsicht dieses Metaphosphat-Anions zwischen Trimetaphosphat und den Polymetaphosphaten hin. Der Übergang in die glasartig erstarrende Polymetaphosphatschmelze findet schon bei einer um ca. 30° C niedrigeren Temperatur statt als bei Trimetaphosphat. Die Säure ist eine starke Mineralsäure in Bezug auf sämtliche H^+-Ionen. Diese Säure wird ebenfalls wie Trimetaphosphorsäure und die Polymetaphosphorsäuren in Ortho- und Pyrophosphorsäure gespalten, jedoch beträgt die Halbwertszeit bei Raumtemperatur ca. das Fünffache derjenigen von Trimetaphosphorsäure.

Der erstgenannte Verfasser möchte an dieser Stelle Herrn Professor A. Ölander, Universität Stockholm, seinen Dank aussprechen für wertvolle Hinweise bei der Durchsicht der Manuskripte der zu diesem Thema veröffentlichten Aufsätze.

Die Untersuchungen wurden mit Mitteln der Königlich Schwedischen Academie der Ingenieurwissenschaften durchgeführt.

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Eingegangen am 22. Dezember 1948.

ACTA CHEMICA SCANDINAVICA 3 (1949) 82—85

Centaur X and Centaur Y. Two Unknown Substances in *Centaurea*-Species

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Spectrographic analysis in the ultraviolet has been carried out on alcohol extracts of the stalks and leaves of a large number of herbs, each of a different family.

Material from the cornflower, *Centaurea cyanus* L., showed a remarkable spectrum with a large number of bands. The same spectrum is also identifiable in *Centaurea jacea* L. and *Centaurea scabiosa* L., *i. e.* the two other *Centaurea*-species common in Sweden. The fraction which gives this spectrum is insolube in water but soluble in ether and has not previously been found in *Centaurea*-species. It is as yet impossible to state whether this fraction is to be found only in *Centaurea*-species. There is, nevertheless, no doubt that its occurrence in the flora is very rare.

It was found that the fraction in question was not uniform. By adsorption experiments on aluminium oxide and activated carbon it could be shown that the spectrum observed consisted of two spectra. The experiments were carried out as follows.

The fresh, finely ground stalks and leaves of *Centaurea cyanus* L. were extracted with ethanol until they were completely free from chlorophyll. After the addition of a large quantity of water the solution was extracted several times with ether. The strongly green-coloured ether solution was washed several times with water and then dried with sodium sulphate *. In this case an ethereal solution was prepared corresponding to 3.2 kg of fresh stalks and leaves per litre.

* A solution prepared in this way and stored for four years showed no change in the characteristic spectrum.

2.5 ml of 5 per cent methanolic potassium hydroxide solution was added to 20 ml of the ethereal solution. The mixture was shaken mechanically for two hours. A large quantity of water was added and the ether layer retained. This was then washed several times with water to remove the saponified chlorophyll; the washing must be done carefully in order to prevent emulsification. The resulting, strongly *yellow* ethereal solution was dried over-night with magnesium sulphate. The ether was evaporated and the residue dissolved in 20 ml of spectroscopically pure hexane. This solution again showed the characteristic spectrum of the original alcohol or ether solution (v. Fig. 1 a**).

As expected, the hexane solution gave a more distinct spectrophotogram than the other two solutions. The position of the absorption maxima of the bands is shown in Table 1, first row.

Table 1. Absorption bands in the ultraviolet of the lipoid-soluble fraction in Centaurea cyanus. Components Centaur X and Centaur Y after chromatographic analysis. The errors in the wave-lengths are about 0.5 mμ.

Hexane solution of	Wave-lengths of absorption maxima mμ								
Total fraction	350	320	305	290	278	269	259	254	244
Centaur X	350	326	307			269	259	254	245
Centaur Y		319	305	291	278				

The hexane solution was chromatographed on an aluminium oxide column (washing solution : pure hexane). The absorption in the ultraviolet of the outflowing solution was controlled by means of the quartz-spectrograph. The carotenoid pigments formed two zones: an upper, yellow, almost immobile zone and a moving reddish-yellow zone. It could be established with the quartz-spectrograph that the ultraviolet spectrum of the *colourless* solution that first issued from the column differed considerably from that of the original hexane solution***.

The two broad bands with maxima at 290 and 278 mμ (the latter band is extremely weak) could not be observed and the broad bands at 320 and 305 mμ were replaced by narrow bands at 326 and 307 mμ. The band at the

** For the photographs a high-voltage hydrogen lamp was used as a source of continuous radiation.

*** The strongly yellow-coloured pigments have nothing to do with the absorption bands described here. As shown later on, the initial spectrum can be separated into two spectra, both belonging to *colourless* fractions. Nor do the common carotenoid pigments absorb at these wave-lengths.

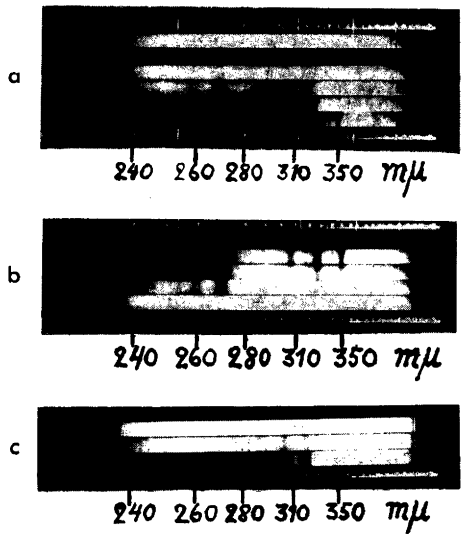

Fig. 1. Absorption spectrum in hexane solution of
a) the lipoid-soluble fraction of Centaurea cyanus,
b) Centaur X,
c) Centaur Y.

longer wave-lenght 350 mμ and the four bands at 269, 259, 254 and 244—245 mμ remained unaltered.

Hence it is obvious that the bands of the original solution cannot be caused by one uniform compound. At least two compounds must have formed the total spectrum of the original solution. Repeated passage of the fraction obtained through an aluminium oxide column did not change the spectral composition. It seems therefore very probable that this spectrum belongs to a uniform compound, which we provisionally call Centaur X. Fig. 1 b gives the spectrum of Centaur X, and the table gives the position of the bands measured.

From the lower half of the chromatogram described, the fraction which corresponds to the remaining part of the original spectrum can be eluated with methanol together with the »moving» lower carotenoid pigment. A spectrophotogram of this fraction dissolved in hexane (the methanol evaporated *in vacuo*) is shown in Fig. 1 c. The compound to which this spectrum belongs is provisionally called Centaur Y. The positions of the absorption maxima of the bands are shown in the table.

If the original hexane solution is treated with activated carbon instead of aluminium oxide, Centaur X is more strongly adsorbed than Centaur Y.

Hence, after shaking the solution with a suitable quantity of activated carbon, the spectrophotogram shows the characteristic picture of Centaur Y. The solution is colourless. Consequently, in addition to Centaur X, the yellow carotenoid pigments are also adsorbed by the carbon (*cf.* footnote ***).

It is worthy of note that the original hexane solution has bands at 320 mμ and 305 mμ, of which the first is a combination of band 326 from Centaur X and band 319 from Centaur Y, and the latter is a combination of band 307 from Centaur X and band 305 from Centaur Y (*cf.* the table).

When shaking an ethereal solution containing Centaur X and Centaur Y with an acid or alkaline aqueous solution, the light absorption is not noticeably altered. It is therefore highly probable that the compounds are neutrals.

SUMMARY

1. A fraction insoluble in water but soluble in ether and hexane and characterized by numerous bands in the ultraviolet region is demonstrated in three *Centaurea*-species commonly found in Sweden.

2. By means of chromatographic analysis it is possible to divide the fraction into two substances, each with characteristic spectra. The two substances are provisionally called Centaur X and Centaur Y.

Received January 12, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 86 – 96

Short Communications

Über die Bildung von Vanillin und Acetaldehyd aus Lignin und Lignosulfonsäure mittels Alkali

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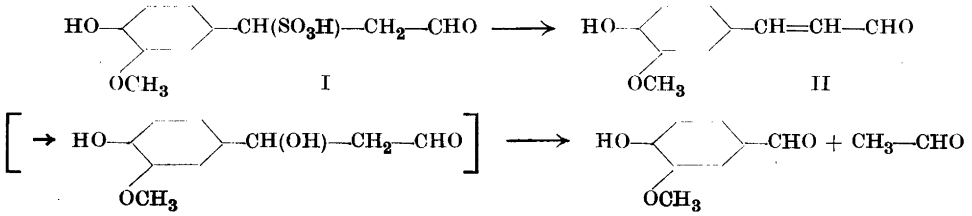
Beim Erhitzen von Sulfitablauge mit Alkali wird, wie zuerst von Grafe[1] festgestellt wurde, Vanillin gebildet; mit 24 %-iger Natronlauge beträgt die Ausbeute nach Tomlinson und Hibbert[2] 4—7 % der eingesetzten Lignosulfonsäure. Bezüglich des Reaktionsmechanismus haben die letztgenannten Autoren die Annahme gemacht, dass durch hydrolytische Abspaltung von Sulfogruppen Seitenketten vom Aldoltypus entstehen und dass diese unter der Einwirkung des Alkalis einer Spaltung unterliegen, welche die Umkehrung der Aldolkondensation darstellt.

Von v. Wacek und Kratzl[3–6] wurde diese Annahme durch Versuche an Modellsubstanzen weitgehend gestützt und ferner die bedeutungsvolle Feststellung gemacht, dess bei der alkalischen Hydrolyse von Lignosulfonsäure neben Vanillin Acetaldehyd als zweites aldehydisches Spaltstück erscheint. Hieraus wurde von Kratzl[6] der Schluss gezogen, dass in der Lignosulfonsäure Gruppen der Struktur I* enthalten sind.

Der von uns erbrachte Nachweis von Coniferylaldehydgruppen (II)* im Lignin bzw. von Coniferylaldehyd-hydrosulfonsäure-gruppen (I)* in Lignosulfonsäure[7] sowie die Ergebnisse ihrer quantitativen Schätzung[8] haben uns veranlasst, die Bedeutung dieser Gruppen für die Vanillin- und Acetaldehydbildung zu untersuchen.

Es zeigte sich dabei zunächst, dass freier Coniferylaldehyd (II) beim Erhitzen in alkalischer Lösung in Vanillin und Acetaldehyd zerfällt. Dies steht in Übereinstimmung mit der Hypothese von Tomlinson und Hibbert[2] sowie mit den von Kratzl und Khautz[4] an ähnlichen Modellsubstanzen erhaltenen Resultaten.



Die Gegenwart von Coniferylaldehydgruppen in Holz und in isolierten Ligninpräparaten[7,8] fordert somit, dass auch diese bei der alkalischen Hydrolyse Acetaldehyd und Vanillin liefern — letzteres allerdings nur in dem Masse, wie es unter

* Die Art und Weise des Einbaus dieser Gruppen in das Lignin- bzw. Lignosulfonsäuremolekül ist in den Formeln I und II nicht berücksichtigt.

den Bedingungen der Hydrolyse aus dem Ligninmolekül herausgespalten wird. Kratzl[5] teilte jedoch kürzlich Versuche mit, wonach in Bestätigung älterer, experimentell nicht näher belegter Angaben nur die Lignosulfonsäure, nicht aber das Lignin im Holze bei der alkalischen Hydrolyse zur Bildung von Vanillin Anlass geben sollen. Zur Klärung dieses Widerspruchs schien uns eine Nachprüfung der Angaben von Kratzl wünschenswert. Wir fanden, dass beim Erhitzen von entharztem Fichtenholzmehl mit 24 %-iger Natronlauge im N_2-Strom sowohl Acetaldehyd wie Vanillin entstehen. Die Ausbeuten an den beiden Aldehyden, die als 2,4-Dinitrophenylhydrazone isoliert wurden, bleiben allerdings weit hinter den mit Lignosulfonsäure erhaltenen zurück; sie entsprechen aber ungefähr denjenigen, die auf Grund der Menge der im Holz vorhandenen Coniferylaldehydreste (1 Coniferylaldehydrest auf je 40—60 OCH_3)[8] erwartet werden können. Auch aus Brauns' »native lignin», d.h. der mittels Alkohol aus Holz (Fichte) extrahierbaren Ligninfraktion wird beim Erhitzen mit Alkali Vanillin und Acetaldehyd gebildet.

Lignosulfonsäuren liefern weit mehr Vanillin und Acetaldehyd, als ihrem Gehalt an (sulfonierten) Coniferylaldehydgruppen (I) entspricht. So erhielten wir z.B. beim Erhitzen einer Lignosulfonsäure, die eine (sulfonierte) Coniferylaldehydgruppe (I) auf je 50 OCH_3-gruppen enthielt, in 24 %-iger NaOH 1 Vanillin auf je 15 OCH_3 und 1 Acetaldehyd auf je 13 OCH_3. Die Annahme von Kratzl[6], wonach die Bildung von Vanillin und Acetaldehyd aus Lignosulfonsäure auf der Anwesenheit von Gruppen des Typus I beruhe, bedarf daher in quantitativer Hinsicht einer Einschränkung. Diese Gruppen können z.B. im vorliegenden Falle nur 1/4 bis 1/3 der gesamten Vanillin- und Acetaldehydausbeute erklären. Die Hauptmenge der gebildeten Aldehyde muss aus Phenylpropaneinheiten anderer Struktur stammen.

Das Vorkommen von zwei Typen vanillin- und acetaldehydliefernder Gruppen in Lignosulfonsäure liess sich experimentell zeigen: Freier Coniferylaldehyd wird bereits von (siedender) 0,1 *N* NaOH mit grosser Geschwindigkeit zu Vanillin und Acetaldehyd gespalten. Ebenso gab auch Lignosulfonsäure schon mit 0,1 *N* NaOH Acetaldehyd, und zwar in einer Ausbeute, die — unter Berücksichtigung der in Versuchen mit reinem Coniferylaldehyd ermittelten Zerstörung von Acetaldehyd in der alkalischen Lösung — dem spektrophotometrisch gefundenen Gehalt des Präparates an (sulfonierten) Coniferylaldehydgruppen (I) entsprach. Parallel mit der — rasch verlaufenden — Bildung des Acetaldehyds verschwanden die für die Coniferylaldehydgruppierung charakteristischen Farbreaktionen, und die Acetaldehydbildung kam zum Stillstand, als keine Coniferylaldehydgruppen mehr kolorimetrisch nachweisbar waren. Wurde die Reaktionslösung nunmehr stark alkalisch gemacht (24 % NaOH), so trat beim Kochen erneut Acetaldehydbildung sowie Bildung von Vanillin ein.

Auch aus entharztem Fichtenholzmehl wird der Acetaldehyd bereits beim Erhitzen mit 0,1 *N* NaOH abgespalten, d.h., er entstammt den in das Lignin — möglicherweise als Endgruppen — eingebauten Coniferylaldehydresten (II).

Bei der Sulfitierung des Lignins werden diese Coniferylaldehydgruppen zu Gruppen der Struktur I sulfitiert. (Die alkalische Aldehydspaltung solcher Seitenketten erfolgt ebensoleicht wie die des freien Coniferylaldehyds, da die Sulfogruppe — wie bei allen 3-Oxo-sulfonsäuren — in alkalischer Lösung unter Regenerierung der Doppelbindung ausserordentlich leicht als Sulfit abgespalten wird.) Darüber hinaus wandelt aber die Sulfitierung eine relativ grosse Anzahl von Phenylpropaneinheiten derart um, dass sie einem unter dem Einfluss von starkem Alkali relativ langsam verlaufenden Abbau zu Vanillin zugäng-

X-Ray Studies on the System Molybdenum Trioxide — Tungsten Trioxide

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The system molybdenum trioxide — tungsten trioxide has been previously studied by Rieck[1], who particularly examined preparations rich in molybdenum trioxide by thermal analysis and noticed that no »mixed crystals» were formed. The observations were controlled by means of X-ray powder photographs.

The starting materials for the present investigation were molybdemun trioxide (Kahlbaum *puriss*), which had been heated at 200° C for 15 hours and tungstic acid (Kahlbaum *purum*), which had been dehydrated at about 500° C. The preparations were obtained by heating weighed mixtures of the trioxides in evacuated silica tubes placed in an electric furnace, giving a rather long range of homogeneous temperature. For heating temperatures exceeding 700° C equilibrium was not reached (*v. infra*)*. Because of this the investigation was confined to a study of the products formed at 700° C. At the latter temperature the heating time was generally about 100 hours.

The preparations were examined by taking X-ray powder photographs with focusing cameras using Cr-K radiation. The phase analysis showed three phases to occur in the system. Preparations $Mo_{0.75}W_{0.25}O_3$ contain reflections of molybdenum trioxide and another phase, while preparations $Mo_{0.4}W_{0.6}O_3$ consist of tungsten trioxide and the same intermediary phase. Samples of $Mo_{0.6}W_{0.4}O_3$ and $Mo_{0.5}W_{0.5}O_3$ do not show any reflections belonging to the trioxides and thus represent fairly pure specimens of the double oxide, the composition of which for this reason may be $MoWO_6$ or approximately such a formula. However, due to the difficulty of detecting small amounts of accompanying contaminations from the powder photographs no further conclusions as to the composition of the intermediary phase can be drawn for the present.

* The silica tubes were strongly attacked when mixtures rich in molybdenum trioxide were heated at 1000° C. Powder photographs showed the tube content to be highly contaminated by cristobalite. Molybdenum trioxide evidently has a pronounced crystallizing effect on silica glass at high temperatures. In fact the white residue mentioned in [2], obtained by dissolving molybdenum trioxide in ammonia after it had been heated in a silica tube at 1050° C for 35 hours, was also found to be cristobalite.

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lich werden. Da sich zeigen liess, dass mit steigendem Sulfitierungsgrad der Lignosulfonsäure nicht nur die Vanillinausbeute[9], sondern auch die Ausbeute an Acetaldehyd steigt, ist anzunehmen, dass auch dieser Typus von aldehydliefernden Gruppen »maskierte» Coniferylaldehydgruppen darstellt; sie müssen allerdings eine andere Struktur besitzen als der Typus der Formel I.

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Eingegangen am 12. Januar 1949.

The unit cell dimensions of molybdenum trioxide have been determined at this Institute[2]. The dimensions $a = 3.966$ Å*, $b = 13.85$ Å, and $c = 3.696$ Å of the orthorhombic unit cell are in good agreement with the figures previously reported by Bräkken[3] and Wooster[4]. The structure of tungsten trioxide has been determined by Bräkken[3] as well. He found it to be triclinic (pseudomonoclinic) and belonging to the space-group C_i^1— $P\,\bar{1}$ with the unit cell dimensions $a = 7.28$ Å, $b = 7.48$ Å, $c = 3.82$ Å, $\alpha = \gamma = \frac{\pi}{2}$ and $\beta \sim \frac{\pi}{2}$. It has now been possible to evaluate powder photographs of this oxide heated in oxygen stream for 20 hours at 870° C. The following dimensions were obtained in good agreement with Bräkken's values:

$a = 7.29$ Å**, $b = 7.54$ Å, $c = 3.85$ Å
$\alpha = \gamma = 90°0$, $\beta = 90°9$.

Powder photographs of the preparations $Mo_{0.75}W_{0.25}O_3$ and $Mo_{0.4}W_{0.6}O_3$ do not show any displacements of the reflections of the trioxides in comparison with the positions of the lines in photographs of the pure trioxides. This is in agreement with Rieck's observations[1]. However, due to the equality of the radii of the metal ions, limited solubilities might naturally cause very slight or even hardly detectable displacements of the lines. (*Cf.* for instance the small dimensional differences connected with a complete interchange of the two metals in MoO_2—WO_2[5], and in molybdates and tungstates with the Scheelite structure[6].) It is thus scarcely possible to confirm Rieck's thermal data from these powder photographs.

The intermediary phase, occurring in preparations $Mo_{0.6}W_{0.4}O_3$ and $Mo_{0.5}W_{0.5}O_3$, forms a yellowish green powder. The powder photographs are rather complicated. No displacements of the lines are detectable in photographs of the preparations containing this phase together with either of the trioxides. The phase is decomposed at higher temperatures as is obvious from powder photographs of preparations heated at 850° C.

In a preparation $Mo_{0.5}W_{0.5}O_3$, heated at 700° C for 45 days, rather long and extremely thin, needle-shaped, green crystals occur. They are often somewhat bent and very fragile. They do not give reflections with hard X-rays so it was not possible to get Laue patterns or single crystal photographs with Mo-*K* radiation. Cu-*K* radiation, however, gave good photographs after long exposure. Rotation and Weissenberg photographs with this radiation were taken with rotation around the needle axis. The identity period parallel to this axis was found to be 3.99 Å. The Weissenberg photographs, resembling those of β-tungsten oxide[2], show doublets or triplets of reflections lying rather close to points corresponding to a fairly simple reciprocal lattice. The Laue symmetry of the sublattice approximates D_{4h}— $4/mmm$, while that of the real lattice is C_{2h}— $2/m$. The a axis of the sublattice is about 5.35 Å. It has not yet been possible to determine the corresponding dimensions of the real structure.

The author is indebted to the *Royal Swedish Academy of Science* for a grant from the *Wallmark fund.*

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Received January 14, 1949.

* Referred to the wave length of Cr-$K\alpha$ = 2.2909 Å. The dimensions given in [2] are in fact expressed in kX units.

** Referred to the wave length of Cr-$K\alpha$ = 2.2909 Å.

The Binary System Zirconium-Boron

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As a part of a study of binary systems, composed of a transition element and boron, the zirconium-boron system has been investigated by X-ray methods. Certain studies on alloys between these elements have been reported [1,2]. The phases, however, do not seem to be pure. Mc Kenna has found a boride of the composition ZrB_2[3], which will be discussed below.

The alloys were prepared in a high frequency vacuum induction furnace[4], using a Philips H. F. generator with an output of 5 kW and a frequency of 800 kc/s as a source of energy. Other methods for preparing the alloys were tried, for instance melting in a vacuum furnace with a graphite tube as heating element and sintering in evacuated silica tubes. The inductive melting in vacuum using high frequency currents, however, was found to be the only possible method of preparation for these pure alloys. The starting materials were zirconium (Foote Mineral Co., Philadelphia) and boron with a purity of about 99 %[5].

X-ray investigations by means of powder methods showed, that small amounts of boron were dissolved by the α-zirconium lattice. The unit dimensions of zirconium (a = 3.229 Å, c = 5.139 Å*) thus increased to the limit a = 3.249 Å, c = 5.203 Å. The solubility limit of boron in α-zirconium seems to be at about 1 atomic % boron. Small amounts of boron caused a great increase in the hardness of the zirconium phase.

Only one intermediate phase was found. This phase, which had the composition ZrB_2, had metallic properties and a considerable hardness. The homogeneity range was narrow. A boride of the composition ZrB_2, containing 1.09 % carbon as impurity, has been reported[3]. The product has been investigated by X-ray methods and the metal atoms found to form a simple hexagonal lattice, the unit dimensions of the cell beeing a = 3.15 and c = 3.53 Å.

Powder photographs of the phase ZrB_2 verified the results. A hexagonal cell was found with unit dimensions:

$$a = 3.169 \text{ Å}, \; c = 3.530 \text{ Å}, \; c/a = 1.11$$

The observed density of 5.64 most closely corresponds to a cell content of one formula unit per cell and the agreement between observed and calculated $p|F|^2$ values is satisfactory, assuming the metal atoms to form a simple hexagonal lattice.

If the metal atoms are situated in 000, the only place for two boron atoms per cell is in $\frac{1}{3}\,\frac{2}{3}\,\frac{1}{2}$; $\frac{2}{3}\,\frac{1}{3}\,\frac{1}{2}$. These positions are compatible with space group D_{6h}^1 — $C\ 6/mmm$ and the boride thus is isomorphous to AlB_2[6] (C 32 type). The boron atoms form a plane hexagonal network, similar to that of the carbon atoms in graphite. The distance boron-boron in the same net will be $a\sqrt{3}/3$ = 1.829 Å, giving a radius of 0.91 Å for the boron atom (assuming the atoms to be spherical and in contact)*. The lattice may be regarded as a sequence of sheets . A H A H A H . . were A are plane hexagonal sheets of zirconium atoms and H are the hexagonal networks of boron atoms. Interpenetrating twins of ZrB_2 were observed, similar to those of AlB_2[6]. The maximum angle between the twins was compatible with (1 1 $\bar{2}$ 2) as twin plane. According to the figures, the same twin plane has been accepted for

* All values given in true Ångström units. 1 Å = 10^{-8} cm = 1/1.00202 kX units.

* The value is slightly greater than the probably correct value 0.87 Å[7]. The larger value found here seems to be caused by the metal atoms beeing in contact.

AlB_2, although it has erroneously been written as $(1\,1\,\bar{2}\,1)$.

This system is composed of a transition element and boron, thus belonging to those, discussed by Hägg[8,9]. The ratio r_B/r_{Zr} is 0.54, assuming the radii to be 0.87 Å for boron and 1.60 Å for zirconium (12-fold coordination). It is less than the critical value 0.59 and in fact the system is very simple. A range of solid solubility of boron in the metallic lattice exists. ZrB_2, the only intermediary phase which has been found, is a typical interstitial compound with a lattice of one of the four simple types, given by Hägg, and has metallic properties. It is of interest to compare this system with the chemically related system titanium-boron with the ratio $r_B/r_{Ti} = 0.60$, for wich a short report has been published[10]. According to this report a range of solid solubility of boron in the titanium lattice exists and a phase TiB_2, isomorphous to ZrB_2 and with metallic properties is to be found. In addition a superlattice, closely related to the titanium lattice exists and further a new phase, TiB, appears. This phase is reported to have »zincblende» structure with definite linkages titanium-boron. Thus it does not belong to the typically interstitial compounds. So far as can be judged from the brief report, the system titanium-boron thus has an intermediate position between simple and complicated systems and in fact the radius ratio is very near the critical value.

The existence of phases MeB_2 of the *C* 32 type seems to be rather usual among the transition elements. In addition to ZrB_2 and TiB_2 the borides CrB_2, CbB_2 and TaB_2 are isomorphous to AlB_2 (Kiessling, unpublished). In the systems molybdenum-boron and tungsten-boron, the *ε*-phases have a lattice, partially composed of a MeB_2 lattice of the type above[7]. This may depend on the tendency of the boron atoms to form plane networks.

On the Action of *Bacillus macerans* Amylase

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Bacillus macerans contains an amylolytic enzyme which is secreted into the culture medium. This enzyme is different from all the other amylases known to us; it converts starch, glycogen *etc.* into the non-reducing Schardingerdextrins (cyclo-amyloses) which can be determined semi-quantitatively by the Tilden-Hudson iodine test [1]. Since the action of the enzyme on starch paste is accompanied by an extremely rapid decrease in viscosity it must be concluded that the enzyme does not only split off end-chains from the amylopectin molecules (under the formation of cycloamyloses) but also ruptures linkages in interior chains between branching points [2]. The action is there-

This investigation has been supported by a grant from *Statens Tekniska Forskningsråd*, which is gratefully acknowledged. I further wish to thank Professor G. Hägg for valuable discussions during the course of the work.

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3. Mc Kenna, P. M. *Ind. Eng. Chem.* **28** (1936) 767.
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9. Hägg, G. *Ibid.* **12** (1931) 33.
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fore, in a way, similar to that of the ordinary α-amylases, but in the case of *macerans* amylase there is, in addition to the hydrolysis, a formation of new 1,4-α-glucosidic bonds, a reaction which probably can be described as a »transglucosidation». Since the interior chains of the amylopectin are relatively short no formation of cycloamyloses from these parts of the molecules can take place. Therefore, the rapid fall in viscosity should be accompanied by an increase in reducing groups. The experiments are in agreement with this view.

The amylolysis by *macerans* amylase does not cease, however, at the stage of the Schardinger dextrins. In some cases at least, the cyclo-amyloses gradually disappear again. Myrbäck and Gjörling [2] found that, on a very extended action of the *macerans* enzyme, the dextrins disappeared completely under the formation of maltose exclusively. Kneen and Beckord [3] also state that components of the *macerans* amylase system cause hydrolysis of the cyclo-amyloses to fermentable sugar. As we have found in new experiments, these observations are probably related to a reversibility of the *macerans* enzyme action. French, Pazur, Levine and Norberg [4] have shown that in solutions of the *macerans* enzyme, cyclo-amyloses and maltose (or certain other sugars) an increase in optical rotation occurs which is explained as due to the formation of higher open-chain saccharides through a reversion of the recognized action of the enzyme: the formation of the cyclo-amyloses from non-cyclic chain molecules. Small amounts of a fraction, probably containing a mixture of such saccharides were recovered.

We have been able to verify the statements concerning the increase in optical rotation. If the explanation given is correct it must be concluded that the open-chain saccharides, which are supposed to be formed, should contain 1,4-α-glucosidic linkages exclusively. If so, they should be broken down to maltose (and possibly small amounts of maltotriose from chains with an uneven number of glucose residues) by β-amylase. In the following experiments we used a solution of the *macerans* enzyme containing one unit per ml. The β-amylase solution had a very high activity: 1 ml in 100 ml 1 % starch solution gave 50 % conversion in about 2 hours. The following mixtures were prepared and stored under toluene at 20°. Pure Schardinger α-dextrin was used.

1)	1 g dextrin,	0.4 g maltose,	5 ml *macerans* enzyme,	5 ml β-amylase
2)	1 g »	0.4 g »	5 ml » »	5 ml water
3)	1 g »	0.4 g »	5 ml water,	5 ml β-amylase

After different incubation times the fermentable sugar was determined with baker's yeast (after removal of toluene). No reaction, or at the utmost a very slight one, took place in exp. 2 and 3. In experiment 1, however, the amount of fermentable sugar increased: after 24 hours 0.69 g maltose was found and after 6 days 1.08 g. The experiments suggest that the »hydrolysis of the Schardinger dextrins» to fermentable sugar is due to a joint action of *macerans* amylase and β-amylase (or probably any other ordinary amylase), and may well be explained on basis of the assumed reversibility of the *macerans* amylase action.

It should be emplasized that, in our experiments, the increase in optical rotation and the hydrolysis were both very slow. Since β-amylase was added in a great excess it must be concluded that the equilibrium between the cyclo-amy-

Antihistamine Agents

III. 2-Imidazolinylmethyl Ethers of Heterocyclic Carbinols

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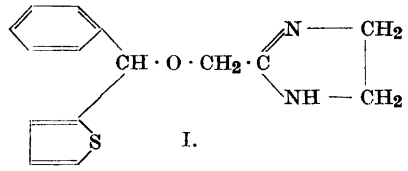
Replacement of a phenyl nucleus of an antihistamine agent by a heterocyclic one often yields compounds with a strongly increased effect. Sometimes, however, the antihistaminic activity is diminished or completely destroyed. For excellent reviews in this field see Loew [1] or Strauss [2].

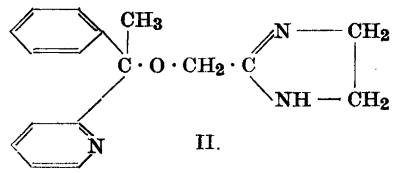
In earlier papers [3, 4] the preparation and the antihistaminic properties of 2-[(diphenylmethoxy)-methyl]-imidazoline and some related compounds have been described. In order to investigate the effect of imidazolinylmethyl ethers of heterocyclic carbinols, the compounds I and II were synthesized and tested. Compound II is an imidazoline analog of Decapryn, a well known histamine antagonist.

The new imidazolines were prepared by condensing equivalent parts of α-thienylphenylcarbinol or phenyl-α-pyridylmethyl-



I.

carbinol with 2-(chloromethyl)-imidazoline in the presence of sodium amide, two equivalents of sodium amide being used to liberate the chloroimidazoline from its hydrochloride. Attempts to use lithium amide or lithium hydride as condensing agents resulted in very poor yields. The compounds were isolated from the reaction mixtures by precipitation with oxalic acid. The free bases could not be obtained in crystalline form and could not be distilled without decomposition even at very low pressures. The bioxalates of the compounds were therefore used in the tests.



II.

Results of preliminary tests of the antihistaminic and antispasmodic activity of the oxalates of these two imidazolines are summarized in Table 1. The tests were carried out on isolated guinea pig ileum*. Activity is expressed in terms of β-dimethylaminoethyl benzhydryl ether hydrochloride (Benadryl) as the unit of activity. According to the tests the antihistaminic and antispasmodic activity of these compounds is rather weak.

Table 1. Effect of 2-imidazolinylmethyl ethers.

Compound	Effect in reducing the spasm produced by: Histamine	$BaCl_2$	Acetylcholine
I	0.15	0.55	0.3
II	0.1	0.1	0.05
Benadryl	1	1	1

loses and the open-chain saccharides is reached in a very slow reaction.

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Received February 6, 1949.

* Acknowledgement is made to Dr. S. Wiedling of Astra's Biological Department for performing these tests.

2-[(α-Thienylphenylmethoxy)-methyl]-imidazoline bioxalate. The starting α-thienylphenylcarbinol was prepared from α-benzoylthiophene by reduction with aluminium isopropoxide. After recrystallisation from light petroleum m.p. 57—58°. Yield 88 %. Minnis [5] who prepared this compound by reduction of the ketone with aluminium amalgam reported the same m.p. This carbinol seemed to be rather unstable. On keeping in air or in vacuum it sometimes decomposed into a tarry mass with a strong smell of sulphur dioxide. The carbinol was therefore used immediately after preparation.

To a suspension of sodium amide in toluene (75 ml), prepared from sodium (4.1 g) and liquid ammonia according to Vaughn, Vogt, and Nieuwland [6], a solution of α-thienylphenylcarbinol (16.2 g) in toluene (75 ml) was added. The reaction mixture was stirred at 70—80° for an hour and left overnight at room temperature. Next day, 2-(chloromethyl)-imidazoline [7] (13.2 g) was added and the mixture warmed at 60° for 1.5 hours. After the mixture had been cooled, the inorganic salts were filtered off. The oxalate was precipitated by the addition of a saturated ethereal solution of oxalic acid to the toluene solution. The crude bioxalate was collected and recrystallised from water. Yield 6.1 g, m.p. 155—156,5°.

$C_{15}H_{16}N_2OS \cdot (COOH)_2$ (362.4)

Calc.	C	56.35	H	4.45	S 8.85
Found	»	56.7	»	4.8	» 9.1

With picric acid a picrate was obtained. M.p. 179—179.5° after recrystallisation from ethanol.

$C_{15}H_{16}N_2OS \cdot C_6H_3N_3O_7$ (501.5)

Calc.	C	50.3	H	3.4	N 14.0
Found	»	50.0	»	3.7	» 14.1

2-[(Phenyl-α-pyridylmethylmethoxy)-methyl]-imidazoline bioxalate. This compound was prepared from phenyl-α-pyridylmethylcarbinol [8] (16.4 g) ans 2-(chloromethyl-imidazoline hydrochloride (12.7 g) in the same way as the preceding imidazoline. In order to remove the oxalate of the starting carbinol the crude oxalate was dissolved in water, made alkaline with sodium bicarbonate and extracted with ether.

The imidazoline base was precipitated from the bicarbonate solution by diluted sodium hydroxide. The oily base was extracted with ether and oxalic acid in ether added until no more precipitate was formed. The bioxalate (3.5 g) was recrystallised from acetone-water 3 : 1. M.p. 197—198° with decomposition.

$C_{17}H_{19}N_3O \cdot (COOH)_2$ (371.4)

Calc.	C	61.4	H	5.7	
Found	»	61.6	»	5.7	

With picric acid a dipricrate was obtained. M.p. 203—205° after recrystallisation from ethanol.

$C_{17}H_{19}N_3O \cdot (C_6H_3N_3O_7)_2$ (739.6)

Calc.	C	47.1	H	3.4	
Found	»	46.8	»	3.4	

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Received February 11, 1949.

Introduction of Substituents in the Aromatic Nucleus. Exploration of its Mechanism by means of Isotopic Hydrogen

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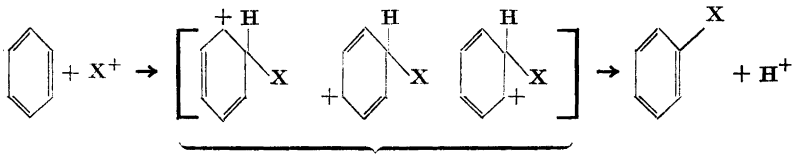
The heavy isotopes of hydrogen, deuterium (^2H) and tritium (^3H), offer a means of determining the slow step of some of the common reactions by which substituents are introduced in the benzene nucleus. Hitherto it has not been settled whether the nitration, bromination, alkylation, *etc.* of benzene and related compounds are true substitutions (of the S_E2 type) or primarily additions of the positive ion to the aromatic nucleus by means of the π electrons of the latter [1]. (Hardly any other type of reaction seems to come into question. It seems quite certain that the substituting agent in these reactions is the corresponding cation.)

If the splitting off of hydrogen takes part in the rate-determining step of the reaction, a position carrying a heavy hydrogen isotope should react more slowly than one carrying protium, owing to the lower zero point energy of the former bond. Generally, the difference in zero point energy between the »activated complexes» of the two reactions only partly counterbalances the corresponding difference for the initial bonds, and the latter being the predominating quantity will cause a measurable difference in reaction velocity.

Thus a substitution of the pure S_E2 type should show such a difference, and if a molecule carries hydrogen atoms of different mass in symmetrical positions, the light-weight atoms should be substituted more frequently than the heavy ones. The product will then contain a higher proportion of the heavy isotope than the starting material.

In the case of an addition mechanism of the »π-complex» type [1] two things may happen. Either of the two steps may be



mesomerism
(written explicitly for the intermediate only)

rate-determining. Since the intermediate has never been caught in these cases, the second step is probably rapid. The first step is likely to determine the rate, and this addition should be practically independent of the mass of the hydrogen atom. According to this mechanism, light and heavy hydrogen should be substituted in the same proportion as they occur in the starting material, and the isotopic ratio should remain unchanged during the reaction.

A necessary condition for the realization of such experiments is that no hydrogen exchange between different reactants occurs.

By means of tracer amounts of tritium and comparison of the specific radioactivity [2] of the reactants and the products, it has been shown that the nitration of toluene [3] and benzene (two nitro groups introduced by means of nitric-

sulphuric acid mixture) follows the π-complex mechanism, as found in quite another way by Ingold *et al.*[4], who very kindly communicated this result to the writer. By the same tritium method it has been found that the introduction of a nitro group in α position in naphthalene by means of nitric acid follows the same mechanism.

Preliminary experiments seem to indicate that the bromination of benzene in the presence of iodine follows mainly the same course.

The chlorination of toluene in α position in the absence of catalyst and in the presence of light, in which chlorine atoms probably remove hydrogen atoms (in any case π-complex formation is impossible in this aliphatic substitution), has been found to prefer light atoms to heavy ones.

Other substitution reactions are being investigated in the same manner.

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Received February 15, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 97—99

New Books

Des Isotopes — Rapports et Discussions. Comptes-Rendus du 7-e conseil de chimie de l'Institut International de Chimie Solvay à Bruxelles en septembre 1947. R. Stoops, Brussells, 1948. 411 pp. 400 Belgian francs; bound, Belgian francs 450.

This book contains the proceedings of the symposium sponsored by the institute mentioned above and held at the University of Brussells from September 22—27, 1947 inclusive. It includes 9 papers, which were sent in advance to the attendants of the conference, and the contributions to the debate during the meeting. The following papers were subjects for discussion: (1) F. Joliot (Paris): Modes de formation, constitution et filiation des isotopes nottament des isotopes artificiels (40 pages; discussion: 3 pages). (2) K. T. Bainbridge (Boston): Some results of mass-spectrum analysis (42 pages; discussion: 4 pages). (3) C. K. Ingold (London): Isotopes in the spectroscopy of polyatomic molecules with special reference to the benzene molecule (51 pages; discussion: 7 pages). (4) M. de Hemptinne (Louvain): Les isotopes comme moyen d'investigation de spectres de bandes (52 pages; discussion: 7 pages). (5) F. A. Paneth (Durham): The preparation of radioactive tracers (17 pages; discussion: 5 pages). (6) A. Langseth (Copenhagen): The preparation of organic deuterium compounds (14 pages; discussion: 10 pages). (7) G. de Hevesy (Stockholm): Application of labelled phosphorus (86 pages; discussion: 8 pages). (8) M. Calvin (Berkeley): Radiocarbon and its application in chemistry and biology (22 pages; discussion: 5 pages). (9) D. Rittenberg (New York): The use of N-15 and D for the study of chemical processes in the living cell (12 pages; discussion: 6 pages).

The papers can be divided into three groups each of which would be of special interest to a certain type of readers.

The introductory contribution of Joliot is easily comprehensible for students. The title is not quite significant and therefore the chapter-headings may be of interest: Introduction [historical]. Constitution, répartition et stabilité des noyaux atomiques. Chimie nucléaire; radioactivité artificielle; unité de radioactivité; isomérie nucléaire. Types de réactions nucléaires provoquées; fissions; transuraniens; production des radioéléments dans les piles. — The contribution of Paneth contains a general description of the following stages for the preparation of a radioactive tracer: a) the selection of a suitable tracer substance, b) the production of the chosen nuclide, c) the analytical separation and concentration of this nuclide, d) the synthesis of the tracer substance incorporating the radioactive atoms, and e) the availability of tracers. The reading of this article will be valuable for anyone interested in the application of tracers and searching for a first approach to the subject.

Another type among the contributions to the symposium is represented by the papers of Bainbridge, Calvin and Rittenberg. These papers are comprehensive reviews with many references and for that reason are a valuable source of information for any scientist. At the same time they are stimulating reading for the specialist,

who can find interesting proposals and ideas in the papers as well as in the contributions to the debate. The article by Bainbridge is in my opinion the best concentrated survey of mass-spectrum analysis, which has been written up to now.

Finally there is a third kind of article represented by the contributions of Ingold, de Hemptinne, Langseth and de Hevesy. These articles are highly specialized and for the specialist only. Ingold's paper for instance summarizes the work of the author and his co-workers concerning the band spectrum of the benzene molecule using partly or completely deuterated molecules. The paper of de Hevesy probably is the most complete account of all tracer work in biochemistry and animal physiology using P-32 hitherto published.

A publication of this kind can serve as an important source of information for the great number of scientists interested in the subject but not able to attend the conference. If, however, the proceedings are not issued until one year later, much of the actual interest will be lost and also it will be unavoidable that some of the papers will not be up to date (in this case for instance the information concerning the transuranium elements or the availibility of tracers). Therefore it would be quite worthwhile to study any means for speeding up the publication of such proceedings, even if this can be reached only with some inconvenience for the reader and at the cost of the appearance of the book. *K. E. Zimen*

Kathleen Lonsdale. *Crystals and X-Rays.* G. Bell & Sons Ltd., London, 1949. 199 pp. 21 s.

As Dr. Lonsdale says in the foreword to this book, it has been designed to interest those who do not now use X-ray crystallography but who might well do so and to instruct those who do use X-ray crystallographic methods without altogether understanding this tool. She also hopes that the book will persuade these two classes of people to pass on to more thorough textbooks.

I think that every reader of the book will find that Dr. Lonsdale has attained these objects in an admirable way. The treatment is elementary and clear although the presentation is very concentrated. Because of this last fact a reader who has had no previous knowledge of X-ray crystallography will not find the text easy. But the stimulating style will certainly awaken the interest of the reader and cause a wish to pursue the subject further. Also people who already are acquainted with X-ray crystallography will find the text useful. In many places one finds various characteristics of the present problems most ably dealt with and the numerous striking metaphors add to the pleasure of the reading. The historical introduction is excellent and reveals incidents and features which have hardly ever been mentioned in earlier textbooks. Another example of an outstanding treatment is the chapter on extra-structural studies.

If any critical remarks should be made they would be connected with the concentrated form of the text. One such remark concerns the treatment of point symmetry and symbols on pp. 56—63. I don't think that a reader without any previous knowledge in crystallography will obtain a sufficient understanding of these pages. The brevity of the text is also the cause of some statements of a too categorical nature. For example it would have been desirable for the author to state on pp. 167—168 that NaCl and KCl form a complete series of solid solutions only at elevated temperatures.

In this journal a book ought to be reviewed with regard to its interest or usefulness to the chemist. I am convinced that Dr. Lonsdale's book will prove quite

valuable to any chemist who wants an introduction to the possibilities of X-ray crystallography in solving chemical problems. It will also facilitate his understanding of papers in structural chemistry. This is a good thing because there is no doubt that many such papers, containing data of great chemical importance, are not understood by most chemists because of the difficult specialized language of X-ray crystallography.

G. Hägg

ACTA CHEMICA SCANDINAVICA 3 (1949) 125—143

Silver Compounds of Acetylene

III. The Equilibrium Constant of the Formation of Silver Carbide from Acetylene

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The formation of symmetric silver carbide, $AgC \equiv CAg$, from acetylene and Ag^+ with liberation of H^+ is a reversible reaction. By use of strong complex formers with Ag^+ like thiourea or cyanide the carbide can be dissolved, regenerating the equivalent amount of acetylene.

The asymmetric form $HC \equiv CAg$ may exist as an intermediate product at the formation or breaking down of symmetric silver carbide, but is not stable at 25° C and moderate acetylene pressure. No formation of the asymmetric carbide can be shown even at an acetylene pressure of 7 atm. If it exists at all the acetylene pressure necessary for its formation at 25° C according to

$$C_2Ag_2(s) + C_2H_2 = 2HC_2Ag(s) \qquad (1)$$

must exceed 7 atm.

The equilibrium can thus be formulated

$$\tfrac{1}{2}\, C_2Ag_2(s) + H^+ = \tfrac{1}{2}\, C_2H_2 + Ag^+ \qquad (2)$$

The thermodynamic equilibrium constant can be defined as

$$K = a_{Ag^+} \cdot P^{\frac{1}{2}}_{C_2H_2} / a_{H^+} \text{ or} \qquad (3)$$

$$pK = pAg - pH + \tfrac{1}{2}\, pP_{C_2H_2} \qquad (3)$$

The activity of solid silver carbide is taken as unity for $a_{C_2Ag_2}$; $P_{C_2H_2}$ is measured in atm.

From simple experiments on splitting and formation of carbide the interval wherein the constant must lie can be roughly determined. The silver carbide is completely split by 0.1 *M* hydrochloric acid (pAg ca 9 and pH ca 1) regenerating acetylene ($pP_{C_2H_2}$ ca. 0) which shows that pK must be less than 8. When passing acetylene ($pP_{C_2H_2}$ca. 0) through an aqueous solution of 0.01 *M* silver perchlorate in 0.1 *M* perchloric acid (pH ca 1) silver carbide precipitates and the silver content in the solution is eventually depressed to $10^{-4} - 10^{-5}$ (pAg 4 — 5), as estimated by a potentiometric titration with 10^{-4} *M* potassium iodide. This gives a lower limit for pK of ca 3. Since pK is larger than 3 the constant can be determined by direct chemical analysis only in systems of high hydrogen ion activity. An analytical method might be used, working with suitable competing complex formers, and would be advantageous if the constants of the complex formers were easily determined. But we have not been able to find a complex former giving a mixture where the silver ions are about equally shared between the two systems. The connection between pAg and pH demanded by the acetylene equilibrium, and the fact that few anions are permissible also considerably limits the choice.

A potentiometric method seems à priori suitable; the H^+- and Ag^+-activities being in no way extreme. Several conditions must however be fulfilled: rather quick occurence of the equilibrium, absence of disturbing side-reactions (*e.g.* some reaction involving a redox process) *etc.* The immediate purpose of our experiments was to find out whether such a method could be used. The three variables to be measured and the experimental conditions generally have therefore been varied over rather a wide range, partly to check that there is a covariation according to the equilibrium formula set up, partly to select the conditions best suited for an accurate determination of the constant. On the whole the results of these experimental variations are satisfactory. There can be no doubt that the measurements actually refer to the equilibrium formulated. In spite of some disturbances, the origin of which is not quite clear, but which are to be discussed, the mean value

$$pK = 3.46 \text{ at } 25^\circ \text{ C}$$

seems to be of satisfactory accuracy and, for the present, sufficient for our investigations. It might however be possible to determine the constant with greater precision.

DISCUSSION OF THE EXPERIMENTAL CONDITIONS

The three quantities required for the calculation of the thermodynamic constant are all *directly measurable.* pAg can be measured potentiometrically as can pH (by means of the glass electrode; in this system hardly any other H/H^+-electrode could be used). $P_{C_2H_2}$ is the partial pressure of acetylene in a gas mixture in equilibrium with the liquid. None of the activities need be computed from an analytically determined concentration; no activity coefficient need be estimated or kept constant.

It is also advantageous that only the difference (pAg-pH) enters into the calculation of pK. This difference should be obtained directly from the potential between a glass and a silver electrode immersed in the same solution, *i.e.* in a cell *without liquid junction,*

$$-\text{glass} \mid \text{A} \mid C_2Ag_2(s),\ Ag\ +$$

where A is a solution in equilibrium with solid silver carbide and with a gas mixture of known acetylene partial pressure. The potential of this cell can be used for the calculation of (pAg — pH) from

$$E = E^\circ - \frac{\ln 10\, RT}{F}(\text{pAg} - \text{pH}) \tag{4}$$

where E° is the standard potential of the electrode couple, *i.e.* the potential of the cell

$$-\text{glass} \left| \begin{matrix} \text{solution} \\ \text{with pAg} = \text{pH} \end{matrix} \right| \text{Ag}\ +$$

Even when using a third electrode as a reference in order to get absolute values for the variables pAg and pH, the diffusion potential to the reference electrode will be eliminated in the calculation of pK.

A third advantage is that the standardization of this system, *i.e.* finding E° for a given glass electrode, is very easy to perform. Measurements of the potential E^s of the cell

$$-\text{glass} \mid \text{B} \mid \text{Ag}\ +$$

where B is an equimolar solution of nitric acid and silver nitrate of total concentration c, show that the potential varies but slightly with c; the reason for this being naturally that the deviation from the standard potential depends only on the rather insignificant difference between the activity coefficients

of silver and hydrogen ions in the same solution. It is easy to extrapolate to $c = 0$, where $E^{\circ} = E^{s}$.

An introductory discussion of foreseen experimental difficulties is justified since the procedure has partly been evolved from such a discussion.

The use of pH-buffered solutions can be supposed to be advantageous; experiments also have proved this. Buffer substances and concentrations can be chosen at will as regards their influence on the activity coefficients as the activities are directly measured. Anions forming insoluble complex salts, *e.g.* of the type $C_2Ag_2 \cdot AgAn$, must however be avoided. An analytical check of the composition of the solid phase may become misguiding, as a stoichiometrically complete transformation of an insoluble complex salt into silver carbide and vice versa is a very slow process. To decide whether silver carbide or a complex salt is the stable solid phase one should know the constants that, according to formulas (3) and (4) in a previous publication [1], characterize the system in the presence of a given anion. But we cannot start with the knowledge of any such system; on the contrary the determination of the silver carbide — acetylene equilibrium is a primary task in such an investigation. It is certain only that *perchlorate* can be regarded as an innoxious anion. This has been proved by the solubility determinations [1] where silver carbide has been shown to be the solid phase in the presence of silver perchlorate of concentrations up to 2 *M*. The possibility that solid complex perchlorates form in the present system is even less. Such a conclusion can be drawn from just the qualitative validity of the formulas referred to. To be safe we have tried to limit our experiments to perchlorate systems. Some experiments with acetate ion present have however given acceptable results.

Identical activity of the silver carbide samples used in the different experiments must be assumed when calculating the equilibrium constant. Even though the identification of the solid phase as silver carbide is certain, the presupposition of constant activity arouses some doubt. The particles of silver carbide are so small that there is a risk that variations in size may cause change in activity. In order to trace such variations silver carbide has been prepared in different ways. No reliable information has been acquired concerning activity variations of importance, still, it might be such variations that limit the accuracy of the measurements. In one case only, a phenomenon appears that may indicate a formation of silver carbide of distinctly different activity: if potentiometer measurements are carried out in such yellow solutions as result from passing acetylene into very dilute silver perchlorate in the absence of other salts,[1, p. 108] values are obtained (not very constant) that indicate a much higher silver carbide activity. Still, we find that such measurements, even though they might be of importance when investigating the nature of

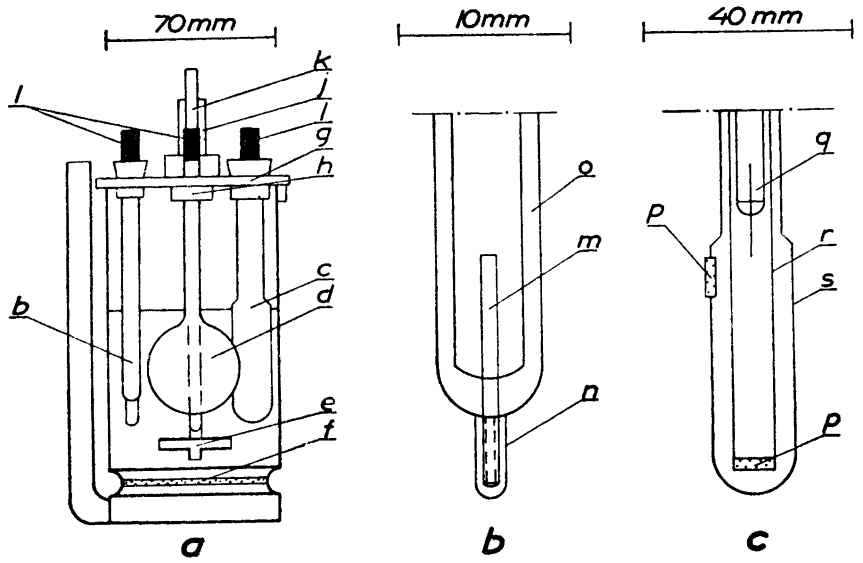

Fig. 1. Apparatus: a Vessel; b Silver electrode; c Reference electrode; d Glass electrode; e Stirrer; f Fritted disk Pyrex M or C; g Lid of plexiglas; h Holder for glass electrode; j K. P. G. bearing; k Shaft of stirrer; l Jackets; m Pt-wire 1 mm Ø, projecting part thread-cut; n Silver sponge; o Jena Normal glass; p Fritted disk Jena G 5/3; q Ag/AgCl-electrode according to Brown; r Internal tube 0.01-M NaCl, 2-M $NaClO_4$; s External tube, 2-M $NaClO_4$.

the »yellow modification», are here irrelevant. Among the experiments described below there is none where the yellow modification was present, except as an unstable intermediate product in the formation of »normal» silver carbide. — Finally it should be remarked that in the potentiometric experiments no inconvenient transformation of the silver carbide occurs, such as that which necessitated repeated addition of fresh silver carbide in the solubility determinations.[1, p. 113]

Apart from the risk of non-reproducible activity of the solid silver carbide the low silver ion activity seems to be the main difficulty of the measurements. Even at pH = 1 and an acetylene pressure of 1 atm. pAg is 4.5 and then increases linearly with pH. In the determination of the solubility products of several insoluble silver salts a common experience seems to be that satisfactory results have been achievable only when using silver electrodes covered, generally by electrolysis, with the silver salt. This method would be advantageous also in our case, especially as it is conceivable that certain disturbances, caused by possible impurities in the continuously introduced acetylene or by some side-

reaction, would be eliminated. We have succeeded in making silver/silver carbide electrodes by electrolysis at a low current density in an ammoniacal solution saturated with acetylene, but these electrodes have not given reproducible potentials.

In the experiments therefore metallic silver electrodes (of »thermal» type) have been used exclusively. The vessel is so constructed that the solid silver carbide can be dispersed with as violent agitation as its explosive tendency permits. Unfortunately only a comparatively small quantity of silver carbide can be used in each experiment*.

EXPERIMENTAL PROCEDURE

The vessel consists of a Pyrex cylinder, $h = 150$ mm, $d = 70$ mm, shaped as in Fig. 1. Gas can be introduced either through the fritted disk in the bottom or through a glass tube (not shown on fig.) passing through the lid and ending just above the surface of the liquid. Several silver electrodes can be introduced. The stirrer consists of a T-tube of 6 mm glass. Precipitate and liquid are drawn from the bottom of the vessel and then thrown out through the horizontal arms. The stirrer is driven through a flexible shaft by a motor so shielded that stirring can go on while measuring. The vessel is immersed in an oil-thermostat where the temperature is 25.00° C ± 0.05.

Glass electrodes of a relatively low resistance (not over 1 megohm) have been used in order to reduce the risk of leakage currents, which is large in this type of experiment where a gas saturated with water-vapour flows through the apparatus. After checks against the hydrogen electrode two slightly different Hartmann & Braun electrodes were selected; one (I) with standard acetate buffer + 0.01 *M* KCl and with Ag/AgCl electrode according to Brown [2]; the other (II) delivered complete with built-in calomel electrode. On the stem of the glass electrode is cemented a ring of plexiglas which can be screwed on to the lid of the vessel.

Silver electrodes are constructed according to Fig. 1 with a threaded platinum rod carrying the silver sponge. We have found these preferable to Brester's [3] spiral type, being more easily covered with an even, complete, and practically crack-free silver sponge. This gives a faster exchange of the solution at the electrode surface, and equilibrium is attained more rapidly. The silver plating and the thermal reduction of silver oxide paste is performed according to Brester's directions. Moderate variations in electrolysing, heating, and cooling do not seem to influence the reproducibility, which is checked by measuring mutual potential differences in 0.001 *M* and 0.1 *M* silver perchlorate. The potentiometer used has a sensitivity of 0.01 mV. The reproducibility of the electrodes, even from different groups, has as a rule been better than ± 0.1 mV and in several cases ± 0.01 mV. Electrodes differing more then 0.1 mV from the average were rejected. We have thus found a remarkable reproducibility but we have not studied more closely the requirements.

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* Wet silver carbide is said not to explode on grinding, but we have convincing experience to the contrary.

Reference electrode (see Fig. 1) consists of an Ag/AgCl-electrode [2] with 2*M* sodium perchlorate in the salt bridge; a rather unsatisfactory arrangement considering the diffusion potential, but contamination by some foreign anion must be avoided. Calibration of the system glass electrode — reference electrode has been performed in the usual manner with buffer solutions, without any greater demand for precision, as only an approximate value of the pH is required.

Potentiometer. Vacuum-tube potentiometer: Radiometer, Copenhagen, Type PHM 3 d, adjusted against Eppley standard cell and calibrated over the measuring range against a compensation bridge NC Jensen, Copenhagen, giving an accuracy of ± 0.2 mV.

Gases and gas mixtures. Acetylene of fair purity has been delivered in cylinders with normal filling (AGA) but without acetone. Nitrogen of commercial quality, in some cases freed from oxygen [4] (No difference in the results has been observed.) For the preparation of gas mixtures of known composition two methods have been used:

(a) Filling acetylene cylinders with an acetylene-nitrogen mixture, the composition checked through analyses. The method is not suited for accurate procedure as the reversible sorption of acetylene in the filling causes a continuous increase in the acetylene content of the gas when emptying the cylinder. The acetylene percentage must be checked before and after each experiment.

(b) Arranging a constant and accurately measured flow of acetylene and nitrogen resp., which are mixed and passed into the vessel. For this purpose a special technique of high precision has been devised, a more detailed description of which will be given elsewhere.

Chemicals. The solid silver carbide and the silver perchlorate have been prepared in the manner already described [1]. Other chemicals of analytical purity.

Procedure of standardization. All standardizations and checks have been performed in the vessel (except the checking of the silver electrodes mentioned) mounted as in the determinations of the acetylene equilibrium. The working conditions of the glass electrode are thus identical in both cases. For the same reason a stream of gas (nitrogen) of approximately the same speed as in the real experiments has been passed through the solution during the standardizations. In this way the atmosphere around the glass electrode is kept at approximately constant humidity.

In the determination of E° for the system

$$- \textit{glass} \mid \textit{solution} \mid Ag +$$

the vessel is charged with 200 ml of water and measured amounts are added of a stock solution which is 1.00 *M* both in silver nitrate and nitric acid, the equimolarity of which has been checked by careful analyses. The volume is adjusted to ca. 200 ml after every addition. E° must be determined in connection with every experiment as the potential of the glass electrode slowly changes.

The procedure for the investigation of the acetylene equilibrium is apparent from the discussion of the results in the following section. The measurements have been carried out in diffuse electric light, strong day-light seems unfavourable. The composition of the solution was usually altered by adding 10 ml of liquid and after mixing again removing 10 ml, thus keeping the volume constant at ca 200 ml. The gas flow rate was about 350 ml/min.

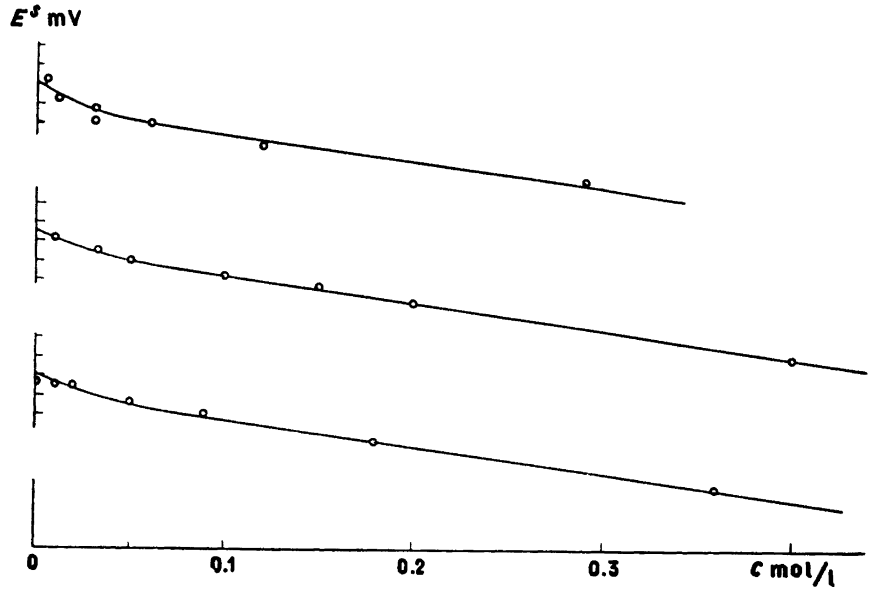

Fig. 2. Determination of E° for the system — glass | liquid | Ag +.

STANDARDIZATIONS AND CHECKS.

Determination of E°. The potential E^s of the cell

$$- \text{ glass } \left| \begin{array}{l} \text{equimolar solution } AgNO_3 + \\ HNO_3, \text{ total concn. } = c \end{array} \right| \text{ Ag } +$$

varies but slightly with the concentration c, as is demonstrated in Fig. 2, giving the results of three typical experiments. (The E-axis units are mV, positive direction upwards.)

For every point the relation

$$E^s = E^\circ + \frac{\ln 10\, RT}{F} (\log f_{Ag} - \log f_H) \qquad (5)$$

should hold. The observed relationship $E^s = F(c)$ should thus express the influence of the concentration on the difference $(\log f_{Ag} - \log f_H)$. With decreasing c both terms tend to zero; but the difference can be neglected where the single terms are still comparatively large. The circumstances thus favour an extrapolation to $c = 0$ where $E^s = E^\circ$.

The experiments show — as demonstrated by the figure — that E^s is a linear function of the concentration when this exceeds ca. 0.05 M. (The deviation from the linear relationship in the interval $0.05 < c < 0.4$ M is in no case greater than the experimental error $\pm$ 0.2 mV.) At concentrations below 0.05 M the curve shows some deviation from the straight line. The measurements become less reproducible at low concentrations; isolated points falling outside the curve connecting the others and the bent part of the curve having a somewhat different shape in different experiments. So far we have interpreted this poor reproducibility as the result of accidental disturbances. To avoid these difficulties we have used the following procedure at each standardization: the straight line is produced to the E-axis and the value thus obtained is increased by 0.7 mV, this being the mean, from several experiments, of the difference between the intercepts on the E-axis of the produced straight line and the curve. The method has the advantage that the determinations are based on well reproducible measurements in the linear interval. A possible error in the value 0.7 mV — hardly, however, more than some tenths of a mV — would appear with the same effect in all experiments.

It is interesting to note that ($\log f_{Ag} - \log f_H$) varies linearly with the concentration (within the range $0.05 < c < 0.4$ M)

$$\log f_{Ag} - \log f_H = f_{\pm AgNO_3} - \log f_{\pm HNO_3} = -0.25 \times c \qquad (6)$$

The assumption, often made [5], that the individual character of an electrolyte can be accounted for by a linear c-term in the function $\log f = F(c)$ seems fully justified in this particular case *. However, the dependence on the concentration is so small that a minor deviation from a linear relationship would hardly be perceptible in spite of the advantage that ($\log f_{Ag} - \log f_H$) is measured directly. A further discussion of these problems is beyond the scope of this article.

Check of $dE/d(pAg\text{-}pH)$ for the cell —glass | solution | Ag + can, within a limited range, be performed by the same dilution technique as has been described for the determination of E^o. If the solution consists of a mixture of silver nitrate and nitric acid in the molar proportion 1 : 100 one gets a potential decreasing linearly with c (= the total concentration). The slope is practically identical with that of the determinations of E^o. In such an ex-

* At the computation of this expression (6) the incomplete dissociation of the nitric acid ought to be taken into consideration. A recalculation using the value of Redlich [6] for the dissociation constant gives but a minor contribution to the linear c-term, about one tenth of the mentioned factor-0.25 and of the same sign.

periment the extrapolated potential for $c = 0$ was — 3.2 mV; a standardization performed on the same occasion gave $E^\circ = +\ 116.0$. Thus

$$dE/d(pAg\text{-}pH) = 59.6$$

which corresponds well with the theoretical value 59.16 at 25° C.

Calibration of the glass electrode and determination of its potential against the hydrogen electrode, E^g has been performed by direct comparison between glass and hydrogen electrodes by the method of MacInnes and Belcher [7] and has given the following results.

Table 1. Determination of E^g *for two glass electrodes. (The* E^g*-values corrected to 1 atm. hydrogen pressure.)*

Solution	pH	E^g	
		Glass I	Glass II
HCl 0.24 *M*	0.7	565.2	719.0
HAc 0.1 M, NaAc 0.1 *M*	4.6	564.8	719.2
$Na_2B_4O_7$ 0.05 *M*	9.2	566.2	735.8

It is evident that the E^g slightly varies with the pH. A considerable deviation appears only at pH 9 for glass II, which has quite a large alkali error. Between pH 7 and 1 constancy of $\pm$ 0.3 mV can be counted on.

The E^g-values of the glass electrodes change somewhat with their aging but so slowly that errors need not be feared; standardizations are made in connection with every series of measurements. The variations in E° are parallel.

As a further check we have computed the standard potential of silver from

$$E^\circ_{Ag} = E^\circ + Eg$$

giving the potential for the cell

$$-\ Pt \mid H_2\,(1\ atm.) \mid a_{H^+} = 1 \,//\, a_{Ag^+} = 1 \mid Ag\ +$$

where the sign // represents a hypothetical electrolyte contact without diffusion potential. In an experiment where E° and E^g were determined on the same day we got

$$E^\circ_{Ag} = +\ 801\ mV$$

in fair agreement with the best values in literature [8] which lie within 799.2—800.2 mV.

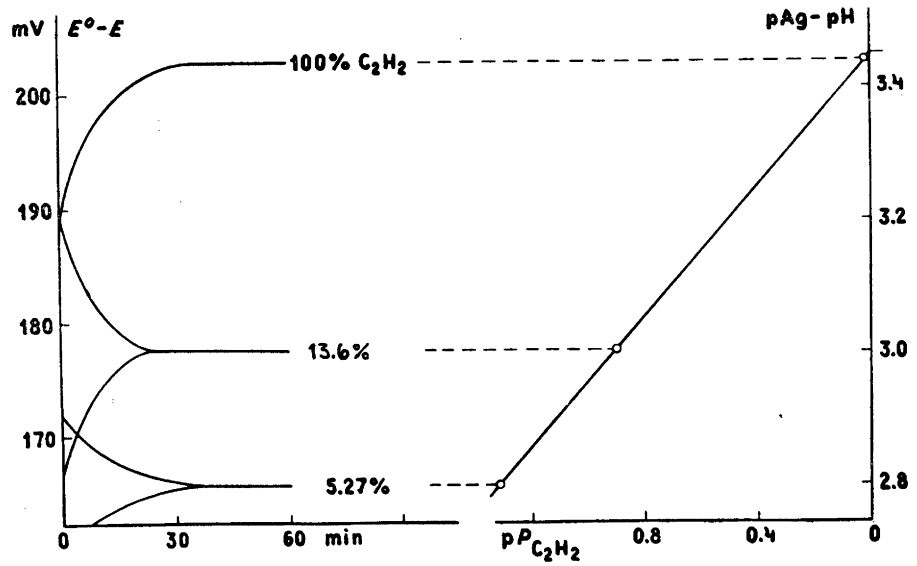

Fig. 3. Variation of $P_{C_2H_2}$ at pH ca. 1.

It should be pointed out that the procedure devised for the determina tio of the standard potential of silver is founded on measurements in a cell wit hou liquid junction — therein differing from earlier determinations. In spite o the limited accuracy of the glass electrode the procedure is of a certain inter est and could possibly be developed into a good means for the determination of the standard potential of silver.

RESULTS

Fig. 3 shows the result of an experiment where the acetylene pressure in the cell

$$-\text{glass} \mid 0.1\ M\ HClO_4, C_2H_2 \mid C_2Ag_2(s), Ag +$$

is varied between 0.05—1 atm. The potential responds rather swiftly to a change in the acetylene pressure and establishes itself at a given value, reproducible for each pressure. As shown by the time curves, identical values appear whether the acetylene pressure is adjusted from above or from below, thus proving a true equilibrium. The shape of these curves naturally depends upon the gas flow rate, particularly in the beginning. A rough estimate shows that the time necessary for the establishment of equilibrium is longer than the

time required for alteration of the concentration of acetylene dissolved in the liquid.

The connection between the values for (pAg — pH) and the corresponding values for $pP_{C_2H_2}$ (calculated from the acetylene content of the gas, the total pressure and the partial pressure of the water vapour) is demonstrated in the right part of the diagram, showing that the variables are correlated as demanded by the formula

$$pK = pAg - pH + \tfrac{1}{2} pP_{C_2H_2}$$

The slope $d(pAg - pH)/dpP_{C_2H_2}$ for the straight line is -0.49_3, in good agreement with the theoretical value $-\frac{1}{2}$. A more accurate value for the slope requires a variation of the acetylene pressure over a wider range. But it is difficult to prepare a gas mixture with an acetylene content below 1% while maintaining the necessary relative accuracy.

The numerical values of pK calculated from the three measuring points are: 3.465, 3.462, and 3.452. In an isolated experiment of this type the pK-value is reproducible within 0.01 units. Divergencies between series at different pH are however often considerably larger.

Experiments at pH greater than 2 frequently give unsatisfactory results. The most obvious trouble is that the equilibrium potential is often very slowly established. In some cases it has been impossible to get a constant value within reasonable time (3—4 h). Evidently such a drawn-out procedure is in itself a disadvantage. Several experiments give the impression that it is possible to differentiate between, on the one hand, the time curve corresponding to the establishment of the acetylene — silver carbide equilibrium, and on the other hand a still slower drift of the potential corresponding to an accumulation of impurities or some similar disturbance. It is all the same impossible to make a correction giving the »true» potential. As a rule values from experiments where a comparatively stable potential is reached only after a long time are obviously faulty. We have however thinned our material only by rejecting experiments not giving a constant potential within 1—1 ½ h. In the table some rejected values, in brackets, are given as examples.

We have not been able to explain the disturbances satisfactorily. The following observations though, contain some suggestions:

(a) At a pH lower than 2 disturbances are insignificant. But in neutral or alkaline solution they occur almost regularly; especially striking if the pH is raised during the course of an experiment by the addition of a base. In order to get satisfactory results in such solutions the experiment must be

Table 2. Determination of pK at different pH.

Measurings a, b, etc. under one number have been performed in sequence, pH-changes effected by addition to the solution.

The pH and composition of the solution are approximately stated. $P_{C_2H_2}$ has been calculated from the acetylene content of the gas, the total pressure and the partial pressure of water vapor (ev. + ammonia). Where two values for $(E°—E)$ are given these correspond to an attainment of the equilibrium from opposite directions according to Fig. 3. Brackets design potentials of unsatisfactory constancy.

No.	Addition of solid C_2Ag_2	Solution mol/l	pH	$P_{C_2H_2}$ atm.	$E° — E$ millivolt	pK
13 a	2 millimole	0.01 $HClO_4$	2.1	0.579	196.5	3.44
					196.2	3.44
b	»	0.05 $HClO_4$	1.5	»	198.4	3.47
					198.3	3.47
c	»	0.35 $HClO_4$	1.1	»	198.8	3.48
					198.8	3.48
d	»	ca 0.01 $HClO_4$ + 0.34 NH_4ClO_4	1.7	»	197.5	3.46
					197.5	3.46
10	—	0.01 $AgClO_4$ + 0.1 $HClO_4$	1.3	0.0674	170.4	3.47
					169.7	3.46
9 a	2 millimole	0.05 NH_3 + 0.05 NH_4ClO_4 + 0.10 NH_4Ac	9.0	0.230	192.7	3.58
(Ag_1)					189.8	3.53
(Ag_2)	»	do	»	»	(201)	(3.72)
					(195)	(3.64)
b	»	0.05 HAc + 0.05 NH_4Ac	4.7	»	188.1	3.49
(Ag_1)	»	+ 0.15NH_4ClO_4			186.1	3.47
(Ag_2)	»	do	»	»	(190)	(3.57)
					(188)	(3.52)
c	»	0.2 NH_4ClO_4 + 0.05 $HClO_4$ + 0.1 HAc	1.2	»	186.7	3.48
(Ag_1)						
(Ag_2)	»	do	»	»	186.7	3.48
8 a	»	0.05 $HClO_4$	1.5	0.190	182.5	3.44
					182.1	3.44
b	»	0.35 $HClO_4$	1.0	»	183.5	3.46
					182.7	3.45
c	»	2.7 $HClO_4$	0.5	»	(185)	(3.49)
					183.8	3.46
7 a	»	0.06 HAc + 0.01 NaAc + 0.04 $NaClO_4$	4.0	0.968	(200)	(3.39)
b	»	do	»	0.164	181.8	3.46
c	»	0.01 $HClO_4$ + 0.1 HAc + 0.1$NaClO_4$	1.4	0.968	206.3	3.49
d	»	do	»	0.164	183.8	3.50

No.	Addition of solid C_2Ag_2	Solution mol/l	pH	$P_{C_2H_2}$ atm.	$E° - E$ millivolt	pK
6 a	2 millimole	0.1 $HClO_4$	1.3	0.155	181.3	3.47
					180.9	3.46
b	»	0.1 $NaClO_4$ + 0.1 HAc	2.1	»	178.6	3.41
					177.6	3.43
c	»	0.1 $NaClO_4$ + 0.05 NaAc + 0.05 HAc	3.6	»	(145)	(2.82)
5 a	»	0.01 $HClO_4$	2.1	0.145	179.4	3.45
					180.4	3.47
b	»	0.1 $HClO_4$	1.3	»	180.4	3.47
					180.4	3.47
c	»	ca 0.01 $HClO_4$ + 0.1 NH_4ClO_4	2.0	»	178.4	3.43
					178.3	3.43
3 a	»	0.1 $HClO_4$	1.3	0.0499	166.4	3.47
					166.4	3.47
b	»	do	»	0.127	178.3	3.46
					178.3	3.46
c	»	do	»	0.953	203.5	3.45
					203.5	3.45

performed by dissolution or precipitation of silver carbide at the intended or higher pH.

(b) If the acetylene pressure is altered during the course of a slow change in the potential, the latter is quickly displaced by an amount fairly well corresponding to the alteration. Afterwards the potential again slowly drifts in the same direction, with practically the same speed as before.

(c) The slow establishment of the potential is associated with some process on the surface of the silver electrode. If, when the potential has become tolerably steady, still another silver electrode is introduced, it is quite a while before this second electrode shows the same potential as the first. Two simultaneously introduced silver electrodes sometimes require unequal times before they show the same potential, although they seem identical according to checks and standardizations. In some cases a residual difference was observed, but disappeared when the pH was reduced to a value below 2 (see Table 2, expt. 9).

Although impurities in the acetylene can be suspected as a source of error we have not been able to get any definite effect either by scrubbing the gas with various absorbents, or by changing the gas flow rate. (Naturally the partial pressure adjusts itself more speedily when the flow rate is higher.)

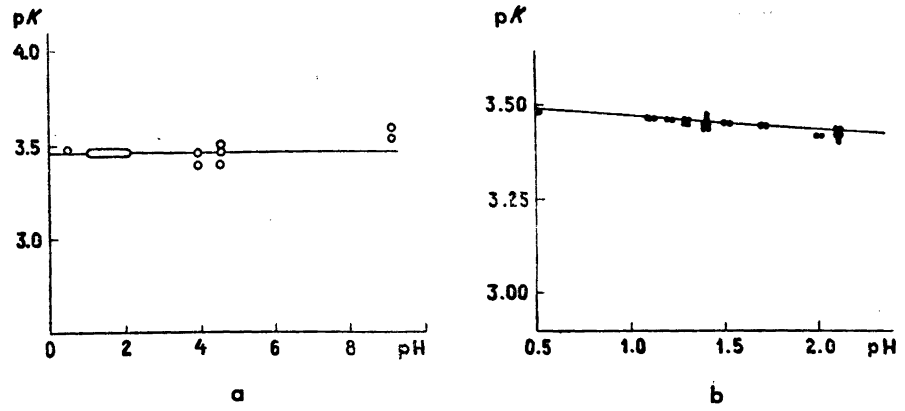

Fig. 4. Values for pK.

Nor has any phenomenon been observed indicating an »over-activity» of acetylene when dispersed in fine bubbles.

Computations of the equilibrium constant should thus be based on measurements performed at a pH less than 2. Isolated experiments with tolerably constant potentials at a higher pH are however satisfactory for the conclusion that pK, according to the equilibrium formula, is in fact independent of pH. *Cf.* Fig. 4 a where the computed pK values are plotted against pH.

But also results from experiments in acid medium give rise to some doubts. All the measurements within the range considered in Fig. 4 b (pH between 0.5 and 2.2) give pK values between 3.4 and 3.5, but there is a certain trend towards higher values at lower pH. Thus it is not permissible to calculate a mean on the assumption that the variations are caused by incidental measuring errors. The mean value of the equilibrium constant

$$pK = 3.46 \text{ at } 25^\circ \text{ C}$$

may therefore be somewhat uncertain in the second decimal place.

THERMODYNAMIC DATA FOR THE REACTION SILVER CARBIDE → ACETYLENE

An estimation of the change in enchresy [9] from the equilibrium constant pK = 3.46 gives

$$\tfrac{1}{2}\, C_2Ag_2 + H^+ = \tfrac{1}{2}\, C_2H_2 + Ag^+ \qquad \Delta G^\circ = +4720 \text{ cal} \qquad (7)$$

If the standard potential of silver at 25° C is assumed to be + 800 mV we get for the reaction

$$\tfrac{1}{2}\,H_2 + Ag^+ = H^+ + Ag(s) \qquad \Delta\,G^\circ = -\,18420\ \text{cal} \qquad (8)$$

and then, for the non-ionic reaction, can be calculated

$$C_2Ag_2(s) + H_2 = C_2H_2 + 2\,Ag(s) \qquad \Delta G^\circ = -\,27400\ \text{cal} \qquad (9)$$

Thermodynamically the conditions for a reduction of silver carbide are favourable, corresponding to the equilibrium constant

$$K = P_{C_2H_2}/P_{H_2} = 10^{20.1} \qquad (10)$$

Two measurements from literature can be used for the calculation of the heat of reaction.

(a) Berthelot and Delépine[10] made calorimetric measurements at the precipitation of silver carbide from an aqueous solution of acetylene + an aqueous solution of silver nitrate with excess ammonia and got

$$\tfrac{1}{2}C_2Ag_2 + NH_4^+ + NH_3 = \tfrac{1}{2}\,C_2H_2\,(\text{diss.}) + Ag(NH_3)_2^+ \qquad \Delta\,H = +\,7780\ \text{cal} \qquad (11)$$

A utilization of Berthelot's results for the computation of Δ H of reactions (7) or (9) demands supplementary heat data. Berthelot's own calculations give, using somewhat different nomenclature

$$\tfrac{1}{2}\,C_2Ag_2 + H^+ = \tfrac{1}{2}\,C_2H_2(g) + Ag^+ \qquad \Delta\,H = 11.300\ \text{cal} \qquad (12)$$

The use of newer values for the data necessary for the recalculation give a difference albeit rather small. The uncertainty of Berthelot's primary determination is however too large to justify such a recalculation. For an estimation of the heat of the non-ionic reaction from the value given above the Δ H-value must be known for $Ag^+ \rightarrow Ag(s)$. The most reliable seems to be Lingane's[11] determination of the temperature coefficient of the standard silver electrode (dE/dT) at 25° C = 0.967 mV/degree. Together with (8) this gives

$$\tfrac{1}{2}\,H_2 + Ag^+ = H^+ + Ag(s) \qquad \Delta\,H = -\,25.070\ \text{cal} \qquad (13)$$

From (12) and (13) we get

$$C_2Ag_2(s) + H_2 = C_2H_2 + 2\,Ag(s) \qquad \Delta\,H = -\,27.600\ \text{cal} \qquad (14)$$

(b) Stadler[12] gives a value for the heat of explosion of silver carbide, as measured in a detonation bomb.

$$C_2Ag_2 = 2\,C\ (\text{graphite}) + 2\,Ag(s) \qquad \Delta\,H = -\,70.300\ \text{cal} \qquad (15)$$

From Stadler's result we obtain directly, using the most reliable (according to Parks and Huffmann [13]) value for acetylene's heat of formation

$$2\,C\,(graphite) + H_2 = C_2H_2 \qquad \Delta H = +\,59.400\ cal \qquad (16)$$

the following value for the change in enthalpy in the non-ionic reaction

$$C_2Ag_2(s) + H_2 = C_2H_2 + 2\,Ag(s) \qquad \Delta H = -\,15.400\ cal \qquad (17$$

and by application of (14) we get for the ionic reaction

$$\tfrac{1}{2}\,C_2Ag_2(s) + H^+ = \tfrac{1}{2}\,C_2H_2 + Ag^+ \qquad \Delta H = +\,17.400\ cal \qquad (18)$$

The difference between the values which can be calculated from Berthelot's — (12) and (14) and Stadler's — (18) resp. (17) — determinations is thus considerable. Only two further quantities are required when using Stadler's values but the result appears as a relatively small difference between the large heats of formation of silver carbide and acetylene. For the present Stadler's value is to be preferred.

For the reaction

$$C_2Ag_2 \rightarrow C_2H_2$$

the following thermodynamic data are thus available

Reaction	$\Delta G°$ cal	ΔH cal	$\Delta S°$ cal/degree
$\frac{1}{2}\,C_2Ag_2(s) + H^+ = \frac{1}{2}\,C_2H_2(g) + Ag^+$	+ 4.720	+ 17.400	+ 42.6
$C_2Ag_2(s) + H_2 = C_2H_2(g) + 2\,Ag(s)$	— 27.400	— 15.400	+ 40.3

COMPARISON WITH OTHER ACETYLIDES

The potentiometric method for determining equilibrium constants for reactions of the type

$$-C\equiv CM + H^+ = -C\equiv CH + M^+$$

offers a possibility of comparing the stabilities of different acetylides.

Experiments in progress on the system cuprous carbide — acetylene have given the preliminary result

$$\tfrac{1}{2}\,C_2Cu_2 + H^+ = \tfrac{1}{2}\,C_2H_2 + Cu^+;\ pK = 5.8 \qquad \Delta G° = +\,7.900\ cal \qquad (19)$$

and for the non-ionic reaction

$$C_2Cu_2 + H_2 = C_2H_2 + 2\,Cu(s) \qquad \Delta G° = -\,8.000\ cal \qquad (20)$$

When comparing with the corresponding value for silver carbide it is evident that the cuprous carbide is essentially more stable

$$C_2Ag_2 + 2\,Cu(s) = C_2Cu_2 + 2\,Ag(s) \qquad \Delta G^\circ = -19.000 \text{ cal} \qquad (21)$$

If the procedure described is to be used for a comparison between acetylene and monoacetylenes, RC≡CH, it must be limited to such monoacetylenes as can be handled in the gaseous state, or at least have a considerable vapour pressure at 25° C. A determination of pK for butenyne gave

$$CH_2 = CH - C \equiv CAg + H^+ = CH_2 = CH - C \equiv CH + Ag^+;\ pK = 0.88$$
$$\Delta G^\circ = +1.200 \text{ cal} \qquad (22)$$

(As in the case of acetylene the activity of the hydrocarbon is measured in atmospheres. Gaseous butenyne is near enough ideal to render this acceptable.) Thus the silver compound of butenyne is considerably easier to split then silver carbide. In a 0.5 *M* silver perchlorate solution saturated with butenyne silver, which, like silver carbide, forms soluble complexes with silver ions, a considerable butenyne pressure can be shown after the addition of acid, whilst such breaking down of silver carbide is not to be observed with ordinary methods.

It is conceivable that all monoacetylenes have p*K*-values, which, like that for butenyne, are considerably lower than for acetylene. The conjugated system C = C — C ≡ C whose resonance must diminish the tendency to acetylide formation, and on the whole the acetylenic character of the terminal hydrogen atom, is however specific for butenyne. A utilization of the experimentally determinable pK-values for an evaluation of the »acidity» of the CH-bond in RC≡CH-compounds, and a systematic investigation of the effect of different substituents, R, would be of great interest. The definition of the standard conditions (e.g. 1 atm. pressure for the hydrocarbons, resp. solid state for the acetylide) involves however an inescapable arbitrariness.

SUMMARY

1. For the determination of the equilibrium constant of the reaction

$$\tfrac{1}{2}\,C_2Ag_2 + H^+ = \tfrac{1}{2}\,C_2H_2 + Ag^+$$

a potentiometer method has been devised, based on measurement of the potential in a cell without liquid junction: — glass | A | C_2Ag_2(s), Ag + , where A is a solution in equilibrium with solid silver carbide and with a gas mixture of known acetylene pressure. A simple method for the determination of the standard

potential of the electrode couple is given. By using perchlorate, which, under the conditions prevailing, does not form insoluble complex salts with silver carbide, it has been possible to perform the experiments over a wide pH-range

2. Only in acid solution are the values well reproducible, giving the mean

$$pK = pAg - pH + \tfrac{1}{2}\, pP_{C_2H_2} = 3.46 \text{ at } 25^\circ C$$

(acetylene activity measured in atm.)

3. The thermodynamic constants (ΔG°, ΔH and ΔS°) have been calculated for the formulated reaction and for the non-ionic reduction of silver carbide to acetylene.

4. The possibility of using a pK-value, obtained by this method for characterization of various acetylene compounds of the type RC $\equiv$ CH has been discussed. The pK-value of butenyne is only 0.88; the difference from acetylene is supposedly dependent on the conjugated system C=C—C$\equiv$C of butenyne.

This work has been supported by a grant from *Statens Tekniska Forskningsråd*, intended for a series of investigations directed by Professor Arne Ölander and fil.lic. Ragnar Vestin. The investigation published here has been planned and performed in collaboration with Professor Ölander. Special thanks are due also to Docent Sven Brohult, LKB Research Laboratory, who has taken great interest in our work.

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Received November 11, 1948.

ACTA CHEMICA SCANDINAVICA 3 (1949) 144—150

Investigations in the Retene Field

II. The Structure of 3-Hydroxy-9-nitroretene and some of its Derivatives

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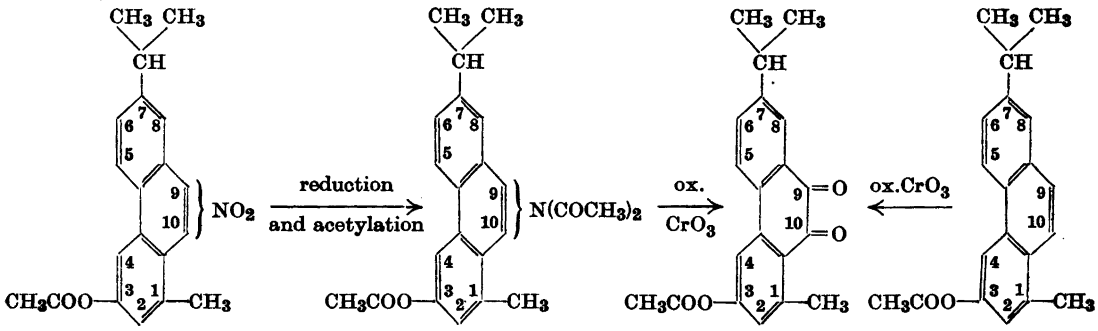
Earlier investigations carried out to throw some light on the reaction between retene and nitric acid, also included some nitrations of retene derivatives [1-3]. In this connection the mononitro-derivatives of the easily obtainable compounds 3-acetoxyretene, 3-hydroxyretene, 3-benzoyloxyretene and 3-ethoxyretene were prepared [2]. Since the structure of these compounds is of interest in the investigation of the nitration products of retene, a determination of their structure will be given here.

Previous investigations have shown that the nitration of 3-acetoxyretene yields, under suitable conditions, a mononitro-3-acetoxyretene. The acetyl-group of this compound is easily split off and the corresponding mononitro-3-hydroxyretene obtained. The latter forms, on benzoylating, the same mononitro-3-benzoyloxyretene which is obtained by nitration of 3-benzoyloxyretene. Thus, the nitro-group occupies the same position in all these derivatives, which are here called x-nitroderivatives. The mononitro-derivative, obtained on mild nitration of 3-ethoxyretene, is named 3-ethoxy-y-nitroretene.

On nitration of retene a few per cent of pure 9-nitroretene can be isolated [4]. Mild nitration of 3-acetaminoretene yields a mixture containing about 60 per cent of 3-acetamino-9-nitroretene and 40 per cent of 3-acetamino-4-nitroretene [5]. Thus, there was some reason to investigate whether the nitro-group might not probably occupy the 9-position in the x- and y-compounds. Oxidation of the derivatives to quinone is a simple method of deciding whether this is the case. A nitro-group in the 9 or 10-position should be eliminated by this reaction. According to experiments reported in the literature, oxidation of the compounds in question does not yield quinones [2]. In the present work,

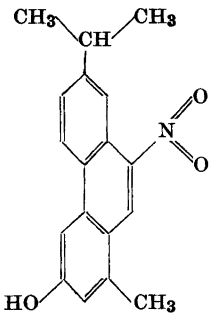
however, it has been found that if the nitro-group is first reduced to an amino-group, a quinone is easily formed on oxidation. The following reactions prove that the nitro-group of 3-acetoxy-x-nitroretene may possibly occupy the 9- or 10-position, since the resulting compound is identical with 3-acetoxyretene-quinone [6]. — To protect the amino-group (if situated in another position than 9 or 10) during the oxidation, the acetylated amine was used. Since the N-diacetyl-compound is easily prepared, it was used in the oxidation experiment.



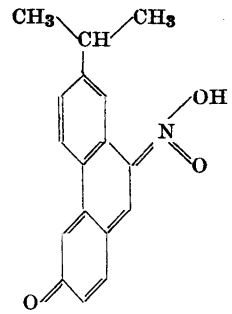
In order to judge between these two possible positions 9 and 10 for the nitro-group, 3-hydroxy-9-nitroretene was prepared from 3-amino-9-nitroretene [3]. This hydroxy-nitroretene is identical with the 3-hydroxy-x-nitroretene, mentioned above, and consequently the nitro-group occupies the 9-position in the latter compound. The benzoyl- and acetyl-derivatives of the two hydroxy-nitroretenes were found to be identical which was only to be expected as the hydroxy-nitroretenes are identical.

On ethylation, 3-hydroxy-9-nitroretene yields an ether which is shown to be identical with the 3-ethoxy-y-nitroretene, obtained on mild nitration of 3-ethoxyretene. Consequently, in this case also the nitro-group occupies the 9-position.

On ethylation — which was carried out in alkaline ethanolic solution — it was observed that the yellow 3-hydroxy-9-nitroretene dissolves in alkalis giving a red colour. The reaction is reversible, for, on acidification, the yellow modification is again obtained. The change of colour may probably be schematically explained in the same way as in other analogous cases by the following structural changes in the molecule.



Neutral and acid solution



Alkaline solution

The reddish-yellow colour of 3-amino-9-nitroretene and 3-amino-4-nitroretene [5] may possibly be explained in a similar way.

It is reported in the literature [7] that reduction of 3-acetoxy-9-nitroretene yields an unstable amine which was isolated as its N-acetyl-derivative. As seen from the description of the synthesis (see the experimental part of the present work), this amine can be prepared by reducing the nitro-compound with $Na_2S_2O_4$ in ethanolic solution. It is obtained as white scales showing no tendency to decompose.

Since there is a possibility that the reduction of 3-acetoxy-9-nitroretene, reported in the literature, yields 3-hydroxy-9-aminoretene (assuming that the acetyl-group was split off during the reduction which was carried out with $SnCl_2$ in a solution containing hydrogen chloride), it was of interest to prepare this hydroxy-aminoretene and investigate its stability. On reducing 3-hydroxy-9-nitroretene with $Na_2S_2O_4$ in ethanolic solution, a white product was obtained which crystallized beautifully as long needles. Analysis of the compound and of its acetyl-derivative showed that 3-hydroxy-9-aminoretene was formed. No decomposition of the compound was observed.

The reported instability of 3-acetoxy-9-aminoretene was therefore probably due to impurities.

The compounds discussed in this paper are listed in Table 1 (p. 147).

EXPERIMENTAL

3-Acetoxy-9-aminoretene

To a boiling solution of 2.0 g of 3-acetoxy-9-nitroretene in 500 ml of ethanol was added in portions over a period of 5 minutes, a solution of 8 g of $Na_2S_2O_4$ in 100 ml of water. The pale yellow solution gradually faded and was at last completely colourless. Inorganic salts are precipitated during the reduction. To the hot reaction mixture 800 ml of water was added. The salts dissolved, and from the clear solution small, white scales

Table 1. Compounds the structure of which are determined in the present work.

(Hydrochlorides and picrates not included).

Name	Formula	M.p. °C *
3-Ethoxy-9-nitroretene	$C_2H_5OC_{18}H_{16}NO_2$	114—114.5
3-Benzoyloxy-9-nitroretene	$C_6H_5COOC_{18}H_{16}NO_2$	145.5—146.5
3-Acetoxy-9-nitroretene	$CH_3COOC_{18}H_{16}NO_2$	197—198
3-Hydroxy-9-nitroretene	$HOC_{18}H_{16}NO_2$	178.5—179
3-Hydroxy-9-aminoretene **	$HOC_{18}H_{16}NH_2$	ca. 230 (decomp.)
3-Acetoxy-9-aminoretene **	$CH_3COOC_{18}H_{16}NH_2$	172—173
3-Acetoxy-9-acetaminoretene	$CH_3COOC_{18}H_{16}NHCOCH_3$	205.5—206.5
3-Acetoxy-9-diacetylaminoretene **	$CH_3COOC_{18}H_{16}N(COCH_3)_2$	186—186.5

began to crystallize. At 0 °C the crystals were filtered off, washed with water and dried. Yield 1.2 g. Recrystallization from ethanol yielded a pure product melting at 172—173 °C.

$C_{20}H_{21}O_2N$	Calc.	C 78.1	H 6.88	N 4.56
	Found	» 78.4	» 6.90	» 4.48

The *hydrochloride* crystallizes as small, white needles when a hot ethanol solution of the amine is diluted with dilute hydrochloric acid and allowed to cool. After washing with ether and drying, the hydrochloride was titrated with 0.1 N sodium hydroxide. Approximate m.p. 235—240 °C (decomp.).

$C_{20}H_{22}O_2NCl$ Calc. HCl 10.6 Found HCl 10.5

The *picrate* was obtained from ethanol as yellow needle-shaped crystals melting at approximately 221 °C (decomp.). The picrate is slightly soluble in ethanol.

$C_{26}H_{24}O_9N_4$	Calc.	C 58.2	H 4.51
	Found	» 58.3	» 4.42

The *N-monoacetyl-derivative* is formed on mild acetylation. The amine was boiled in acetic anhydride for one minute and after decomposition of the anhydride with water, the white reaction product was crystallized from ethanol. 3-Acetoxy-9-acetaminoretene forms small, white, needle-shaped crystals with m.p. 205.5—206.5 °C.

$C_{22}H_{23}O_3N$	Calc.	C 75.6	H 6.65
	Found	» 76.0	» 6.53

* The melting points are approximately corrected.
** Compound not published before.

The *N-diacetyl-derivative* is obtained when the amine is boiled in acetic anhydride for 30 minutes. It crystallizes from propanol as short, white prisms. M.p. 186—186.5 °C.

$C_{24}H_{25}O_4N$	Calc.	C 73.6	H 6.44	N 3.58
	Found	» 73.6	» 6.50	» 3.64

This derivative can also be prepared by boiling 3-hydroxy-9-aminoretene in acetic anhydride for 30 minutes.

3-Acetoxyretenequinone

This compound is usually prepared by oxidation of 3-acetoxyretene [6]. Here it is shown that it can be prepared from N-diacetyl-3-acetoxyretylamine-(9). 0.6 g of the latter compound was suspended in 10 ml of glacial acetic acid, and to this suspension 1.2 g of CrO_3 was added. At the beginning of the reaction the temperature was 40 °C but the heat of the reaction caused it to rise. However it was kept below 70 °C. When the reaction was finished, 5 ml of water was added and the solution allowed to cool. The crystallized quinone, thus obtained, was filtered off and recrystallized from glacial acetic acid. It forms flat, orange-coloured, needle-shaped crystals with m. p. 200—201 °C. Yield 0.2 g. Admixture of 3-acetoxyretenequinone, prepared from 3-acetoxyretene, does not cause any change in the melting point.

$C_{20}H_{18}O_4$	Calc.	C 74.5	H 5.63
	Found	» 74.3	» 5.70

3-Hydroxy-9-nitroretene

2.0 g of 9-nitro-3-retylamine hydrochloride were suspended in a mixture of 180 ml of glacial acetic acid, 30 ml of water and 2 ml of conc. sulphuric acid. The amine was diazotized at 20 °C with a solution of 1 g of $NaNO_2$ in 10 ml of water. To the pale yellow diazonium salt solution 60 ml of dilute sulphuric acid (1 volume of water and 1 volume of conc. sulphuric acid) were added, followed by an excess of urea. The solution was slowly heated to 60 °C and kept at this temperature for three or four hours. After the addition of 100 ml of water, the solution was allowed to cool. The precipitated hydroxy-compound was filtered off washed with water and dried. Yield crude product 1.6 g. Two recrystallizations from glacial acetic acid yielded pure product. M.p. 178.5—179 °C. If the compound is mixed with the nitroretenol which is obtained by splitting off the acetyl-group from nitrated 3-acetoxyretene, no change in the melting point can be observed.

$C_{18}H_{17}O_3N$	Calc.	C 73.2	H 5.80
	Found	» 73.0	» 5.72

3-Acetoxy-9-nitroretene was prepared by boiling the hydroxy-compound in acetic anhydride for 45 minutes. The 3-acetoxy-9-nitroretene thus obtained crystallizes as pale yellow scales with m.p. 197—198 °C.

$C_{20}H_{19}O_4N$	Calc.	C 71.2	H 5.67
	Found	» 70.7	» 5.59

On nitration, 3-acetoxyretene yields a mononitro-derivative with the same melting point 197—198 °C. A mixture of this compound with the 3-acetoxy-9-nitroretene described above melts at 197—198 °C.

3-Benzoyloxy-9-nitroretene was prepared by treating 3-hydroxy-9-nitroretene with benzoyl chloride in pyridine solution. From ethanol, it crystallizes as long, yellow needles with m.p. 145.5—146.5 °C.

$C_{25}H_{21}O_4N$	Calc.	C 75.2	H 5.30
	Found	» 75.0	» 5.30

On nitration, 3-benzoyloxyretene yields a mononitro-3-benzoyloxyretene which after some recrystallizations from ethanol (boiling the ethanol solution with some charcoal is recommended) is quite pure and has the same melting point as 3-benzoyloxy-9-nitroretene. A mixture of the two compounds also melts at 145.5—146.5 °C.

3-Ethoxy-9-nitroretene was prepared in the following way. 0.5 g of 3-hydroxy-9-nitroretene was dissolved in 8 ml of ethanol and a solution of 1 g of KOH in 5 ml of water was added. An intensely red-coloured solution was formed. At 5 °C, 1.5 ml of diethyl sulphate was added and the mixture was stirred at this temperature for ten hours. Gradually the ethyl ether precipitated as a yellow crystalline product and the red colour became weaker. Two recrystallizations from propanol yielded a pure substance, crystallizing as long, thin, yellow needles with m.p. 114—114.5 °C.

$C_{20}H_{21}O_3N$	Calc.	C 74.3	H 6.55
	Found	» 74.3	» 6.56

On mild nitration of 3-ethoxyretene, a mononitro-3-ethoxyretene is formed. Its melting point is the same as that of 3-ethoxy-9-nitroretene. A mixture of these two compounds also gave the melting point 114—114.5 °C.

3-Hydroxy-9-aminoretene

A solution of 15 g of $Na_2S_2O_4$ in 75 ml of water was slowly added with stirring to a solution of 7.5 g of 3-hydroxy-9-nitroretene in 200 ml of dilute ethanol (75 per cent). As the reduction progressed, 3-hydroxy-9-aminoretene precipitated and the reaction mixture was practically decolourized. After the addition of 200 ml of water and cooling to 20 °C, the reaction product was filtered off, washed with water and dried. Yield crude product 6.0 g. After recrystallization from ethanol, 3-hydroxy-9-aminoretene was obtained as long, white, needle-shaped crystals. The compound melts within an interval of some degrees at approximately 230 °C (decomp.).

$C_{18}H_{19}ON$	Calc.	C 81.5	H 7.23	N 5.28
	Found	» 81.6	» 7.18	» 5.24

The *hydrochloride*. To a hot solution of 3-hydroxy-9-aminoretene in ethanol, dilute hydrochloric acid was added. On cooling the hydrochloride was obtained as white needles. It was filtered off, washed with ether and dried at 100 °C for 15 minutes. The amount of HCl was determined by titration with 0.1 N sodium hydroxide.

$C_{18}H_{20}ONCl$ Calc. HCl 12.1 Found HCl 12.2

On heating, the hydrochloride decomposes and shows no definite melting point. The decomposition product melts within an interval of some degrees at approximately 275—280 °C.

The *picrate* crystallized from ethanol solution as yellow scales. Like the hydrochloride it decomposes on heating. When the temperature exceeds 200 °C the picrate turns dark, but does not melt.

$C_{24}H_{22}O_8N_4$	Calc.	C 58.3	H 4.49
	Found	» 58.1	» 4.62

The combustion must be carried out carefully, otherwise the picrate decomposes very rapidly.

SUMMARY

Previously prepared mononitro-derivatives of 3-ethoxyretene, 3-benzoyloxyretene, 3-acetoxyretene and 3-hydroxyretene are shown to have the nitro-group in the 9-position. 3-Hydroxy-9-aminoretene and its acetyl-derivatives have been prepared. 3-Acetoxy-9-aminoretene, reported in the literature as an unstable amine, has been isolated and shows no tendency to decompose.

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Received January 13, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 151—156

Syntheses and Properties of some Acetylated Alkyl Glucosides

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In connection with an investigation of the action of strong acids on acetylated glucosides, it was necessary to prepare a number of acetylated alkyl glucosides. This paper deals with the syntheses and properties of these substances. Most of them are already known but have in some cases been prepared by new methods. All specific rotations have been measured in chloroform solution (2 %), because this seems to be the solvent most generally used. The specific rotations of many of the previously known substances had been measured in other solvents.

The molecular rotations (Tables 1 and 2) show certain regularities. Methanol is a »super» primary alcohol and its glucosides stand in a class by themselves, but for the four other primary alkyl β-glucosides the molecular rotation is remarkably constant. Also the secondary alkyl β-glucosides have about the same molecular rotations with one exception, cyclopentyl β-glucoside. This is not unexpected. One must assume that the cyclopentane ring, with its rigid, planar configuration, will deviate considerably from open, aliphatic chains in its light absorbing properties. The molecular rotations of the other cycloalkyl β-glucosides are also somewhat different from those with open chains but here the deviations are much smaller, being surprisingly small for cyclobutyl β-glucoside. The β-glucosides of the two optically active butanols can not be compared directly with the other ones, but the mean value of their molecular rotations (— 9,800°) agrees well with the rest of the series studied.

For the β-glucosides in which the alkyl group contains chlorine, hydroxyl, alkoxyl or acetoxyl groups, the molecular rotation is higher than for the unsubstituted alkyl β-glucosides.

Rather few alkyl α-glucosides have been prepared. The transformation of the β-glucosides into the α-form is not particularly diffitl,cu but the acetylated

Table 1. Tetraacetyl-β-alkyl glucosides.

Alkyl group	M.p. °C *	$[\alpha]_D^{20}$	$[M]_D^{20}$
Methyl	104—105	— 18.7°	— 6,800°
Ethyl	106—107	— 22.7	— 8,500
Propyl	102—103	— 20.6	— 8,000
n-Butyl	65.5—66.5	— 20.2	— 8,200
iso-Butyl	120—121	— 19.8	— 8,000
iso-Propyl	136—137	— 24.4	— 9,500
l-sec. Butyl	125—126	— 34.4	— 13,900
d-sec. Butyl	101—103	— 14.2	— 5,700
Pentyl (3-)	109.5—110.5	— 21.5	— 9,000
Cyclobutyl **	124—126	— 26	— 10,500
Cyclopentyl **	134.5—135.5	— 33.6	— 14,000
Cyclohexyl	120—121	— 23.8	— 10,200
Cycloheptyl **	108.5—109	— 22.0	— 9,800
tert. Butyl	144—144.5	— 11.6	— 4,700
Allyl	89.5—90	— 24.2	— 9,400
Benzyl	96—97	— 53.2	— 23,300
$ClCH_2CH_2$-	117.5—118	— 13.8	— 5,600
$HOCH_2CH_2$-	103—103.5	— 7.6	— 3,000
$AcOCH_2CH_2$- **	53.5—54.5	— 14.4	— 6,200
$H_5C_2OCH_2CH_2$-	65—66	— 19.5	— 8,200
$HOCH_2CH_2CH_2$-	97—98	— 17.0	— 6,900

Table 2. Tetraacetyl-α-alkyl glucosides.

Alkyl group	M.p. °C *	$[\alpha]_D^{20}$	$[M]_D^{20}$
Methyl	100—101	+ 131°	+ 47,400
Ethyl	60.5—61.5	+ 132	+ 49,700
iso-Propyl	85.5—86.5	+ 143	+ 55,800
Cyclopentyl **	46—47	+ 140	+ 58,200
Cyclohexyl	40—41	+ 122	+ 52,300
tert. Butyl	69—70	+ 129 ***	+ 52,100***
Allyl	51—52	+ 130	+ 50,400
Benzyl	109.5—110.5	+ 142.5	+ 62,400

* All melting points uncorrected.
** These substances are new.
*** The values reported in a previous communication [10] are erroneous.

α-glucosides of this type have low melting points and it is often very difficult to obtain them in crystalline form. This is unfortunate, because when both the α- and β-glucosides are known, one can determine the Hudson values, which are more informative than the molecular rotations.

The 2B values (Table 3) are, in correspondance with the theory, rather constant. Their mean value is about 43,000. The 2A value shows much greater variation. It increases in the series methyl < ethyl < *iso*propyl. For cyclohexyl it is about the same as for *iso*propyl, while the value for cyclopentyl is much higher. The low value for tertiary butyl is unexpected; from the series above one would expect a higher value. The benzyl glucosides have the highest 2A value, but also here the 2B value is quite normal.

Table 3. Hudson values for tetraacetyl alkyl glucosides.

Alkyl group	2A	2B
Methyl	54,200	40,600
Ethyl	58,200	41,200
iso-Propyl	65,300	46,300
Cyclopentyl	72,200	44,200
Cyclohexyl	62,500	42,100
tert. Butyl	56,800 ***	47,400 ***
Allyl	59,800	41,000
Benzyl	85,700	39,100

EXPERIMENTAL PART

The mercuric acetate method

Some tetraacetyl-β-alkyl glucosides were prepared by a modification of the method of Zemplén [1] for alkyl cellobiosides.

Acetobromoglucose (8.22 g, 20 mmole) was dissolved in absolute benzene (40 ml). The anhydrous alcohol in question (16 ml) and mercuric acetate (3.03 g, 9.5 mmole) were added and the mixture was boiled for 15 minutes on the steam bath. When cold the solution was washed with water, dried over calcium chloride and concentrated under reduced pressure. The residue was recrystallized from ethanol-water, 1 : 1.

The glucosides prepared by this method are listed in Table 4.

From Table 4 it is evident that the mercuric acetate method is satisfactory for the preparation of tetraacetyl-β-alkyl glucosides. The yields are rather good, 50 % or better, and it is quicker than the method of Koenigs and Knorr. The reaction time is much shorter and commercially available mercuric acetate is used instead of the silver oxide or carbonate which must be freshly prepared. When, however, the alcohol is expensive,

Table 4. Glucosides prepared by the mercuric acetate method.

Alkyl group	M.p. °C	Yield %	Previous preparation Reference	Yield %
Methyl	104—105	57	2	51
Ethyl	106—107	56	2	70
n-Propyl	102—103	63	3	76
iso-Propyl	136—137	66	3	57
n-Butyl	65.5—66.5	51	4	71
sec.-Butyl	Mixture of d- and l-forms	56	4	—
iso-Butyl	120—121	59	4	53
tert. Butyl	144—144.5	45	4	39
Allyl	89.5—90	69	5	80
$ClCH_2CH_2$-	117.5—118	59	6	50
$H_5C_2OCH_2CH_2$-	65—66	50	9	82

the method is out of the question. In this case the method of Koenigs and Knorr, improved by Helferich and Goerdeler [5], is to be preferred.

The characteristic data of the substances prepared by this method correspond well with those of other authors. The only exception is tetraacetyl-β-(β-chlorethyl) glucoside. Coles, Dodds and Bergeim [6] report that their substance melted at 114° and contained 8.36 % of chlorine. The substance prepared in this laboratory melted at 117.5—118°.

$C_8H_{11}O_6Cl\,(OCCH_3)_4$ (410.7) Calc. Cl 8.63 Acetyl 41.9
Found » 8.69 » 41.9

The yield of *tert.* butyl glucoside was raised from 29 to 45 % when the reaction was carried out in tertiary butanol without any benzene.

The crude mixture of the tetraacetyl-β-butyl (2-) glucosides was fractionated by recrystallization from ethanol-water. The pure *l*-derivative (configuration determined by Veibel and Lillielund [4]) melted at 125—126° and had a specific rotation of — 34.4°. The corresponding data for the *d*-derivative were: M.p. 101—103° $[\alpha]_D^{20}$ — 14.2°.

Tetraacetyl-β-cyclopentyl glucoside

Tetraacetyl-β-cyclohexyl glucoside and tetraacetyl-β-benzyl glucoside were prepared by the method of Fischer and Helferich [7]. Tetraacetyl-β-cyclopentyl glucoside was prepared analogously.

Acetobromoglucose (30 g) was dissolved in a mixture of absolute ether (400 ml) and absolute cyclopentanol (100 g) and freshly prepared silver oxide (15 g) was added. The mixture was shaken for 5 hours and then centrifuged, filtered and concentrated under reduced pressure to a sirup which was submitted to steam distillation. When most of the

cyclopentanol had been removed, the steam distillation was discontinued. The glucoside, a slight yellow sirup, soon crystallized. It was recrystallized from ethanol.

Yield 21 g (69 %) M.p. 134.5—135.5°. $[\alpha]_D^{20}$ — 33.6°.

$C_{11}H_{16}O_6(OCCH_3)_4$ (416.2) Calc. Acetyl 41.3 Found Acetyl 41.5

Tetraacetyl-α-cyclopentyl glucoside

Tetraacetyl-α-cyclohexyl glucoside was prepared by the method of Pacsu [8]. Tetraacetyl-α-cyclopentyl glucoside was prepared analogously. Titanium tetrachloride (4.5 g) in absolute chloroform (30 ml) was added to a solution of tetraacetyl-β-cyclopentyl glucoside (10 g) in absolute chloroform (100 ml). The mixture was boiled under reflux in the absence of moisture for 75 minutes. It was then allowed to cool and poured into ice-water. The colorless chloroform solution was washed with aqueous potassium bicarbonate and with water, dried over calcium chloride and concentrated under reduced pressure. The residue was dissolved in light petroleum. After several weeks in the refrigerator the substance crystallized. Yield 7.5 g. M.p. 46—47°. $[\alpha]_D^{20}$ + 140°.

$C_{11}H_{16}O_6(OCCH_3)_4$ (416.2) Calc. Acetyl 41.3 Found Acetyl 41.4.

Tetraacetyl-β-cycloheptyl glucoside

Tetraacetyl-β-cycloheptyl glucoside was prepared by the method of Helferich and Goerdeler [5].

Acetobromoglucose (7.5 g) was dissolved in a mixture of cycloheptanol (6 ml) and absolute chloroform (100 ml). Drierite (7 g) was added and the mixture was shaken for 30 minutes. Then, freshly prepared silver oxide (4 g) was added and the shaking continued for 10 hours. The mixture was worked up in the same way as for the β-cyclopentyl glucoside.

Yield 6.4 g. (79 %) M.p. 108.5—109.5°. $[\alpha]_D^{20}$ — 22°.

$C_{13}H_{20}O_6(OCCH_3)_4$ (444.2) Calc. Acetyl 38.9 Found Acetyl 38.9

By the same method the β-glucosides of cyclobutanol and pentanol (3-) were prepared.

Tetraacetyl-β-cyclobutyl glucoside

No yield can be specified because one of the starting materials, cyclobutanol, was not pure and the amount available was too small to permit any purification. The resulting glucoside was recrystallized from ethanol until the melting point and the specific rotation were constant. M.p. 124—126°. $[\alpha]_D^{20}$ — 26°.

$C_{10}H_{14}O_6(OCCH_3)$ (402.2) Calc. Acetyl 42.8 Found Acetyl 43.0

Tetraacetyl-β-pentyl(3-) glucoside

Previously prepared by Veibel. [11]. Yield 75 %. M.p. 83—85°. $[\alpha]_D^{20}$ — 21.5°

$C_{11}H_{18}O_6(OCCH_3)$ (418.2) Calc. Acetyl 41.1 Found Acetyl 41.0

Pentaacetyl-β-glycol glucoside

Tetraacetyl-β-glycol glucoside was prepared according to Karjala and Link [9]. Pentaacetyl-β-glycol glucoside was prepared by acetylation of the tetraacetyl derivative with acetic anhydride in pyridine. Yield 100 %. The substance was recrystallized from ether — light petroleum. M.p. 53.5—54.5°. $[\alpha]_D^{20}$ — 14.4°.

Tetraacetyl-β-trimethyleneglycol glucoside was prepared by the method of Karjala and Link [9].

The syntheses of the α-glucosides of methyl-, ethyl-, *iso*propyl-, *tert.* butyl-, allyl- and benzyl-alcohol have been described in earlier papers [10].

SUMMARY

A number of acetylated alkyl glucosides, five of which are new, have been prepared. Eleven glucosides have been prepared by the mercuric acetate method, which was found to be a satisfactory method. The molecular rotation of the substances is discussed.

The author wishes to thank *Statens Naturvetenskapliga Forskningsråd* for a grant and Mr. L. Asp for skilful assistance.

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Received January 31, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 157—162

Enzymatic Breakdown of Polymetaphosphate. III

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In previous papers [1,2] it has been shown that polymetaphosphates of very high molecular weight are broken down by enzymes from moulds such as *Aspergillus niger*, *Aspergillus oryzae* and *Penicillium expansum*. In these experiments preparations of polymetaphosphate were used having molecular weights of more than one million. Thus in the latter experiments a colloid of purely inorganic character was broken down by enzymatic means. (Concerning the earlier investigations on metaphosphatases see the references in our previous articles.)

During the course of continued investigation attention has also been paid to the occurrence of polymetaphosphate degrading enzymes in other organisms than those mentioned above. In this paper some experiments carried out on enzyme extracts from some different kinds of fungi and bacteria will be described.

As substrate for these experiments a high molecular polymetaphosphate $(KPO_3)_n$ has been used; the preparation is designated K 15. The substance, the molecular weight of which was determined, as 1,200,000, has been described in a previous paper [2]. When preparing the substrate solutions this polymetaphosphate has been dissolved in solutions containing an excess of sodium ions.

With some exceptions (see below) the organisms have been cultivated under sterile conditions on suitable nutrient solutions or agar-plates. The enzyme extracts have been prepared by grinding up the organisms in water or in a suitable buffer. (For more details concerning the enzyme preparation reference is made to [1].) In some cases the organisms have been treated with acetone for a short time before the enzyme extraction in order to remove lipoid substances. The extracts have been dialyzed in cellophane bags against water at some degrees above zero for some days. Before being used for experiments the extracts have been centrifugated and filtered.

As previously the breakdown has been studied at 25 °C by means of viscosity measurements in a capillary viscosimeter according to Ostwald. The experiments have been performed in acetate and phosphate buffers of different pH-values in order to determine the pH-optima of the enzymes. As a measure of the enzyme activity for comparing experiments the z-values defined in an earlier paper have been used [2]

$$z = (\eta_{sp})_{t=0} \cdot \frac{d(1/\eta_{sp})}{dt} \qquad (1)$$

(η_{sp} = specific viscosity, t = time)

The z-values are usually suitable as a relative measure of the enzyme activity (the substrate and the substrate concentration being the same), as the z-values are rather independent of the variations i the salt concentrations which may occur. (The viscosity of a polymetaphosphate solution is not only dependent on the concentration of the colloid but also on the concentration and species of the low molecular salts in the solution.)

The experiments have been carried out in the following manner: 1 ml of the enzyme solution has been added to 5 ml of a 0.5 % polymetaphosphate (K 15) solution in a suitable buffer and the decrease of the viscosity with time has been recorded. (The ionic strength of the buffers generally has been 0.3 of which 0.2 is due to NaCl). $1/\eta_{sp}$ was plotted as a function of time and from the slopes of the straight lines obtained, the z-values were calculated.

EXPERIMENTS WITH FUNGI

These organisms were cultivated under sterile conditions with the exception of *Collybia velutipes* and *Tricholoma equestre*, found in the forest (in the month of October). The extracts of *Saccharomyces cerevisiae* were prepared from commercial baker's yeast. The mould *Penicillium chrysogenum* was obtained in a frozen state from the penicillin plant of Kärnbolaget, Stockholm.

In the cases of inactive extracts (extraction with water or buffer) the process was generally repeated after undergoing treatment with acetone, yet without obtaining active preparations. In the case of baker's yeast the extracts showed a much greater activity if treatment with acetone preceded the extraction. The *A. niger* extract used for these experiments was from the same culture as that used during a previous work [2], but this time the mycelium was treated with acetone. However, the pH-optimum was not affected by this modified method of preparation.

For the sake of brevity the results have been collected in Table 1. As is seen from the table the *Ascomycetes* (hitherto investigated) have enzymes which break down the high molecular polymetaphosphate.

Table 1. Experiments with enzyme extracts from fungi.

	Organism	pH-interval investigated	Activity	pH-optimum
Phyco-mycetes	*Phycomyces Blakesleeanus*	4.4 — 8.0	—	
	Rhizopus nigricans	4.4 — 8.0	—	
Asco-mycetes	*Penicillium expansum*	3.6 — 5.8	+	4.5
	Penicillium chrysogenum	3.6 — 6.3	+	4.8
	Penicillium funiculosum	3.7 — 5.4	+	4.5
	Aspergillus niger	4.0 — 7.0	+	5.7
	Aspergillus oryzae	6.6	+	—
	Saccharomyces cerevisiae	5. 2— 9.0	+	7.2
Basidio-mycetes	*Marasmius graminum*	4.4 — 7.3	—	
	Marasmius ramealis	4.2 — 6.2	—	
	Merulius domesticus	4.2 — 6.2	—	
	Polyporus betulinus	4.2 — 6.2	—	
	Collybia velutipes	3.9 — 8.0	—	
	Tricholoma equestre	3.9 — 8.0	—	

Neither from the *Basidiomycetes* nor from the *Phycomycetes* has it been possible to obtain extracts of polymetaphosphate degrading enzymes.

In Figs. 1 and 2 the z-values obtained have been plotted as a function of pH.

EXPERIMENTS WITH BACTERIA

Extracts from a number of different bacteria have also been investigated in a similar way. The organisms were cultivated on agar plates under sterile conditions. After being removed from the plates the bacteria were treated with acetone, dried at room temperature and extracted by being ground up with sand in water or a suitable buffer.

Hitherto it has only been possible to obtain active extracts from *Proteus vulgaris*.

The results have been collected in Table 2. In Fig. 3 the z-values have been plotted as a function of pH (*Proteus vulgaris*). As seen the enzyme activity has its optimum at pH $\sim$ 4.7.

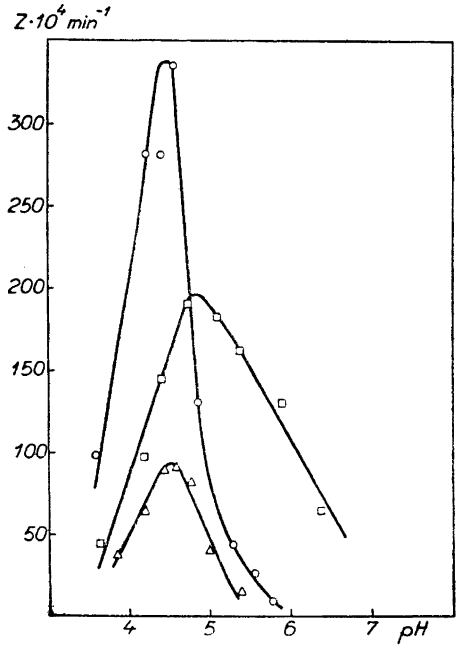

Fig. 1. The influence of pH upon the activity.
- ○ *Pencillium expansum*
- △ *Pencillium funiculosum*
- □ *Penicillium chrysogenum*

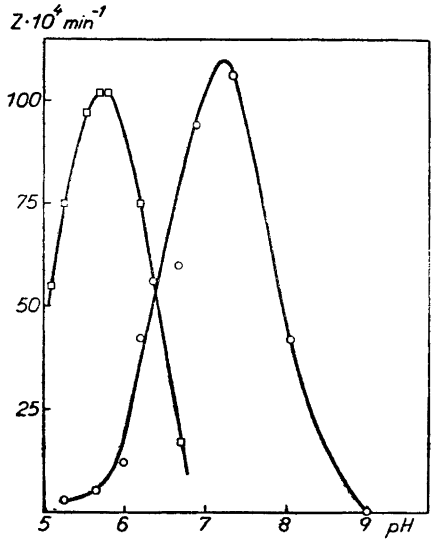

Fig. 2. The influence of pH upon the activity.
- □ *Aspergillus niger*
- ○ *Saccharomyces cerevisiae*

Table 2. Experiments with enzyme extracts from bacteria.

Organism	pH-interval investigated	Activity	pH-optimum
Proteus vulgaris	3.9 — 5.8	+	4.7
Bacillus subtilis	4.5 — 8.0	—	
Micrococcus lysodeiticus	4.2 — 8.0	—	
Pseudomonas aeruginosa	4.2 — 7.3	—	
Sarcina lutea	4.2 — 8.0	—	
Cellvibrio fulva	4.5 — 8.0	—	
Escherichia coli	4.2 — 7.3	—	
Bacillus mesentericus	4.2 — 6.9	—	
Bacillus prodigiosus	4.2 — 7.3	—	

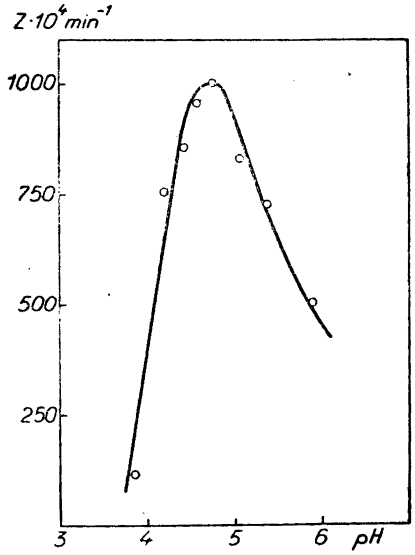

Fig. 3. The influence of pH upon the activity. Proteus vulgaris.

DISCUSSION

As seen from the tables enzyme extracts degrading high molecular polymetaphosphate have been obtained from some fungi belonging to the *Ascomycetes* and from one bacterium *viz. Proteus vulgaris.* In the cases of inactive extracts it may of course be difficult to decide whether the organism does not possess such an enzyme or whether the enzyme preparation has failed for some reason. However, it must be pointed out that in many cases the experiments have been repeated and the results have still been negative. However, low molecular metaphosphate, *e.g.* sodiumtrimetaphosphate $Na_3(PO_3)_3$, considered to be of cyclic structure, can be broken down by extracts from some organisms which do not seem to possess enzymes capable of breaking down the high molecular colloid polymetapohsphate. Thus, for instance, the authors have studied the degradation of sodiumtrimetaphosphate to orthophosphate by enzyme extracted from liver of rabbit and cow. These experiments will be published in another paper.

Perhaps an investigation of the presence or absence of polymetaphosphate degrading enzymes may be of value in classifying microorganisms. The position of the pH-optimum or other physical constants may perhaps also be of importance in this connection.

SUMMARY

Investigations of the occurrence of enzymes degrading colloid polymetaphosphates have hitherto shown that such enzymes are found in some fungi belonging to *Ascomycetes* and in the bacterium *Proteus vulgaris*. The enzymatic breakdown has been studied by means of viscosity measurements and the pH-optima of the enzymes have been determined.

The authors wish to thank Prof. A. Tiselius and Prof. T. Svedberg for the privilege of carrying out this work in their laboratories. We are grateful for the valuable technical assistance rendered by Miss A. Karlsson and Mrs M. Hårsta. The investigation was supported by a grant from *Statens Naturvetenskapliga Forskningsråd*.

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Received January 24, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 163—173

The Crystal Structure of Ramsdellite, an Orthorhombic Modification of MnO_2

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In the course of an investigation of the hollandite minerals from the Ultevis district[1] attempts were made to synthesize products isomorphous with these minerals. In connection with this work there appeared a phase that was found to be a modification of MnO_2, previously described by Glemser[2] as γ-MnO_2. It was considered of great interest to investigate the structure of this simple manganese dioxide before any further investigation was made of the more complicated manganese oxide minerals. Since the substances obtained before had given rather diffuse X-ray patterns, and as it was desirable to have crystals large enough for single crystal investigation, the syntheses were directed with a view to giving macrocrystals. γ-MnO_2 was prepared in ways 1) and 2) described by Glemser[2] and the preparations obtained were then treated in the following ways.

a) Hydrothermally at various temperatures; the preparations were placed in a platinum crucible, enclosed in a steel bomb. The products obtained all gave the X-ray patterns of pyrolusite.

b) The preparations were heated in sealed glass tubes with nitric acid and sulphuric acid of various concentrations, at temperatures from 120° C to 170° C. When sulphuric acid was used the products gave X-ray patterns of γ-MnO_2, somewhat sharpened compared with the initial ones. With nitric acid pyrolusite was obtained except in one case where a new phase appeared, which, however, gave only two reflections in the powder pattern. Neither of these reflections belonged to pyrolusite or γ-MnO_2.

In no case was there any sign of macroscopical crystals and in order to get products giving sharp powder patterns at least, the preparations were boiled for several days with either nitric or sulphuric acid with the use of

reflux. They were also heated for various lengths of time up to 200° C, but the experiments were not very successful.

In 1947 Cole, Wadsley and Walkley [3] published a study on manganese dioxide. They had synthesized preparations of γ-MnO_2 giving good X-ray powder patterns. By comparison they found that in intensities and spacings these agreed closely with the powder patterns of ramsdellite as given by Fleischer and Richmond [4]. In a collection of manganese oxide minerals, which Mr. Fleischer had kindly placed at the disposal of the Geological Survey of Sweden for identification of the Swedish manganese oxide minerals, there was a sample of ramsdellite from Lake Valley, New Mexico. As this mineral was exactly what I had tried to prepare, I took the liberty to use it for further investigations.

The mineral consisted of aggregates of small subparallel needle crystals, which gave it a platy appearence, as first described by Ramsdell [5]. It fell easily to pieces and it was difficult to find a crystal to mount on a goniometer. The needles were to small to be handled by themselves, but a fragment of parallel needles could be used to give Weissenberg photographs. That the fragment used was not composed of absolutely parrallel needle crystals, was probably the reason why a few discrepancies appeared in the intensities of the Weissenberg photographs, as will be seen later. Rotation and Weissenberg photographs were made, rotating the crystal around two axes at right angles. The symmetry was found to be orthorhombic, as had already been stated by Fleischer and Richmond [4], and preliminary cell dimensions were determined. * These were later used to index the powder photographs, from which more accurate values were obtained. The powder photographs were taken with focusing cameras with both Fe-K and Cr-K radiation. They gave the cell dimensions $a = 4.533 \pm 0.005$ Å, $b = 9.27 \pm 0.01$ Å and $c = 2.866 \pm 0.005$ Å, ($a = 4.524$ kX, $b = 9.25$ kX and $c = 2.860$ kX), which as we see, hold a simple relation to those of pyrolusite ($a = 4.40$ Å, $c = 2.87$ Å): the c-axis is of the same length, the a-axis is slightly longer and the b-axis a little more than double. Consequently, the cell volume of ramsdellite is practically twice that of pyrolusite, and as there are two formula units of MnO_2 in the tetragonal cell, there ought to be four in the orthorhombic cell. Then the calculated specific gravity is 4.84 and in good agreement with the observed value 4.83. Like most pyrolusites occuring in nature, ramsdellite, too, contains water and various other oxides. The foreign elements amount to nearly 5% in the

* Mr Richmond had also made an approximate determination of the unit cell, $a = 4.5$ Å, $b = 9.2$ Å and $c = 2.83$ Å, which was privately communicated to me, when this work was already completed.

examined mineral — (SiO_2 — 0.8%, TiO_2 — 0.2%, Al_2O_3 — 0.8%, Fe_2O_3 — 1.2%, CaO — 0.2% and H_2O — 1.3%). It cannot be decided if the mineral contains these substances merely as impurities, or if the manganese atoms are partly replaced by some of the other metal atoms. In any case, the structure factors would not differ appreciably and in the following calculations the mineral is treated as pure MnO_2.

The Weissenberg photographs obtained registered the reflections *hk*0, *hk*1, 0*kl* and 1*kl*. There are no systematic extinctions for the reflections *hkl* or *hk*0, but those of the type *h*0*l* are only present for $h+l = 2n$, and those of the type 0*kl* for $k = 2n$. This is characteristic for space groups D_{2h}^{16} and C_{2v}^{9}. For the positions of the atoms the space group D_{2h}^{16} — P*bnm* will be considered, the axes being changed as compared with the diagram in the *International tables* (1935). The relation between the axes is *a'b'c'* = *cab*, where *a'b'c'* are the axes here chosen. There are four manganese atoms and eight oxygen atoms to place, and preliminary calculations show that the two sets of fourfold positions without parameters are altogether out of the question. If the four metal atoms are placed in 4 : (*c*) $xy\frac{1}{4}$; $xy\frac{3}{4}$; $\frac{1}{2}+x$, $\frac{1}{2}-y$, $\frac{3}{4}$; $\frac{1}{2}-x$, $\frac{1}{2}+y$, $\frac{1}{4}$, there is an agreement between the values observed and calculated for the parameters $2\pi x = 10°$ and $2\pi y = 50°$. On account of lack of space the eight oxygen atoms cannot be placed in 8 : (*d*), for if one atom is placed in *xyz* there is another one in $xy\frac{1}{2}-z$, and the distance between the two oxygen atoms in the *c* direction is only $\frac{1}{2}c = 1.43$ Å. By placing the oxygen atoms in two fourfold positions in 4 : (*c*), one arrives at a very feasible arrangement of these around the manganese atoms, six oxygen atoms forming an octahedron with a manganese atom at its centre. This is a coordination structure of the same kind as is found in pyrolusite. The *x* and *y* parameters of the oxygen atoms were determined, and to give a good agreement between the values observed and calculated, the parameters of the manganese atoms had to be adjusted a little. The following arrangement was arrived at:

4 Mn	in 4 : (*c*)	with	$2\pi x_1 = 8°$	and	$2\pi y_1 = 49°$	where	$x_1 = 0.022$, $y_1 = 0.136$
4 O_1	in 4 : (*c*)	»	$2\pi x_2 = 120°$	and	$2\pi y_2 = 99°$	»	$x_2 = 0.333$, $y_2 = 0.275$
4 O_2	in 4 : (*c*)	»	$2\pi x_3 = -76°$	and	$2\pi y_3 = -12°$	»	$x_3 = -0.211$, $y_3 = -0.033$

A projection of the structure upon (001) is shown in Fig. 1 a.

Every manganese atom is surrounded by six oxygen atoms distributed at the corners of a distorted octahedron. Two oxygen atoms are at a distance of 1.86 Å, two at 1.92 Å, one at 1.91 Å and one at 1.89 Å, which gives an average Mn—O distance of 1.89 Å. The shortest Mn — Mn distance is 2.91 Å.

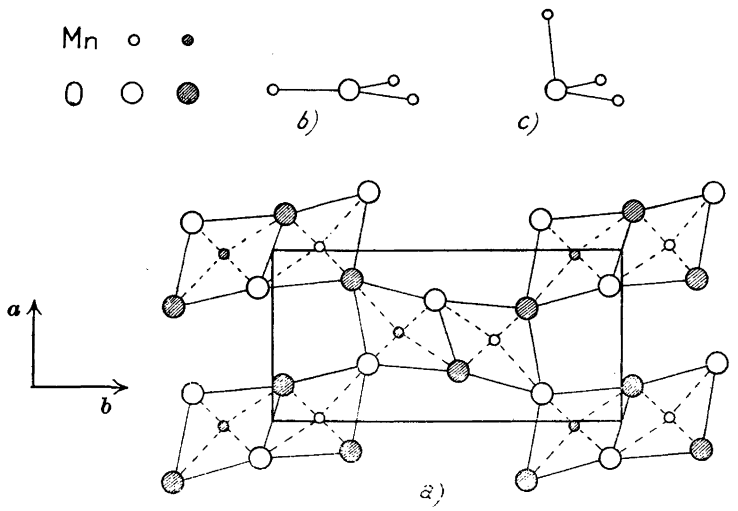

Fig. 1. Ramsdellite, orthorhombic MnO_2. a) Projection upon (001) at the level of $\frac{1}{4}c$. The filled circles indicate the atoms at the level of $\frac{3}{4}c$. b) The arrangement of the Mn atoms around the O_1 atoms. c) The arrangement of the Mn atoms around the O_2 atoms.

Every oxygen atom is surrounded by three manganese atoms, but there is a difference in the way in which these are arranged around the O_1 and O_2 atoms. The O_1 atom is at the centre of an almost equilateral triangle, which has the metal atoms at its corners, Fig. 1 b. This is like the arrangement in rutile. The O_2 atom is placed at the apex of a trilateral pyramid, the base corners of which hold Mn atoms, Fig. 1 c. The shortest O — O distance is 2.48 Å, which seems very short but not improbable, as will be seen later.

The structure is built up of distorted oxygen octahedra, each with four shared edges. The distortion is due to a contraction of the shared edges, a fact which Pauling [6, 7] has pointed out for all TiO_2 structures. I cite from Pauling [6, p. 247]: »Just such a distortion is to be expected, for the Coulomb repulsion of the two quadrivalent metal ions brought near each other when an edge is shared will cause the titanium — titanium distance to increase until the repulsion of the two oxygen ions defining the shared edge becomes large enough to counteract the effect.» The octahedra in the structure under consideration are linked together by sharing edges in two different ways:

a) In the direction of the *c*-axis the octahedra are linked together by sharing opposite edges, thus producing continuous strings running in the *c* direction.

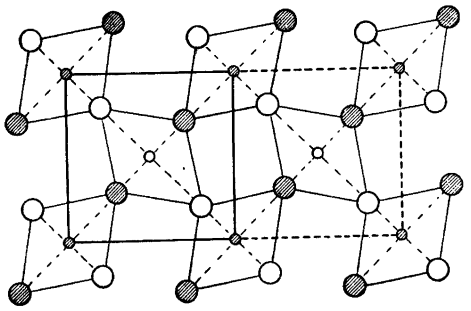

Fig. 2. Pyrolusite, tetragonal MnO_2. Projection upon (001) at zero level. The filled circles indicate atoms at the level $\frac{1}{2}c$.

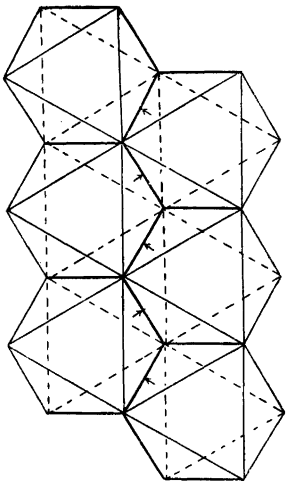

Fig. 3. A double string of undistorted octahedra. Shared edges are drawn in thick lines, and the edges by which two single strings are linked together are indicated by arrows.

b) Two such strings are linked together, one string being displaced half of the *c*-axis in relation to the other, so that an octahedron from one string shares an edge with each of two octahedra from the other string.

Under a) two manganese ions are brought together and cause a contraction of the shared edge. The O — O distance defining this shared edge is 2.58 Å. Under b) three manganese ions repel each other and the contraction of the shared edges is still larger with the O — O distance of 2.48 Å. Fig. 3 shows two strings of undistorted octahedra linked together in the way just described. The shared edges are drawn in thick lines and those shared between octahedra from two different strings are marked with arrows as well. To complete the structure the octahedra in the double strings are connected with octahedra from other double strings by sharing the corners that do not belong to the edges with the largest contraction.

For the Weissenberg photographs the observed and calculated intensities are given in Table 1. They show good agreement except for a few reflections. Thus 120 and 140, which have almost the same calculated intensities — 3.3 and 3.8 resp — show very different intensities when observed on the photograph of the zero level, with the *c* axis as the rotation axis, where 140 is much stronger than 120. On the photograph, however, of the first level, with the *a* axis as the rotation axis, they have about equal intensities, in accordance with the calculated values. For the reflection 101 a similar discrepancy is observed. The photograph from the first level, around the (100) direction,

Table 1. Weissenberg photographs of ramsdellite, orthorhombic MnO_2. Comparison between observed and calculated intensities. Fe-K radiation.

hkl	*I* obs.	*I* calc.	*hkl*	*I* obs.	*I* calc.	*hkl*	*I* obs.	*I* calc.
020	w.+	4.9	340	w.	0.9	231	w.	3.3
040	st.	14	350	m.+	6.5	241	v.w.—	0.6
060	w.	2.3	360	m.+	6.1	251	—	0.0
080	st.	13	370	v.st.	57	261	w.	6.0
						271	—	0.1
110	v.st.+	170	400	st.	15			
120	w.	3.3	410	m.	5.9	301	m.	5.6
130	v.st.	48	420	v.w.—	0.1	311	st.	15
140	m.	3.8	430	—	0.0	321	v.w.	0.1
150	w.	2.8	440	st.	24	331	w.	2.4
160	v.w.—	0.1				341	w.	1.1
170	st.	12	021	v.st.	39	351	m.	7.0
180	m.	3.4	041	w.	3.0	361	w.	1.2
190	w.	1.5	061	v.st.	31			
			081	w.+	5.0	411	m.	6.7
200	st.	18				421	st.	25
210	—	0.0	101	v.w.	0.7			
220	w.	1.9	111	st.	23	002	v.st.	39
230	m.	5.5	121	st.	11	022	v.w.	0.4
240	v.st.	32	131	st.	14	042	m.	14
250	w.	1.6	141	v.w.—	0.4	062	w.	3.0
260	w.	2.6	151	st.	20			
270	v.w.—	0.1	161	v.w.—	0.2	112	st.	9.4
280	st.	22	171	w.	2.0	122	w.	0.5
			181	v.w.	0.8	132	st.	12
310	w.	3.3				142	w.	0.9
320	w.	2.2	211	v.w.—	0.3	152	w.	1.5
330	st.	11	221	v.st.	28	162	v.w.—	0.1

v.w. = very weak, w. = weak, m. = medium, st. = strong, v.st. = very strong.

shows a strong spot, while that from the first level, around the (001) direction, shows a very weak spot, which is in agreement with the calculated intensity, 0.7. Such anomalies must be due to imperfections of the crystal.

In Table 2 the values of the powder photographs are registered. The agreement between observed and calculated intensities is not so good as for the Weissenberg photographs. The divergences, however, are of a systematic nature. Thus the reflections $0k0$ and $1k0$ are much more intense than the other reflections. Comparing for instance 121 and 140, which have about the same observed intensities and angles of reflection close to each other,

Table 2. Powder photographs of ramsdellite, orthorhombic MnO_2. Comparison between observed and calculated $sin^2\Theta$ values and intensities. Fe-K radiation.

hkl	*Sin²Θ* obs.	Sin²Θ calc.	*I* obs.	*I* calc.	*hkl*	Sin²Θ obs.	Sin²Θ calc.	*I* obs.	*I* calc.
120	0.0885	0.0892	m.	9.5	340	—	0.5848	—	0.9
130	0.1430	0.1437	v.st.	104	132	0.6010	0.6003	w.	25
021	0.1569	0.1577	st.	77	331	—	0.6226	—	4.8
101	—	0.1596	—	0.1	042	—	0.6310	—	15
111	0.1702	0.1706	st.	92	202	—	0.6390	—	7.4
040	0.1739	0.1744	st.	14	212	—	0.6499	—	0.0
200	0.1825	0.1824	v.w.—	16	142	—	0.6764	—	1.8
210	—	0.1933	—	0.0	222	—	0.6826	—	0.7
121	0.2028	0.2033	st.	40	350	—	0.6829	—	6.6
140	0.2196	0.2200	st.	6.5	261	0.6873	0.6889	v.w.	12
220	—	0.2250	—	3.4	171	—	0.6938	—	3.3
131	0.2569	0.2578	st.	45	080	0.6983	0.6976	w.+	6.9
230	0.2798	0.2805	m.	16	341		0.6989		2.3
041	0.2879	0.2885	v.w.	4.6	270	—	0.7165	—	0.1
211	—	0.3074	—	0.9	400	—	0.7297	—	6.8
150	0.3171	0.3181	w.+	3.5	232	—	0.7371	—	4.5
141	—	0.3341	—	1.0	410	—	0.7406	—	6.8
221	0.3401	0.3401	st.	85	180	0.7449	0.7432	w.	3.1
240	0.3567	0.3568	st.	44	420	—	0.7733	—	0.1
060	0.3919	0.3924	w.	1.9	152	—	0.7747	—	4.1
231	0.3948	0.3946	w.	10	351	—	0.7970	—	13
310	—	0.4213	—	4.8	360	—	0.8028	—	5.6
160	—	0.4380	—	0.1	081	—	0.8117	—	5.9
151	0.4319	0.4322	st.	53	242	0.8143	0.8141	w.+	56
320	—	0.4540	—	2.6	430	—	0.8278	—	0.0
250	—	0.4549	—	1.9	271	—	0.8306	—	1.0
002	0.4568	0.4566	m.	24	062	—	0.8490	—	2.8
241	—	0.4709	—	1.4	411	—	0.8547	—	12
022	—	0.5002	—	0.4	181	—	0.8573	—	1.6
061	0.5060	0.5065	st.	34	312	—	0.8779	—	9.5
330		0.5085		12	280	0.8809	0.8800	w.	17
112	0.5130	0.5131	v.w.	21	421	—	0.8874	—	37
301	0.5254	0.5245	v.w.—	6.3	162	—	0.8946	—	0.1
311	0.5358	0.5354	w.	35	440	—	0.9041	—	19
122	—	0.5458	—	1.3	322	—	0.9108	—	7.3
161	—	0.5521	—	0.5	252	—	0.9115	—	5.7
321	—	0.5681	—	0.2	361	—	0.9169	—	3.0
251	—	0.5690	—	0.0	190	—	0.9285	—	1.5
260	—	0.5748	—	2.8	431	—	0.9419	—	18
170	0.5794	0.5796	m.	13	370	0.9445	0.9445	w.diff.	40

v.w. = very weak, w. = weak, m. = medium, st. = strong, v.st. = very strong, diff. = diffuse.

Table 3. Comparison between ramsdellite, a synthetical product of γ-MnO_2 and pyrolusite. Cr-K radiation. The β-reflections are omitted, except when they coincide with α-reflections.

	Ramsdellite			γ-MnO_2		($MnO_{1.967}$)	Pyrolusite			
		$Sin^2\Theta$	I		$Sin^2\Theta$	I			$Sin^2\Theta$	I
d	hkl	obs.	obs.	hkl	obs.	obs.	hkl	$h2kl$	obs.	obs.
4.10	110	0.0777	v.st.	110	0.08	st.diff.				
3.232	120	0.1250	w.	120	0.13	v.w.—	110	120	0.1346	st.
2.535	130	0.2001	v.st.							
2.436	021	0.2200	st.	021	0.2213	st.	011	021	0.2259	m.
2.343	111	0.2379	m.	111	0.2400	m.(br.)				
2.329	040	0.2429	m.	040			020	040	0.2716	w.
2.143	121	0.2843	st.	121	0.2878	st.	111	121	0.2954	st.
2.058	140	0.3080	st.	140	0.3070	w.	120	140	0.3393	m.
1.903	131	0.3609	st.	131	0.38	v.w.				
1.796	041	0.4044	w.							
1.713	150	0.4450	m.							
1.670	141	0.4688	v.w.							
1.656	221	0.4767	st.	221	0.4885	st.(br.)	211	221	0.4989	v.st.
1.616	240 β 151	0.5002	v.st.	240	0.5091	m.	220	240	0.5427	st.
1.542	060	0.5490	v.w.							
1.537	231	0.5533	w.							
1.479	151	0.6050	v.st.	151	0.59	w.diff.				
1.429	002	0.6398	m.	002	0.6459	m.	002	002	0.6374	st.
1.357	330 061	0.7088	v.st.				130	160	0.6790	m.
1.348	112	0.7190	w.							
1.320	311	0.7510	w.+							
1.270	170	0.8108	m.							
1.246	132	0.8416	m.							
1.216	042	0.8841	w.—							

v.w. = very weak, w. = weak, m. = middle strong, st. = strong, v.st. = very strong, br. = broad, d. = spacings in kX.

we find that the calculated intensity for 121 is 40 and that for 140 is only 6.5. Similarly the calculated intensities for $0k0$ and $1k0$ are in general only one sixth of those calculated for the general reflections with the same observed intensities. This must be due to preferred orientations of the crystallites, in spite of the fact that the material was well ground before it was used for powder photographs. Why 280 appears — with a calculated intensity of 17 — and not 421, with a calculated intensity of 37, is a little difficult to explain, for there does not seem to be any constant strengthening of the $2k0$ reflections. Probably overmuch importance should not be attached to this fact, as the

last part of the film from the outer focusing camera is rather diffuse, especially for reflections with $\sin^2\Theta$ values greater than 0.7.

Table 3 contains X-ray data for a synthetically prepared γ-MnO_2. The products obtained all had very similar powder patterns and the lines were not so sharp that it was possible to decide whether differences in the observed $\sin^2\Theta$ values were due to changes in the cell dimensions or should be ascribed to different measurings of the lines. Therefore, data for only one product are shown in the table. This γ-MnO_2 was prepared by oxidising a solution of $MnSO_4$ with ammoniumpersulphate. The precipitate was well washed with hot water and then boiled for ten hours with sulphuric acid, washed and dried at 115° C till constant weight was obtained. The proportions of manganese and available oxygen were determined. The following values were obtained: MnO — 79.75 % and O — 17.70 %, giving the total 97.45%. The missing per cents are probably water which was not determined, as it was not possible to decide whether it enters the structure or is absorbed water. Without counting the water, the formula is $MnO_{1.967}$, and the corresponding value for ramsdellite is $MnO_{1.971}$. As a comparison, the observed reflections of ramsdellite are given in Table 3, together with the intensities and the $\sin^2\Theta$ values for unfiltered Cr-K radiation. The β-reflections are omitted except when they coincide with α-reflections. In the pattern of γ-MnO_2 we do not find strong reflections from the planes 130, 131, 151 and 330—061 as are found in the pattern of ramsdellite. There is only a shading of the film where the reflections might be expected. The sharps reflections that appear are the very ones that are common for ramsdellite and pyrolusite, indexed with a double b-axis. This is elucidated in Table 3. in which next to the column with the correct indices of pyrolusite, there is a column where all the k-indices are doubled. The cell dimensions of the three substances are:

	a	b	c
pyrolusite	4.40 Å	4.40 Å	2.87 Å
γ-$MnO_{1.967}$	4.43	9.36	2.85
ramsdellite	4.53	9.27	2.86

It is probable that the synthetic γ-MnO_2 is an intermediate product between pyrolusite and ramsdellite and that the γ-MnO_2 here prepared is very closely related to pyrolusite, while the γ-MnO_2 prepared by Cole and co-workers [3] has taken a further step towards ramsdellite, for in their pattern 130 also appears. A projection of pyrolusite upon (001) is shown in Fig. 2.

When the structure of ramsdellite had already been determined Mr. Fleischer drew my attention to the remarkable similarity that Mr. Richmond

had found between the powder patterns of ramsdellite and diaspore, $AlHO_2$. A closer comparison shows that as a matter of fact ramsdellite belongs to the same type of structure $-EO_2-$ as diaspore. They have the same space group, D_{2h}^{16}, almost the same cell dimensions, and their parameters differ but little, as can be seen from the table below, where the corresponding values for goethite are also listed. The values for diaspore and goethite are from Hoppe [8,9].

		Parameters								
		a	b	c	x_{Mn}	y_{Mn}	x_{O_1}	y_{O_1}	x_{O_2}	y_{O_2}
ramsdellite	MnO_2	4.53Å	9.27Å	2.87Å	+0.022	0.136	0.17	—0.23	—0.21	—0.033
diaspore	$AlHO_2$	4.42	9.44	2.84	—0.048	0.146	0.29	—0.20	—0.20	—0.056
goethite	$FeHO_2$	4.59	10.00	3.03	—0.045	0.146	0.31	—0.20	—0.20	—0.047
groutite	$MnHO_2$	4.56	10.70	2.85						

It is interesting to compare the interatomic distances in the structures of ramsdellite and diaspore, particularly as the Mn^{4+} and the Al^{3+} ions are of the same size. All the corresponding interatomic distances except one are equal; the one exception being the distance between oxygen atoms marked A and B in Fig. 1 a. For diaspore this distance is only 2.65 Å, which is very short to be between oxygen atoms belonging to different octahedra. Ewing [10] explained this short distance as due to a hydrogen bond and Hoppe [9] came to the same conclusion. For ramsdellite which does not contain any hydrogen atoms the distance A—B is 3.34 Å, which is quite normal. The divergency of the two distances A—B in diaspore and ramsdellite may be another proof that the proposed positions for the hydrogen atoms in diaspore are correct. Anyhow, it is evident that the hydrogen atoms are not essential for the structure type of diaspore.

Recently Gruner [11] has described a new mineral, groutite, $MnHO_2$, which he preliminarily determined to belong to the diaspore-goethite group. Cell dimensions for groutite are listed in the table above. Further investigation will show if this mineral really has the same structure as diaspore, in which case ramsdellite, MnO_2, and groutite, $MnHO_2$, are also isostructural, rather a unique occurrence.

SUMMARY

The structure of ramsdellite, MnO_2, has been investigated. The symmetry is orthorhombic and the cell dimensions are $a = 4.533$ Å, $b = 9.27$ Å and $c = 2.866$ Å. Possible space groups are D_{2h}^{16} and C_{2v}^{9}. Good agreement between observed and calculated values is found for the following arrangement of the atoms in D_{2h}^{16} — $Pbnm$

4 Mn	in 4 : (c) :	$2\pi x_1 =$	8°	$2\pi y_1 =$	49°
4 O_1	in 4 : (c) :	$2\pi x_2 =$	120°	$2\pi y_2 =$	99°
4 O_2	in 4 : (c) :	$2\pi x_3 =$	− 76°	$2\pi y_3 =$	− 12°

Each manganese atom is surrounded by six oxygen atoms distributed at the corners of an octahedron with an average Mn — O distance of 1.89 Å. Each oxygen atom is surrounded by three manganese atoms. The oxygen octahedra are linked together by sharing edges, thus producing double strings running in the *c* direction; in pyrolusite there are single strings. By sharing corners the octahedra are further linked together to complete the structure. The relations between pyrolusite, γ-MnO_2 and ramsdellite are shown and cell dimensions are given for a synthetically prepared γ-MnO_2.

A comparison between the structures of ramsdellite and diaspore is also made, and indicates that they belong to the same type of structure, as well as the new mineral groutite $MnHO_2$. The contraction between oxygen atoms in diaspore, which is due to the hydrogen bond, is not found in ramsdellite.

Some of the technical equipment has been provided out of a grant from *Statens Naturvetenskapliga Forskningsråd* to Mr. A. Byström for investigations of some oxide systems, and I wish to thank Mr. Byström for valuable discussions in the course of the investigation. I am also indebted to Mr. M. Fleischer for supplying the mineral and for his kind observation of the similarity between diaspore and ramsdellite.

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Received January 4, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 174—178

Studies on Copper(I)cyanides

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The biological antagonism between copper(II)ions and cyanide was discovered by Chistoni in 1925[1]. Later it has been demonstrated that cyanide inactivation of certain enzyme systems can be reversed by means of copper(II)-ions (see *e. g.* Kubowitz[2]). Thereby one of the following reactions may take place:

$$2\ Cu^{\cdot\cdot} + 4\ CN' = 2\ CuCN + (CN)_2 \quad (1)$$

$$4\ Cu^{\cdot\cdot} + 8\ CN' = 2\ CuCN,Cu(CN)_2 + (CN)_2 \quad (2)$$

$$2\ Cu^{\cdot\cdot} + 6\ CN' = 2\ Cu(CN)_2' + (CN)_2 \quad (3)$$

$$2\ Cu^{\cdot\cdot} + 7\ CN' = 2\ Cu(CN)_3'' + CNO' \quad (4)$$

At very high cyanide concentrations higher complexes may also be formed but in 0.005 *M* KCN there is equilibrium at equal amounts of $K_3Cu(CN)_4$ and $K_2Cu(CN)_3$ (Kunschert[3]). As the cyanide concentrations of interest are usually below 0.005 *M* the equations 1—3 may describe the actual reactions. The experiments described below were carried out in order to test this. In series I copper cyanide precipitates were studied and in series II mixtures of copper(II)-ions and cyanide ions in different proportions were examined. III. In connection with the precipitate studies copper(I)cyanide was crystallized in two forms.

I. PRECIPITATE EXPERIMENTS

Two gions of cyanide per gatom copper were mixed under nitrogen as 1 *M* solutions and the precipitate washed several times with distilled water, alcohol and ether on a glass filter and finally dried over sulfuric acid. The complex precipitate was suspended in redistilled water at 25° C for 8—10 hours. The clear saturated supernate was then decanted off. The precipitate was again suspended in water. The whole procedure of saturation was repeated until no changes occurred in the supernate (conveniently controlled by the electrical conductivity).

The mother liquor and washings from the precipitation and the saturated solutions prepared from the precipitate were analysed for cyanide and copper content, and for potential in a copper half-cell. Copper was determined photometrically with diethyl-dithiocarbamate (Braun and Scheffer[4]). Cyanide was estimated by means of electrometric titration with silver sulfide as indicator electrode. In the mother liquor also cyanate was determined with the method of Lebouc q[5]. The copper potentials were measured under nitrogen at 25° C using a brightly polished rod of electrolytic copper as indicator electrode. The rod was polished anew before each measurement. The valve potentiometer was read off to $\pm$ 1 mV.

The results are summarized in Table 1. Obviously the mother liquor contains complex compounds of the type CuCN,n·KCN where n is great. For a normal potential $E_0 = 522$ mV (Latimer)[6] the copper(I)ion activity corresponds to $2.9 \cdot 10^{-7}$ M.

Table 1. Copper potentials and concentrations of copper and cyanide in supernatants from suspensions of copper(I)cyanides.

Test solution	No. of experiments	E_{Cu} mV	CN′ mmol per liter	Cu mmol per liter	gions CN′ / gatom Cu
Mother liquor	2	135	565—627 *	17.2—22.6	28—33
Washings nos. 2—5	5	—158 to —176	5.95—27.4	4.34—10.3	1.15—3.36
First saturation	8	—230 to —271	20.8—35.3	15.6—26.2	1.19—1.35
4th or later saturations	7	213 to —83	0.149—0.335	0.174—0.339	0.73—0.99

In the washings the copper activity has sunk by about 5 powers of 10 and the ratio CN:Cu corresponds on an average to the compound $KCu(CN)_2$ (equ. 3) which according to Spitzer[7] has a solubility of 0.04 mol per liter at 18° C.

In the first saturation with the precipitate the copper activity has sunk further a good power of 10. The ratio CN:Cu is less than 2, and evidently the main part of the $KCu(CN)_2$ has been removed, while some cupri-cupro-cyanide $CuCN,Cu(CN)_2$ still remains.

In the fourth and the following saturations the conductivity is constant at 10^{-5} r. o. The more soluble complex copper(1)cyanides seem here to have been

* CNO′: 1 — 20 % of the cyanide concentration found.

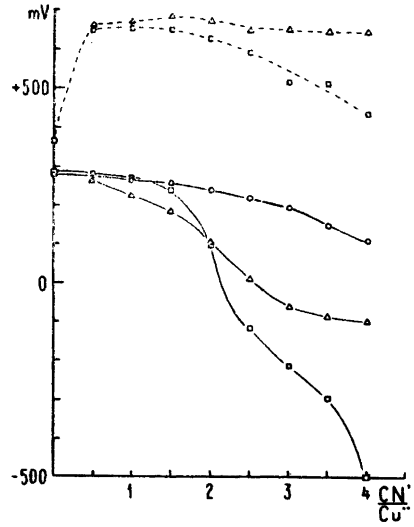

Fig. 1. Copper potentials (full lines) and redox potentials (dotted lines) for mixtures of coppersalts and cyanide.

□ *potentials in 0.005 M $CuCl_2$*
△ » » *0.0005* » *$CuSO_4$*
○ » » *0.00005* » »

entirely removed, and the remaining precipitate is supposed to be pure CuCN, whose solubility under the prevailing conditions is 0.1—0.3 mmol per liter. The copper potentials are here very labile and thus do not permit any conclusions.

II. MIXING EXPERIMENTS

Solutions of $CuCl_2$ or $CuSO_4$ were mixed with solutions of KCN so that the molar proportions between cyanide and copper were between 0.5 and 4.0 in even steps of 0.5. The concentrations were selected so that the final concentration of each mixture in a series was the same regarding one of the ions. Determinations were made of the potential in the copper half-cell as well as of the oxido-reduction potential. Typical experiments are reproduced in Fig. 1. When the copper concentration is 0.5 m*M* or higher the copper activity changes rapidly when the CN:Cu ratio rises to 1.5 and over indicating complex formation. In weaker solutions, on the other hand, one obtains no such sudden changes of potential, but the reduction of activity takes place evenly within the measured region. This supports the assumption that complex compounds of the type CuCN,*n* KCN are only stable in solutions that in respect of copper are 0.5 m*M* or more. In weaker solutions they disintegrate or are not formed.

Also the redox potentials are changed in a similar way to the copper potentials, which confirms the conclusions concerning the complex formation.

As the cyanide-content in the blood from different kinds of animals killed by inhalation of HCN does not amount to 0.5 m*M*, copper treatment should

thus give rise only to CuCN (equ. 1). Hence the full detoxicating copper dose is to be calculated on the basis of 0.5 gatom copper per gion cyanide (*cf.* Agner[8]).

III. CRYSTALLIZATION OF COPPER(I)CYANIDE

In the course of the foregoing experiments it was observed that in flasks from the first saturation tests that had been allowed to stand for some time two kinds of crystals arose, dark green and dark red. Systematic experiments showed that besides potassium-containing complex compounds, dark green crystals appeared at room temperature in a suspension of the washed precipitate (see under I). At 40° C, on the other hand, mostly dark red crystals were formed. These, however, may also arise at room temperature. The red and green crystals thus prepared were indistinguishable from those first observed.

Both kinds of crystals were difficultly soluble, but with $H_2O_2 + H_2SO_4$ or HNO_3 it was possible to dissolve them completely. For analysis crystals were weighed out and put into a 30 ml distillation flask containing some water. When the air had been driven out by boiling, concentrated HNO_3 + some H_2O_2 was added, and all cyanide was driven out by continued heating. The cyanide was collected in 0.005 *N* $AgNO_3$, whereupon the excess of silver was determined by electrometric titration. The residue in the distilling flask was analysed for copper iodometrically according to Bitskei[9]. Some analytic values are given in Table 2.

Despite the dispersion in the analytic values, one is probably justified in assuming that both the dark green and the dark red crystals have the gross formula CuCN. At least the green crystals are certainly different from the

Table 2. Analyses of copper(I)cyanide crystals.

Dark green crystals				Dark red crystals			
Weight mg	Micromols CN	Micromols Cu	Cu/CN	Weight mg	Micromols CN	Micromols Cu	Cu/CN
1.76	15.3	18.8	1.23	1.48	12.9	11.6	0.90
1.26	12.5	11.4	0.91	1.78	11.8	13.4	1.14
1.23	12.8	9.6	0.75	1.64	14.5	10.7	0.74
1.57	15.4	11.6	0.75	1.70	14.2	12.3	0.87
1.25	9.6	10.6	1.13	1.52	11.0	10.8	0.98
1.49	9.8	6.5	0.66	1.38	11.8	11.8	1.00
Mean value			0.91				0.94

copper(I)cyanide crystals obtained by Wöhler[10] from lead copper cyanide after precipitation with sulfuretted hydrogen and subsequent evaporation, and crystallographically examined by Dauber[11].

Preliminary X-ray investigations show that the red modification is monoclinic with the following dimensions of the elementary cell: $a = 17.78$ kX, $b = 6.79$ kX, $c = 21.10$ kX and $\beta = 99°$. The green modification has a rhombic elementary cell with $a = 17.80$ kX, $b = 6.79$ kX and $c = 21.85$ kX *.

* We are indebted to Dr. Frans E. Wickman of the Mineralogical Department of the Stockholm University for the X-ray investigations.

SUMMARY

The reactions between copper(II)ions and cyanide ions have been investigated, in which connection it has emerged that complex copper cyanides are formed or exist only if the copper concentration is $\geqq 0.5$ mM. In connection herewith, we observed the formation of 2 new copper cyanide crystals that according to the analyses are 2 modifications of copper(I)cyanide.

For the copper treatment of cases of hydrogen cyanide poisoning our results imply that the dose should be calculated on the basis of, at the most 0.5 gatoms of Cu per gion of cyanide.

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Received December 22, 1948.

ACTA CHEMICA SCANDINAVICA (1949) 179—189

Spectrophotometric Study on Complex Formation between Cupric and Sulphate Ions

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Recently [1] methods have been developed for the determination of all complexity constants of a complex system. The primary assumption in these methods is the possibility of carrying out the investigation at a constant concentration of a highly dissociated neutral salt and at a low concentrations of the complex forming substances. Thus the activity coefficients remain constant and the classical law of mass action can be applied. In systems of slight complexity, this condition cannot be fullfilled because of a high and greatly varying ligand concentration. In such cases the variation of the activity coefficients must be taken into consideration. An attempt in this direction has been made by J. Bjerrum [2] who made some approximate assumptions in regard to activity coefficients, which are reasonable at high ligand concentrations. Thus this method cannot be used for determinations at lower ionic strengths, which were carried out in this study. Therefore a new method was developed which permits the determination of the first complexity constant from the light absorption measurements over wide limits of ionic strength. The method is applied in regard to the cupric sulphate system.

CALCULATION OF THE FIRST COMPLEXITY CONSTANT

The first complexity constant of cupric sulphate is defined by

$$k_1 = [CuSO_4]/[Cu^{++}][SO_4^{=}] \quad (1)$$

If the first complex is the only one in the system the following stoichiometric equations are valid:

$$c_{Cu} = [CuSO_4] + [Cu^{++}] \quad (2)$$

and

$$c_{SO_4} = [SO_4^{=}] + [CuSO_4] \tag{3}$$

where c_{Cu} and c_{SO_4} are the stoichiometric molarities of the cupric and sulphate ions. From equations (1), (2) and (3) the familiar expression follows:

$$y/c_{Cu} = x/(x + K_1) \tag{4}$$

where $x = [SO_4^{=}]$, $y = [CuSO_4]$ and $K_1 = 1/k_1$. On the other hand according to Beer's law

$$\varepsilon = e/c_{Cu} = (E - \varepsilon_{Cu}[Cu^{++}] - \varepsilon_{SO_4}[SO_4^{=}])/c_{Cu} = \varepsilon_{CuSO_4} y/c_{Cu} \tag{5}$$

where E is the extinction (d = 1 cm), and ε_{Cu}, ε_{SO_4}, ε_{CuSO_4} are molar extinction coefficients. The elimination of y/c_{Cu} from equations (4) and (5) leads to the expression

$$\varepsilon = \frac{\varepsilon_{CuSO_4}\, x}{x + K_1} \tag{6}$$

or

$$\log\varepsilon = \log\varepsilon_{CuSO_4} + \log \frac{x}{x + K_1} \tag{7}$$

The differentiation of equation (7) gives

$$\varphi = \frac{\mathrm{d}\log \varepsilon}{\mathrm{d}\log x} = \frac{K_1 - x(\mathrm{d}K_1/\mathrm{d}x)}{x + K_1} \tag{8}$$

This equation can be put into the form

$$\varphi = \frac{K_1(1 - \psi_1)}{x + K_1} \tag{9}$$

where $\psi_1 = \mathrm{d}\log K_1/\mathrm{d}\log x$. The solution of equation (9) for K_1 gives

$$K_1 = \frac{\varphi\, x}{1 - \varphi - \psi_1} \tag{10}$$

The first complexity constant can be calculated by means of this equation. When the composition of the solution can be held nearly constant during the measurement K_1 is constant and we can put $\psi_1 = 0$. Thus in this case K_1 can be calculated very simply from the experimental φ-values. When, however, this condition is not fulfilled the computation is much more difficult. Differentiation of the Debye-Hückel equation

$$\mathrm{p}K_1 = \mathrm{p}K_{1.0} - \frac{4.05\sqrt{I}}{1 + a\sqrt{I}} + B\,I \tag{11}$$

used as the interpolation equation, gives

$$\frac{\mathrm{dp}K_1}{\mathrm{d}px} = 6.909\,x\,(1 - \frac{1}{3}\frac{\mathrm{d}y}{\mathrm{d}x})\left(\frac{2.03}{\sqrt{I}\,(1 + a\sqrt{I})^2} - B\right) \tag{12}$$

where

$$I = 3c_{\mathrm{Cu}} + 3x - y \tag{13}$$

and

$$\frac{\mathrm{d}y}{\mathrm{d}x} = c_{\mathrm{Cu}}K_1\,(1-\psi_1)/(x + K_1) \tag{14}$$

From equation (10) and (11) we obtain

$$F = \mathrm{p}K_{1.0} - \frac{4.05\sqrt{I}}{1 + a\sqrt{I}} + BI - p\varphi - px + p(1-\varphi-\psi_1) = 0 \tag{15}$$

For every x-value we get an equation (15) with tree unknowns $\mathrm{p}K_{1.0}$, a and B. When rough values for the unknowns have been found the final solution can be obtained by successive approximations using the method of least squares.

In a system with only a complex the derived equations are valid at all ligand concentrations. In systems with more than a complex the equations are very nearly correct in low ligand concentrations but incorrect at high ligand concentrations. The above equations can, however, easily be extended to systems with higher, additive nonabsorbing complexes. Instead of equation (10) we obtain in this case

$$K_1 = \frac{\varphi\,x}{1-\varphi-\psi_1-\sum_2 (\nu-1+\varphi+\psi_1-\psi_\nu)x^\nu/K_\nu} \tag{16}$$

where the constants K_ν are defined by

$$1/K_\nu = k_\nu = \frac{[Cu(SO_4)_\nu^{(2\nu-2)-}]}{[Cu^{++}][SO_4^{=}]^\nu} \tag{17}$$

and $\psi_\nu = \mathrm{d}pK_\nu/\mathrm{d}px$.

Equation (16) can further be generalized to a system with an absorbing complex $Cu(SO_4)_\nu^{(2\nu-2)-}$.

EXPERIMENTAL

Cupric perchlorate was prepared by dissolving cupric oxide, Kahlbaum for analysis, in perchloric acid and recrystallizing several times.

Lithium sulphate, B. D. H. laboratory reagent, was recrystallized many times.

The other chemicals were the best qualities obtainable and were used without purification.

The light absorption measurements were made with a Beckman Quartz Spectrophotometer Model DU. The slit width at ultraviolet wavelengths was 0.20—0.24 mm (272—320 mμ) corresponding to a spectral band width 0.8—1.1 mμ. The increase or decrease of the slit width by 50 per cent did not affect the results. The temperature of the room where the measurements were made was maintained at 25° C. Because the photometer lamp increases the temperature in the cell compartment the extinction measurements were made so rapid that the temperature of the solution measured did not find time to change. Later the »mounting block» was equipped with a refrigerator coil soldered into the block. The block was held at constant temperature with the aid of a »contact thermometer» which was put into a hole bored in the block.

RESULTS

A typical measurements series is represented in Fig. 1. In this series the cupric perchlorate concentration was constant ($c_{Cu} = 0{,}01003$) and the wavelength 272 mμ. In the figure log ε is plotted against the logarithm of the sulphate ion concentration x. Several similar series were made at wavelengths 272—320 mμ. The curves obtained proved to be parallel within experimental errors if the cupric perchlorate concentration was not raised so high that the composition of the salt medium changed considerably. The own absorption of cupric ions is at these wavelengths negligible. It is interesting that the slope of the curves seems to be the same also at the red and the beginning of the infrared wavelengths within experimental errors which, admittedly, are considerable because of the great own absorption of cupric ions. These results seem to prove that this system has only a complex which absorbs light and still more they make it probable that the system has only this complex. The complex in question is obviously the first one, $CuSO_4$.

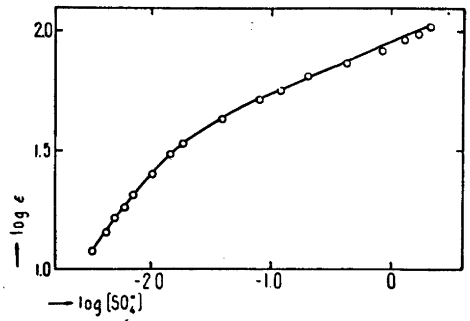

Fig. 1. The effect of lithium sulphate additions on light absorption of cupric perchlorate solutions. $c_{Cu} = 0.01003$, $\lambda = 272\ m\mu$, $\varepsilon_{Cu} \approx 0$.

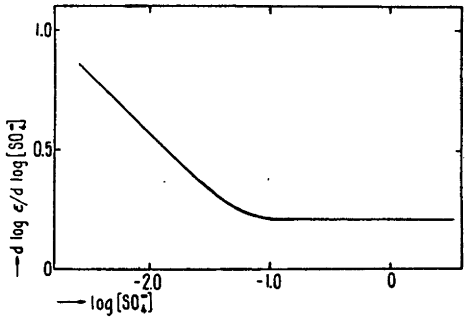

Fig. 2. The dependence of dlogε/dlogx on the sulphate ion concentration.

For the calculation of the first complexity constant, the quantity $\varphi = \mathrm{d}\log\varepsilon/\mathrm{d}\log x$ must first be determined. With this intention the experimental data of $\log\varepsilon$ were fitted at high ligand concentrations to a polynomial of the first order and at lower ligand concentrations to two polynomials of the second order. These polynomials were then differentiated. The results were checked by graphical means. The mean value of the quantity φ obtained from the data at different wavelengths is plotted in Fig. 2 against $\log x$.

In Table 1 the calculations of the constant K_1 are represented at some ionic strengths. In this table pK_1 means the values calculated by means of equation (18), $p\overline{K}_1$ the values obtained with the aid of equation (10) and ΔpK_1 is their difference. The calculations carried out by the method of least squares in the manner described above gave the result

Table 1. Calculation of the first complexity constant of cupric sulphate system.

$\sqrt{I}$	x	φ	ψ_1	pK_1	$p\overline{K}_1$	ΔpK
0.205	0.004	0.765	0.142	1.466	1.482	− 0.016
0.274	0.015	0.486	0.354	1.323	1.341	− 0.018
0.387	0.040	0.280	0.528	1.133	1.234	− 0.101
0.574	0.100	0.212	0.616	0.901	0.910	− 0.009
0.693	0.150	0.212	0.616	0.792	0.734	+ 0.058
0.883	0.250	0.212	0.581	0.659	0.592	+ 0.067
1.110	0.400	0.212	0.497	0.549	0.536	+ 0.013
1.560	0.800	0.212	0.282	0.428	0.475	− 0.047
1.940	1.245	0.212	0.0688	0.393	0.436	− 0.043
2.300	1.750	0.212	− 0.162	0.400	0.409	− 0.009
2.600	2.240	0.212	− 0.373	0.428	0.389	+ 0.039

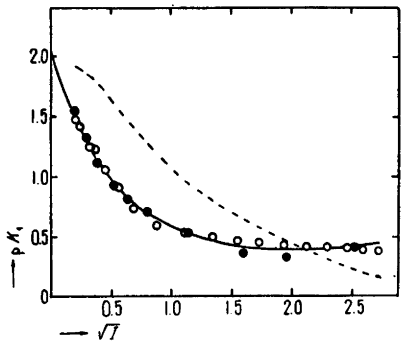

Fig. 3. The first complexity constant of cupric sulphate in lithium sulphate solutions.

$$\log k_1 = pK_1 = 2.099 - \frac{4.05\sqrt{I}}{1 + 1.618\sqrt{I}} + 0.0520\ I \qquad (18)$$

In Fig. 3 pK_1 is plotted against $\sqrt{I}$. The hollow circles in the figure mean the values calculated with the aid of equation (10) and the full circles the values calculated by means of equation (25). The dotted line represents the values obtained by means of equation (10) assuming that the activity coefficients do not vary and thus $\psi_1 = 0$. In the final calculations twenty points were used.

Some experiments were carried out concerning the effect of added perchloric acid on the extinction of cupric sulphate solution. The results are represented in Fig. 4. The shapes of the curves come obviously from the formation of bisulphate ions. When sulphuric acid instead of perchloric acid was added the extinction of cupric sulphate solution remains nearly constant until relatively high sulphuric acid concentrations. It is therefore obvious that cupric and bisulphate ions form nonabsorbing complexes as $CuHSO_4^+$.

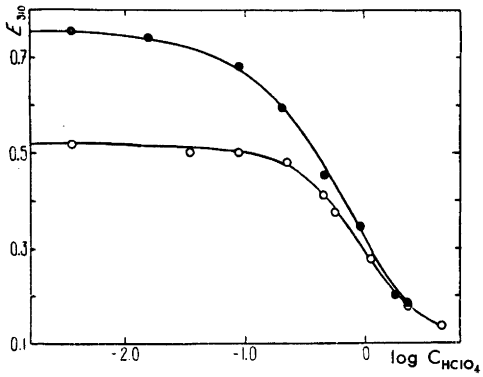

Fig. 4. The effect of perchloric acid on the extinction of cupric sulphate solutions. Upper curve: $c_{CuSO_4} = 0.487$, Lower curve: $c_{CuSO_4} = 0.243$, $c_{Li_2SO_4} = 0.619$; $\lambda = 31\ m\mu$.0

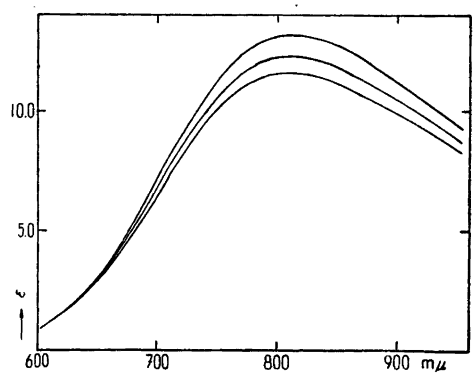

Fig. 5. Absorption spectra of some cupric salt solutions. Upper curve: $c_{Cu(ClO_4)_2} = 0.0823$, $c_{Li_2SO_4} = 2.13$, *Middle curve:* $c_{CuSO_4} = 0.0605$, *Lower curve:* $c_{Cu(ClO_4)_2} = 0.0600$.

The formation of these complexes takes place of course also on addition of perchloric acid.

As is known cupric perchlorate solution has two absorption maxima, the one at extreme ultraviolet wavelengths, the other at about 820 mμ. It is interesting that by adding sulphate ions to cupric perchlorate solution the extinction also increases at longer wavelengths. Also the extinction of cupric sulphate solution is greater than that of a cupric perchlorate solution at equal concentrations. In Fig. 5 absorption spectra of some cupric perchlorate and sulphate solutions are represented.

It is noteworthy that the extinction of cupric sulphate solution seems to increase linearly with the concentration at longer wavelengths. This fact has sometimes been presented as evidence of the complete dissociation of cupric sulphate. This linear relation can, however, be comprehended even if complex formation occurs. According to Beer's law the extinction of cupric sulphate solution is

$$E = \varepsilon_1 [Cu^{++}] + \varepsilon_2 [CuSO_4] \tag{19}$$

or

$$E = \varepsilon_1 c_{Cu} + (\varepsilon_2 - \varepsilon_1)[CuSO_4] \tag{20}$$

When $\varepsilon_2 - \varepsilon_1 > 0$ the extinction is greater than that of an equally concentrated perchlorate solution. If $\varepsilon_2 - \varepsilon_1$ is relatively low compared to ε_1 the latter term in (20) is small compared with the former and the non-linear relation between $[CuSO_4]$ and c_{Cu} has only a slight effect on the extinction. Moreover the relation between $[CuSO_4]$ and c_{Cu} is also roughly linear as seen from the equation

$$[CuSO_4] = c_{Cu}^2 / (K_1 + 2c_{Cu} - [CuSO_4]) \tag{21}$$

which follows from equations (1) and (2). It is therefore very difficult to ascertain experimentally the non-linear character of this relation. At ultraviolet wavelengths from about 250 mμ towards longer wavelenths, ε_1 is very small and therefore the relation between the extinction and the concentration is more distinctly non-linear and this can also be verified experimentally.

DISCUSSION

The fact that the slopes of the curves representing log ε as a function of log x do not depend on the wavelength between 272 and 320 mμ within experimental errors seems to prove that in the cupric sulphate system only a complex exists. The numerical values of the slopes indicate that the dominating complex is the first one. We can also study this question by means of the extended equation (16). Putting $\nu = 2$ we obtain from this equation

$$K_1 = \varphi x / \{1 - \varphi - \psi_1 - (1 + \varphi + \psi_1 - \psi_2)\, x^2 / K_2\} \qquad (22)$$

We can further assume that $\psi_1 = \psi_2$. When $K_2 = \infty$ we have equation (10). It is now obvious that the effect of the second complex appears more at high ligand concentrations. Therefore we can assume that $pK_{1,0}$ and a in equation (18) have correct values. The only unknown is B. For each value K_2 the corresponding value of B can now be calculated by means of equations (22), (18) and (12). The solution can be performed by graphical means. Now tree possibilities exist as represented in Fig. 6 where the difference of the pK_1 values calculated by means of equation (11) and (22) is plotted against B. The upper curve represents the case when no solution exists with positive K_2 values. In this case higher absorbing complexes obviously belong to the system. The lower curve represents the case when solutions with positive K_2 values can be found. The middle curve represents the case when no higher complex can exist in the system. A solution can be found in this case only when $K_2 = \infty$. Experimental data gave so slight departures from the case 2 that we can say the system has no higher complexes. A rough estimation gave for the second complexity constant the upper limit $k_2 < 0.05$.

That the first complex $CuSO_4$ is the absorbing one in the system appears from the numerical values of the quantity φ. Supposing that the complex $Cu(SO_4)_i^{(2i-2)-}$ is the only one, we obtain instead of equation (10)

$$K_i = 1/k_i = \varphi x^i / (i - \varphi - \psi_i) \qquad (23)$$

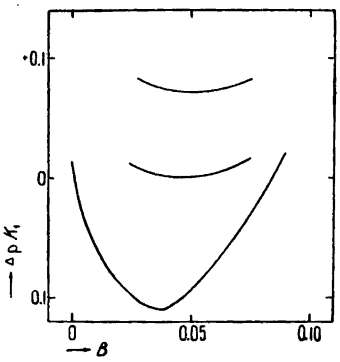

Fig. 6. Calculation of the coefficient B by graphical means.

Using this equation with $i > 1$ we obtain from the experimental data an unreasonably steep curve representing the relation between pK_i and $\sqrt{I}$.

The accuracy of the values obtained for the first complexity constant appears from Fig. 3 where the hollow circles mean the values calculated with the aid of equation (10). Another way of estimating the accuracy is as follows. We calculate first the values of the constant K_1 by means of equation (18) with the experimental values of c_{Cu} and c_{SO_4}. The corresponding values of $[CuSO_4]$ can then be calculated with the aid of equation

$$[CuSO_4] = c_{Cu}c_{SO_4}/(K_1 + c_{Cu} + c_{SO_4} - [CuSO_4]) \qquad (24)$$

which follows from equations (1), (2) and (3). Now values for the molar extinction coefficient of the complex $CuSO_4$ can be calculated by means of equation (5). The values obtained should be equal but are not because of errors made in determining the quantity φ. Now the values of constant K_1 can be calculated by means of equation

$$K_1 = \frac{(c_{Cu} - e/\varepsilon_{CuSO_4})\ (c_{SO_4} - e/\varepsilon_{CuSO_4})}{e/\varepsilon_{CuSO_4}} \qquad (25)$$

The calculations were carried out with such a value $\varepsilon_{CuSO_4} = 120$ ($\lambda = 272$ mμ) that the values of K_1 obtained fit as well as possible with the K_1 values from equation (18). These values are represented in Fig. 3 with full circles. As seen in Fig. 3 the values calculated by means of equation (25) do not deviate more from the values calculated by means of equation (18) than the values calculated by means of equation (10).

The dissociation and light absorption of cupric sulphate solution have often been investigated. Mecke and Ley [3] as well as Ley and Heidbrink [4]

have proved that cupric sulphate solutions »obey Beer's law» at the red but not at ultraviolet wavelengths. They consider complex formation as possible. Several authors have investigated this system with methods which are based upon the more or less arbitrary selection of some relations to represent the behaviour of the hypothetical completely dissociated electrolyte [5]. Therefore the results obtained cannot be very reliable. In this way Davies [6] as well as Owen and Curry [7] have obtained for K_1 the value 0.005 and 0.0043 respectively from conductance measurements. For all that these values do not deviate very much from the value obtained in this paper for the thermodynamic constant $K_{1,0} = 0.008$. Recently Fronaeus [8] has studied the cupric sulphate system by means of potentiometric and spectrophotometric methods using sodium sulphate — sodium perchlorate solutions with ionic strength 1 and assuming that the activity coefficients do not change with the composition. The values obtained for the first complexity constant, spectrophotometrically $k_1 = 5$ and potentiometrically $k_1 = 10$, are not very much different from the present value at this ionic strength $k_1 = 4$. On the contrary the values obtained for higher complexity constants, $k_2 = 10$—17 and $k_3 = 200$, seem to be too high. This can be understood because the variation of activity coefficients has not been taken into account. A clear view of the importance of considering the change of activity coefficients is given in Fig. 3 where the dotted line represents the results without considering the change of activity coefficients.

SUMMARY

A method for the study of complex formation in systems with slight complexity, taking into account the change of activity coefficients, is described.

The first complexity constant of cupric sulphate has been determined in lithium sulphate solutions as the function of ionic strength by means of light absorption measurements.

The possibility of the existence of other complexes in the system is discussed. Their effect can appear only at high ionic strengths and is obviously slight.

It is proved that the light absorption of a cupric sulphate solution is greater than that of a equimolar cupric perchlorate solution even at red wavelengths. This, as well as the fact that cupric sulphate solutions »obey Beer's law» at these wavelengths is proved to be in agreement with complex formation.

The effect of perchloric and sulphuric acid on the light absorption of cupric sulphate solutions has been investigated. It is obvious that complex formation between cupric and bisulphate ions occurs.

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Received March 16, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 190—200

On the Equilibrium State of a Branched Molecule

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It appears at the present time to be rather unanimously assumed that the molecules of amylopectin and glycogen have a ramified structure, in which the glucose residues are united by α-glucosidic 1,4- or 1,6-linkages. The latter are in a minority, constituting only 5—10 per cent of the total number and representing the branching points of the chain molecules. As to the pattern of ramification (whether quite irregular or possessing some sort of regularity) opinions differ somewhat [1].

Recent investigations have proved that the biological synthesis of the polysaccharides in question is effected by two phosphorylases, one reversibly synthesizing 1,4-linkages, the other 1,6-linkages, the starting material being in both cases glucose-1-phosphate [1–3]. The first enzyme is the phosphorylase studied by Hanes and others which, when acting alone, yields unbranched polysaccharide molecules of the amylose type. This enzyme is called »*P*-enzyme» by Haworth *et al.* [4] Bernfeld[5] and Meyer *et. al.* [6] use the name »phosphorylase» for this enzyme. The enzyme synthesizing 1,6-linkages is termed »isophosphorylase» by Meyer. The isophosphorylase seems, on the whole, to be identical with the Haworth group's »Q-enzyme» and with Cori's »branching factor».

The present authors tried some years ago to calculate the pattern of a polysaccharide formed by the simultaneous action of both enzymes [7]. It was assumed that the two enzymes act at random, causing a ramified molecule to grow from an original »germ». As a simplication of the problem an *irreversible* synthesis was assumed. The calculation yielded, among others, two results which could be compared with experimental data: it predicted that β, the »degree of ramification» (see below), should for large molecules be independent of the molecular weight, and it gave — as a function of β only — values for ζ, the fraction of the glucose units of the polysaccharide,

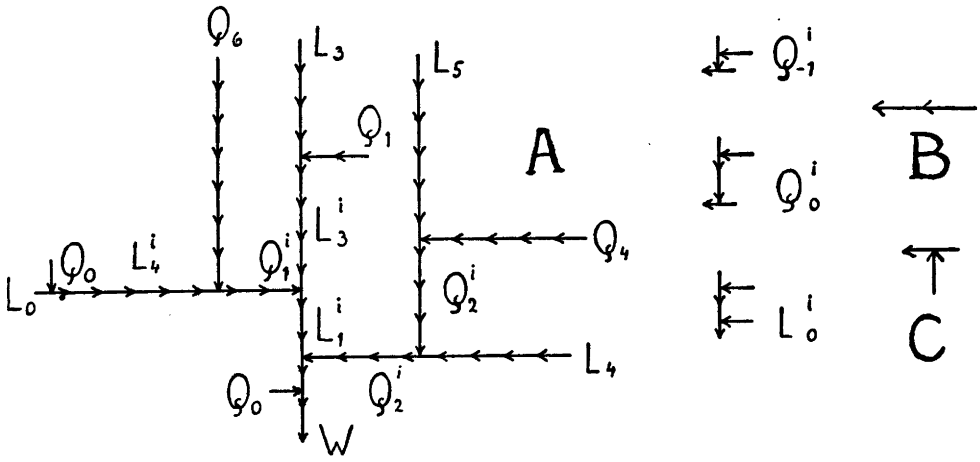

Fig. 1. A, part of a branched molecule, showing the different types of chains, Q_n, L_n, Q_n^i, and L_n^i. B, maltose; C, isomaltose.

which are split off as maltose on treatment of the polysaccharide with β-amylase; this enzyme attacks the end chains only [1]. The calculated values proved to agree reasonably well with the experiments.

Bernfeld [5] and Meyer [6] *et al.* emphasize the reversible nature of the synthesis and seem to assume that the final state of the polysaccharide molecules formed is really one of equilibrium. In the following we shall try to calculate the pattern of a molecule formed through a *reversible* synthesis by joint action of phosphorylase and isophosphorylase in such a way that the polysaccharide is in equilibrium with a solution containing simple glucose units.

Our calculations seem to show that an equilibrated branched molecule would give much lower yields of maltose on treatment with β-amylase than those actually found for amylopectin; the assumption of *irreversible* synthesis seems thus to be nearer the truth for *amylopectin*. On the other hand, it seems quite possible that *glycogen* represents an *equilibrium* state. The usual yields of maltose from glycogen, when treated with β-amylase, are of the calculated magnitude, and the larger yields occasionally reported by Meyer *et. al.* (which were once taken by us as a check for the irreversible synthesis mechanism) may possibly have been caused by previous degradation of the polysaccharide.

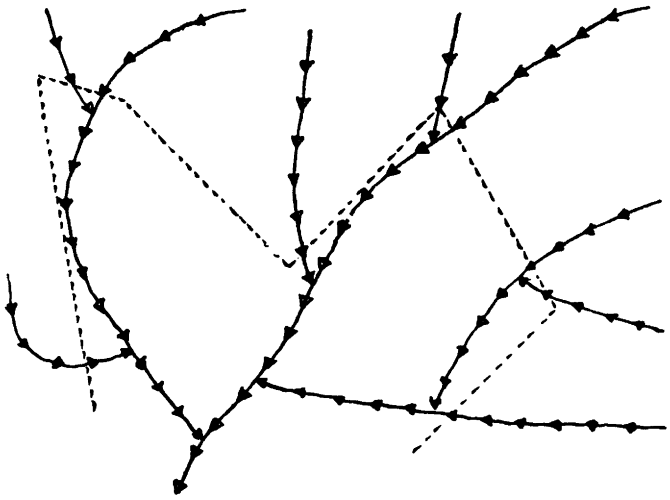

Fig. 2. Part of a ramified molecule of the amylopectin-glycogen type. Parts outside dotted line are split off as maltose by β-amylase.

THEORETICAL

Notations

Fig. 1 A gives a schematic picture of a small branched molecule. The glucose units are represented as arrows, the arrow point representing the 1-position. The 4-position is at the rear, the 6-position at the middle of the arrow shaft. Thus Fig. 1 B represents maltose (with a 1,4-linkage) and Fig. 1 C isomaltose (with a 1,6-linkage).

If the total number of glucose units in a molecule is N, and the number of 1,6-linkages (ramifications) is B, the ratio $\beta = B/N$, the *degree of ramification*, is a characteristic of the molecule, which can be estimated in several ways. Another characteristic quantity is ζ, the fraction of the total number of glucose residues that can be cut off by the action of β-amylase. This enzyme is believed to attack end chains only and to cut off the glucose units in pairs, yielding maltose (Fig. 2).

In the subsequent discussion it is convenient to introduce symbols for the different types of glucose chains: end chains of types Q_n and L_n, and inner chains of types Q_n^i and L_n^i. The numbers n are so chosen (see Fig. 1 A) that all glucose units are counted except those taking part in 1,6-links (two for every branching point). If the number of each type of chains is represented by the corresponding small letter, then

$$B = \beta N = \sum_0^\infty q_n + \sum_0^\infty l_n \tag{1}$$

$$N = \sum_0^\infty n q_n + \sum_0^\infty n l_n + \sum_{-1}^\infty n q_n^{\mathrm{i}} + \sum_0^\infty n l_n^{\mathrm{i}} + 2B \tag{2}$$

If it is assumed that the β amylase can split off maltose from Q_n and L_n so that finally only Q_0 or L_0 is left for even n and Q_1 or L_1 for odd n, then the yield will be

$$\zeta_2 N = 2\,(l_2+q_2+l_3+q_3) + 4\,(l_4+q_4+l_5+q_5) + 6\,(\ldots\ ) = \sum_1^\infty 2m\,(l_{2m}+ q_{2m}+l_{2m+1}+q_{2m+1}) \tag{3}$$

If, however, the end chains with even n are broken down only to Q_2 and L_2 the yield is

$$\zeta_3 N = \zeta_2 N - 2(l_2+q_2+l_4+q_4+\ldots) = \zeta_2 N - 2\sum_1^\infty (l_{2m}+q_{2m}) \tag{4}$$

Irreversible synthesis

The present authors have tried previously the following simple assumptions [7]. The branched molecule grows from a solution containing glucose, in the form of glucose-1-phosphate, and the enzymes. The probability that between the times t and $t+\mathrm{d}t$ a certain end chain will grow by the linking of a new glucose unit at the free 4-position is $k\mathrm{d}t$. The probability that in the same time a new branch will be formed on a certain glucose residue with a free 6-position is $\gamma k\mathrm{d}t$. The quantities k and $k\gamma$ are the same for all units with free 4- or 6-positions in the macromolecule. Even if k varies with time (*e. g.* because of irregular input of fresh glucose-1-phosphate) the ratio γ is assumed to be constant.

Under these assumptions we found that β tends to the value for which

$$\gamma = \beta^2\,(1-\beta)^{-2} \tag{5}$$

and, moreover,

$$q_n = N\,c_n\,(1-\beta)$$

$$l_n = N\,c_n\,(n+1)\,\beta$$

$$q_n^{\mathrm{i}} = N\,c_n\,(1-\beta)^3\,(1+n\beta)^{-1}\,[1+(n+1)\,\beta]^{-1}$$

$$l_n^i = q_n^i [(n+1)^2 \beta^2 + (n-1)\beta + 2] (1-\beta)^{-1} [1 + (n-1)\beta]^{-1} \quad (6)$$

where

$$c_0 = \beta^2 (1 - \beta + \beta^2)^{-1}; \; c_n = c_{n-1} (1-\beta)^2 [1 - \beta + (n+1)\beta^2]^{-1} \quad (7)$$

For the yield of maltose with β-amylase we found

$$\zeta_2 = 0.6557 - 1.4371\beta + 0.3151\beta^2 + 0.3154\beta^3 + \ldots . \quad (8)$$

if the end chains are broken down to $n = 0$ or 1, and

$$\zeta_3 = 0.6557 - 2.4371\beta + 1.8151\beta^2 + 1.8154\beta^3 + \ldots . \quad (9)$$

if they are broken down to $n = 2$ or 1. *E.g.* for amylopectin with $\beta = 0.056$ we calculated $\zeta_2 = 0.576$; $\zeta_3 = 0.525$.

Reversible synthesis

We shall consider a branched macro molecule which is in prolonged contact with a solution containing glucose-1-phosphate and the two phosphorylases. We assume, as is also done by Bernfeld and Meyer *et. al.*, that the enzymes can act only by linking new glucose units from the solution, one by one, to free 4- or 6-positions in the macromolecule, or by the reversal of these reactions: splitting off the outermost glucose units, one by one, by breaking a 1,4- or 1,6-link. Thus we assume that the enzymes cannot split off molecules with two, three or more glucose units, nor can they synthetize such molecules in the solution. (Haworth *et. al.*[4] assume that the Q-enzyme is able to attach ready made »unit chains» of considerable length to the polysaccharide.)

As an approximation it is moreover assumed here that all free 4-positions in the macromolecule are equally accessible so that the same velocity and equilibrium constants apply to all. Similarly all free 6-positions are assumed to be equivalent.

Now the problem is to find the distribution of the glucose units over the various types of chains in the equilibrium state of the molecule which is obtained when the reversible processes have been acting on the macro molecule for a very long time.

The problem can be attacked in several ways. It can be treated kinetically, as was the irreversible synthesis in our previous papers; terms for the reversed reactions are included in the time derivatives for the numbers of different types of branches, and the derivatives are equated to zero at equilibrium.

It can also be treated by applying the law of mass action, thus by assuming one constant, K_q, for the formation of 1,6-links and one, K_1, for the formation of 1,4-links.

However, the simplest line of approach seems to be the statistical; of course it gives the same results as the others. Let us regard a macro molecule at equilibrium. The number of 1,6-links is $N\beta$, that of 1,4-links is $N(1-\beta)$ and that of end chains is $N\beta$. Then for any one glucose residue ➡ in the macro molecule the probability that its

1-position forms a 1,4-link ➡→ is $1-\beta$

» forms a 1,6-link ➡↓ is β

4-position forms no link ➡ is β

» forms a 1,4-link →➡ is $1-\beta$

↓

6-position forms a 1,6-link ➡ is β

» forms no link ➡ is $1-\beta$.

Now the numbers of the different types of branches can be calculated. Let us begin with q_n.

The number of glucose units, the C_1 of which forms a 1,6-link is $N\beta$. The probability that a chain of n residues, united by 1,4-linkages, is tied to a glucose unit of this kind with a 1,4-link is $(1-\beta)^n$; that the last glucose unit has a free 4-position is β; and that all the $(n+1)$ units have their 6-positions free is $(1-\beta)^{n+1}$. Thus we find the number of Q_n chains

$$q_n = N\beta\ (1-\beta)^n\ \beta(1-\beta)^{n+1} = N\beta^2\,(1-\beta)^{2n+1}$$

$$q_0 = N\beta^2\,(1-\beta) \tag{10}$$

In the same way we find, as can easily be controlled by means of Fig. 1,

$$l_n = N\beta\,(1-\beta)^n\,\beta(1-\beta)^n = N\beta^2\,(1-\beta)^{2n}$$

$$l_0 = N\beta^2 \tag{11}$$

$$l_n^i = N\beta\,(1-\beta)^{n+1}\,\beta\,(1-\beta)^n = N\beta^2\,(1-\beta)^{2n+1}$$

$$l_0^i = N\beta^2\,(1-\beta) \tag{12}$$

$$q_n^i = N\beta\,(1-\beta)^{n+1}\,\beta\,(1-\beta)^{n+1} = N\beta^2\,(1-\beta)^{2n+2}$$

$$q_{-1}^i = N\beta^2 \tag{13}$$

These formulas can by elementary summation be found to agree with (1) and (2).

For the yield of maltose with β-amylase we find using (3) and (4):

$$\zeta_2 N = \sum_1^\infty 2m(l_{2m} + q_{2m} + l_{2m+1} + q_{2m+1}) =$$

$$= N\beta^2\,(2-\beta)\,[1+(1-\beta)^2]\,2\sum_1^\infty m(1-\beta)^{4m} = \frac{2N\beta\,(1-\beta)^4}{1-(1-\beta)^4}$$

and

$$\zeta_2 N - \zeta_3 N = 2\sum_1^\infty (l_{2m} + q_{2m}) = 2N\beta^2\,(2-\beta)\sum_1^\infty (1-\beta)^{4m} =$$

$$= \frac{2N\beta^2\,(2-\beta)\,(1-\beta)^4}{1-(1-\beta)^4}$$

from which

$$\zeta_2 = \frac{2\beta\,(1-\beta)^4}{1-(1-\beta)^4} = 0.5 - 1.25\beta + 0.625\beta^2 + 0.3125\beta^3 + 0.03125\beta^4 + \ldots. \tag{14}$$

$$\zeta_3 = \frac{2\beta\,(1-\beta)^6}{1-(1-\beta)^4} = 0.5 - 2.25\beta + 3.625\beta^2 - 2.1875\beta^3 + 0.03125\beta^4 + \ldots. \tag{15}$$

(Incidentally these equations happen to be the same as those obtained if it is assumed that the synthesis is irreversible but that branching can take place only at the last units of the end chain/»Case E»/[7].)

The equilibrium constants

The process of forming a new 1,4-link can be written:

$$C_6H_{11}O_5PO_4H^- + \overset{4}{H}OC \ldots\ldots \rightleftharpoons C_6H_{11}O_5\overset{4}{O}C \ldots\ldots + H_2PO_4^-$$

or

$$C_6H_{11}O_5PO_4H^- + \overset{\cdot}{\longrightarrow} x \rightleftharpoons \longrightarrow\overset{\cdot}{} x + H_2PO_4^- \tag{16}$$

Here $\overset{\cdot}{\longrightarrow} x$ stands for any *specified* type of end chain which is of course *the same* in both membra of (16). At the x at C_1 there may be a 1,6-link or a chain

of a certain number of glucose residues coupled by 1,4-links and ended by a 1,6-link. Similarly at the point at the 6 position there may or may not be a 1,6-link.

The equilibrium constant for the process is

$$K_1 = \frac{\left\{\longrightarrow\overset{\cdot}{\longrightarrow} x\right\}\left\{H_2PO_4^-\right\}}{\left\{\longrightarrow\overset{\cdot}{} x\right\}\left\{C_6H_{11}O_5PO_4H^-\right\}} = \frac{\left\{\longrightarrow\overset{\cdot}{\longrightarrow} x\right\}}{\left\{\longrightarrow\overset{\cdot}{} x\right\} g} \tag{17}$$

We shall consider the quantity g introduced here. If K is the thermodynamic equilibrium constant for the formation of glucose-1-phosphate from dihydrogen phosphate ion and glucose,

$$C_6H_{12}O_6 + H_2PO_4^- \rightleftharpoons C_6H_{11}O_5PO_4H^- + H_2O \tag{18}$$

then g is related to the activities of the reacting substances by

$$g = \frac{\left\{C_6H_{11}O_5PO_4H^-\right\}}{\left\{H_2PO_4^-\right\}} = K\frac{\left\{C_6H_{12}O_6\right\}^*}{\left\{H_2O\right\}} \tag{19}$$

The asterisk (*) signifies that the *glucose* activity is *virtual* (the activity glucose would have had in equilibrium with glucose-1-phosphate and phosphate at the prevailing concentrations) and need not bear any relation to the actual concentration of glucose, since the equilibrium (18) is generally not realized.

One might also consider g as a measure of the virtual activity $\left\{C_6H_{10}O_5\right\}^*$ of glucose residues in the solution.

Of course, one might equally well use one of the ratios $\frac{\left\{C_6H_{11}O_5PO_4H_2\right\}}{\left\{H_3PO_4\right\}}$ or $\frac{\left\{C_6H_{11}O_5PO_4^{2-}\right\}}{\left\{HPO_4^{2-}\right\}}$, which always bear a constant ratio to g.

From the foregoing (compare *e.g.* q_n and q_{n+1} in (10) or l_n and l_{n+1} in (11) and use (17)) it is evident that

$$K_1 = (1-\beta)^2 g^{-1} \tag{20}$$

The process of forming a new 1,6-link is

$$\cdot\longrightarrow x + C_6H_{11}O_5PO_4H^- \rightleftharpoons \quad \cdot\underset{\downarrow}{\longrightarrow} x + H_2PO_4^- \tag{21}$$

where $\cdot\longrightarrow x$ stands for any glucose unit with a free C_6. Now the number of such units in the macro molecule is $N - B = N(1-\beta)$. The group in

the right member of (21) is a Q_0 chain; using (10) we find for the equilibrium constant K_q of (21)

$$K_q = \frac{q_0}{(N - B)g} = \beta^2 g^{-1} \tag{22}$$

Because of (20) and (22) both β and g are uniquely determined at equilibrium by K_q and K_1. On the other hand, since β and g can both in principle be determined experimentally, it should be possible to measure K_1 and K_q separately.

Let us call the (bimolecular) velocity constant for the formation of new 1,4-links k_{1+} and that for new 1,6-links k_{q+}. The (bimolecular) constants for the breaking of the same links are denoted by k_{1-} and k_{q-}. The equilibrium constants for (18) and (21)

$$K_q = \frac{k_{q+}}{k_{q-}}\,;\; K_1 = \frac{k_{1+}}{k_{1-}} \tag{23}$$

are based on thermodynamics; these ratios are thus independent of the enzyme concentrations. On the other hand the ratio

$$\gamma = \frac{k_{q+}}{k_{1+}} \tag{24}$$

which occurred in our calculations on irreversible synthesis, may be different in different enzyme mixtures, and there is no reason why there should be any relation between γ, K_q and K_1.

DISCUSSION

We shall now see whether the equations derived for the equilibrium state can apply to the branched molecules of amylopectin and glycogen. If we insert the value $\beta = 0.056$ for amylopectin, we find $\zeta_2 = 0.432$ from (14) and $\zeta_3 = 0.385$ from (15). The experimental values are actually much higher, between 0.53 and 0.62, and agree rather well with those calculated from (9) for the case of irreversible synthesis. Thus it seems very improbable that amylopectin corresponds to an equilibrium state. Of course, the existence of long, almost entirely unbranched amylose chains is also quite incompatible with the assumption of equilibrium with a solution containing both 1,4- and 1,6-linking enzymes.

On the other hand, for glycogen with $\beta = 0.090$, we calculate $\zeta_2 = 0.393$ and $\zeta_3 = 0.325$ from (14—15). The experimental values of ζ are generally between 0.35 and 0.45. In a few exceptional experiments, Meyer obtained

ζ values for glycogen as high as 0.53. However, in these cases the glycogen had been treated repeatedly with alkali hydroxide solutions so that it does not seem improbable that a certain disruption of the inner chains had taken place which would of course increase ζ.

The hypothesis that amylopectin is chiefly formed by an irreversible process whereas glycogen is an equilibrium product is not incompatible with the different modes of formation. The starch is generally formed by a oneway process and stored for a long time, whereas the glycogen is formed as a reserve for short times of delivery and is continuously changing its amount in the animal (or yeast) organism. It is also possible that the differences between amylopectin, amylose and glycogen may be related to the growth of the starch granules [8].

It is possible that under certain conditions the organism can form glycogen so rapidly that there is no time for equilibrium. In such samples of glycogen the relation between β and ζ would deviate from (14 or 15) and instead tend towards (8 or 9). Thus at least one of β or ζ would differ from the values for normal, equilibrated glycogen.

If glycogen with $\beta = 0.09$ corresponds to the thermodynamic equilibrium between 1,4- and 1,6-links, we can then calculate

$$\frac{K_q}{K_1} = \frac{\beta^2}{(1-\beta)^2} = \frac{(0.09)^2}{(0.91)^2} \approx 0.01$$

SUMMARY

The paper deals with the pattern assumed by a branched macromolecule such as amylopectin or glycogen in equilibrium with a solution containing glucose-1-phosphate and the two phosphorylases causing the formation and breaking of α-glucosidic 1,4- and 1,6-linkages. Formulas (10—13) are derived for the partition of the glucose residues over different types of inner chains and end chains.

A quantity especially suited for comparison with experiments is ζ, the fraction of the glucose residues split off by the action of β-amylase.

For the case of equilibrium (reversible synthesis) equations (14, 15) are derived, giving ζ as a function of β, the degree of ramification. The authors have previously derived (8, 9), giving ζ (β) for the case of completely irreversible synthesis. When these equations are tested with experimental values of β and ζ, it seems that amylopectin is not in an equilibrium state but rather approaches the case of irreversible synthesis. The values for glycogen on the

other hand seem to agree rather well with the formula for equilibrium. The difference between amylopectin and glycogen may be understood from the different way in which they are formed.

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   Myrbäck, K., and Sillén, L. G. » » » **55** (1943) 311;
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Received January 19, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 201—212

Short Communications

The Structure of Divinyl Ether

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The study of the diethyl and divinyl ether by Fischer and Ehrenberg [1, 2] has given rise to a discussion of the structure of the gaseous divinyl ether. Usually the structure of this compound is explained by assuming a resonance which gives a certain degree of double bond character to the C—O-bonds [3]. The molecule should accordingly be expected to be planar. On the other hand the short $H_2—H_3$-distance in the planar model is unfavourable and will counter-act the tendency to coplanarity. The van der Waals distance between two hydrogen atoms is 2.4—2.5 Å, while the $H_2—H_3$-distance for an undistorted model would be about 2.0 Å.

In order to decide whether one of the two effects predominates or an intermediate equilibrium position might exist, we have carried through an electron diffraction investigation by the aid of the sector method.

In Fig. 1 the $\sigma(r)$-curve of divinyl ether is given. The peaks with Roman numerals are due to the C—C- and C—O-distances. The other maxima are mainly caused by the C—H- and O—H-distances which give a relatively important contribution in this case. These maxima are, however, also somewhat influenced by the diffraction effect.

We are not able to determine separately the C—C- and C—O-bond distances from the $\sigma(r)$-curve. The same difficulty also occurs for the distances $C_1—O$ and $C_2—C_3$. An accurate determination of the parameters in the molecule can therefore not be expected. The following bond distances

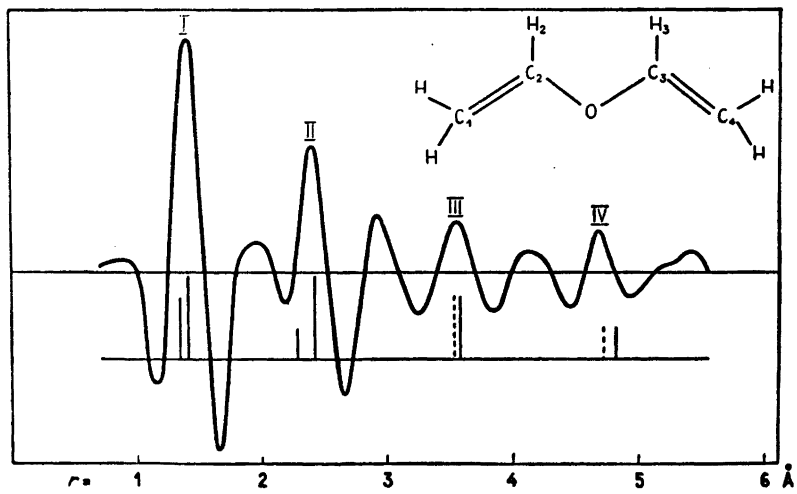

Fig. 1. $\sigma(r)$-curve for divinyl ether.

and valency angles seem, however, to give best agreement with the $\sigma(r)$-curve:

C_1C_2	1.35 Å
C_2O	1.42 Å
$\angle\ C_2OC_3$	107°
$\angle\ C_1C_2O$	123°

The C—C and C—O-distances occuring in a planar molecule with these parameters are indicated below the $\sigma(r)$-curve by a line diagram. (Full-drawn lines). The maximum IV does not fit with the $C_1—C_4$-distance. If we give up the claim of coplanarity, however, and for instance rotate the vinyl groups about the C—O-bonds an angle of 20° in such a way that the C_1- and C_4-atoms move in the same direction, a relatively good agreement may be obtained. (Dotted lines in the line diagram.) Because of the uncertainty in the determination of the bond distances and valency angles an accurate determination of the magnitude and form of the deviation from coplanarity is impossible. By varying the distances and angles within reasonable limits we have, however, not been able to find a planar model which can be brought in accordance with the $\sigma(r)$-curve. We feel therefore justified in concluding that the deviation from coplanarity is real and that this deviation must be ascribed to the short $H_2—H_3$-distance in the undistorted planar model.

In this connection another question related to our problem may be mentioned. Wheland [3] has drawn attention to the surprising fact that the resonance energy of ethyl vinyl ether seems to be slightly *greater* than that of divinyl ether; it would have been expected to be somewhat *smaller* since only a single unstable structure takes part in the resonance in the ethyl vinyl ether molecule, while *two* such structures take part in the resonance of the divinyl ether molecule. This fact might be brought in relation to the unfavourable $H_2—H_3$-distance in the planar divinyl ether molecule model. If our assumption of a deviation from coplanarity is correct, the π-orbitals will overlap less completely causing a reduction of the resonance energy. But even if the molecule was assumed to be strictly planar the unfavourable $H_2—H_3$-distance would have decreased the stability of the divinyl ether molecule. In the case of the ethyl vinyl ether, on the other hand, the planar configuration is stabilized by the favourable $H_2—H_3$-distances. In an ethyl vinyl ether molecule with a planar carbon-oxygen skeleton the $H_2—H_3$-distances are approximately 2.3 Å. If any of the carbon atoms are brought out of the plane, one of the $H_2—H_3$-distances will decrease and might therefore oppose the deviation from coplanarity.

We wish to express our gratitude to fil.mag. Inga Fischer, University of Stockholm, who has suggested this investigation and placed a sample of divinyl ether at our disposal. We also wish to thank Prof. O. Hassel for having read the manuscript.

1. Ehrenberg, L., and Fischer, I. *Acta Chem. Scand.* 2 (1948) 657.
2. Fischer, I., and Ehrenberg, L. *Acta Chem. Scand.* 2 (1948) 669.
3. Wheland, G. W. *The theory of resonance* (1947) p. 61.

Received March 7, 1949.

On the Identification of α-Aminoadipic Acid by Paper Chromatography

SUNE BERGSTRÖM and KARIN PÄÄBO

Department of Physiological Chemistry, University of Lund, Lund, Sweden

Recent work has shown that α-aminoadipic acid is an important metabolite. It has been isolated from *Cholera vibrio* [1] and the acid has been found active in transamination [2] in arginine formation [3] and also shown to be an intermediate in the lysine metabolism in mammals [4] and in *Neurospora* [5].

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Borsook *et al.*[4] reported that they were unable to separate α-aminoadipic acid from glutamic acid by paper chromatography and had to resort to the more cumbersome chromatography on starch when investigating the natural occurance of this compound.

Due to the possible practical importance to workers in this field we wish to report an observation made in connection with work on lysine requiring mutants of *Ophiostoma*[6], some of which has been found to grow with α-aminoadipic acid[7]. We have found that this amino acid can be clearly separated from glutamic acid by chromatography on paper with an acid medium similar to that described by Edman[8].

On Munktells filter paper no. 0B[9] with a medium consisting of 45 % *n*-butyric, 45 % isovaleric and 10 % water by volume, a mixture of aspartic, glutamic and α-amino adipic acid was separated after 12 hours in well developed spots with R^F values of respectively 0.18, 0.26 and 0.33, the descending solvent front having moved 41 cm.

In more complex amino acid mixtures two dimensional developments with this medium in combination with phenol, butanol or amyl alcohol-pyridine make identification possible.

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9. Supplied by J. A. Munktells Pappersfabriksaktiebolag, Grycksbo, Sweden. Dimensions: 50 × 50 cm.

Received March 4, 1949.

The Crystal Structure of Tetrachlorocyclohexane of m.p. 174° C

O. HASSEL and E. WANG LUND

Universitetets Kjemiske Institutt, Blindern—Oslo, Norway

The structure formula of the compound $C_6H_8Cl_4$ obtained by direct chlorination of cyclohexane[1] and melting at 174° C has not been determined. On treating cyclohexane or chlorocyclohexane with chlorine at room temperature in artificial light and separating the deposited crystals from the liquid, this substance is easily obtained. We also prepared it from 1,2-dichlorocyclohexane using the same procedure, a fact which seems to indicate that at least two of the chlorine atoms occupy 1,2-positions. An electron diffraction investigation of the vapour carried out in our laboratory some time ago led to the assumption that the molecule has the configuration 1 ε, 2 ε, 4 κ, 5 κ. A measurement of the dipole moment[2] supported this view, indicating that the tetrachloride has a configuration corresponding to that of the tetrabromocyclohexane of m. p. 185° earlier investigated[3].

In order to check the result of the electron diffraction work a complete X-ray crystallographic analysis has been carried out, and an extract of the results will be given here. We intend to bring an account of both the electron diffraction investigation and the crystal analysis with full details shortly in order to demonstrate the close agreement between the results obtained in these two independent investigations.

The crystals of tetrachlorocyclohexane belong to the orthorhombic sphenoidal class. Laue and Weissenberg photographs show that the *c*-axis has pseudo-tetragonal symmetry. The lattice constants are:

$a = 7.60$ Å, $b = 7.54$ Å, $c = 7.72$ Å

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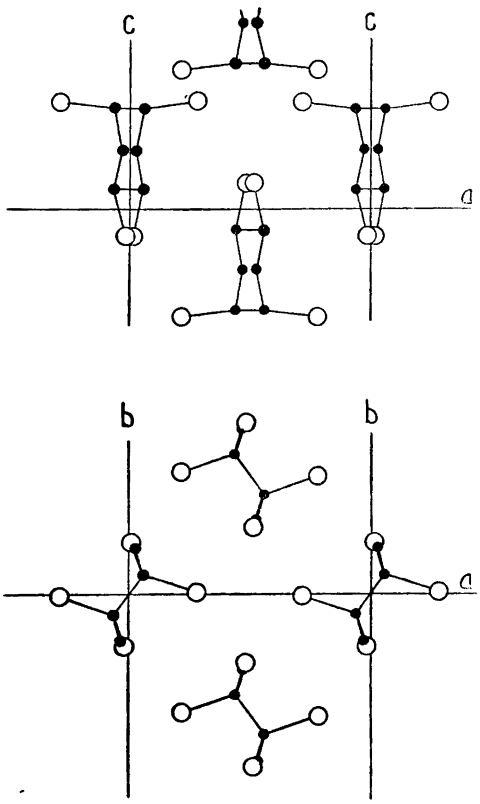


Fig. 1. Projection showing the position of the molecules in the unit cell. The upper part gives the projection in the (010)- plane, the lower part in the (001)-plane. Open circles represent chlorine atoms, filled circles carbon atoms. C—C-distance 1.54 Å, C—Cl distance 1.79 Å.

The unit cell contains *two* molecules. From the fact that the space group is D^3_2—$P2_12_12$ it follows that the molecules are crystallographically equivalent and possess a twofold axis of symmetry running parallel to the *c*-axis. This axis must of course bisect the distance between say the carbon atoms 1 and 2 and the carbon atoms 4 and 5.

Intensity data obtained from Weissenberg photographs by means of the multiple film method served as a basis for the two-dimensional Fourier analyses, of which one gave the projection of the electron density in the (001)-plane, a second in the (010) plane. In order to find the correct sign of the structure factors the 1ε, 2ε, $4\varkappa$, $5\varkappa$ structure was assumed to be correct. The Fourier maps, showing well defined maxima of the expected heights, fully justified this procedure. The parameters of the chlorine atoms could be determined with a considerable degree of accuracy:

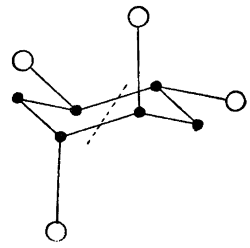


Fig. 2. Shape of the tetrachlorocyclohexane molecule.

$$x_1 = 0.282,\ y_1 = -0.008,$$
$$z_1 = 0.437,\ x_2 = 0.015,$$
$$y_2 = -0.215,\ z_2 = -0.105.$$

The peaks due to the carbon atoms are less marked and partly overlapped by those of the chlorine atoms, but approximate parameters may still be given:

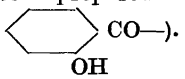
$$x_3 = 0.062,\ y_3 = -0.081,\ z_3 = 0.408,$$
$$x_4 = 0.062,\ y_4 = -0.081,\ z_4 = 0.082,$$
$$x_5 = 0.028,\ y_5 = -0.194,\ z_5 = 0.245.$$

Fig. 1 gives a projection of the structure in the (010)-plane (upper half) and in the (001)-plane (lower half). The form of the molecule is shown in Fig. 2. It is easily seen that the configuration is really 1ε, 2ε, $4\varkappa$, $5\varkappa$, but that the valency angles are not strictly tetrahedral. This deviation from an ideal structure is most strikingly demonstrated by the fact that the

Some Substituted Amides of Salicylic Acid

KAI ARNE JENSEN and
S. CHRISTIANSEN LINHOLT

Chemical Laboratory, University of Copenhagen, Copenhagen, Denmark

In connection with investigations on heterocyclic substituted amides of 4-aminosalicylic acid some amides of salicylic acid have been prepared (Table 1, R = ).

These compounds were prepared by the reaction of the appropriate amino compound with acetylsalicylic acid chloride in pyridine.

By the preparation of the first-mentioned compound 0.01 mole of acetylsalicylic acid chloride was added under cooling to a solution of 0.01 mole of 2-aminothiazole in 5 ml of pyridine. After standing for two hours at room temperature the solution was diluted with 50 ml of water and the precipitate which separated was filtered after 24 hours and recrystallized from ethanol. Compounds nos. 2 and 3 were prepared in a similar way; the thiadiazole compound was recrystallized from a mixture of ethanol and pyridine and subsequently treated with boiling ethanol.

The compounds nos. 1 and 2 form long, white needles which are readily soluble in ethanol; compound no. 3 which is almost insoluble in ethanol, separates in very small crystals.

For the preparation of compounds nos. 4—6, 0.01 mole of the amino compound was dissolved in the necessary amount of pyridine and 0.01 mole (resp. for no. 4: 0.02 moles) of acetylsalicylic acid chloride was added with cooling and stirring. After standing for 24 hours the solutions were diluted with their own volume of ethanol, neutralized with sodium carbonate, filtered and the filtrate poured into 200 ml of water. In the case of nos. 4 and 6 a white precipitate was obtained, which was filtered and recrystallized from ethanol. The derivative of sulphathiazole, however, separated as an oil, which was turned into solid form by dissolving in 25 ml of 1 *N* NaOH and precipitating with hydrochloric acid. The yield of this compound was small. The yields of the other compounds were about 80 %.

The sulphanilamide derivative has previously been prepared by essentially the same method by v. Euler and Hasselquist [1]. For comparison we prepared this compound in the same way as the first three compounds. The two products were found to be identical.

---

two ε-bonds are not parallel, each of them making an angle of about 7° with the principal axis of the carbon ring. We may add that this deformation was observed also in the electron diffraction examination.

The proof has thus been given, that the tetrachlorocompound of m. p. 174° corresponds to the tetrabromocyclohexane of m. p. 185° [3]. It is highly probable that the substance will be the chief reaction product when a solution of chlorine is added to a solution of 1,4-cyclohexadiene in a suitable organic solvent.

The shape of the (optically active) molecule is not altered by a conversion of the carbon ring. A study of the optical antipodes should therefore be possible even in solution.

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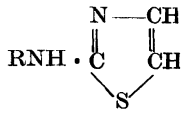
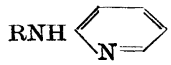
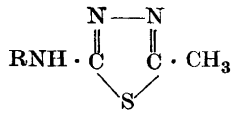
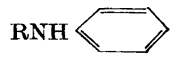
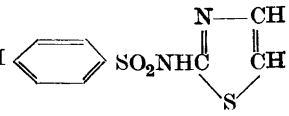
Received March 12, 1949.

Table 1.

	M. p. °C	(% N) calc.	found
1. N-Salicyloyl-2-aminothiazole 	258	12.73	12.92
2. N-Salicyloyl-2-aminopyridine 	210	13.10	13.32
3. N-Salicyloyl-2-amino-5-methyl-1,3,4-thiadiazole 	298	17.90	17.74
4. N^4-Salicyloyl-sulphanilamide 	257	9.60	9.50
5. N^4-Salicyloyl-sulphathiazole 	268	11.20	11.40
6. bis-(4-Salicyloylamino)-diphenylsulphone 	265	5.74	5.87

During these processes the acetyl group of acetylsalicylic acid is split off. Compound no. 1 was also prepared by the reaction of 2-aminothiazole with benzoylsalicylic acid chloride; in this case, too, the unacylated compound resulted. Nos. 1 and 2 were further prepared by the reaction of salicylic acid chloride with the amine in pyridine solution. In addition to the inconvenience of working with the unstable salicylic acid chloride, this method gave only small yields. The identity of the products prepared by the different methods was shown by analyses and mixed melting points.

Acetylsalicylic acid chloride was prepared by heating equivalent amounts of acetylsalicylic acid and thionyl chloride at 60° for 30 minutes and distilling the product *in vacuo* (b. p. 140° at 15 mm). Yield almost 100 %.

Salicylic acid chloride was prepared by the method of Kirpal [2].

The compounds prepared were tested for antibacterial effect against *Diplococcus pneumoniae* (type I), *Eberthella typhosa*. *Staphylococcus aureus* and *Escherichia coli*, but were found to be without effect in the concentration 1 : 5000.

We thank Dr. K. Kjerulf Jensen for carrying out the bacteriological tests.

1. v. Euler, H., and Hasselquist, H. *Arkiv Kemi, Mineral. Geol.* **A 24** (1947) no. 9.
2. Kirpal, A. *Ber.* **63** (1930) 3190.

Received March 8, 1949.

p-Hydroxybenzenesulphonamides

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The announcement [1] that 2-*p*-hydroxybenzenesulphonamidothiazole (»Darvisul») has an effect on poliomyelitis virus has suggested to us the preparation of some related compounds (Table 1).

These were prepared by diazotation of the corresponding amino derivatives: The sulphanilamide (0.005—0.01 mole) was dissolved in 100 ml of dilute hydrochloric acid (4 *N*) and cooled at 0—5°; the hydrochloride often separated, but regardless of this the calculated amount of sodium nitrite was added gradually with stirring. The diazonium chlorides separate as beautiful, yellow crystalline precipitates. The suspension of the diazonium salt was added gradually to 150 ml of boiling water; when all had been added, the solution was boiled for half an hour, activated carbon was added, and the hot solution was filtered and chilled. In most cases the hydroxyl compound separated directly, but in some cases the solution had to be neutralized (nos. 5, 8 and 9). The quinoline derivative (no. 5) separates in neutral solution as hydrochloride. The sulphone, no. 9 (analogue of Promizole), separates in acid solution as hydrochloride, but as free amine by addition of excess of ammonia. The precipitates were filtered off and recrystallized from water under addition of activated carbon.

Table 1.

No.	Name	Formula	M. p. °C	% N calc.	% N found
1.	2-(*p*-Hydroxybenzenesulphonamido)-thiazole	$C_9H_8O_3N_2S_2$	221—22	10.93	11.06
2.	2-(*p*-Hydroxybenzenesulphonamido)-benzthiazole	$C_{13}H_{10}O_3N_2S_2$	292	9.15	9.10
3.	2-(*p*-Hydroxybenzenesulphonamido)-5-methyl-thiazole	$C_{10}H_{10}O_3N_2S_2$	231	10.37	10.35
4.	2-(*p*-Hydroxybenzenesulphonamido)-5-methyl-1,3,4-thiadiazole	$C_9H_9O_3N_3S_2$	217—18	15.49	15.05
5.	6-Methoxy-8-(*p*-hydroxybenzenesulphonamido)-quinoline-hydrochloride	$C_{16}H_{15}O_4N_2SCl$	268	7.64	7.53
6.	N-(3,4-Dimethylbenzoyl)-*p*-hydroxybenzenesulphonamide	$C_{15}H_{15}O_4NS$	187	4.59	4.84
7.	6-(*p*-Hydroxybenzenesulphonamido)-coumarin	$C_{15}H_{11}O_5NS$	230—31	4.41	4.72
8.	*p*-Hydroxybenzenesulphonyl-guanidine	$C_7H_9O_3N_3S$	160—62	19.52	19.48
9a.	*p*-Hydroxyphenyl-2-aminothiazolyl(5)-sulphone	$C_9H_8O_3N_2S_2$	260	10.93	10.76
9b.	do. -hydrochloride	$C_9H_9O_3N_2S_2Cl$	247	9.58	9.33

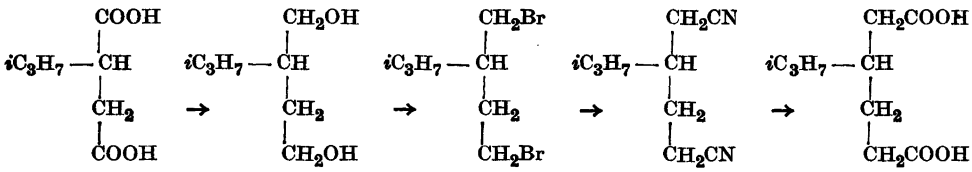
The compounds were tested for bacteriostatic activity on *Diplococcus pneumoniae* (type I), *Eberthella typhosa*, *Staphylococcus aureus* and *Escherichia coli*, but were found to be inactive in the concentration 1 : 5000. Jensen and Schmith [2] have previously found *p*-hydroxybenzenesulphonamide to be inactive on pneumococci even in the

The Steric Connections of β-*iso*-Propyladipic Acid

ARNE FREDGA

Chemical Institute, University of Uppsala, Uppsala, Sweden

Optically active β-*iso*-propyladipic acid is in several cases obtained on degradation of terpenoid compounds, *e. g.* limonene [1] and phellandral [2], and its steric connections are therefore of special interest for the stereochemistry of the terpene group. Von Braun and coworkers have resolved the racemic acid into the optical antipodes [1] and made several attempts to relate it to the *iso*-propylsuccinic acid [3]. It had previously been found, that (+)-β-methyladipic acid could be degraded to (+)-methylsuccinic acid [4], but corresponding experiments with the *iso*-propyl derivative were unsuccessful. They also tried to go the opposite way:



The initial reduction could be accomplished either by sodium in alcohol or catalytically with copper chromium oxide catalyst at high temperature and pressure. Both methods are, however, known to cause racemisation of an asymmetric carbon atom in α-position and the products obtained were completely inactive.

Reduction of carboxylic acids to primary alcohols can now be performed very smoothly with *lithium aluminium hydride* in ethereal solution [5]. This reaction could be expected to proceed without loss of activity. Racemic and dextro-rotatory *iso*-propylsuccinic acid were therefore treated with lithium aluminium hydride according to Nystrom and Brown [5]. The resulting diols were, without further purification, successively converted to dibromides, dicyanides and dicarboxylic acids using familiar methods. The ultimate yields were rather poor, but the acids obtained could be identified as racemic and dextro-rotatory β-*iso*-propyladipic acid. A closer investigation of the different steps might lead to a better yield.

16 g of racemic *iso*-propylsuccinic acid yielded 1.85 g of crude β-*iso*-propyladipic acid. After recrystallisations from water, benzene and hydrochloric acid, there remained 1.52 g with m. p. 82.5—83.5°.

0.1207 g: 12.11 ml 0.1060 *N* NaOH. — 27.63 mg: 58.25 mg CO_2 and 21.14 mg H_2O.
$C_9H_{16}O_4$ (188.2)
Calc. Equiv. wt 94.1 C 57.43 H 8.57
Found » » 94.0 » 57.49 » 8.56

8 g of pure (+)-*iso*-propylsuccinic acid having $[\alpha]_D^{25} = +22.9°$ in aqueous solution [6] gave 0.91 g of crude β-*iso*-propyladipic acid. After recrystallisations from water and hydrochloric acid, there remained 0.55 g having m. p. 71—73°.

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concentration 1 : 200 and *p,p'*-dihydroxydiphenylsulphone to possess only a slight bacteriostatic activity (1 : 4000).

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2. Jensen, K. A., and Schmith, K. *Z. Immunitätsforsch.* **102** (1942) 276.

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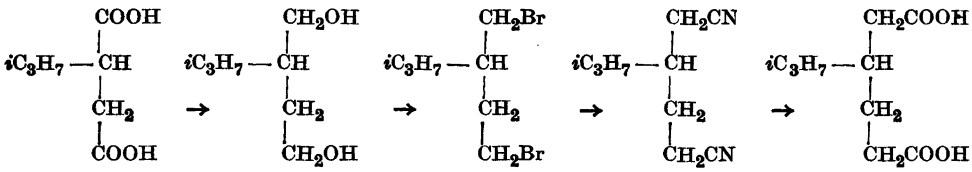

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Equiv. wt. Calc. 94.1 Found 94.2
$[\alpha]_D^{25} = +5.2°$; $[M]_D^{25} = +9.8°$.

On comparison with the values given by Von Braun and Werner [1], this preparation seems to contain about 90 % of (+)-acid and 10 % of (—)-acid. As the rotatory power is very low and the data of these authors refer to much more concentrated solutions, this estimation is very approximate. It can however be stated that an optically active carboxylic acid can in this way be converted to a higher homologue without material loss of activity. As the asymmetric carbon atom is not involved in the reactions, (+)-*iso*-propylsuccinic acid and (+)-*β*-*iso*-propyladipic acid must be sterically related.

The latter acid may be obtained from (+)-limonene [1], and this terpene is thus sterically connected to the alkylsuccinic acids. On the other hand, (+)-limonene is sterically related to (+)-fenchone and (+)-camphor [7, 8], which have previously been connected to the alkylsuccinic acids by aid of *α*-*iso*-propylglutaric acid [9]. These earlier results are corroborated by the present investigation. A further discussion of these matters and a more detailed account of the experiments will be published later.

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Electron Diffraction of Cyclooctatetraëne Vapour

O. BASTIANSEN AND O. HASSEL

Universitetets Kjemiske Institutt, Blindern—Oslo, Norway

The most direct way for determining the molecular structure of cyclooctatetraëne would be the use of interferometric methods based either on X-rays or electrons. Reports on interferometric investigations were published in 1947 by the present authors [1] and by Kaufman, Fankuchen and Mark [2]. The conclusions, however, to which the present authors were led using the electron diffraction sector method were quite different from those reached by Kaufman *et al.* using X-ray crystallographic methods.

In order to check our results two new analyses were carried out, the first with the same material used in our previous investigation (a sample prepared by prof. A. Langseth), the second using new material obtained from The British Oxygen Company. The computation work associated with the second of these new analyses was carried out by a new member of the staff who had no knowledge about the results of the two first analyses. In Fig. 1 the $\frac{\sigma(r)}{r}$-curve obtained in the very first analysis (A) is compared with those of the two new analyses (B and C). The C-C-C-angle is determined by the ratio of the r-values of the two first high maxima of the

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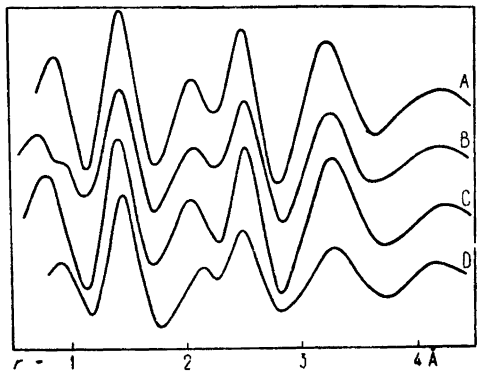

Fig. 1. $\frac{\sigma(r)}{r}$*-curves of cyclooctatetraëne A, B, C experimental curves, D theoretical curve based on the »crown» model.*

$\frac{\sigma(r)}{r}$-curve. The angles calculated from the three individual curves are A) 120°, B) 122°, C) 122.5°.

We feel quite safe when drawing the conclusion that the C-C-C-angle is 121.5° ± 2°. The r-value of the first high maximum was found to be 1.42_5 Å, on making exposures of cyclooctatetraëne and benzene during the same run and assuming the C-C bond length in benzene to be 1.40 Å — a value obtained in our earlier work.

We are unable, however, to decide from our investigations whether all the C-C bond distances are equal or if two alternate C-C distances occur, the mean value being 1.42_5 Å. In the latter case we regard it very improbable, however, that distances corresponding to normal single and double bonds are present as postulated by Kaufman *et al.*

Sets of theoretical $\frac{\sigma(r)}{r}$-curves have been computed based both on a planar arrangement of the carbon atoms, and on the nonplanar »tub» and »crown» forms of the molecule. The first alternative is excluded by the fact that the C-C-C-angle is about 122°. The corresponding theoretical curves are also incompatible with such an assumption. A decision between the two nonplanar arrangement just mentioned could in fact be made by comparing the curves, the »crown» model only being able to explain the details of the experimental curve. The theoretical curve D is computed on the basis of the »crown» model.

The $\frac{\sigma(r)}{r}$-curves obtained using material of different origin are in excellent agreement, a fact which seems to exclude the possibility that the materials are chemically different.

A determination of the positions of the hydrogen atoms will be very difficult. We may mention, however, that there are signs indicating a C-H bond distance smaller than that observed in benzene.

Contrary to the opinion expressed by Kaufman *et al.* we think it proved by thermochemical experiments that the resonance energy of the cyclooctatetraëne molecule must be considerable. It seems obvious also, that the structure derived from our electron diffraction work is in good agreement with conclusions drawn from Raman spectroscopical observations[3].

We wish to express our gratitude towards The British Oxygen Company for having placed at our disposal quantities of very pure cyclooctatetraëne.

1. Bastiansen, O., and Hassel, O. *Tids. Kjemi, Bergvesen Met.* **7** (1947) 55; see also *Nature* **160** (1947) 128.
2. Kaufman, H. S., Fankuchen, I., and Mark, H. *Nature* **161** (1948) 165.
3. Lippincott, E. R., and Lord, R. C. *J. Am. Chem. Soc.* **68** (1946) 1868, *J. Chem. Phys.* **16** (1948) 548.

Received April 1, 1949.

A Note of the Preparation of Terephthalic Acid

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From the time of the discovery of terephthalic acid by Hofmann [1] in 1856, to the present [2], the only practical method of obtaining this substance from *p*-cymene has been oxidation with chromic acid in sulphuric acid solution.

$$C_{10}H_{14} + 4\ H_2Cr_2O_7 \rightarrow C_6H_4(COOH)_2 + 4\ H_2O + 2\ CO_2$$

This method is both slow and inefficient. In two separate runs, using 80 g $K_2Cr_2O_7$ and 65 ml conc. H_2SO_4 in 350 ml of water for 10 ml cymene, the following yields of terephthalic acid were obtained:

	Yield after 12 h refluxing	Yield after 36 h refluxing
Run I	18.0 %	—
Run II	13.5 %	24.8 %

It can thus be seen that the yield, even after 36 hours, is far from satisfactory, to say nothing of the expenditure in time required.

This oxidizing procedure utilizes a two phase system, *i. e.*, the cymene is insoluble in the chromic acid mixture. It was believed that if a suitable solvent could be found, the reaction could be made to take place in a one phase system, and the procedure vastly improved. Such a solvent was found in acetic acid.

The following procedure was followed in the single phase oxidation of cymene:

Approximately 30 g of CrO_3 was dissolved in 40 ml of water. To this was added a mixture of 160 ml glacial acetic acid and 50 ml of conc. sulphuric acid. This oxidizing mixture was then placed in a flask equipped with a reflux condenser. Two ml cymene were dissolved in 50 ml of glacial acetic acid and this solution was slowly dropped into the heated oxidizing mixture. Uusually 25 to 30 minutes were allowed for the complete introduction of the cymene solution.

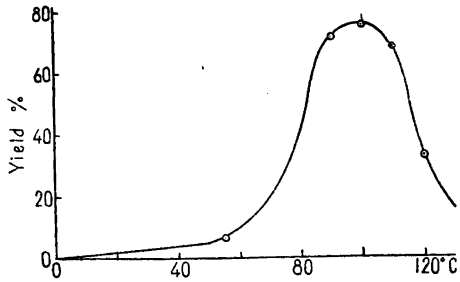

Fig. 1. Yield of terephthalic acid at various temperatures.

The excess chromic acid was then completely reduced by sulphurous acid, and the solution was shaken with ether, in which terephthalic acid is insoluble, and filtered. The precipitate was washed with hot water, dried at 105° C for one hour, and weighed.

The following results were obtained by oxidation at various temperatures:

Run	Temp. °C	Yield %	Titration equivalent
I	55	6.4	87.8
II	90	71.5	84.2
III	100	75.6	83.2
IV	110	68.5	83.2
V	120	33.0	86.0

These results, when plotted, show that the maximum yield of terephthalic acid is obtained at approximately 100° C.

In conclusion it may be stated that the single phase oxidation of *p*-cymene to terephthalic acid using acetic acid as a solvent, decreases the time required by the older method by 95 % and, at the same time, increases the yield by over 400 %.

1. Hofmann, A. W. *Ann.* **97** (1856) 206.
2. Kirjakka, P. *Suomen Kemistilehti* **A 13** (1940) 62.

Received February 11, 1949.

New Book

P. Gilard *Traité de Physico-chimie des Silicates.* »Les Etudes des Composés Siliceux», Edition—Publicité—Impression, Soc. An. Bruxelles, 1948. 307 s.

P. Gilard ist Professor für Silikatchemie an der Universität Liège.

Der Verfasser hat die Absicht eine Behandlung sämtlicher Zweige der silikatchemischen Industrie vorzunehmen. Die theoretischen Grundlagen sind im Teil I (387 S.) gegeben. Der Inhalt dieses Teils entspricht einer Erweiterung und Vertiefung seiner an der Universität gehaltenen Vorlesungen. Nach einer geochemischen Einleitung folgt die Beschreibung von physikalischen, strukturkristallographischen und chemischen Eigenschaften der Kieselsäure und der vom mineralogischen und technischem Gesichtspunkt wichtichsten Silikate. Die auch für die Praxis auf diesem Gebiete wichtigen Umsetzungen der festen Phasen erhält eine eingehende Behandlung, die in die immer noch recht knappe silikatchemische Lehrbuchslitteratur wohl zum ersten Mal zusammenfassend introduziert wird.

Nicht ganz klar scheint mir allerdings die Darstellung der Spring'schen Druckversuche, wo der Druck nach der Untersuchung von Masing keine andere Rolle gespielt hat als kontaktverbessernd so lange die Phasen im festen Zustand blieben.

Zum Schluss wird in diesem Teil auch solche Probleme, wie Diffusion, Einwirkung von anwesenden Gasen, strukturabhängige Adsorptions- oder Austauschreaktionen, die für eine tiefere Kenntnis der technischen Arbeit notwendig sind, behandelt.

Der zweite Teil dürfte als ein Lehrbuch für Glasfabrikation bezeichnet werden können. Unter dem Begriff Lehrbuch ist dabei zu verstehen, dass nicht bloss die technischen Verfahren und Glaseigenschaften beschrieben werden, sondern auch die wissenschaftlichen Grundlagen. Gerade bei einer so alten und rezeptbetonten Fabrikation ist dies sehr willkommen und für den Bedarf der modernen Technik effektiv und unvermeidlich. Der Referent hätte gern gesehen, dass die Bedeutung der Strukturuntersuchungen von Dietzel und Zachariassen noch mehr hervorgehoben wären.

Es ist sehr zu begrüssen, dass wir durch französische Literatur einen viel zu lange nicht sehr guten Kontakt verbessert bekommen. Auf diesem Gebiet ist eigentlich nichts erschienen seit Le Chareliers' La Silice et les silicates. Die Chemie in Belgien und Frankreich ist heute auch auf diesen Gebieten ausserordentlich lebhaft. Der Kongress für Silikatchemie in Bruxelles 1947 und für die Reaktionsfähigkeit des festen Zustandes in Paris 1948 haben dieses Verhältnis in effektivster Weise illustriert.

Y. Arvid Hedvall

ACTA CHEMICA SCANDINAVICA 3 (1949) 213—230

On the Rôle of Adenylpyrophosphatase in Alcoholic Fermentation and on the Occurrence of Trehalose during Fermentation with Maceration Juice *

RAGNAR NILSSON and FRITHIOF ALM

Institute of Microbiology, Agricultural College of Sweden, Uppsala, Sweden

The differences between the course of the fermentation of the sugar in the living yeast cell, on the one hand, and in zymase preparations, on the other, have for many years been the object of exhaustive studies at this Institute. By using dried preparations of bottom yeast, which have been produced so cautiously that the entire glycolytic enzyme system of the living yeast cell has remained intact, while the permeability hindrances in the cell wall have been removed, it has been possible to place the experiences from the maceration juice fermentation in relation to the fermentation conditions in the living cell.

On the basis of the investigations a comprehensive series of treatises [1—10] have been published. The two most important results of those therein reported will be dealt with in this paper.

In what way does the zymase system of the living yeast cell differ from that of the maceration juice?

The living yeast cell contains a fermentation principle, presumably a dephosphorylating enzyme, which is wanting in yeast maceration juice, and

* This paper was received by me July 21st, 1948, for publication in the *Archives of Biochemistry*. By agreement with professor Karl Myrbäck it was intended to precede a paper by Elander and Myrbäck: »Isolation of Crystalline Trehalose after Fermentation of Glucose by Maceration Juice», with which it is connected. In consequence of certain proposals from the American Editor, with which the authors, from different reasons, could not comply, the paper by Nilsson and Alm is presented here. The paper by Elander and Myrbäck will appear in the *Archives of Biochemistry*.

H. Theorell

which is essential for the rapid fermentation of the entire amount of sugar added. For a detailed discussion of this phenomenon, the reader is referred in particular to a recapitulatory lecture given in 1942 [10] *.

With the result briefly related above are connected the investigations recently published by Meyerhof [13, 14], concerning the rôle of adenylpyrophosphatase in fermentation. It is, however, incorrect, when Meyerhof considers that he is able to explain all our observations in the following way [13, p. 118]. »All observations of Nilsson are easily explained by the greater or smaller destruction of the yeast apyrase in his different preparations.» This should soon be quite clear to anyone taking the trouble to study our communications. We will first discuss Meyerhof's view of the different periods in fermentation. In the publication [13, pp. 117 and 118] already cited, he writes: »The most extensive work devoted to this problem is that of Nilsson, who, between 1936 and 1942, investigated yeast preparations which either showed the usual break when half of the sugar was fermented or did not show it (intakte Trockenhefe). In the latter case addition of cytolytic agents, dyestuffs, *etc.*, induced the appearance of this break.»

The break in the fermentation curve that is induced by the interventions in question, does not appear after half of the sugar has been fermented to alcohol and carbon dioxide, but when an amount of CO_2 has been produced which, according to Harden's equation, corresponds to the amount of free phosphate at the beginning of the fermentation [6]. What takes place between this break and the break occurring later when half of the sugar is fermented, constitutes a period in the fermentation process with which Meyerhof is unfamiliar. We will return to this later in this paper (pp. 216 and 229).

The problems that our publications have introduced into the literature dealing with alcoholic fermentation can in the main only partially be further elucidated by experiments made in fermenting mixtures of the kind used by Meyerhof and with the methods employed by him. The differences in the arrangement of the experiments are much too great. Thus, for instance in Meyerhof's experiments, the substrate/enzyme ratio is incomparably smaller than in our experiments. We have already shown [2, p. 391] what the consequences of this can be. Further, Meyerhof studies the rate of fermentation during various sections of time, whereas we follow the entire fermentation process from beginning to end.

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* The same theme was also treated in a lecture given on April 27th, 1946, at Helsinki University. Here special emphasis was laid upon certain facts affording support for the view that the new enzyme is of general significance for the normal metabolism of all living cells. Evidence in favour of this is supplied, *inter alia*, by the investigations made by Virtanen *et al.* [11, 12] into the formation of lactic acid in dried preparations of lactic acid bacteria.

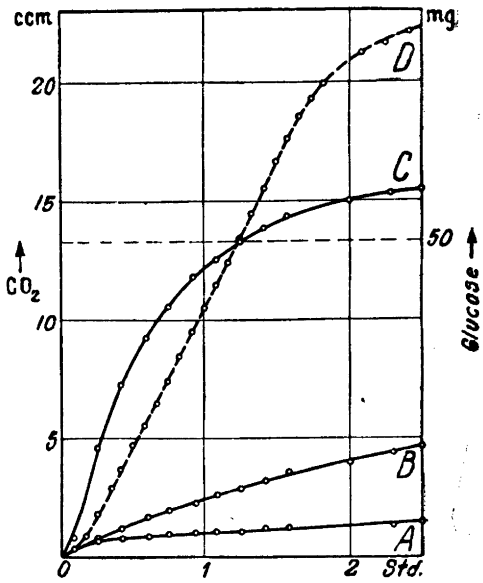


Fig. 1. Vergärung von Hexosediphosphorsäure durch intakte Trockenhefe. Gärtemperatur 30° C.

In the matter of all details we refer the reader to our publications already cited, while we in the following illustrate in a few fundamental respects the differences between Meyerhof's opinion and our own.

It can thus in the first place be pointed out that Meyerhof's experiments provide no explanation whatever of the profound influence exerted upon the fermentation by the agents studied by us, *e.g.* alcohol, toluene, bile acids, *etc.* [6, 8, 10] *.

According to Meyerhof, the slow fermentation period is to be explained exclusively by the fact that the fermentation system lacks a sufficient amount of adenylpyrophosphatase for the fermentation of the hexosediphosphate [14, p. 143]. »If potato enzyme is added to a yeast extract fermenting free sugar at a time where the speed of the phosphate period has fallen down to the low level of the second period, the rate rises again to the level of the phosphate period. *By mere addition of enough purified ATP-ase the fermentation type of yeast extract is changed into the fermentation type of living yeast.*«

One wonders then why, under the same conditions, no fermentation of the accumulated hexosediphosphate can be induced by the addition of intact dried yeast, which completely ferments glucose without the occurrence of

* Meyerhof himself, as we know, even used alcohol in preparing adenylpyrophosphatase from yeast.

any other, slower fermentation period. We reproduce in this connection the following experiment published [10, pp. 29 and 30] by us earlier.

»Dieser Versuch wurde so ausgeführt, dass zunächst in einem Macerationssaft 200 mg Glucose + die äquivalente Menge Phosphat bis zum Knick der Gärkurve vergoren wurden. Die CO_2-Entwicklung hat dann praktisch aufgehört (Kurve A), und die Hälfte des Zuckers (100 mg) liegt als Hexosediphosphorsäure vor. Jetzt wird in einem Gäransatz intakte Trockenhefe zugegeben (Kurve B). Ein zweiter Gäransatz bekommt sowohl intakte Trockenhefe als auch 100 mg Glucose (Kurve C). Ein Vergleich der Kurven B und C zeigt, wie ersichtlich, dass auch in der permeablen, intakten Trockenhefe die unveresterte Glucose mit einer ungleich grösseren Geschwindigkeit vergoren wird als die Hexosediphosphorsäure. Kurve D zeigt die Vergärung von 100 mg Glucose mit intakter Trockenhefe in einem Gäransatz ohne vorangehende Macerationssaftgärung. Die maximale Gärgeschwindigkeit ist hier etwas niedriger als in dem Gäransatz C, was sich durch den Zuschuss an Zymase erklärt, den letzterer Ansatz in Form von Macerationssaft bekommen hat.«

The investigations carried out by Meyerhof with adenylpyrophosphatase obviously offer no explanation of the phenomena here demonstrated.

To the remarkable fact that, as shown by Curve C, glucose added to this system produces only half of the calculated amount of CO_2 with intact dried yeast, we will return later (p. 229).

On the occurrence of a sugar deficit during fermentation in maceration juice and on the chemical character of this deficit

If sugar is fermented with maceration juice * in a fermenting mixture containing a smaller number of moles of phosphate than moles of hexose, two breaks occur in the fermentation curve. The first break occurs when an amount of CO_2 has been evolved that, according to Harden's equation, corresponds to the amount of free phosphate in the fermenting mixture at the onset of fermentation. The second break occurs — irrespective of the amount of phosphate — when half of the sugar has been fermented to alcohol and carbon dioxide [2, pp. 378–382]. In order to illustrate this fact we reproduce the following, previously published [2, p. 381] experiment.

»Besonders schön kommt das verschiedenartige Verhalten der intakten Trockenhefe und des Mazerationssaftes in den bei 40° ausgeführten Gärversuchen zum Vorschein.

* Or with any zymase system whatever that lacks the new fermentation principle mentioned in the foregoing.

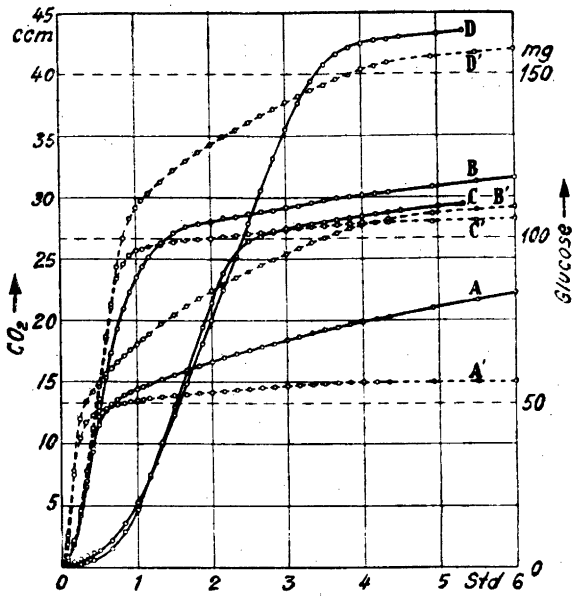


Fig. 2.

Der zu diesem Versuch benutzte Mazerationssaft enthält pro 1 ccm 14.0 mg P_2O_5 (ausschliesslich als Orthophosphat). Der Phosphatgehalt des Mazerationssaftes wird bei der Berechnung der in den Reaktionsmischungen vorhandenen Phosphatmenge berücksichtigt.

Zu jeder Gärungsprobe (Gesamtvolumen 2 ccm): 0.1 ccm Zymophosphatlösung + 1 Tropfen 2.5 %-ige Acetaldehydlösung (in den Proben C und D jedoch kein Zymophosphatzusatz).

In den Proben A, B, C und D 200 mg Trockenhefe H; in A', B', C' und D' 1 ccm Mazerationssaft.

In Probe	A : 100	mg	Glucose	+	Phosphat	äquivalent	mit	100	mg	Glucose
» »	B : 200	»	»	+	»	»	»	100	»	»
» »	C : 200	»	»	+	»	»	»	200	»	»
» »	D : 300	»	»	+	»	»	»	200	»	»
» »	A': 100	»	»	Phosphatgehalt		38.6	mg	P_2O_5		
» »	B': 200	»	»	»		38.6	»	»		
» »	C': 200	»	»	»		79.6	»	»		
» »	D': 300	»	»	»		79.6	»	»		

Reaktionstemperatur 40°.

Bei der intakten Trockenhefe tritt, wie wir schon früher gefunden haben, innerhalb weiter Grenzen unabhängig von der Phosphatkonzentration auf der Gärkurve ein Knick auf, wenn 50 % des Zuckers zu Kohlensäure vergoren sind (Kurve A, B, C und D).

Im Mazerationssaft (Kurve A', B', C', D') ist die Lage des ersten Knickes der Gärkurve durch die *Hardens*che Gleichung festgelegt (aus dem Phosphatgehalt der Reaktionsmischungen berechnet sich nach *Hardens* Gleichung die Lage des ersten Knickpunktes in den Kurven A', B', C' und D' zu 13,1 bzw. 13,1, 26,7 * und 27,0 ccm CO_2). Nach dem Eintreten dieses ersten Knickes schreitet bei Überschuss von Zucker in der Reaktionsmischung die Gärung mit herabgesetzter Geschwindigkeit fort, um noch weiter abzunehmen, etwa wenn der Punkt erreicht wird, wo der Zucker zu 50 % zu Kohlensäure vergoren ist. Ein scharf markierter Knick kann diesmal in Anbetracht der verhältnismässig niedrigen Gärgeschwindigkeit während der zweiten Gärungsphase nicht erwartet werden (Kurve B' und D').»

The phosphate in the fermenting mixture is completely esterified already at the first break and remains esterified during the second, slower fermentation period up to the second break. Nevertheless a transformation takes place during this period in the phosphoric ester (or possibly in the mixture of esters) produced up to the first break. This ester, which is rather difficultly hydrolysable, is gradually transformed into hexosediphosphate, a process that appears to be terminated when the second break is reached. Parallel with this, the content of free sugar in the fermenting mixture decreases and is practically 0 at the second break [7, pp. 66 and 67].

As we have often pointed out in earlier publications [*cf.* 7, pp. 68—70], this peculiar circumstance that we have observed in maceration juice fermentation means that the original amount of sugar in the fermenting mixture cannot, at the second break, be accounted for in the form of known products formed. After deduction of half the amount of sugar, which recurs as alcohol and carbon dioxide, and the amount of hexosediphosphate produced, there remains a sugar deficit. In a suitably chosen composition of the fermenting mixture, this deficit can constitute a very considerable part of the amount of sugar originally added.

From our investigations into the chemical character of this deficit, we have hitherto in our publications only briefly mentioned some results. Here we will confine ourselves to the following quotation [7, p. 70]: »Bei dem zweiten Knick der Gärkurve sammelt sich nämlich in der Gärmischung eine phosphor-

* Die Phosphatmenge ist in dieser Reaktionsmischung dem Zucker gegenüber etwas im Überschuss. Die Lage des Knickpunktes wird somit aus der Zuckermenge berechnet.

freie, nicht reduzierende Substanz an, die bei Erhitzung in n H_2SO_4 ein stark reduzierendes Produkt gibt. Nach Versuchen zu beurteilen, die in letzter Zeit zusammen mit M. Elander ausgeführt wurden, findet unter diesen Versuchsbedingungen ausserdem eine Kondensation vom Typus der Acyloinkondensation statt.»

It is not clear to what extent the sugar deficit observed by us can be placed in relation to the formation of a glycogen-like polysaccharide during the fermentation that has earlier been observed by Harden and Young [15] and further studied by Naganishi [16]. The second break occurring in the fermentation curve after half of the sugar has been fermented to alcohol and carbon dioxide, was unfamiliar to these authors, and Harden assumes that the phosphate is esterified in the form of hexosediphosphate already at the first break. (At that time hexosemonophosphate had not been discovered.) Naganishi finds that the polysaccharide synthesis is promoted by the addition of phosphate to the fermenting mixture. The formation of the substance studied by us is suppressed, on the other hand, by the addition of phosphate (See, for instance, Table 2, p. 222).

In order completely to elucidate the question of the chemical nature and mode of formation of the »sugar deficit», we have made comprehensive investigations, over which a very considerable experimental material is available. The problem has been attacked along two different lines. As already mentioned, it has been examined whether the deficit can be explained by means of an acyloin condensation taking place between the first and the second break in the fermentation curve. Although it was possible to establish such a continuous condensation, this was nevertheless not of such magnitude as to enable the deficit to be accounted for in this way, it seemed. The subsequent investigations were therefore entirely concentrated upon the non-phosphorylated, non-reducing substance which, as we had already found, arises during this fermentation period. As has been mentioned, on hydrolysis with N H_2SO_4 this gives rise to a strongly reducing product. It was found that the hydrolysis product is fermented in the same way as glucose. We further established that the non-hydrolysed substance has an optical rotation corresponding to the disaccharide trehalose. The quantitative conditions prevailing at its formation did not appear to us, however, to be sufficiently investigated for it to be considered fully demonstrated that the sugar deficit established by us consists of trehalose. Accordingly our work was continued with the purpose of clarifying this point completely and of ascertaining the mechanism of the formation of the trehalose. Owing to external circumstances beyond our control these fermentation investigations had to be discontinued in 1941 and the question of their continuance left for the time being. While

waiting for the time when the work could be resumed, we refrained from publishing the investigations that had already been made. From a private communication made to us by Professor K. Myrbäck we have, however, learnt that the question of the chemical nature of the sugar deficit has recently been the object of investigation in his laboratory, crystallized trehalose having been isolated from fermenting mixtures of the kind described by us in the foregoing. This causes us now to single out from our research material and to publish certain of our earlier observations with regard to this matter.

Methods. The maceration juice used in the experiments was prepared according to Nilsson and Alm [17]. For each fermentation sample was used 1 ml maceration juice with addition of 0.05 ml of a 2.5 % solution of acetaldehyde and 0.1 ml of a c. 6 % solution of sodium hexosediphosphate to suspend the induction period. Phosphate was added as a mixture of KH_2PO_4 and $Na_2HPO_4 \cdot 2\,H_2O$, pH≃6.3. The amounts of glucose and phosphate added are indicated in each particular case. The total volume of the fermentation samples was 2 ml, unless otherwise stated.

For the purpose of studying the reaction products formed during the process of fermentation, a series (exactly uniform) of fermenting mixtures in 2 ml amounts (unless otherwise stated) was mechanically shaken in a water thermostat and connected to separate gas burettes for determination of the CO_2 evolved. At different points on the fermentation curve 2 fermentation samples (with some few exceptions) were taken out each time and immediately diluted with 10 ml *aq. dest.* and placed in a boiling water bath for 2 minutes. This causes the protein to coagulate, and after filtering a perfectly clear solution is obtained.

For precipitation of inorganic phosphate and phosphoric esters, an aliquot of the clear filtrates was mixed with $BaCl_2$ in slight excess, made alkaline to phenolphthalein, and absolute ethanol added to 50 per cent of the volume of the solution. After filtration clear solutions were obtained.

Table 1. Reducing substance after Ba precipitation.

Curve	Minutes	CO_2 ml	Reducing substance, mg		Difference	Sugar deficit calc. mg
			Before hydrolysis	After hydrolysis		
	0	0	2.22	5.05	2.83	
	0	0	2.13	4.52	2.39	
A	350	1.30	3.33	6.04	2.71	
	350	1.00	3.13	5.97	2.84	
	0	0	101.7	100.1	−0.6	
	0	0	99.8	99.1	−0.7	
B	350	15.25	5.64	24.40	18.76	27.7
	350	15.15	5.54	24.40	18.86	28.1

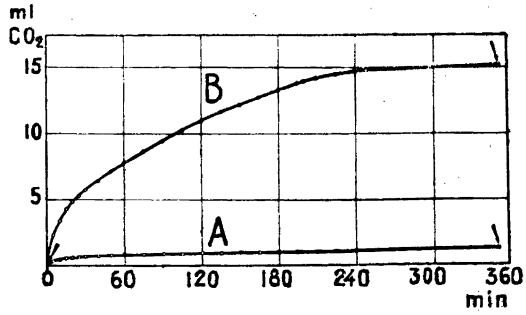


Fig. 3. Fermentation in maceration juice without addition of phosphate. Temp. 40° C.The maceration juice contains an amount of inorganic P equimolar to 30.5 mg of glucose. Curve A: Without additions. Curve B: 100 mg glucose. Analyses in Table 1. (Methods p. 220).

The orthophosphate was determined according to Embden [18], the glucose according to Schaffer-Somogyi [19]. All the reduction values were calculated as glucose and were always computed for the entire reaction mixture (2 ml, unless otherwise stated). The glucose determinations were made partly direct in the filtrate from the Ba precipitation and partly after hydrolysis of the filtrate in *N* H_2SO_4 in a boiling water bath for 3 hours.

From Fig. 3 and the accompanying Table 1, one is inclined to draw the following conclusion. At the second break in the fermentation curve a part of the sugar has been changed into a product (x), which is not itself reducing, but which yields a reducing hydrolysis product (y).

The substance in question (x) does not consist of phosphoric esters, as these were removed with Ba and alcohol before the analyses were made. In the majority of our experiments we have besides satisfied ourselves that the Ba precipitated preparations containing the substance in question (x) were free from inorganic phosphate and after 3 hours' hydrolysis in *N* H_2SO_4 (y)

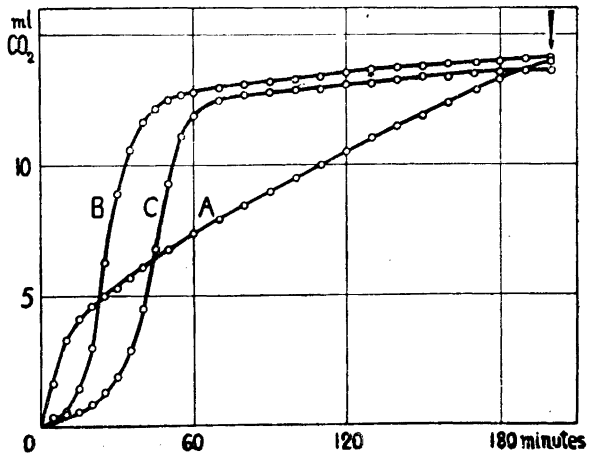


Fig. 4. Fermentation of 100 mg glucose in maceration juice with different amounts of phosphate. Temp. 40° C. The maceration juice contains an amount of inorganic P equimolar to 30.5 mg of glucose. The total amount of inorganic P is in Curves A, B, and C equimolar to 30.5, 80.5, and 130.5 mg of glucose respectively. Analyses in Table 2. (Methods p. 220).

did not give determinable amounts of phosphate. After combustion with $H_2SO_4 - H_2O_2$ it was established that the preparations contained an insignificant amount of total P, not exceeding 1 P in 6 $C_6H_{12}O_6$.

The relation between the hydrolysable substance found (x) and the sugar deficit discussed in the foregoing pages, is illustrated by experiments with varying amounts of phosphate in the fermenting mixture (Fig. 4 and Table 2).

Table 2. Reducing substance after Ba precipitation. Fermentation period 200 minutes.

Curve	CO_2 ml	Reducing substance, mg		Difference	Sugar deficit calc., mg
		Before hydrolysis	After hydrolysis		
A	13.95	3.0	20.4	17.4	32.6
	14.00	3.0	20.4	17.4	32.4
B	14.15	1.3	12.2	10.9	6.9
	14.15	1.2	11.9	10.7	6.9
C	13.55	1.2	5.4	4.2	0
	13.65	1.2	4.7	3.5	0

Even though it was not possible to establish a quantitative agreement, it is nevertheless clearly conceivable that the sugar deficit consists just of the hydrolysable substance found (x).

In order to characterize the substance formed (x), the fermentability of its hydrolysis product (y) was first studied. A larger fermenting mixture, containing per 2 ml 100 mg glucose without addition of phosphate was fermented to the end (Fig. 5, Curve A). After precipitation with Ba and alcohol, and evaporation of the alcohol from the filtrate this was hydrolysed for 3 hours in *N* H_2SO_4 and neutralized. The solution then obtained contained 2.12 mg reducing substance (y) per ml. It is termed preparation 1. The corresponding value before hydrolysis was 0.56 mg per ml. The fermentability of the reducing substance was tested with living brewer's yeast (Fig. 6).

20 ml of preparation 1 contain 42.4 mg reducing substance calculated as glucose.

It appears from Fig. 6 that the hydrolysis product (y) is fermented in exactly the same manner as glucose by living brewer's yeast.

By means of one preparation (Fig. 5, Curve B), made in the same way as in previous experiments, was obtained (after more intensive evaporation) a preparation 2 a and after hydrolysis of a part of this a hydrolysed preparation 2 b. To preparation 2 a was added Na_2SO_4 to the same sulphate con-

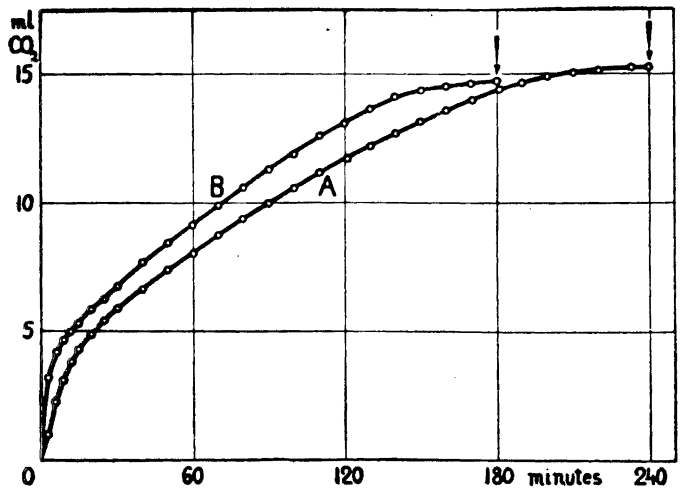
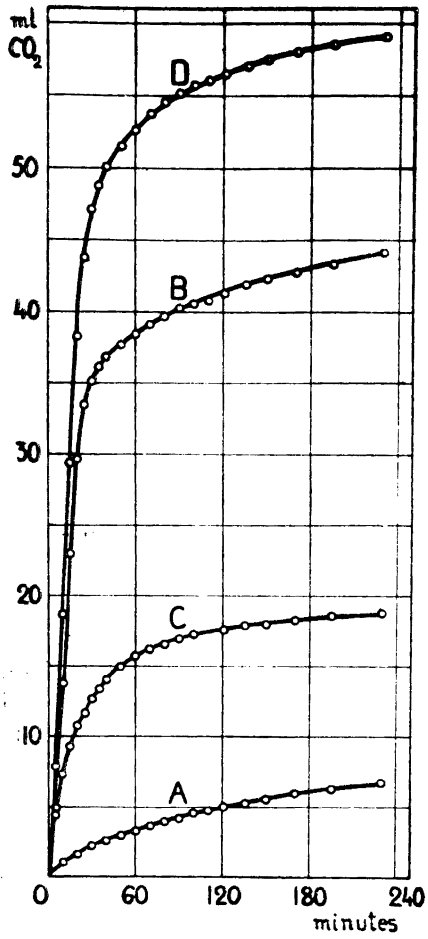


Fig. 5. Control curves of preparations 1, 2 a, and 2 b, p. 222. Temp. 40° C. (Methods p. 220).

centration as in preparation 2 b. The preparations contained 4.05 and 18.38 mg of reducing substance (y) per ml respectively. The fermentability was tested with living brewer's yeast (Fig. 7) as well as with maceration juice (Fig. 8).

The results shown in Fig. 7 confirm those obtained from the experiment just mentioned (Fig. 6) that the reducing substance (y) is fermented in the same way as glucose by living yeast. That this is also the case in fermentation with maceration juice is apparent from Fig. 8. In the latter case, as always in fermentation in maceration juice with a sufficient amount of phosphate, a break occurs when 50 % of the sugar has been converted into alcohol and CO_2.

Judging from the results obtained, the hydrolysable substance found (x) should consist of some kind of condensation product of glucose. In order to gain an idea of the molecular size of this condensation product, its dialysability through a collodion membrane was studied. The preparation used in this case was produced in the way already described. However, the fermenting mixture contained per 2 ml 150 mg of glucose as compared with 100 mg in the earlier experiments. 20 ml of the preparation (solution A) were dialysed in a collodion sack for 19 hours against 40 ml *aq. dest.* The solution outside the collodion sack is termed solution B. The dialysis was continued against running distilled water (13 l) during 24 hours. The remainder in the collodion sack after this time is termed solution C. The reducing substance was determined



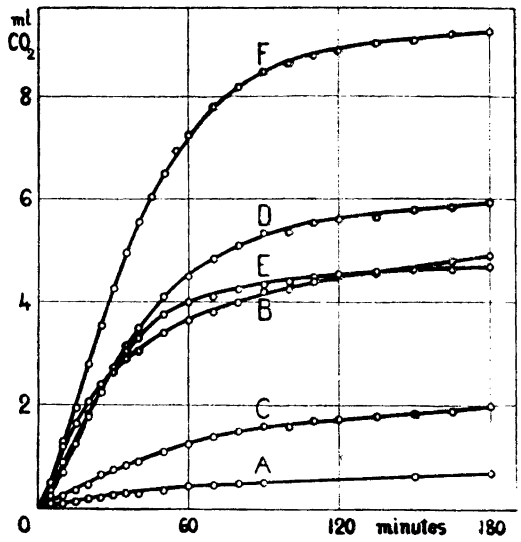


Fig. 7. Fermentation of preparations 2 a and 2 b with living brewer's yeast. Temp. 30° C. To each sample (total volume 2.6 ml) was added 1 ml of yeast suspension (40 g pressed yeast + 100 ml aq. dest.). Curve A: Without additions. Curve B: 20 mg glucose. Curve C: 1 ml of preparation 2 a. Curve D: 1 ml of preparation 2 a + 20 mg of glucose. Curve E: 1 ml of preparation 2 b. Curve F: 1 ml of preparation 2 b + 20 mg of glucose. (Methods p. 220).

Fig. 6. Fermentation of preparation 1 with living brewer's yeast. Temp. 30° C. To each sample were added 3 g of pressed yeast. Curve A: 20 ml aq. dest. Curve B: 20 ml aq. dest. + 200 mg glucose. Curve C: 20 ml preparation 1. Curve D: 20 ml preparation 1 + 200 mg glucose. (Methods p. 220).

in solutions A, B, and C partly before and partly after 3 hours' hydrolysis in N H_2SO_4. The results obtained appear in Table 3, where the total amount of reducing substance (calculated as glucose) in the entire solution is given.

It is evident from the experiment results given in Table 3 that the hydrolysable substance (x) must be low molecular.

In order further to characterize the hydrolysable substance (x), the optical rotation before and after hydrolysis was determined in a solution from the

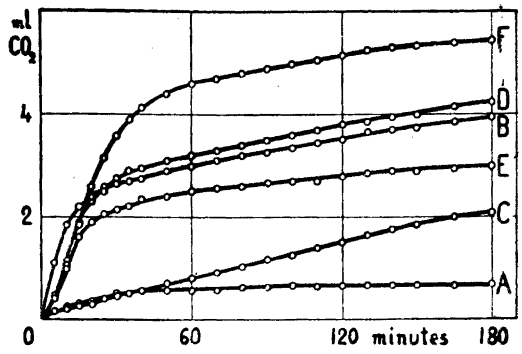


Fig. 8. Fermentation of preparations 2 a and 2 b with maceration juice. Temp. 30° C. To each sample (total volume 2.6 ml) was added 1 ml of maceration juice. Other additions as in Fig. 7. (Methods p. 220).

Table 3. Dialysis experiment.

Solution	Reducing substance, mg	
	Before hydrolysis	After hydrolysis
A	56.8	138.8
B	37.0	112.7
C	0	0

same preparation as was used in the previous experiment. Before hydrolysis the solution already contained a considerable amount of glucose. Through hydrolysis the solution was diluted in the ratio 1 : 2.44. The rotation determinations will be found in Table 4, together with the values for the hydrolysed solution multiplied by the dilution factor.

Table 4. Optical rotation of the hydrolysable substance (x) and its hydrolysis product (y).

Solution	Reducing substance, mg/ml	Optical rotation/dm
Before hydrolysis	2.74	+ 0.64°
After hydrolysis	2.23	+ 0.11°
After hydrolysis corrected with regard to the change in volume	5.44	+ 0.27°

From Table 4 $[\alpha]_D$ can be computed for the hydrolysable substance (x). Of the remaining glucose in the solution there arises an optical rotation of

$52.3° \times 2.74 \cdot 10^{-3} = 0.14°$. The residue, $0.64° - 0.14° = 0.50°$, comes from the substance that after hydrolysis gives in addition $5.44 - 2.74 = 2.70$ mg of reducing substance. The specific rotation for the hydrolysable substance (x) is, assuming it to be a disaccharide, thus: $[\alpha]_D = \frac{0.50°}{2.70} \cdot \frac{360}{342} \cdot 10^3 = 195°$.

This is in good agreement with $[\alpha]_D$ for trehalose, which, according to Schukow [20], has a specific rotation of $[\alpha]_D = + 197.1°$. The specific rotation of the substance obtained after hydrolysis, $[\alpha]_D = \frac{0.11°}{2.23} \cdot 10^3 = 49°$, agrees well with $[\alpha]_D$ for glucose.

In consequence of the results obtained, it now thus seems possible to explain the above-discussed sugar deficit on fermentation in maceration juice by means of the occurrence of a low molecular, non-reducing substance, which has the optical rotation of trehalose and which on hydrolysis yields a product with the optical rotation of glucose, which with living yeast as well as maceration juice is fermented in the same way as glucose.

That the hydrolysable substance (x) consists of trehalose cannot be seriously doubted. The completion of the investigation by the isolation of the trehalose was postponed for the time being, as we felt it to be most important first to obtain a sound basis for judging whether the sugar deficit observed can be entirely traced back to a formation of trehalose. That the trehalose found can exist preformed in the maceration juice in a free state seems to be out of the question in view of the observations made here (pp. 220 and 222). On the other hand, there might be a possibility of the maceration juice containing a trehalose phosphate, which during fermentation transfers its phosphate to the glucose added. What is of importance in this connection is that the maceration juice already on direct hydrolysis without preceding Ba precipitation gives a certain reduction value. This value is of the same magnitude as that obtained after Ba precipitation and hydrolysis at the second break of the fermentation curve when 100 mg of glucose are fermented.

The reducing substance obtained on direct hydrolysis of the maceration juice is, at least partially, fermentable. Our research material is in this respect, unfortunately, insufficient to enable us to form a definite opinion of the significance of these observations. It is, however, clear that a certain cautiousness is not out of place when it is a matter of setting the sugar deficit in relation to the trehalose formed during fermentation.

The sugar deficit in the fermentation is dependent upon the sugar/phosphate ratio at the onset of fermentation and increases if this ratio is increased. If the sugar deficit consists of trehalose, the same must be true for

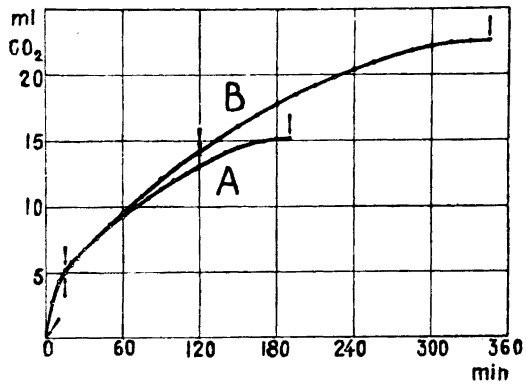


Fig. 9. Fermentation in maceration juice of varying amounts of glucose without addition of phosphate. Temp. 40° C. Curve A: 100 mg glucose. Curve B: 150 mg glucose. Analyses in Tables 5 and 6. (Methods p. 220).

the formation of trehalose during fermentation. How matters stand is illustrated by the following experiment (Fig. 9).

Table 5. Reducing substance after Ba precipitation in the fermentation of 100 mg glucose.

Minutes	CO_2 ml	Reducing substance, mg		Difference	Sugar deficit calc., mg
		Before hydrolysis	After hydrolysis		
0	0	109.5	104.3	— 5.2	
0	0	109.9	104.5	— 5.4	
15	5.55	60.7	64.6	3.9	
15	5.35	60.2	61.1	0.9	
190	15.15	5.3	28.4	23.1	28.1
190	15.10	5.3	28.5	23.2	28.3

Table 6. Reducing substance after Ba precipitation in the fermentation of 150 mg glucose.

Minutes	CO_2 ml	Reducing substance, mg		Difference	Sugar deficit calc., mg
		Before hydrolysis	After hydrolysis		
0	0	154.4	—	—	
0	0	155.1	151.3	— 3.8	
15	5.50	—	—	—	
15	5.10	108.0	99.1	— 8.9	
120	14.25	53.7	69.4	15.7	
120	14.20	55.0	70.7	15.7	
345	22.45	6.2	44.2	38.0	50.8
345	22.55	6.2	43.9	37.7	50.4

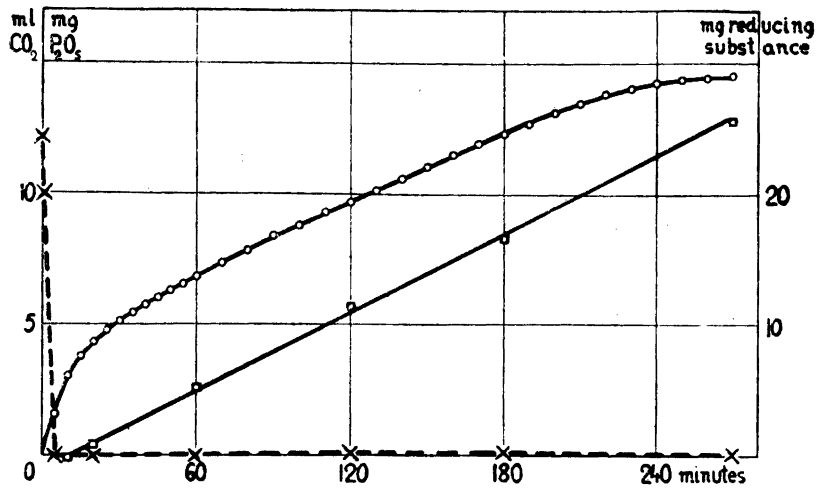


Fig. 10. Fermentation in maceration juice of 100 mg of glucose without addition of phosphate. Temp. 30° C. (Methods p. 220).

○ ——— ○ *Evolution of CO_2.*

× ——— × *Disappearance of inorganic phosphate.*

□ ——— □ *Formation of reducing substance.*

It appears from Fig. 9 and the accompanying Tables 5 and 6 that the amount of trehalose formed is augmented with increasing amounts of sugar. No quantitative agreement exists between the amount of trehalose formed and the sugar deficit calculated. The experiment nevertheless provides support for the view that the trehalose is formed from the sugar added and does not consist of trehalose that in one state or another is preformed in the maceration juice.

The formation of trehalose during the fermentation process is illustrated by Fig. 10.

As will be seen from Fig. 10, the formation of trehalose sets in only after the first break in the fermentation curve has been reached. The phosphate is then already practically completely esterified, and no splitting off of the phosphate, measurable with the analytic method employed here, takes place during the entire period of the experiment. The formation of trehalose proceeds along a practically straight line. This appears to us to remove to an essential degree the suspicion that the trehalose found might in some state be preformed in the maceration juice. If the maceration juice contained trehalose phosphate, the transphosphorylation of its phosphate to the glucose present in great excess in the fermenting mixture should not reasonably, purely from the kinetic point of view, proceed along a straight line.

Finally, we will return in this connection to the experiment reported in Fig. 1 (p. 215). As has already been pointed out (p. 216), added glucose produces with intact dried yeast only half of the calculated amount of CO_2 in the fermenting mixture there used (Curve C). Here we are dealing with an equimolar mixture of glucose and hexosediphosphate, and it is natural to assume that a transphosphorylation here takes place between the hexosediphosphate (or the triosephosphates in equilibrium with it) and the glucose added. It seems possible that the sugar deficit, which in this case amounts to half of the sugar added, consists of trehalose. The senior author has with M. Elander made preliminary experiments on the fermentation mechanism in such mixtures of sugar and esters. The present authors hope for the opportunity of resuming this investigation.

As has been mentioned above (p. 219) our earlier experiments do not sufficiently illustrate the mechanism at the earlier described production of trehalose. The following possibility should be closer investigated:

$$\text{hexosemonophosphate} + \text{glucose} \rightleftharpoons \text{trehalose} + \text{phosphate}$$

In this connection ought to be mentioned the reaction studied by Doudoroff [21]:

$$\text{glucose-1-phosphate} + \text{fructose} \rightleftharpoons \text{sucrose} + \text{phosphate}$$

With the phosphorylase used by Doudoroff could, however, no phosphorolysis of trehalose be received; nor could fructose be replaced by glucose in that system.

SUMMARY

1. Starting from our earlier investigations into the mechanism of the alcoholic fermentation in the living yeast cell, Meyerhof's researches on the rôle of adenylpyrophosphatase in fermentation are discussed. It is demonstrated that several of our observations are given no explanation through Meyerhof's investigations and cannot agree with the view that the zymase system of the living yeast cell differs from that of the maceration juice only by a higher content of adenylpyrophosphatase.

2. It is demonstrated that in fermentation in maceration juice with an excess of sugar versus phosphate a continuous formation of a low molecular, non-reducing substance takes place between the first and the second break in the fermentation curve. This substance has the optical rotation of trehalose, and on hydrolysis yields a product with the optical rotation of glucose, which with living yeast as well as with maceration juice is fermented in the

same way as glucose. It seems probable that this can provide an explanation of the »sugar deficit» established and discussed in our earlier publications, that is present at the second break. The quantitative conditions are not yet sufficiently clear, however, for it to be regarded as definitely proved that the sugar deficit is entirely covered by the trehalose formed.

3. The conditions prevailing in fermentation in mixtures of sugar and esters are discussed.

4. The mechanism of the formation of trehalose is discussed.

Our investigations have been carried out with the aid of grants from the Wenner-Gren Foundation and the Royal Swedish Academy of Sciences, for which we wish to express our sincere gratitude.

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Received January 15, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 231—246

Some Adsorption Experiments with Amino-Acids and Peptides, Especially Compounds of Tryptophan

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We describe here some model experiments carried out in the hope of devising methods for the separation of peptides containing different numbers of aromatic amino-acid residues from one another and from similar purely aliphatic compounds. Such methods would be useful for analysing the complicated mixture of peptides of leucine, valine and tryptophan encountered in partial hydrolysates of gramicidin [1], the more so because peptides containing tryptophan residues do not show very striking differences of behaviour from corresponding leucine or valine compounds in partition chromatographic procedures so far described [2, 8]. Adsorption procedures employing charcoal seemed promising for the purpose, in view of its powerful selective adsorption of tryptophan and other aromatic amino-acids (Tiselius [3, 4]). This effect has been utilized by various authors for group separations of free aromatic amino-acids (for bibliography see Martin and Synge [5], Tiselius [6], Tiselius, Drake and Hagdahl [7], Fromageot, Jutisz and Lederer [25]).

It did not seem too much to hope that, using charcoal adsorption columns, a group separation of the peptides in a partial hydrolysate might be achieved as follows: —

A. Peptides devoid of aromatic residues.

B. Peptides with one aromatic residue per molecule.

C. Peptides with two or more aromatic residues per molecule.

Such a separation procedure might have a wide field of use in the study of proteins and peptides.

At one stage an alternative possibility was suggested by Moore and Stein's [8] observation of anomalous retardation, especially of aromatic amino-acids,

on starch partition chromatograms. We found that similar effects occur on filter paper, and by developing filter paper chromatograms with water, it was possible to demonstrate retardation of tryptophan (travelling as a well-defined spot having R_F 0.6—0.7) relative to such aliphatic amino-acids as leucine and valine, which moved as spots near the water front ($R_F = 1$). If Traube's rule were obeyed, tryptophyltryptophan should be still more strongly adsorbed, and this was found to be the case, it giving a somewhat elongated streak (R_F 0.25—0.45), permitting ready differentiation and separation of tryptophan and tryptophyltryptophan in mixtures. However, it soon became apparent that the desired group separation could not be realised by simple adsorption chromatography on paper or starch, since the addition of such solvents as ethanol (which are necessary for rendering peptides of the type to be studied sufficiently soluble in aqueous media) led to marked alteration in the relative R_F values of the various tryptophan compounds (see Experimental Section). The adsorption effects on paper do not seem to be strong enough to predominate over the changes in the solubilities of the various compounds (*cf.* Cohn and Edsall[9], Tiselius[10]) and/or incipient 'partition' effects caused by the addition of ethanol. The changes of this type thus appear to be rate-determining. In this way the relative positions on paper chromatograms of tryptophan and tryptophyltryptophan are actually reversed on passing from water to 60% *v/v* aqueous ethanol as developing solvent.

With adsorption on charcoal, effects due to modifying the solvent do not dominate the adsorption effects, and the relative behaviour of the different classes of compound is not greatly altered. (Compare the separations in the Experimental Section with water and with 50% *v/v* aqueous ethanol). Accordingly we recommend the use of charcoal for group separations of this type, especially with unknown compounds. Nevertheless, for particular separations, adsorption chromatography on starch or paper using elution development by aqueous media may prove advantageous, particularly in view of the ease of elution.

The separation of two mixtures of model substances has been mainly studied: (a) leucylglycine-glycyltryptophan; (b) leucine-tryptophan-tryptophyltryptophan. Clear-cut separations could be obtained in both cases, and it seems fairly well established that the content of aromatic residues per molecule is the dominant factor, and other effects subsidiary. Thus, although insertion of a peptide bond (glycine $\longrightarrow$ glycylglycine) or lengthening of an aliphatic chain (glycine $\longrightarrow$ leucine, glycylglycine $\longrightarrow$ leucylglycine) leads to marked increases in the adsorption by charcoal (Tiselius[3, 4]) such effects are not very noticeable in the series (tryptophan $\longrightarrow$ glycyltryptophan $\longrightarrow$ leucyltryptophan). Indeed, all three of these compounds were found to have similar retention volumes in adsorption on charcoal from aqueous solution, and

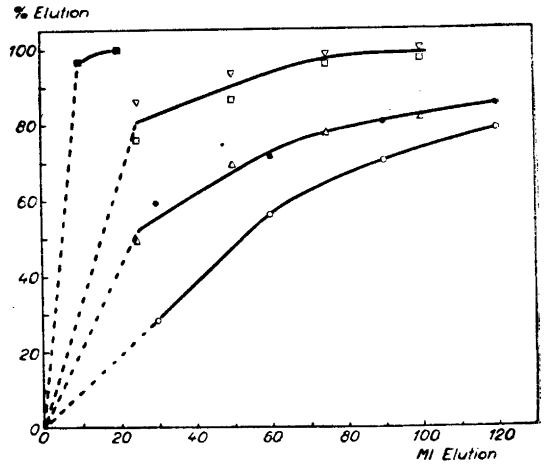

Fig. 1. Elution curves for DL-*leucylglycine from charcoal columns under various conditions (see text).*

○ *Untreated charcoal (water)*

●△ *Two batches St 2 charcoal (water)*

▽□ *Two batches St 6 charcoal (water)*

■ *St 6 charcoal (0.2 % w/v aqueous phenol)*

a mixture of the three showed no definite separation of fronts during frontal analysis, either in the presence or absence of phenol. By contrast, tryptophyltryptophan was found to be much more strongly adsorbed on charcoal than tryptophan, mixtures of the two substances giving well defined 2-step diagrams on frontal analysis, and the differences in adsorption became still more marked in the presence of phenol, which acts as a displacing-eluting agent. These observations lend strength to the view that the different components of tyrocidine recognised by Synge and Tiselius [11] (*cf.* Pedersen and Synge [12]) differ chiefly in the number of tryptophan residues per molecule. Certain eluting or displacing agents, namely stearic acid, phenol and cetylpyridinium bromide have been found of value for refining the separations obtained. It is convenient to discuss their use separately, in view of the different methods of introducing them. In many of our experiments stearic acid and phenol have been used concurrently with good results.

Stearic acid. This had been found useful as a displacing-eluting agent in experiments with tyrocidine on charcoal in ethanol solution [11]. The present experiments were done with aqueous solvents, and it was found practical to adsorb known amounts of stearic acid on to the charcoal in bulk from ethanol

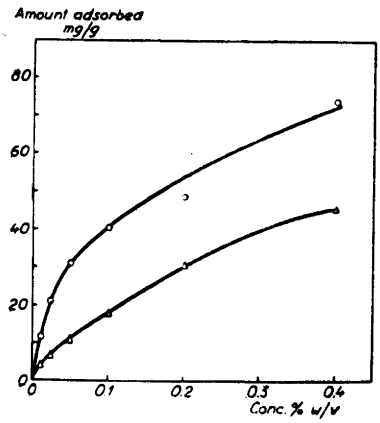

Fig. 2. Adsorption isotherms for aqueous DL-*valylglycine on charcoal before and after treatment with stearic acid.*
○ *Untreated charcoal*
△ *St 6 charcoal*

solution, and then to wash away the ethanol with water. There was no sign of elution of this adsorbed stearic acid from the charcoal except where high concentrations of ethanol and phenol were used on the chromatograms.

The charcoal treated with stearic acid showed better elution characteristics than untreated charcoal, as is apparent from the recoveries at different stages of elution development shown in the Experimental Section. This effect is reflected at least in part by a change in the form of the adsorption isotherms, illustrated by the adsorption isotherms for valylglycine on treated and untreated charcoals, (see Fig. 2). Presumably the stearic acid blocks adsorption centres at which very intense or irreversible adsorption would otherwise occur.

A further useful effect of the stearic acid treatment for the present work was apparent from a study of its effect on the retention volumes for a number of relevant compounds (see Table 2). The adsorption of the aliphatic compounds is depressed to a uniformly greater extent (40—55 %) than that of the aromatic compounds (18—27 %). The selectivity of the charcoal for the purpose in hand is thus increased, while at the same time specificity of adsorption within the two groups is not markedly affected. It seems therefore that pretreatment of charcoal with stearic acid can be recommended as a routine procedure when working with amino-acids and peptides in aqueous media (*e.g.* for such separations as those referred to immediately below). Similar effects were noted by Tiselius and Hahn [13] in adsorption separation of saccharides on charcoal pre-treated with ephedrine.

Since the present work was done, Weiss [14] has described the treatment of charcoal with fatty acids, and higher alcohols. In addition to ion exchange and 'secondary adsorption effects' (*cf.* Steenberg [15]) Weiss observed diminution of the adsorption affinity of the treated charcoal for 5-aminoacridine.

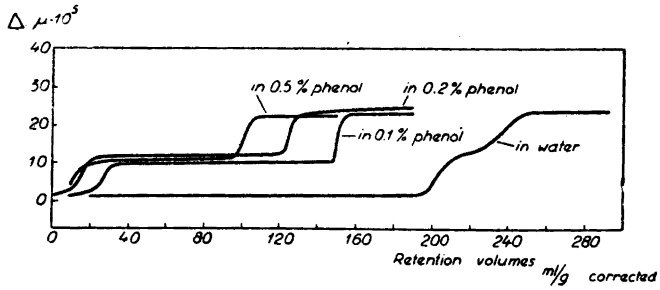

Fig. 3. Frontal analysis diagrams for 0.1 % w/v glycyl-L-tryptophan on St 6 charcoal columns in the presence of water and varying concentrations of phenol.

Phenol and cetylpyridinium bromide. Tiselius [6, 16] used phenol as a displacing agent in displacement analysis of mixtures of aliphatic peptides on charcoal. Frontal analysis experiments (see Fig. 3) on the effect of phenol on the adsorption of tryptophan showed that such low concentrations as 0.1—0.5% *w/v* had effects on the retention volumes for tryptophan, glycyltryptophan and leucyltryptophan very slight in comparison with their displacement-elution effects on purely aliphatic peptides. Similarly, higher concentrations of phenol, up to 5 % *w/v*, that had marked eluting effects on tryptophan (*cf.* Schramm and Primosigh[17], Tiselius, Drake and Hagdahl [7]) were found to have relatively slight effects in eluting tryptophyltryptophan (Table 3). Tentative evidence has been obtained that cetylpyridinium bromide (which, with similar substances, has been shown by Tiselius and Hagdahl[18] to be a useful agent for displacing tryptophan from charcoal) has very little power to elute tryptophyltryptophan from charcoal. Indeed, no satisfactory agent for this purpose has so far been found.

It is perhaps worth commenting briefly on the rationale of chromatographic development in which the solvent passing through the column is repeatedly changed or in which increasing concentrations of an eluting agent are incorporated in the solvent. This procedure (sometimes called 'flowing' chromatography) formed part of Tswett's original technique, and has been widely used for as long as chromatography has been practised. Although in some respects it is a cruder technique than the procedures of frontal analysis, displacement development and elution development (without change of solvent) it may be regarded in essence as the introduction of a displacing agent whose affinity for the adsorbent is intermediate between those of the substances which it is wished to separate. One of these is displaced, and the other is not. The development of the column would, in the absence of the displacing agent,

proceed more slowly and with considerable tailing of the first band into the second—perhaps with some displacement of the first substance by the second, but with no clear gap between them. The effect of introducing the intermediate displacing agent is to introduce a space between the two substances which it is wished to separate, filled by the displacing agent, which can then easily be separated from either substance by other means (with phenol, by volatilisation). The process is analogous to the introduction of extraneous substances to form azeotropes or as carriers in distillation of mixtures that are otherwise difficult to fractionate on account of similarity of boiling point or the small amount of material in relation to the apparatus.

The adsorption behaviour of tryptophan and its compounds on charcoal does not appear to be markedly affected by pH. In several experiments tryptophan hydrochloride was used instead of tryptophan. Cl' ions (as HCl) were found to pass through the columns without significant retardation, the retention of the tryptophan being unaffected by the presence of the acid.

We conclude that charcoal adsorption procedures suggested by the experiments described here may prove generally useful for group separations involving aromatic compounds.

EXPERIMENTAL

Technique

Amino-acid and peptide preparations. With the exception of tryptophyltryptophan (see below), the preparations studied had all been characterised, and conformed to the usual criteria of purity, including homogeneity in paper chromatograms. Optical rotations of optically active compounds fell in the accepted range. The peptide specimens were the same as those studied by Synge [19], with the addition of glycylglycylglycine (kindly given by Mr. N. W. Pirie), D-leucyl-L-tryptophan (synthesised according to H. Fischer [20]) and D-leucylglycylglycine (Hoffmann La Roche).

Preparation of tryptophyltryptophan by partial hydrolysis of tryptophan anhydride. 600 mg of L-tryptophan methyl ester (prepared according to Abderhalden and Kempe [21]) was heated overnight in a sealed evacuated tube at 180°. The crystals melted at first to a colourless liquid which gradually became more viscous and yellower. By morning the hot tube contained rosettes of crystals which subsequent work indicated to be *tryptophan anhydride.* These could be recrystallised with poor recovery from methanol or ethanol, but this did not readily eliminate impurities running slowly on *n*-butanol-H_2O paper chromatograms, and giving a positive Ehrlich colour reaction (possibly tryptophan polypeptide esters). Tryptophan anhydride gives a positive Ehrlich

reaction and runs fast under the same conditions. The slow-moving impurities were precipitated by dissolving the melt in 20 ml hot ethanol, cooling, and adding 200 ml ether. The resulting precipitate was filtered off after storing the mixture at 0° overnight. The filtrate was almost free from Ehrlich-positive impurities. It was evaporated to dryness *in vacuo*, and dissolved in 15 ml glacial acetic acid. 15 ml 10 *N* HCl was added, and the mixture was kept for 10 days at 35° in a sealed evacuated vessel. Preliminary experiments had shown that after this time little or no unchanged anhydride remained. Paper chromatography (*n*-butanol-H_2O) showed trytophyltryptophan to be the main Ehrlich-positive component of the mixture, while some free tryptophan had been formed. Some impurities giving yellow-green colours with Ehrlich's reagent, and moving more slowly than tryptophan on the chromatograms, were also present. Further hydrolysis with hot HCl caused complete hydrolysis, with disappearance of the tryptophyltryptophan and an increase in free tryptophan (for quantitative data see below).

The crude 10 day partial hydrolysate was evaporated to dryness *in vacuo*, and the syrupy, partly crystalline reddish-brown product was redissolved in 50% *v/v* aqueous acetic acid (30 ml). This solution contained 2.24 mg N/ml (Kjeldahl-Friedrich), of which 3.2 % was carboxyl-N (by ninhydrin-CO_2 reaction — see below) and 20.4 % was amino N (Van Slyke, 4 min reaction time). After hydrolysis in 5 *N* HCl for 24 h in a sealed evacuated tube at 110°, the carboxyl-N rose to 23% of the total N. Suitable quantities of the solution were evaporated to dryness *in vacuo* before being used in the adsorption experiments. The solution was assumed to contain 1.05 mg tryptophan and 10.7 mg *tryptophyltryptophan* ($C_{22}H_{22}O_3N_4$) per ml (both as the respective hydrochlorides). In the frontal analysis, a low step, due to the free tryptophan, was always observed running ahead of the main front. Other impurities were undoubtedly present, which fluoresced in ultra-violet light on the paper chromatograms, but did not give a positive Ehrlich reaction. Presumably racemisation had also occurred in the course of the preparation.

Preparation of charcoal. A single batch of charcoal ('*Carbo activ*') was used throughout this work, made from '*Carbo activatus purus siccus*' (Merck, Darmstadt) by washing, drying, mixing and storing exactly according to Claesson's [22] directions.

Conduct of charcoal adsorption experiments. Many of the adsorption runs on columns were followed with optical control in the interferometric apparatus of Tiselius and Claesson according to the general procedure described by Claesson [22]. In some experiments, particularly elution experiments, the packed filters were used without connecting them to the interferometer. In most experiments other than simple frontal analyses, whether or not the inter-

ferometer was used, the effluent fractions were subjected to further confirmatory tests or to analyses. Unless otherwise stated, volumes of solvent passing the filters are given 'corrected'. Experiments were carried out at 25°. A. R. phenol was used throughout.

Ninhydrin-CO_2 determinations were done according to Van Slyke *et al* [23], using 100 mg pH 4.7 citrate buffer and 50 mg ninhydrin in water at a total volume of 3 ml.

Ehrlich colour reaction for tryptophan and its derivatives. At least an equal volume of 0.1% *w/v* *p*-dimethylaminobenzaldehyde in 10 *N* HCl was added to the sample to be analysed (in water or acetic acid). It was found desirable to remove ethanol or phenol by prior evaporation to dryness. The resulting blue colour was allowed to develop for up to 24 h at room temperature (see also the following paragraph).

Paper chromatography. One-dimensional paper chromatography was much used throughout the work for qualitative and rough quantitative tests on the composition of effluents from the filters. The pairs of substances whose separation on charcoal was studied were to some extent selected so that they could be readily identified on paper chromatograms. The substances were commonly applied to the paper as 5 μl. portions of the 1% *w/v* solutions. The various amino-acids and peptides were detected on the paper chromatograms by spraying with ninhydrin in the usual way and also, for distinguishing or demonstrating the tryptophan derivatives, by spraying with 0.1% *w/v* *p*-dimethylaminobenzaldehyde in 10 *N* HCl. After spraying with this reagent the papers were hung up wet at 37° in a closed vessel over 10 *N* HCl for a few hours, during which time the tryptophan derivatives gave a permanent blue colour, which increased for some time after removal from the vessel and drying.

Tryptophyltryptophan coloured rather slowly under these conditions, taking several days after drying to reach its maximum intensity. This compound gave a very faint colour with ninhydrin on paper, compared with other peptides studied. Munktell OB filter paper was used throughout the present work. Semi-quantitative comparisons were made by running different known amounts of the same compound as parallel chromatograms on the same sheet of paper with the specimen it was desired to assay.

Behaviour of amino-acids and peptides on paper chromatograms in different solvents

In work with *n*-butanol-H_2O chromatograms it was found that the R_F values for tryptophan in relation to leucine and valine and for glycyltryptophan relative to leucylglycine were lower than those recorded by Consden,

Gordon and Martin [2, 24]. It is reasonable to attribute this to higher adsorptive capacity of the Munktell paper used in the present work compared with the Whatman paper used by Consden, Gordon and Martin. However, experiments with recent batches of Whatman no. 1 paper have given similar results to those recorded below for Munktell paper. Table 1 gives R_F values for representative compounds in various solvents.

Table 1. R_F values for a series of amino-acids and peptides on paper.

Authors	Consden, Gordon and Martin [2, 24]	Present work		
Paper	Whatman no. 1	Munktell OB	Munktell OB	Munktell OB
Solvent mixture	*n*-butanol-H_2O	*n*-butanol-H_2O	60 % *v/v* aqueous ethanol	water
Addition	cupron	HCN	—	—
DL-Leucine	0.38	0.40	—	1
DL-Valine	0.20	0.21	—	1
L-Tryptophan	0.35	0.27	0.5	0.6—0.7
DL-Leucylglycine	0.23	0.25	—	—
Glycyl-L-tryptophan	0.29	0.16	0.6	0.8
L-Leucyl-L-tryptophan ..	0.60	0.6—0.7	1	0.9
Tryptophyltryptophan ..	—	0.4—0.6	0.8—0.9	0.25—0.45

Some theoretical implications of the data recorded in Table 1 have been mentioned above. All the components listed except tryptophyltryptophan gave fairly compact spots. Tryptophyltryptophan under all conditions gave a more elongated spot, the R_F values given in the table defining its limits.

For routine differentiation of leucylglycine and glycyltryptophan in effluents from charcoal chromatograms, development with butanol-HCN was employed. For leucine, tryptophan and tryptophyltryptophan development was with water.

Treatment of charcoal with stearic acid and its effect on adsorption phenomena

50 g of *Carbo activ* was mixed with a solution in 200 ml ethanol of the amount of stearic acid (Kahlbaum) which it was desired to incorporate with the charcoal. After stirring at intervals for an hour, water was added gradually with stirring until the volume was 2 l. The charcoal was then allowed to settle from the clear supernatant, and was washed by decantation with ten

further changes of distilled water, after which it was filtered off and dried in a vacuum desiccator over H_2SO_4 and soda lime before storing. The resulting products looked in no way different from the untreated charcoal, and were, if anything, more readily wetted by water. Various batches were prepared containing 2% by weight of stearic acid on the weight of charcoal taken (St 2) and 6% (St 6) respectively.

Table 2 shows the retention volumes on charcoal before and after treatment with stearic acid for a number of substances in 0.1% *w/v* aqueous solution, as determined by frontal analysis. The data for untreated charcoal fall into a sequence very similar to that given by Tiselius [16] for 0.5% aqueous solutions on a different type of charcoal with different adsorption capacity. The present data extend this series to some hitherto unstudied peptides. The *diminution* of the originally very low adsorptive capacity for glycine indicates that the stearic acid treatment does not confer ion-exchanging properties on the charcoal, at least in respect of monoamino-monocarboxy-compounds (*cf.* Weiss [14], Steenberg [15]). The last column in the Table shows clearly the different extent to which aliphatic and aromatic compounds are affected by the stearic acid treatment.

The data for tryptophan and tryptophyltryptophan referring to frontal analyses as hydrochlorides are for break-through of the amino-acid or peptide fronts, demonstrated by steps in the interferometer curves and by the simultaneous appearance of a positive Ehrlich reaction in the effluent. The Cl' fronts appeared on the diagrams as low steps, almost without retardation; their nature was confirmed by testing the effluent with $AgNO_3$.

Procedure for study of the separation of leucylglycine and glycyltryptophan

A number of comparative experiments were carried out as follows: — A 250 π mm^3 filter was packed with charcoal (0.36—0.39 g) and washed thoroughly with water. Exactly 3.0 ml of an aqueous solution containing 11.7 mg DL-leucylglycine and 9.45 mg glycyl-L-tryptophan was then pressed into the filter from a micro-burette. The micro-burette was then replaced with a larger burette, supplying distilled water, with which development was continued. The effluent was collected in successive fractions of known volume (measured from the beginning of pressing in) which were analysed for N by the Kjeldahl procedure, Ehrlich tests having shown that at no stage had any glycyltryptophan emerged from the filter during the development with water. A check on the completeness of elution of the leucylglycine, revealed by the cumulative Kjeldahl figures (see Fig. 1) was afforded by substituting

Table 2. Retention volumes (ml/g) for 0.1 % w/v aqueous solutions of amino-acids and peptides on charcoal before and after treatment with stearic acid.

Charcoal	Carbo activ	St 2	% of retention vol. with untreated charcoal	St 6	% of retention vol. with untreated charcoal
Aliphatic, etc. amino-acids					
Glycine	0.9	0.6	67	—	—
DL-Alanine	1.1	—	—	—	—
DL-Valine	9.3	—	—	—	—
DL-Leucine	27	19.9	74	13.7	51
L-Proline	5.4	—	—	—	—
Aliphatic peptides					
Glycylglycine	5.3	—	—	3.2	60
Glycylglycylglycine	30	23	77	14	47
Glycyl-DL-alanine	7.4	—	—	—	—
DL-Alanylglycine	8.9	—	—	—	—
Glycyl-DL-valine	25	—	—	12.6	50
DL-Valylglycine	40	—	—	18	45
Glycyl-DL-leucine	60	—	—	32	53
DL-Leucylglycine	68	51.5	76	37	54
D-Leucylglycylglycine	96	—	—	—	—
Aromatic compounds					
DL-Phenylalanine	155	—	—	—	—
L-Tryptophan	235	177	75	171	73
do (as HCl)	—	—	—	187	—
Glycyl-L-tryptophan	300	246	82	222	74
D-Leucyl-L-tryptophan	258	—	—	212	82
Tryptophyltryptophan (as HCl)	—	—	—	290	—
Phenol	172	—	—	137	80

5% *w/v* aqueous phenol for the eluting water (*cf.* Tiselius, Drake and Hagdahl[7]). 30 ml of this eluted any remaining leucylglycine from the filter as well as 60—70% of the glycyltryptophan. The 5% phenol eluate was evaporated to dryness *in vacuo* and the amount of leucylglycine in it was determined semi-quantitatively by paper chromatography with *n*-butanol-H_2O (see above). The glycyltryptophan was recovered undegraded and unchanged as far as could be judged from the paper chromatograms, on which it always gave a simple spot.

Elution behaviour of charcoal before and after treatment with stearic acid

The cumulative water elution curves for DL-leucylglycine according to the above procedure are plotted in Fig. 1 for charcoal of 5 different batches. The marked effect of treatment with stearic acid in improving the elution is manifest. The paper chromatograms on the phenol eluate, made after elution of St 6 columns with 90 ml of water indicated that as little as 0.5% of the leucylglycine used had remained uneluted.

Adsorption isotherms for charcoal before and after treatment with stearic acid

Adsorption isotherms were constructed from retention volume (frontal analysis) data obtained for a range of concentrations of DL-valylglycine in water both with untreated charcoal and with St 6 charcoal (*cf.* Claesson [22]). The data obtained are shown graphically in Fig. 2.

Retention volumes of tryptophan compounds: effects of phenol and ethanol

Retention volumes were ascertained for 0.1% *w/v* aqueous solutions of tryptophan and its compounds by frontal analysis on St 6 charcoal in the presence of varying concentrations of phenol. The procedure was modified as follows to deal with the presence of a second solute. The filter, packed dry with a known weight of charcoal, was washed with at least 3 times as much of the aqueous phenol solution as would be required to saturate the charcoal (see Table 1). A 0.1% *w/v* solution of the amino-acid or peptide to be studied (dissolved in the same phenol solution) was supplied from a burette, and frontal analysis with optical control was commenced in the usual way, except that the reference channel of the interferometer was filled with the appropriate aqueous phenol solution instead of pure water. Fig. 3 shows a family of curves obtained in this way for glycyl-L-tryptophan in the presence of different concentrations of phenol. The early step on each curve must represent a rise in concentration of phenol owing to its displacement by the glycyltryptophan. The Ehrlich reaction only became positive in the effluent with the second step in each curve. It seems from the curves that in each case the amount of phenol displaced is directly proportional to the amount of peptide adsorbed. The retention volumes, given by the position of the appropriate steps, are shown in Table 3. The figures show a marked difference between the behav-

iour of tryptophyltryptophan and the other tryptophan compounds containing only one tryptophan residue per molecule. These data served as an indication for the use of rather high concentrations of phenol (5% *w/v*) for separating tryptophan from tryptophyltryptophan (see below).

Table 3. Retention volumes for 0.1% w/v solutions of tryptophan compounds on St 6 charcoal in various aqueous phenol solutions (ml/g).

(The figures in brackets give the retention volume in a given phenol solution as a percentage of that in pure water).

Concn. of phenol in solvent (% *w/v*)	0	0.1	0.2	0.5	1.0%
L-Tryptophan	171	—	—	82 (48%)	—
do (as HCl)	187	—	—	103 (55%)	77(41%)
Glycyl-L-tryptophan	222	149 (67%)	124 (56%)	100 (45%)	—
D-Leucyl-L-tryptophan .	212	—	—	126 (59%)	—
Tryptophyltryptophan .. (as HCl)	290	—	—	250 (86%)	220 (76%)

Frontal analysis of mixed solutions of tryptophan, glycyltryptophan and leucyltryptophan (each 0.03% *w/v*) on St 6 charcoal both in water and in 0.5% *w/v* aqueous phenol gave no well-defined steps, nor did paper chromatography of the effluent fractions (*n*-butanol-H_2O with HCN) indicate any marked separation of the three compounds. On the other hand, frontal analysis of a mixture of tryptophan and tryptophyltryptophan (each 0.1% *w/v*) as hydrochlorides in water gave two well-defined steps (192 and 250 ml per g charcoal respectively). Paper chromatography (water) of the effluent fractions indicated clearly that the first step corresponded to tryptophan and the second to tryptophyltryptophan. When the same experiment was done, without interferometric control, in 50% *v/v* aqueous ethanol, paper chromatographic tests on the effluent indicated a retention volume for tryptophan of approx. 57 ml per g charcoal and for tryptophyltryptophan in the range 190—360 ml per g.

Phenol in the separation of leucylglycine and glycyltryptophan in aqueous solution

The separation procedure described above for these compounds was carried out on St 6 charcoal (previously saturated with 0.2% *w/v* aqueous phenol), 0.2% *w/v* aqueous phenol being used as solvent for applying the peptide

mixture and for the development. The elution data (Kjeldahl N. — see Fig. 1), showed that 99.7% of the leucylglycine had emerged in the first 20 ml of effluent. Development with this solvent was continued up to 50 ml. No leucylglycine whatsoever could be detected contaminating the glycyltryptophan in the further effluent obtained by elution with 30 ml 5% *w/v* aqueous phenol.

Phenol and cetylpyridinium bromide in the separation of leucine, tryptophan and tryptophyltryptophan in aqueous ethanol

The retention volume data for tryptophan and tryptophyltryptophan in 50% *w/v* aqueous ethanol given above suggested that the substitution of this mixture for water should, in elution development, favour the separation of tryptophan from tryptophyltryptophan. However, the marked lowering effect of the ethanol on the retention volume of tryptophan suggested that the separation of aliphatic compounds from tryptophan etc. might be impaired. This was found to be the case in the elution experiment described below, and accordingly we recommend that ethanol concentrations should be kept as low as solubility factors permit, at least during the stage of separating purely aliphatic peptides from aromatic ones. Conditions may thus be approximated to those described in the preceding paragraph for the separation of leucylglycine and glycyltryptophan.

A 500 π mm^3 filter was packed dry with St 6 charcoal (0.711 g) and was washed with 100 ml 0.1% *w/v* solution of phenol in 50% *v/v* aqueous ethanol. 15 mg each of D-leucine, L-tryptophan and tryptophyltryptophan (all as hydrochlorides) was then introduced by pressing in 3 ml of a solution of the mixture in the same phenol-ethanol-water mixture. Development was continued with this same solvent mixture. Successive portions of effluent (measured from the beginning of pressing in) were collected. Tryptophan broke through in the fraction 6—9 ml and Kjeldahl determinations showed that about 90% of the leucine had previously emerged from the column. When altogether 45 ml of the eluting mixture had passed, about 50% of the tryptophan had been eluted. Elution was continued with 75 ml of 5% *w/v* phenol in 50 % *v/v* aqueous ethanol. This eluted approximately a further 15 % of the tryptophan and a certain amount of stearic acid. Paper chromatograms (water) on this last eluted material showed that tryptophyltryptophan (and of course leucine) were completely absent from it.

In view of the successful results of Tiselius and Hagdahl[18] in eluting tryptophan from charcoal with cationic detergents, the same experiment was

repeated using as eluting agent 0.5% *v/v* 'Fixanol C' (a preparation of cetylpyridinium bromide kindly given by Imperial Chemical Industries Ltd., Manchester) in 50% *v/v* aqueous ethanol. This had the expected displacing-eluting effect on tryptophan, but no indication was obtained of the elution of tryptophyltryptophan even after the passage of 150 ml of the eluting solution through a filter previously saturated with a solution of 'Fixanol C' at the same concentration before adsorbing the mixture for analysis.

The presence of cetylpyridinium bromide in the eluates interfered with the recognition of tryptophyltryptophan by paper chromatography. However, control experiments showed that 'Fixanol C' did not interfere with the ninhydrin-CO_2 reaction of tryptophan and did not prevent the determination of tryptophyltryptophan by hydrolysing it with hot 6 *N* HCl in a sealed evacuated tube, evaporating the product to dryness, and carrying out ninhydrin-CO_2 determinations on the residue. A large excess of 'Fixanol C' was present throughout all these operations. In none of the eluate fractions was there noted an increase in the ninhydrin-CO_2 figure after applying the acid hydrolysis.

SUMMARY

Experiments are described which indicate that adsorption analysis on charcoal in aqueous solution can be used for the group separation of aliphatic from aromatic peptides, and for the further separation of the latter according to the number of aromatic amino-acid residues incorporated in a molecule. Separations of this kind should prove useful in analysing mixtures such as partial hydrolysates of gramicidin. Effects of stearic acid, phenol, ethanol, cetylpyridinium bromide and hydrochloric acid on the adsorption phenomena have been studied and in some cases utilised for improving the separations.

Some new data are presented on the relative roles of partition and adsorption phenomena in chromatography on filter paper.

We are grateful to Mr. S. Gerstedt and Miss Eileen Fallows for their technical assistance.

Part of the expenses for this investigation were defrayed by grants from the Swedish Natural Science Research Council.

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Received February 5, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 247—255

Antihistamine Agents

IV. Piperidino- and Morpholinoalkyl Derivatives of Phenothiazine

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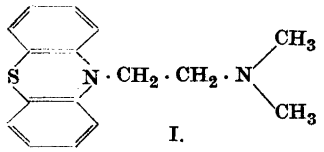
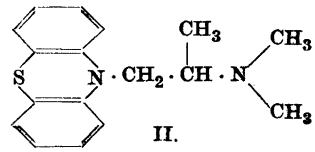
Dimethylaminoalkyl derivatives of phenothiazine were reported by Halpern and Ducrot [1] to possess excellent antihistaminic activity. Thus 10-(β-dimethylaminoethyl)-phenothiazine (I) was found to protect guinea pigs against 400 lethal doses of histamine and 10-(β-dimethylaminopropyl)-phenothiazine (II) protected against 1500 lethal doses, whereas Neo-Antergan, the most potent antihistamine agent known before, protected against only 80 lethal doses under similar conditions. Recently the synthesis of a series of 10-[β-(N-pyrrolidyl)-alkyl]-phenothiazines has been described [2]. One of these compounds, 10-[β-(N-pyrrolidyl)-ethyl]-phenothiazine (III), exerted a very strong antihistaminic activity [3].



I.



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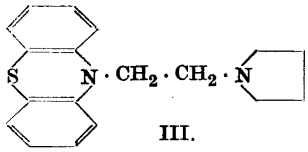
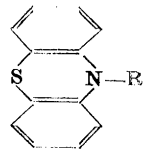


III.

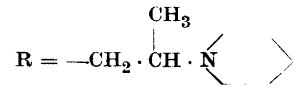
In connection with earlier works on synthetic antihistamine agents in this laboratory some piperidino- and morpholinoalkyl derivatives of phenothiazine have been prepared. These compounds (IV—VII) were obtained by condensing the hydrochlorides of β-(N-piperidino)-ethyl chloride, β-(N-morpholino)-ethyl chloride, α-(N-piperidino)-β-chloropropane and α-(N-morpholino)-β-

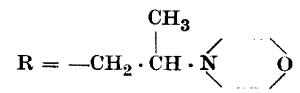
chloropropane with phenothiazine in the presence of sodium amide. In order to liberate the aminoalkyl chloride from its hydrochloride two equivalents of the condensing agent was used.



IV. R = $-CH_2 \cdot CH_2 \cdot N$ 

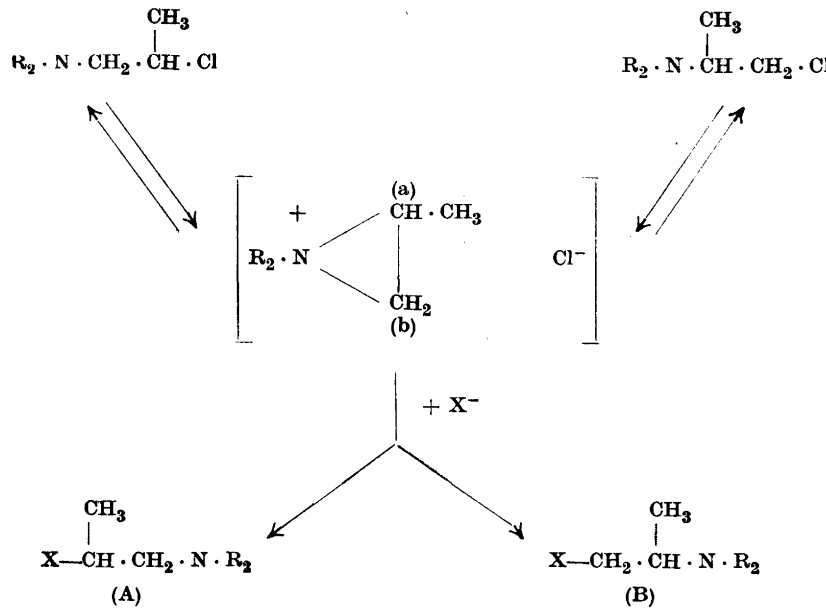
V. R = $-CH_2 \cdot CH_2 \cdot N$ 

VI. R = $-CH_2 \cdot CH(CH_3) \cdot N$ 

VII. R = $-CH_2 \cdot CH(CH_3) \cdot N$ 

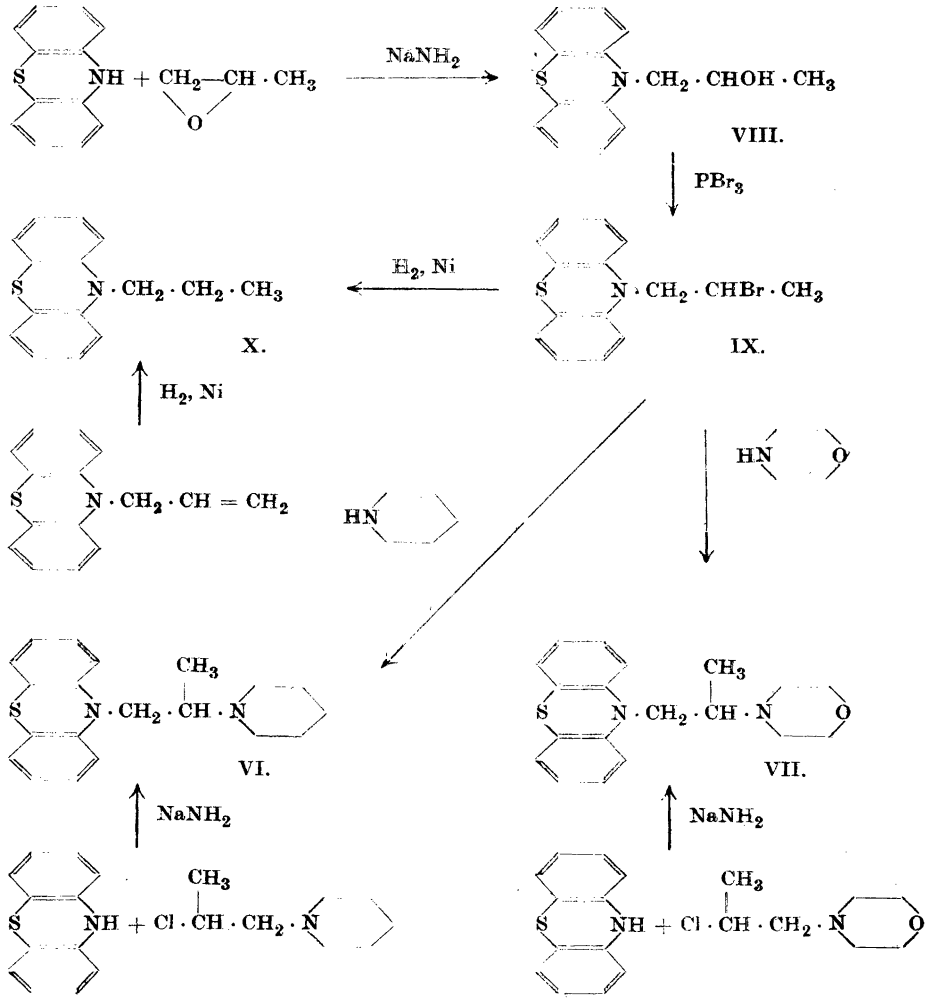
Pharmacological data concerning these compounds will be published elsewhere.

It is known that in the use of α-alkylamino-β-chloropropanes as alkylating agents a rearrangement takes place, the reaction product being a mixture of the expected product (A) and the rearranged one (B) [4–6]. The same result is obtained in alkylations with the isomeric α-chloro-β-alkylaminopropanes [7, 8].



It has been proposed [4, 5, 9] and shown [10] that under the influence of alkaline condensing agents the alkylaminochloropropanes give rise to a cyclic imonium ion, which then reacts with an anion X^- to yield two isomeric compounds (A and B), depending on whether the reagent X^- attacks the ethylene imonium ion at (a) or (b). Since the position (b) is least substituted, it is probable that it will be the favored point of attack, the main product thus having the structure (B).

In the reaction between phenothiazine and alkylaminochloropropanes, however, only one product seems to have been isolated. Charpentier [8] showed that α-dimethylamino-β-chloropropane and α-chloro-β-dimethylaminopropane



yielded the same compound and he was able to prove, that it had the structure represented by (B) above. The American investigators [2] found only one isomer too in the reaction between phenothiazine and α-(N-pyrrolidyl)-β-chloropropane. They gave it the structure (B) without proof.

In the present investigation only one reaction product could be isolated in the reaction between phenothiazine and α-(N-piperidino)-β-chloropropane or α-(N-morpholino)-β-chloropropane. It could be shown that rearrangements had occurred in both reactions and that the compounds obtained possessed the structure VI and VII, respectively. This was performed by preparing these two compounds in a quite different way. Phenothiazine reacted smoothly with propylene oxide in the presence of sodium amide, giving 10-(β-hydroxypropyl)-phenothiazine (VIII). On treating this compound with phosphorus tribromide 10-(β-bromopropyl)-phenothiazine (IX) was formed. The structure of VIII and IX was established by hydrogenation of IX, which yielded a compound identical with 10-propylphenothiazine (X) prepared from 10-allylphenothiazine. On treating 10-(β-bromopropyl)-phenothiazine with piperidine and morpholine, respectively, two compounds were obtained (VI and VII), which must be 10-[β-(N-piperidino)-propyl]- and 10-[β-(N-morpholino)-propyl]-phenothiazine.

These two amines proved to be completely identical with the products obtained on treating phenothiazine with α-(N-piperidino)-β-chloropropane and α-(N-morpholino)-β-chloropropane.

EXPERIMENTAL

10-[β-(N-Piperidino)-ethyl]-phenothiazine

To a mechanically stirred suspension of sodium amide in toluene (100 ml), prepared from sodium (4.6 g) and liquid ammonia according to Vaughn, Vogt, and Nieuwland [11], was added phenothiazine (19.9 g) in toluene (50 ml). The mixture was refluxed for two hours, and β-(N-piperidino)-ethyl chloride hydrochloride [12] (18.4 g) was added. After stirring for fifteen hours at the refluxing temperature the mixture was cooled and the inorganic salts filtered off. To the toluene solution was added a saturated solution of oxalic acid in ether, until no more precipitate was formed. The crude, crystalline oxalate (32.2 g) was crystallised twice from 50 % ethanol. M. p. 198—199° with decomposition.

$C_{19}H_{22}N_2S \cdot (COOH)_2$ (400.5)	Calc.	C 63.0	H 6.0
	Found	» 63.5	» 6.1

The base was obtained by suspending the oxalate in water and adding N sodium hydroxide. This yielded an oil, which was extracted with ether. The ether was dried over calcium chloride and distilled off. The residue distilled at 0.25 mm at 240° in the

bath giving a colourless oil, which crystallised in a couple of days. M. p. 39—40°. After recrystallisation from light petroleum the base melted at 43—44°.

$C_{19}H_{22}N_2S$ (310.5)	Calc.	C 73.5	H	7.1	N	9.0
	Found	» 73.4	»	7.05	»	9.0

The hydrochloride was prepared by adding a solution of hydrogen chloride in ether to an ether solution of the base. M. p. 165—166° after recrystallisation twice from acetone.

$C_{19}H_{22}N_2S \cdot HCl$ (346.9)	Calc.	C 65.8	H	6.7	Cl	10.2
	Found	» 65.2	»	6.7	»	10.2

10-[β-(N-Morpholino)-ethyl]-phenothiazine

This compound was prepared from phenothiazine (26.8 g) and β-(N-morpholino)-ethyl chloride hydrochloride [13] (24.8 g) in the same way as described above. The compound was isolated from the filtered reaction mixture by adding oxalic acid in ether. The oxalate (27.5 g) was recrystallised twice from acetone. M. p. 194—195° with decomposition.

$C_{18}H_{20}N_2OS \cdot (COOH)_2$ (402.5)	Calc.	C 59.7	H	5.5
	Found	» 59.8	»	5.4

The base obtained from the oxalate distilled at a bath temperature of 225° at 0.25 mm. It soon hardened into white crystals with the m. p. 70—71°. Crystallisation from light petrol raised the m. p. to 74—74.5°. This compound has also been prepared by Gilman and Shirley [14] by treating 10-(β-chloroethyl)-phenothiazine with morpholine. They obtained the m. p. 74.5—75.5°.

$C_{18}H_{20}N_2OS$ (312.4)	Calc.	C 69.2	H	6.45	N	9.0
	Found	» 69.2	»	6.4	»	9.0

The hydrochloride could be prepared in the usual manner. It was however very hygroscopic and was difficult to obtain in a pure state.

10-(β-Hydroxypropyl)-phenothiazine

To a stirred suspension of sodium amide in toluene (150 ml), prepared from sodium (6.9 g), phenothiazine (60 g) was added. The mixture was refluxed for ten hours. After cooling to 60° propylene oxide (26.1 g) dissolved in toluene (150 ml) was added in portions. The mixture was then refluxed for further two hours, cooled to room temperature, filtered, and washed twice with water (50 ml). The solvent was then evaporated and the residue was distilled in vacuum. B. p. 192—196°/0.3—0.5 mm. The distillate (53.4 g) consisted of a colourless almost glassy mass.

$C_{15}H_{15}NOS$ (257.3)	Calc.	C 70.0	H	5.9
	Found	» 70.0	»	5.9

10-(β-Bromopropyl)-phenothiazine

A mixture of 10-(β-hydroxypropyl)-phenothiazine (10 g), phosphorus tribromide (20 g), and chloroform (20 ml) was refluxed for one hour. After cooling the solution was shaken with sodium bisulfite solution, dried over calcium chloride and evaporated to dryness. The crystalline residue (12.2 g) was recrystallised twice from ethanol. M. p. 125—126°.

$C_{15}H_{14}BrNS$ (320.3)	Calc.	Br 25.0	N 4.4
	Found	» 25.4	» 4.3

10-Propylphenothiazine

A. 10-Allylphenothiazine [14] (5.0 g), dissolved in ethanol (50 ml), was hydrogenated at room temperature and normal pressure with Raney nickel as a catalyst. The calculated amount of hydrogen was added in twenty minutes. The solution was filtered and the solvent evaporated. The oily residue distilled at 162—165°/0.02 mm, yielding an almost colourless oil (4.1 g), which solidified in two weeks. Recrystallisation from ethanol yielded white crystals melting at 49.5—50°.

$C_{15}H_{15}NS$ (241.3)	Calc.	C 74.6	H 6.3
	Found	» 74.6	» 6.3

B. 10-(β-Bromopropyl)-phenothiazine (1.1 g) and potassium hydroxide (0.2 g) were dissolved in ethanol (50 ml) and shaken with hydrogen at ordinary temperature and pressure in presence of Raney nickel. After twenty minutes the calculated amount of hydrogen was consumed. The solution was filtered and concentrated in vacuum to about 5 ml. On cooling, white needles (0.6 g) melting at 49.5—50° separated. A mixed melting point with the 10-propylphenothiazine prepared from 10-allylphenothiazine showed no depression.

10-[β-(N-Piperidino)-propyl]-phenothiazine

A. In a manner identical with that described above phenothiazine (24.5 g), α-(N-piperidino)-β-chloropropane hydrochloride [15], and two equivalents of sodium amide in toluene (100 ml) were refluxed for fourteen hours. The reaction mixture was filtered and extracted with 2.5 N hydrochloric acid (100 ml). A thick, brown oil separated at the bottom of the separatory funnel. The oil and the water layer were combined and extracted with ether to remove ether soluble material. The mixture was then made alkaline with diluted sodium hydroxide and the resulting oil extracted with ether. The ether layer was dried over calcium chloride, the solvent was distilled off and the residue distilled in vacuum. B. p. 190—200°/0.3—0.4 mm. Yield 11.2 g of a colourless, very viscous oil. On treatment with light petroleum the oil solidified, giving crystals (6.1 g) melting at 98—102°. After four recrystallisations from light petroleum: acetone m. p. 119—120°.

$C_{20}H_{24}N_2S$ (324.5)	Calc.	C 74.0	H 7.45	N 8.6
	Found	» 73.6	» 7.4	» 8.4

The great loss of material in treating the oily base with light petroleum and the difficulty of obtaining a constant m. p. suggested the presence of the isomeric reaction product. No attempts were made at the present to isolate any remaining isomer.

From the base the hydrochloride was obtained in the usual way. M. p. 256—257° after recrystallisation from ethanol.

$C_{20}H_{24}N_2S \cdot HCl$ (360.9)	Calc.	C 66.55	H 7.0	Cl 9.8
	Found	» 65.6	» 7.0	» 9.8

B. Crude 10-(β-bromopropyl)-phenothiazine (6.1 g) and piperidine (10 g) were dissolved in benzene (25 ml). A little copper powder was added and the mixture was heated in a sealed glass vessel at 100° for 48 hours. After cooling the separated piperidine hydrobromide was filtered off and the filtrate washed thoroughly with water. The benzene solution was dried over calcium chloride, and oxalic acid in ether was added until no more precipitate was formed. The crude oxalate (4.0 g) was suspended in water and the base liberated with N sodium hydroxide. The oily base was extracted with ether and an ethereal solution of hydrogen chloride added. The hydrochloride obtained in this way melted at 256°—257° after crystallisation from ethanol. Mixed m. p. with the hydrochloride prepared according to (A) showed no depression.

The base obtained from the hydrochloride melted at 120—121° after recrystallisation from light petroleum: acetone. Mixed m. p. with base from (A) 119—120°.

For further comparison the picrate was prepared. After crystallisation from ethanol m. p. 172.5—173° with decomposition.

$C_{20}H_{24}N_2S \cdot C_6H_3N_3O_7$	Calc.	C 56.4	H. 4.9	
	Found	» 56.6	» 5.1	

The picrate obtained from the base from (A) melted at 172.5—173° with decomposition. Mixed m. p. 172.5—173° with dec.

10-[β-(N-Morpholino)-propyl]-phenothiazine

A. α-(N-Morpholino)-β-chloropropane hydrochloride, which was to be used as starting material, did not seem to have been reported in the literature. It was prepared in the following way.

Morpholine (60.0 g) and propylene chlorohydrine (31.6 g) were heated on the water bath for three hours. After cooling, the mixture was poured out into water and made strongly alkaline with 40 % sodium hydroxide. The amino alcohol was extracted with chloroform, the solvent was evaporated and the residue distilled in vacuum. The pure *α-(N-morpholino)-β-hydroxypropane* (36.0 g) boiled at 99—101°/16 mm.

$C_7H_{15}NO_2$ (145.2)	Calc.	C 57.9	H 10.4
	Found	» 58.2	» 10.2

α-(N-Morpholino)-β-hydroxypropane (35.0 g) was dissolved in chloroform (25 ml) and thionyl chloride (25 ml) in chloroform (25 ml) was cautiously added. The mixture

was then refluxed for an hour. On cooling, *α-(N-morpholino)-β-chloropropane hydrochloride* (37.0 g) separated. M. p. 177—177.5° after recrystallisation from acetone.

$C_7H_{14}ClNO \cdot HCl$ (200.1)	Calc.	C 42.0	H 7.6	Cl 35.4
	Found	» 42.2	» 7.5	» 34.8

The reaction between phenothiazine (19.9 g) and α-(N-morpholino)-β-chloropropane hydrochloride (19.8 g) was carried out in the usual way. The base was isolated from the reaction mixture by means of oxalic acid. The crude oxalate (24.2 g) was recrystallised twice from acetone. M. p. 195—196° with decomposition.

$C_{19}H_{22}N_2OS \cdot (COOH)_2$ (416.5)	Calc.	C 60.55	H 5.8
	Found	» 60.7	» 6.0

The base prepared from the oxalate distilled at 0.2—0.3 mm at a bath temperature of 240° giving a light yellow very viscous oil. By dissolving the material in hot light petroleum and cooling the solution, white crystals melting at 92—93° were obtained.

$C_{19}H_{22}N_2OS$ (326.5)	Calc.	C 69.9	H 6.8	N 8.6
	Found	» 70.3	» 6.9	» 8.6

The hydrochloride prepared in the usual manner melted at 250—252° after repeated recrystallisations from acetone: light petroleum.

$C_{19}H_{22}N_2OS \cdot HCl$ (362.9)	Calc.	C 62.9	H 6.4
	Found	» 62.4	» 6.4

B. The reaction between 10-(β-bromopropyl)-phenothiazine (6.1 g) and morpholine (10 g) was carried out as described for the piperidino compound. The crude oxalate (2.5 g) was recrystallised from acetone. M. p. 194.5—196° with decomposition. No depression was obtained with oxalate from (A). The base prepared from the oxalate melted at 91.5—92° after recrystallisation from light petroleum. Mixed m. p. with base from (A) 91.5—92.5°.

SUMMARY

Four piperidino- and morpholinoalkyl derivatives of phenothiazine have been prepared.

It has been shown that α-(N-piperidino)-β-chloropropane and α-(N-morpholino)-β-chloropropane undergo rearrangements similar to that observed in other α-alkylamino-β-chloropropanes.

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Received March 9, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1948) 256—258

Monosubstitution Derivatives of Cadalene. II *

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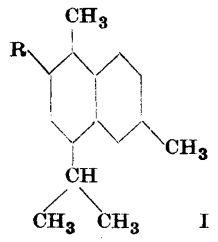
In a previous paper [1], one of us has shown that the nitration, Friedel-Crafts-acetylation, and bromination of cadalene lead to products with the substituent in the same position. By degradation reactions it was also shown that the entering substituent most probably occupies the 2-position (I; R = NO_2, CH_3CO, Br resp.), although the 3-position could not be definitely excluded.



I

After that work had been published, a paper by Campbell and Soffer [2] came to our knowledge, where the synthesis of 2-methylcadalene (I; R = CH_3) is described. By converting one of the substituting groups NO_2, CH_3CO or Br into CH_3 it would be possible to procure unequivocal evidence of the 2-position for these substituents.

This has now been done by treating the Grignard-reagent from bromocadalene with ethyl orthoformate and hydrolysing the acetal (I; R = $CH(OC_2H_5)_2$), which was not isolated, to the aldehyde [3] (I; R = CHO). This was reduced by Clemmensen-reduction to methylcadalene (I; R = CH_3). This compound was an oil, but was characterised as the picrate, the trinitrobenzolate, and the styphnate. These had m. p:s in good agreement with the values given by Campbell and Soffer [2] for the corresponding derivatives of

* Part I, Gripenberg, J. *Ann. Acad. Sci. Fennicae Ser. A.* **59** (1943) no. 14.

their 2-methylcadalene. Professor M. D. Soffer was kind enough to carry out mixed melting point determinations on these derivatives, for which we are grateful. He reported the following m. p:s.

	Present authors	Campbell and Soffer	Mixed melting point
Picrate	140—140.5°	138.5—139°	139.5—140.5°
Styphnate	170°	170°	170°
Trinitrobenzolate	167—168°	169—169.5°	167—169.5°

(The m. p:s of the picrate and the trinitrobenzolate are somewhat lower than the m. p:s reported in the experimental part. This is probably due to partial decomposition of the products on standing.)

As no depression of the m. p:s was observed, it seems safe to assume that the parent hydrocarbons were identical.

Briggs, Gill, Lions and Taylor [4] have, in a somewhat different way, also been able to connect the derivatives obtained by direct substitution of cadalene with the synthetic 2-methylcadalene.

The constitution originally assigned to nitro-, acetyl- and bromocadalene and the products obtained from them [1] can hence be regarded as correct.

EXPERIMENTAL

2-Cadalenealdehyde. The Grignard-reagent was prepared with 1.1 g Mg from bromocadalene (13 g) and then ethyl orthoformate (6 g) was added. The ether was distilled off, and the mixture was heated for half an hour on a water-bath. The thick oil obtained was poured into water, a small amount of acetic acid was added, and the acetal extracted with ether. The ether solution was warmed with 2 *N* hydrochloric acid on a water-bath. The ether was then evaporated and the remaining oil fractionated in a vacuum. The following fractions were obtained:

I	—170°/8 mm	1.1 g
II	170—180°/8 mm	1.4 g
III	190—200°/8 mm	3.5 g
Residue		3.0 g

Fraction III solidified and was recrystallised from light petroleum, m. p. 85.5—86.5°.

$C_{16}H_{18}O$ (226.2)	Calc. C 84.88	H 8.04
	Found » 85.00	» 7.55

The semicarbazone was prepared in the usual way and had after recrystallisation from alcohol m. p. 222—223°.

$C_{17}H_{21}ON_3$ (283.2)	Calc. C 72.06	H 7.47
	Found » 71.87	» 7.31

2-Methylcadalene. 2-Cadalenealdehyde (3 g) was reduced with amalgamated zinc (10 g) and hydrochloric acid (100 ml; 1 : 1) in the usual way. The reduction product was extracted with ether and steam distilled. 1 g of a colourless oil was obtained. This was without further purification converted into the picrate, styphnate and trinitrobenzolate.

The picrate, red needles from alcohol, had m. p. 143—144°.

The styphnate, orange red needles from alcohol, had m. p. 169—170°.

The trinitrobenzolate, yellow needles from alcohol, had m. p. 170—170.5°.

SUMMARY

The conversion of bromocadalene into 2-methylcadalene is described. The previously proposed structure for bromocadalene, and other derivatives of cadalene is thereby verified.

The analyses were carried out by Mr. K. Salo, University of Helsinki.

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Received February 16, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 259—271

Polarographische Analyse mit festen Elektroden

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Vorliegende Arbeit wurde 1944 ausgeführt, um die Brauchbarkeit der festen Elektroden für die polarographische Analyse zu untersuchen. Sie wurde durchgeführt ohne Kenntnisse der bereits von Kolthoff und Mitarbeiter [1,2,3] auf demselben Gebiet veröffentlichten Arbeiten, die zum Teil auch dieselben stofflichen Systeme behandeln und mit deren Ergebnisse sie in völligem Einklang steht.

Bekanntlich fusst die polarographische Analyse nach Heyrovský auf folgende Vorteile der tropfenden Quecksilberelektrode:

1. Das fliessende Elektrodenmaterial beschränkt die chemische Polarisation der Elektrodenoberfläche auf ein Minimum.

2. Die durch das Tropfen des Quecksilbers bewirkte Rührung der Lösung ist bei jedem Tropfen die gleiche, sodass sich auch die für die Stromstärke massgebende Diffusionsschicht jedesmal in dergleichen Weise neu bildet.

3. Das Quecksilber hat dem Wasserstoff gegenüber eine besonders hohe Überspannung und erlaubt daher Arbeiten bei stark negativem Elektrodenpotential.

Eine bequemere Arbeit mit einer festen Elektrode oder die Anwendung einer solchen für Aufgaben, bei denen die tropfende Quecksilberelektrode nicht benutzt werden kann, muss auf jeden Fall durch Verzicht auf einige dieser Vorteile der Tropfelektrode erkauft werden.

An einer Blechelektrode in einer Lösung ohne Konvektion ist die jeweilige Stromstärke nach dem Einschalten der Polarisationsspannung noch eine Funktion der Zeit, indem in immer grösseren Räumen Konzentrationsverarmung auftritt und damit Konzentrationsgefälle sowie Sättigungsstromdichte fortlaufend absinken und zwar umgekehrt proportional der Quadratwurzel aus der Zeit [1, s. 21]. In ungerührter Elektrolytlösung wäre grundsätzlich eine Konzentrationsbestimmung aus der Sättigungsstromstärke nur dann möglich, wenn eine bestimmte Polarisationszeit hinreichend genau eingehalten würde.

Die Zeitabhängigkeit kann vermieden werden, wenn die Lösung in definierter Weise durchgerührt wird. Dann erhält man an der Elektrode eine Flüssigkeitsschicht mit wesentlich laminarer Strömungsform. Durch die effektive Dicke dieser Grenzschicht und durch die Konzentration der Lösung ist das Konzentrationsgefälle und damit die Menge an zudiffundierendem Stoff sowie die Stromstärke eindeutig definiert, wenn unmittelbar an der Elektrode durch ein hinreichend negatives Potential die Konzentration des zu bestimmenden Stoffes klein gegenüber der Konzentration in der Lösung gemacht wird. Auf dieser Grundlage ist ein Verfahren von Mueller [4] ausgearbeitet worden.

Auch ohne Rührung erhält man an festen Elektroden einen nahezu zeitunabhängigen Sättigungsstrom, wenn eine kleine kugelähnliche Elektrode (z. B. eingeschmolzener und kurz abgeschnittener Platindraht) verwendet wird.

Auf eine Wiedergabe der von Wagner durchgeführten, unveröffentlichten Ableitung der Zeitabhängigkeit des Sättigungsstromes bei kugelförmigen Elektroden wird verzichtet, da sie der von Kolthoff [1, S. 29] gleichzeitig ausgeführten Ableitung weitgehend analog ist.

Mit R als Kugelradius und $q = 4\pi R^2$ als Elektrodenoberfläche, D als Diffusionskoeffizient, c_L als Konzentration in der Lösung, c_0 als Konzentration an der Elektrode, ν als Zahl der Elementarladungen, die für die Reduktion eines Ions bzw. einer Molekel benötigt werden, und $F = 96{,}500$ Coulomb ergibt sich für die Stromstärke:

$$i = 4\pi R^2 \cdot c_0 \cdot \left(\sqrt{\frac{D}{\pi t}} + \frac{D}{R}\right) \cdot \nu \cdot F \qquad (1)$$

Für grössere Zeiten wird praktisch das 2. Glied innerhalb der Klammer allein massgebend, und man erhält einen zeitunabhängigen Grenzwert der Stromstärke:

$$i = 4\pi R D \nu \mathrm{F}(c_L - c_0) \qquad \left(\text{Für } t \gg \frac{R^2}{D}\right) \qquad (2)$$

Ebenso wie in der Polarographie nach Heyrovský ist die Stromstärke proportional der Konzentration auf Grund des 1. Fickschen Gesetzes und unter Voraussetzung, dass die Konzentration c_0 an der Elektrode klein ist gegenüber der Konzentration c_L in der Lösung (d. h. praktisch: Unabhängigkeit der Stromstärke von der Spannung = Sättigungsstrombereich).

$$i = 4\pi R D \nu \mathrm{F} c_L \qquad \left(\text{Für } t \gg \frac{R^2}{D} \text{ und } c_0 \ll c_L\right) \qquad (3)$$

2. Berechnungen zur Wahl der zweckmässigsten Elektrodengrösse

Aus Gleichung (1) ist ersichtlich, dass an einer punktförmigen Elektrode unmittelbar nach dem Einschalten der Polarisationsspannung die Stromstärke zunächst wie an einer ebenen Elektrode umgekehrt proportional der Zeit fällt und sich asymptotisch dem Grenzwert nach Gleichung (2) nähert. Für das Verhältnis von jeweiliger Stromstärke i zum Grenzwert $i_{(t=\infty)}$ gilt:

$$\frac{i}{i_{(t=\infty)}} = 1 + \frac{R}{\sqrt{\pi D t}} \tag{4}$$

Je kleiner der Kugelradius R, nach desto kürzerer Zeit ist der Grenzwert praktisch erreicht. Mit $D = 10$ cm²/sec als Durchschnittswert für Diffusionskoeffizienten in wässriger Lösung und $R = 0{,}0025$ cm erhält man folgenden zeitlichen Gang der Stromstärke:

t (s)	5;	15;	30;	60;	120;	300
$\frac{i}{i_{(t=\infty)}}$	1,20	1,12;	1,08;	1,06;	1,04;	1,026

Bereits nach 30 s ist der zeitliche Gang nur gering, wenn auch die Annäherung an den endgültigen Grenzwert verhältnismässig grosse Zeiten verlangt. Wenn eine Annäherung an den Grenzwert bis auf 1 % gefordert wird, beträgt die erforderliche Wartezeit bereits eine halbe Stunde. Praktisch kommen derartige Zeiten nicht in Frage, da die unvermeidbare Konvektion der Flüssigkeit die Gültigkeit der Gleichungen (1) und (4) bei grösseren Zeiten beschränkt. Auch bei Ausschluss von äusseren Störungen und von Dichteunterschieden infolge lokaler Temperaturdifferenzen tritt zwangsläufig eine Auftriebsströmung auf, indem die Flüssigkeit in der Umgebung der Elektrode durch Bildung und Verbrauch von einzelnen Molekel- oder Ionenarten ihre Dichte ändert. Bei Wartezeiten von 1 Minute und bei Konzentrationen von 0,01 Mol/l oder darunter dürfte diese Störung noch unerheblich sein. Bei grösseren Konzentrationen und längeren Wartezeiten können jedoch Komplikationen auftreten, worauf gelegentliche Beobachtungen und überschlagsmässige Rechnungen hindeuten.

Bereits hier sei mitgeteilt, dass für Elektroden, deren Grösse ungefähr einem Radius $R = 0{,}0025$ cm entspricht, sich in der Praxis eine Wartezeit von 1 Minute zur Erzielung reproduzierbarer Ergebnisse bewährt hat, wie in Tabelle 1 (S. 265) an Hand von Versuchsdaten erläutert ist.

Übergang zu Elektroden mit geringeren Abmessungen würde die Einstellzeit verkürzen. Hierbei verringern sich jedoch gleichzeitig auch die Stromstärken, sodass die Möglichkeit zur Untersuchung verdünnter Lösungen beschränkt oder aber die Benutzung von Galvanometern mit sehr hoher Stromempfindlichkeit erforderlich wird. Mit $R = 0{,}0025$ cm und $D = 10^{-5}$ cm^2/sec erhält man z. B. aus Formel (2) für $c_L = 10^{-2}$ Mol/l $= 10^{-5}$ Mol/cm^3 und $\nu = 1$ als zeitlichen Grenzwert der Sättigungsstromstärke: $i = 3 \cdot 10^{-7}$ A. Dieser Wert der Stromstärke ist ungefähr zwei Zehnerpotenzen geringer als der zeitliche Mittelwert der Stromstärke an einer Quecksilbertropfelektrode üblicher Grösse. Die Benutzung noch kleinerer fester Elektroden erscheint somit trotz kleinerer Einstellzeiten nicht empfehlenswert.

3. Konstruktive Gestaltung der Elektrode und elektrische Schaltung

Zur praktischen Herstellung einer angenähert halbkugelförmigen Elektrode, die im folgenden auch als Punktelektrode bezeichnet wird, wurde ein Platindraht von 0,005 cm Durchmesser in ein Glasrohr eingeschmolzen und möglichst dicht über der Einschmelzung mit einer Rasierklinge abgeschnitten. Auch wenn die Oberflächengestalt von einer Kugel oder Halbkugel im einzelnen abweicht, so entspricht dennoch das Konzentrationsfeld in einiger Entfernung dem Fall einer Kugelelektrode, und es ist insbesondere ein zeitlicher Grenzwert des Sättigungsstromes zu erwarten.

Die Einschmelzstelle des Platindrahtes wurde nach Fig. 1 in ein weiteres Glasrohr als Schutzhülle gegen Strömungen der Flüssigkeit eingeschmolzen. (Messungen ohne Schutzrohr sind zwar möglich, aber nur mit geringer Genauigkeit.) Durch das Schutzrohr konnte von unten ein Kohlendioxyd- oder Stickstoff-Gasstrom zwecks Durchmischung mit der äusseren Flüssigkeit geleitet werden. Gleichzeitig diente der Gasstrom zur Entfernung von Luftsauerstoff.

Zwecks Verringerung des Spannungsanteiles der chemischen Polarisation der zu untersuchenden Elektrodenvorgänge wurde der aus der Einschmelzung herausragende Platindraht platiniert oder mit Kupfer, Silber oder Quecksilber überzogen.

Nach vorangegangener kathodischer Entfettung wurden folgende Bedingungen zur Herstellung der genannten Metallüberzüge benutzt:

a) *Platinierung.* Bad: 3 g Platinchlorwasserstoffsäure + 0.02 g Bleiacetat + 100 ml Wasser. Elektrolyse mit 16 V und 700.000 Ω Vorschaltwiderstand während 10 Minuten. Anschliessend kurzzeitig kathodische Wasserstoffentwicklung in 2 N Schwefelsäure mit 16 V und 700.000 Ω Vorschaltwiderstand.

b) *Verkupferung.* Bad: 125 g Kupfersulfat (5 aq) + 1000 ml 1 N Schwefelsäure. Elektrolyse mit 16 V und 10^6 Ω Vorschaltwiderstand während 15 sec und anschliessend mit 10^7 Ohm während 6 Minuten.

c) *Versilberung.* Bad: 9 g Silberchlorid + 9,5 g Kaliumcyanid + 250 ml Wasser. Elektrolyse mit 2 V und $2 \cdot 10^6$ Ω Vorschaltwiderstand während 1 Minute und anschliessend mit $2 \cdot 10^7$ Ω während 45 Minuten oder länger.

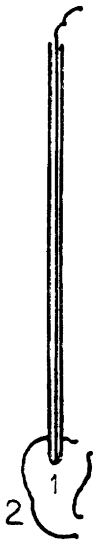

Fig. 1. Elektrode zur polarographischen Analyse mit eingeschmolzenem, kurz abgeschnittenem Platindraht (Punktelektrode). (1 Platindraht; 2 Glaskappe.)

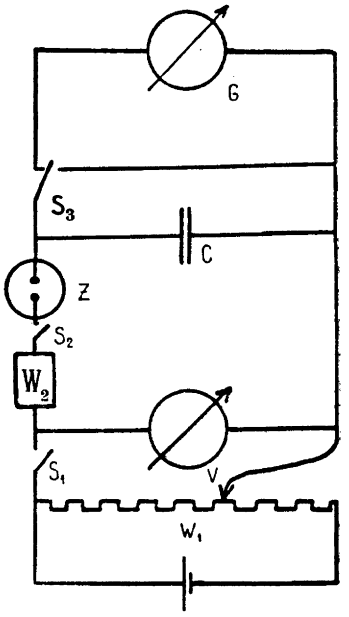

Fig. 2. Schaltung zur Aufnahme von Strom-Spannungskurven. W_1 Widerstand 20 Ohm als Potentiometer; V Voltmeter; Z Zelle; S_1, S_2, S_3 Schalter; C Kondensator, 1 μF; G Spiegelgalvanometer (1 Skalenteil = 2,85 · 10^{-9} A); W_2 Widerstand 50 000 Ohm (oder gegebenfalls kleiner) zum Schutz des Galvanometers.

d) *Verquickung.* Bad: 3 g Sublimat + 25 ml Salpetersäure (65 %) + 1000 ml Wasser. Elektrolyse mit 2 V und 2 · 10^6 Ω Vorschaltwiderstand während 1 Minute und anschliessend mit 2 · 10^7 Ω während 30 Minuten oder länger.

Es hat sich als zweckmässig erwiesen, die Elektroden jeweils einige Tage vor Gebrauch herzustellen und in destilliertem Wasser aufzubewahren. Bei ungeeigneter Vorbehandlung treten leicht Störungen auf.

Als Gegenelektrode diente teilweise ein platiniertes Platinblech, dessen Potential durch das zu messende Reduktions-Oxydations-System festgelegt wurde, teilweise auch eine Silber-Silberchloridelektrode, deren Potential gegen die Normalwasserstoffelektrode zu + 0,29 V für $[Cl^-]$ = 0,1 Mol/l gegeben ist.

Die elektrische Schaltung zeigt Figur 2.

Sofern Arbeitselektrode und Gegenelektrode von gleicher Art sind (z. B. platiniertes Platin), wird die Zelle vor Beginn einer Messungsreihe kurz geschlossen (Schalter S_1 geöffnet; Schalter S_2 geschlossen; Schalter S_3 nach rechts). Hierdurch gleichen sich etwa vorhandene Unterschiede der beiden Elektroden aus und die Messung beginnt mit einem stromlosen Anfangszustand. Zur Aufnahme einer Strom-Spannungskurve wird der Ab-

griff am Potentiometerwiederstand W_1 zunächst ganz links eingestellt, Schalter S_1 geschlossen und Schalter S_2 geöffnet. Alsdann wird der Abgriff an W_1 stufenweise nach rechts verschoben, um vorgegebene Spannungswerte einzustellen, die am Voltmeter V abgelesen werden. Schalter S_1 wird eingeschaltet und gleichzeitig eine Stoppuhr in Gang gesetzt. Nach einigen Sekunden kann der Kurzschluss des Galvanometers G aufgehoben werden, indem der Schalter S_3 nach links gelegt wird. Um hierbei den Stromfluss in der Zelle nicht zu unterbrechen, ist der Kondensator C eingebaut. Als Zeitspanne bis zum Ablesen der Stromstärke hat sich im allgemeinen 1 Minute als zweckmässig erwiesen. Vor einer weiteren Erhöhung der Spannung ist der Schalter S_3 nach rechts zwecks Kurzschluss des Galvanometers umzulegen, da bei Änderung des Potentials einer platinierten Platinelektrode grosse Anfangsstromspitzen infolge Aufladung der Polarisationskapazität der Elektrode auftreten.

Wenn die Zelle neben einer Arbeitselektrode aus anderem Metall eine Silber-Silberchloridelektrode in einer Lösung mit vorgegebener Konzentration an Cl^--Ionen als Gegenelektrode enthält, ist die Zelle vor Beginn der Messungen nicht kurzzuschliessen. Der Anfangswert der vorzugebenden Spannungswerte ist gleich der Differenz des Gleichgewichtspotentials des zu untersuchenden Redoxsystems gegenüber der Gegenelektrode zu wählen.

Die einzelnen Stromstärkenwerte wurden als Skalenausschläge eines Spiegelgalvanometers abgelesen (Prinzip der Polarometrie). Auf die photographische Registrierung (Plarographie) wurde für den vorliegenden Zweck einer Untersuchung prinzipieller Möglichkeiten verzichtet.

Für jedes einzelne Stoffsystem wurden nach Möglichkeit folgende Untersuchungen ausgeführt.

1. Aufnahme einer Strom-Spannungskurve für die in Aussicht genommene Grundlösung ohne den zu bestimmenden Stoff, insbesondere zur Ermittelung der Potentialwerte, bei denen eine merkliche Wasserstoff- oder Sauerstoffentwicklung einsetzt.

2. Aufnahme von Stromspannungskurven mit ausgewählten Konzentrationen des zu bestimmenden Stoffes zwecks Ermittelung des Spannungsbereiches, in dem die Stromstärke praktisch unabhängig von der Spannung wird (Sättigungsstrombereich).

3. Messung der Stromstärke bei festgehaltenem Spannungswert etwa entsprechend der Mitte des nach 2 erhaltenen Sättigungsbereiches bei stufenweiser Zugabe des zu bestimmenden Stoffes und Prüfung auf Proportionalität zwischen Stromstärke und Konzentration.

Der Gang der Stromstärkenwerte innerhalb der ersten Minute nach dem Einschalten der polarisierenden Spannung zeigt Tabelle 1. Die Zahlenangaben sind Mittelwerte aus jeweils 10 Einzelbeobachtungen. Die nachfolgend stehenden mittleren Schwankungen der Einzelbeobachtungen geben einen Eindruck von der Reproduzierbarkeit. Bei höheren Konzentrationen und entsprechend grösseren Galvanometerausschlägen wachsen die Schwankungen mit der Wartezeit an. Diese Tatsache dürfte auf die von Laitinen und Kolthoff[2] diskutierten Konvektionsströmungen zurückzuführen sein.

Tabelle 1. Anodische Oxydation von Eisen(II)-Salz an einer Punktelektrode aus platiniertem Platin bei 0,4 V Spannung. (1 Skalenteil Galvanometerausschlag = 2,85 · 10^{-9} A; Zusammensetzung der Lösung wie in Abschnitt 6).

$[Fe^{++}]$	Galvanometerausschlag nach Wartezeiten von			Mittlere Schwankung der Einzelbeobachtung nach Wartezeiten von		
	15 s	30 s	60 s	15 s	30 s	60 s
0,00385	20,2	19,2	18,6	± 0,3	± 0,3	± 0,3
0,00754	37,5	35,8	34,9	± 0,3	± 0,4	± 0,6
0,0111	53,6	51,2	49,9	± 0,2	± 0,3	± 0,3
0,0145	70,0	67,2	65,7	± 0,2	± 0,4	± 0,6
0,0179	85,2	81,5	79,9	± 0,3	± 0,2	± 0,5

Im Mittel ist die Stromstärke bei einer Wartezeit von 15 s um 7 % höher als für 60 s. Dieser Unterschied entspricht den Abschätzungen an Hand der theoretischen Gleichung (4) für eine ideale Kugelelektrode mit $R = 0{,}0025$ cm.

Auf Grund dieser Versuche wurde für die Mehrzahl der Versuche eine Wartezeit von 1 Minute eingehalten, da hierbei Abweichungen vom Sollwert der Wartezeit nur noch wenig ins Gewicht fallen und da die Erhöhung der mittleren Schwankung gegenüber kleineren Wartezeiten nicht wesentlich erscheint.

4. Die kathodische Reduktion von Brom

Zur Prüfung der grundsätzlichen Eignung der hier vorgesehenen Arbeitsweise wurde zunächst die kathodische Reduktion von Brom untersucht. Zusammensetzung der Grundlösung: 50 ml 2 *M* Salzsäure + 5 ml 1 *M* Kaliumbromid. Hierzu wurden wechselnde Mengen 0,1 *N* Kaliumbromat-Lösung gegeben, wodurch Brom gebildet wird. Arbeitselektrode und Gegenelektrode bestanden aus platiniertem Platin. Bei Konzentrationen an Br_2 zwischen 0,0005 und 0,025 Mol/l wurde Unabhängigkeit der Stromstärke von der Spannung von 0,3 bis 0,8 V mit einer Genauigkeit von ± 2 % erhalten. Die in diesem Bereich gemessene Sättigungsstromstärke ist mit sehr guter Genauigkeit der Konzentration proportional (vgl. Tabelle 2). Das System bietet besonders geringe Schwie-

Tabelle 2. Kathodische Reduktion von Brom an einer Punktelektrode aus platiniertem Platin bei 0,5 V Spannung. (Wartezeit 1 Minute; Galvanometerempfindlichkeit: 1 Skalenteil = 2,85 · 10^{-9} A.)

$[Br_2]$	Galvanometerausschlag s	$\frac{s}{[Br_2]} \cdot 10^{-2}$
0,0054	54,1	100
0,0108	109,2	101
0,0155	156,6	101
0,0204	203,8	100
0,0250	249,0	100

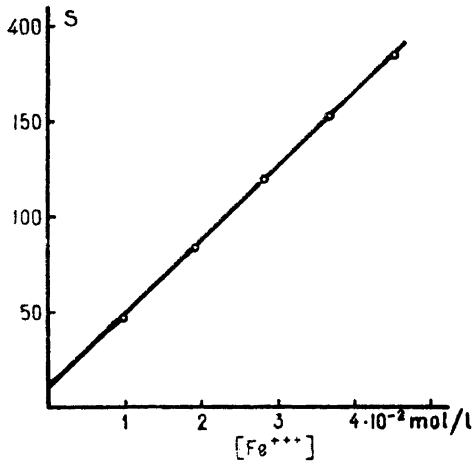

Fig. 3. Galvanometerausschlag s für die kathodische Reduktion von Eisen (III)-Salz bei 0,6 V als Funktion der Konzentration. (1 Skalenteil = 2,85 · 10⁻⁹ A).

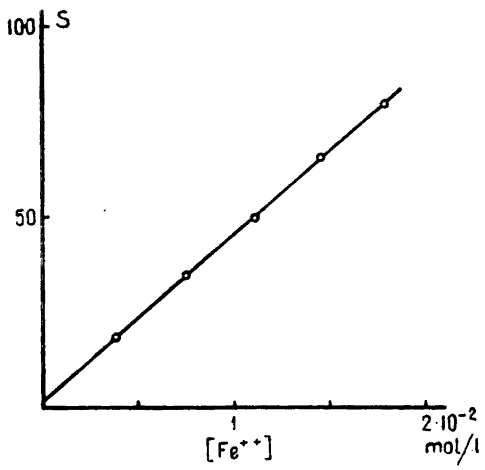

Fig. 4. Galvanometerausschlag s für anodische Oxydation von Eisen(II)-Salz bei 0,4 V als Funktion der Konzentration (1 Skalenteil = 2,85 · 10⁻⁹ A).

rigkeiten, da das Brom-Gleichgewichtspotential genügend weit vom Potential des nächstfolgenden kathodischen Vorgangs der Wasserstoffabscheidung entfernt ist.

5. Die kathodische Reduktion von Eisen (III)-Salz

Grundlösung: 50 ml einer Lösung mit 2 *N* Schwefelsäure und 0,1 *N* Ferrosulfat. Hierzu wurden wechselnde Mengen einer Lösung mit 2 *N* Schwefelsäure und 0,5 *M* Ferriammoniumsulfat gegeben. Kathodischer Vorgang: $Fe^{+++} + e^- = Fe^{++}$. Arbeitselektrode und Gegenelektrode bestanden aus platiniertem Platin. Das Potential der Gegenelektrode ist durch das Fe^{+++}—Fe^{++}-Potential festgelegt. Sättigungsstrom wurde für einen Gehalt an Ferriionen von 0,01 bis 0,05 Mol/l zwischen 0,2 und 0,6 Volt erhalten. Der Sättigungsstrom ist mit guter Näherung eine lineare Funktion des Zusatzes an Eisen-(III)-Salz (Vgl. Fig. 3). Bei Extrapolation auf dem Abszissenwert null in Figur 3 verbleibt ein gewisser Anfangsausschlag, der durch einen geringen Gehalt der Grundlösung an reduzierbaren Ionen oder Molekeln entstanden oder durch einen elektrischen Nebenschluss bedingt sein kann.

6. Die anodische Oxydation von Eisen (II)-Salz

Grundlösung 50 ml einer Lösung mit 2 *N* Schwefelsäure und 0,5 *M* Ferriammoniumsulfat. Hierzu wurden wechselnde Mengen einer Lösung mit 2 *N* Schwefelsäure und 0,2 *M* Ferrosulfat gegeben. Anodischer Vorgang: $Fe^{++} = Fe^{+++} + e^-$. Arbeitselektrode und Gegenelektrode bestanden aus platiniertem Platin. Sättigungsstrom wurde für ein

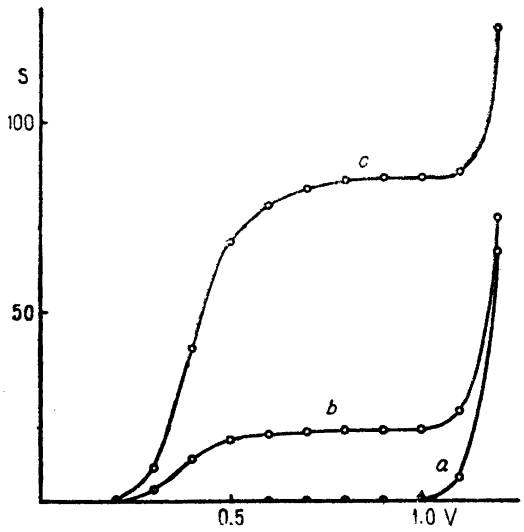

Fig. 5. Stromspannungskurven für die kathodische Reduktion von Sauerstoff in alkalischer Lösung (0,1 M Kaliumchlorid + 0,01 M Kaliumhydroxyd) an platiniertem Platin gegenüber einer Silber-Silberchlorid-Elektrode. (1 Skalenteil = 2,85 · 10^{-9} A).
a) Gereinigter Stickstoff.
b) Luft.
c) Sauerstoff.

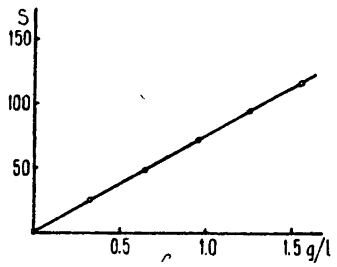

Fig. 6. Galvanometerausschlag s für die anodische Oxydation von Ascorbinsäure an platiniertem Platin gegenüber einer Silber-Silberchlorid-Elektrode bei 0,84 V als Funktion der Konzentration c (1 Skalenteil = 2,2 · 10^{-9} A).

Gehalt an Ferro-Ionen von 0,004 bis 0,018 Mol/l zwischen 0,2 und 0,5 V erhalten. Der Sättigungsstrom ist mit guter Näherung eine lineare Funktion des Zusatzes an Eisen(II)-Salz (vgl. Figur 4).

7. Die kathodische Reduktion von Sauerstoff

Es wurde die kathodische Reduktion von Sauerstoff an Punktelektroden aus platiniertem Platin, Silber und Kupfer untersucht. Als Gegenelektrode diente eine Silber-Silberchlorid-Elektrode; bei einer Konzentration an Chlorid von 0,1 Mol/l liegt das Potential bei + 0,29 V gegenüber der Normalwasserstoffelektrode. Der Sauerstoffgehalt der Lösung wurde durch Einleiten von Luft mit 20,9 Volumenprozent Sauerstoff sowie von reinem Sauerstoff vorgegeben. Sauerstoffreie Lösungen wurden durch Einleiten von besonders gereinigtem Stickstoff erhalten. Unabhängigkeit der Stromstärke von der Spannung sowie Proportionalität zwischen Sättigungsstromstärke und Sauerstoffgehalt wurden in alkalischer Lösung (0,1 *M* Kaliumchlorid und 0,01 *M* Kaliumhydroxyd) für alle drei Elektrodenarten erhalten. Die Konstanz des Sättigungsstromes ist für eine Elektrode aus platiniertem Platin am besten (vgl. Fig. 5). Hinreichende Konstanz des

Sättigungsstromes wurde auch für neutralsalzhaltige ungepufferte Lösungen erhalten, indem hier Hydroxylionen durch den Elektrodenvorgang entstehen. In gepufferter Lösung mit pH = 6,81 ist der Spannungsbereich des Sättigungsstromes für Sauerstoff von einer Atmosphäre nicht hinreichend gross. Beim Durchleiten von Luft (Sauerstoffdruck = 0,21 at) wurde an versilberter und verkupferter Elektrode Sättigungsstrom zwischen 0,7 und 1,5 V erhalten. Versuche mit verquickter Elektrode in saurer Lösung ergaben keinen Bereich der Spannung mit genügend konstanter Stromstärke.

8. Die kathodische Reduktion von Nitrobenzol

An verkupferter, versilberter und verquickter Punktelektrode wurden Sättigungsströme in ungepufferter 0,1 *M* Kaliumchloridlösung bei Spannungen von etwa 0,8 bis 1 V gegen eine Silber-Silberchlorid-Elektrode erhalten. Die Frage nach der chemischen Natur des Reaktionsproduktes ist offen. Die Stromstärken sind der Konzentration an Nitrobenzol weitgehend proportional. Die Löslichkeit von Nitrobenzol in Wasser bei Raumtemperatur wurde zu 0,191 g Nitrobenzol/100 g Lösung bestimmt. Der Wert stimmt mit den Angaben von Davis [5] und von Gross und Saylor [6] überein.

Es wurde festgestellt, dass auf diese Weise Nitrobenzol auch in nahezu gesättigter Anilinlösung bestimmt werden kann und dass somit eine Bestimmung von Nitrobenzol in Anilin möglich ist. Die Erfassungsgrenze dürfte bei 0,05 % Nitrobenzol in Anilin liegen.

9. Die anodische Oxydation von Ascorbinsäure

Es wurde ein anodischer Sättigungsstrom an platiniertem Platin in einer Lösung mit 2 *N* Schwefelsäure und 0,02 *N* Salzsäure bei Potentialen von + 0,8 bis + 1,0 V gegen eine Silber-Silberchlorid-Vergleichselektrode erhalten. Die Stromstärken sind den Konzentrationen weitgehend proportional (vgl. Fig. 6).

10. Versuche über die kathodische Reduktion von Kupfer- und Zinksalzlösungen

Über die Möglichkeiten einer Auswertung der Stromstärkenwerte für die Reduktion von Metallionen zu Metall an Punktelektroden wurden nur vereinzelte Erfahrungen gesammelt. In Anlehnung an die Analysenvorschrift zur Untersuchung von Messing an der Quecksilbertropfelektrode [7] wurden Stromspannungskurven für ammoniakalische Kupfer-Zink-Salzlösungen aufgenommen.

Grundlösung: 5 g Ammoniumchlorid + 5 ml NH_3 (d = 0,91) + 45 ml Wasser. Hierzu wurden vorgegebene Mengen Kupferchlorid- und Zinkchlorid-Lösung zugegeben. Als Arbeitselektrode diente platiniertes Platin, das sich naturgemäss im Laufe der Messung mit Kupfer und Zink überzog. Die Stromspannungskurve zeigt Fig. 7. (Wartezeit für jeden Messpunkt 15 s.) Es sind zwei deutlich nacheinander auftretende Spannungsbereiche mit Sättigungsstrom zu erkennen. Bei 0,05 V wird Kupfer allein abgeschieden, bei 0,4 V Kupfer und Zink zusammen. Die bei diesen Spannungswerten gemessenen Stromstärken sind weitgehend proportional der vorgegebenen Konzentration (vgl. Fig. 8). Damit ist prinzipiell die Möglichkeit einer Anwendung der Punktelektrode für

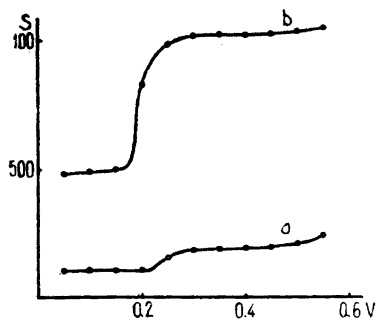

Fig. 7. Strom-Spannungskurven für die kathodische Reduktion von ammoniakalischen Kupfer-Zink-Salzlösungen mit Kupfer als Gegenelektrode. (1 Skalenteil = 2,85 · 10⁻⁹ A).

a) Cu^{++} = 0,00098; $[Zn^{++}]$ = 0,00098 Mol/l

b) Cu^{++} = 0,0045; $[Zn^{++}]$ = 0,0045 Mol/l

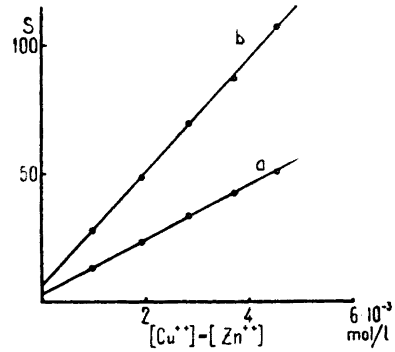

Fig. 8. Galvanometerausschlag s für die kathodische Reduktion von ammoniakalischen Kupfer-Zink-Salzlösungen mit Kupfer als Gegenelektrode. (1 Skalenteil = 2,85 · 10⁻⁹ A).

a) Spannung 0,05 V (Abscheidung von Kupfer)

b) Spannung 0,4 V (Abscheidung von Kupfer und Zink).

Vorgänge dieser Art gezeigt. Nach Messung jeder der angegebenen Lösungen wurde der gebildete Kupfer-Zink-Niederschlag durch anodische Polarisation mit 0,5 V abgelöst. Grundsätzlich ist zu beachten, dass während der Messung der Radius der Elektrode nach Massgabe der Metallabscheidung wächst. Unter den angegebenen Bedingungen, insbesondere bei Beschränkung auf 15 sec, fällt dieser Fehler noch nicht ins Gewicht.

11. Versuche mit Drahtelektroden

In einer bisher unveröffentlichten Arbeit von Wagner wurde berechnet, dass die Stromstärke an einer Drahtelektrode im Gebiete der Konzentrationspolarisation zeitlich fortlaufend sinkt, also keinen Grenzwert erreicht. Der Gang für grössere Zeiten ist jedoch nur noch schwach (etwa umgekehrt proportional dem Logarithmus der Zeit). Somit ist zu erwarten, dass im Spannungsbereich des Sättigungsstromes die Stromstärke nach Vorgabe ungefähr festliegender Wartezeiten im wesentlichen proportional der Konzentrationen der an den Elektroden umsatzfähigen Stoffe ist.

Die Konstruktion der Drahtelektrode entsprach der der Punktelektrode (Fig. 1); nur wurde hier der Platindraht nicht abgeschnitten, sondern ausgespannt und in den unteren Teil des Glasschutzrohres 2 eingeschmolzen.

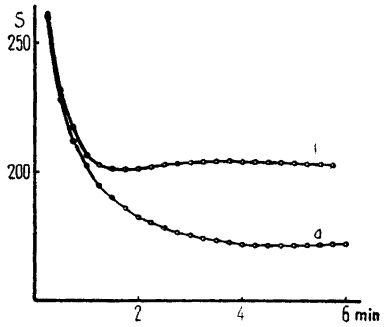

Fig. 9. Galvanometerausschlag s für die kathodische Reduktion von Brom an einer platinierten Platin-Drahtelektrode bei 0,3 V als Funktion der Zeit.
a) Br_2 = 0,0046 Mol/l; (Skalenteil = $5{,}3 \cdot 10^{-8}$ A)
b) Br_2 = 0,025 Mol/l; (1 Skalenteil = $3{,}1 \cdot 10^{-7}$ A)

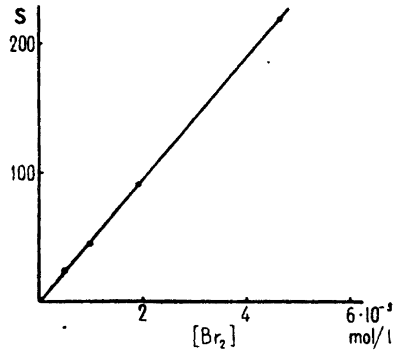

Fig. 10. Galvanometerausschlag s für die kathodische Reduktion von Brom an einer platinierten Platin-Drahtelektrode bei 0,3 V als Funktion der Konzentration (1 Skalenteil = $5{,}3 \cdot 10^{-8}$ A).

Vorläufige Versuche haben ergeben, dass der zeitliche Gang der Stromstärke bei Wartezeiten von zwei Minuten oder darüber sogar noch geringer ist, als die theoretischen Diffusionsformeln erwarten lassen (vgl. Figur 9); wahrscheinlich erfolgt hier ein zusätzlicher Stofftransport durch Konvektion der Flüssigkeit (vgl. S. 264).

Da die Oberfläche einer Drahtelektrode entsprechend dem Verhältnis von Drahtlänge zu Durchmesser grösser ist, sind mit Drahtelektroden wesentlich grössere Stromstärken (etwa zwei Zehnerpotenzen) zu erhalten, sodass die Erfassungsgrenze zu kleineren Konzentrationen verschoben wird.

Entsprechend den Erwartungen wurde gefunden, dass in geeigneten Systemen die Stromstärke nach einer Wartezeit von einer Minute mit guter Genauigkeit proportional der Konzentration ist (vgl. Fig. 10). Im ganzen scheint jedoch die erreichbare Genauigkeit geringer als für die in Abschnitt 4 bis 10 benutzten Punktelektroden. Die Verwendung von Drahtelektroden dieser Ausführnng dürfte trotz grösserer Stromstärkenwerte für die Praxis nicht besonders zu empfehlen sein, da der Zeitfluss wesentlich mehr als bei Punktelektroden ins Gewicht fällt.

ZUSAMMENFASSUNG

Es wurden die Möglichkeiten einer polarographischen Analyse mit festen Elektroden untersucht. Die Zeitabhängigkeit des durch die Diffusionsgesetze

bestimmten Sättigungsstromes ist unerheblich, wenn kurz abgeschnittene Drahtenden benutzt werden (Prinzip der Punktelektrode). Als Elektrodenmaterial wurde vorzugsweise platiniertes Platin benutzt, in einzelnen Fällen auch Platin mit Silber-, Kupfer- oder Quecksilberüberzug. Hinreichende Bereiche des Sättigungsstromes und Proportionalität mit der Konzentration wurden für folgende Systeme gefunden:

1. Kathodische Reduktion von Brom.
2. Kathodische Reduktion von Eisen(III)-Salz.
3. Anodische Oxydation von Eisen(II)-Salz.
4. Kathodische Reduktion von Sauerstoff.
5. Kathodische Reduktion von Nitrobenzol.
6. Anodische Oxydation von Ascorbinsäure.
7. Kathodische Reduktion von Kupfer- und Zinksalz.

Für Anregung und Beistand bei der Arbeit bin ich Herrn Professor Dr. Carl Wagner sehr zu Dank verpflichtet.

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Eingegangen am 16. Februar 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 272—278

Effect of Decrease in the Protein Content of Cells on the Proteolytic Enzyme System

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In the previous works from this laboratory [1, 2] enormous changes were noted to take place in the enzymatic activity of *Escherichia coli* if the protein content of the cells was lowered by growing the bacteria in a nutrient solution containing plenty of sugar but little nitrogen. The nitrogen content of *E. coli* grown in a normal nutrient solution is about 13 % of the dry matter, but when *E. coli* is grown in a nitrogen-deficient medium, the nitrogen value can easily fall to 9—10 % and in some cases to half of the normal nitrogen content. The cells with different nitrogen content have very different enzymatic machineries; the activity of certain enzymes in low-nitrogen cells remains fairly similar to that in normal cells, while the activity of other enzymes is enormously lower. On the basis of the results it seems that the *indispensable enzymes, i. e.*, the enzymes without which the cell is unable to live whatever the composition of nutrient, retain their activity very well even though the nitrogen content is lowered. On the contrary, the *dispensable enzymes i. e.*, the enzymes which expand the living conditions of the cell, enabling it to utilize several nutrients but not being necessary in all nutritional conditions, lose most or all of their activity when the nitrogen content is decreased. For instance, the activity of saccharase lowered to about 10 % of the maximum when the nitrogen content of the cell dry matter fell from about 13 % to about 10 %, whereas the activity of catalase did not lessen. The enzymes which improve the living conditions of the cell are mostly adaptive, as for instance, lactase, maltase, and the major part of saccharase of our *E. coli* strain. Only a few per cent of the maximum saccharase activity is retained in the cells produced in saccharose-free nutrient solutions. The great dependence of the adaptive enzymes on the protein content of the cells has later been confirmed in regard to many

adaptive enzymes of *E. coli* (Virtanen and Winkler [3] in regard to lactase, De Ley [4] in regard to enzymes mentioned below).

The starting point for these investigations was the hypothesis advanced by Virtanen [5] that the proteins of the active young cells are practically entirely or at least for the major part enzyme proteins. If this be true, the lowering of the protein content of the cells brings about either a weakening of the activity of all the enzymes in proportion to the protein content of the cells *or* a strong decrease in certain enzymes while the others remain unchanged. The experimental results are in agreement with the latter concept.

De Ley [4, 6] has continued in Ghent the studies started in this laboratory on the correlation of the nitrogen content and respiration in *E. coli*. His thesis contains some very noteworthy results. The rate of respiration is relatively little lessened by the fall in the protein content of the cells. Only in cells with an extremely low protein content (abt. 4 % N) has the rate of respiration fallen to half or a little more. The fall of respiration is similar on different substrates. On the other hand, the anaerobic fermentation (acid formation) ceases entirely when the nitrogen content of the cells is lowered to below 8 %, and is already weak, abt. 10 % of the maximum, when the nitrogen content of the cells is still 10 %. Thus the lowering of the protein content of the cells makes the system of fermentation enzymes incomplete. In aerobic conditions the acid formation is only slightly lessened with the lowering of the protein content, and the curves representing the respiration and aerobic fermentation run parallel. New possibilities have thus been opened for elucidation of the correlation between the mechanism of respiration and anaerobic fermentation.

The adaptive formic hydrogenlyase, which catalyses the formation of H_2 and CO_2 from formate, disappears in N deficiency [4]. Cells with 10 % N of dry matter are already completely devoid of this enzyme. Decarboxylating enzymes disappear in low-nitrogen cells.

The results which so far have been obtained in regard to the dependence of the enzyme activity on the protein content of the cells open many new aspects. Fig. 1 illustrates these results graphically.

The present paper gives an account of our findings on the dependence of the proteolytic enzyme system on the nitrogen content of cells. The test organism used was the same strain of *E. coli* (K_3) as in the previous experiments of this laborbtory.

METHODS

E. coli which had been cultivated for 290—315 times in a saccharose nutrient solution containing 8 g $(NH_4)_2SO_4$ per 5 l, was grown for the experiment in a nutrient solution of the same composition as in the previous invest-

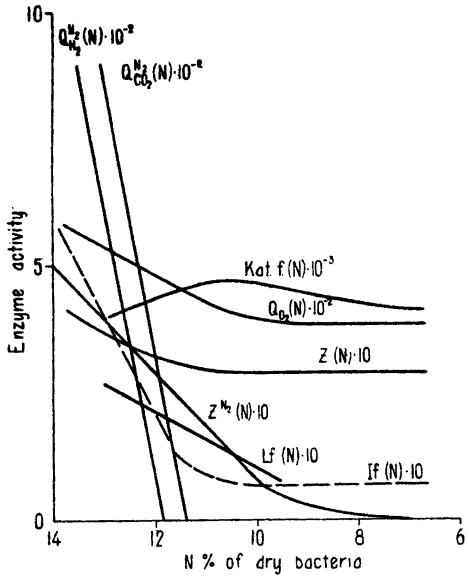

Fig. 1. Changes in the activity of different enzymes as affected by the N-content of E. coli. All activities calculated per N-content of cells.
Kat. f. (N) · 10^3 Catalase effect (Virtanen and De Ley); If (N) · 10 Saccharase effect (Virtanen and De Ley); Q_{O_2} (N) · 10^{-2} Respiration (De Ley); Z (N) · 10 Aerobic acid formation (De Ley); Z^{N_2}(N) · 10 Anaerobic acid formation (De Ley); $Q^{N_2}_{H_2}$(N) · 10^{-2H} H_2 formation from formic acid (De Ley); $Q^{N_2}_{CO_2}$ (N) · 10^{-2} CO_2 formation from formic acid (De Ley); Lf (N) · 10 Lactase effect (Virtanen and Winkler).

igations, *viz.*, 25 g saccharose, 25 g K_2HPO_4, 10 g NaCl, 100 mg $MgSO_4$, H_2O to 5 l. The amount of $(NH_4)_2SO_4$ ranged in different flasks from 8 to 0.1 g per 5 l. Since the yield of bacteria in higher N-concentrations was greater than in lower ones, only 1 litre cultures were used in ammonium sulphate concentrations of 8 to 1 g per 5 l, and 5 litre cultures in lower concentrations. In this way, sufficient bacteria were produced in all N-concentrations and an equal amount of bacterial dry matter could be used in each experimental series for determination of enzyme activity. In order to prevent losses in nitrogen, ammonium sulphate solutions were sterilized separately and added to the main solution just before inoculation. The pH was kept at about 6.5 by neutralizing with NaOH, the temperature of growth was 31° C, and duration 60 h.

Bacteria were separated by centrifuging, washed once, and suspended in 40 ml distilled water. Determinations of dry matter at 96° C and of nitrogen were made on the suspension. For each experiment a sample was taken containing 40 mg bacteria (calculated as dry bacteria). This was added to 120 ml of 2 % casein solution. First a 10 % casein (technical) solution was prepared with pH about 10. It was diluted to 2 %, and the pH lowered to 6.5. Toluene was added to all solutions.

Hydrolysis of casein was observed by determining amino-N with the Cu-method[7] (coeff. 0.28). In one experiment, besides, soluble N was determined as well as amino N according to van Slyke. Determination of soluble N was

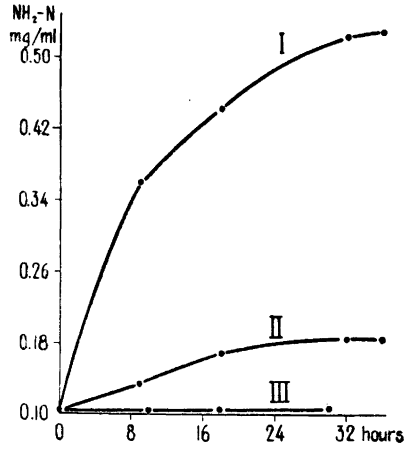

Fig. 2. Formation of amino-nitrogen from casein by E. coli.

Curve I: E. coli suspension plus casein
» *II: Casein*
» *III: E. coli suspension.*

made as follows. The pH of the solution was lowered to 4.6 with 1 % acetic acid, the flask was heated for 3 min in a boiling saturated solution of NaCl and let stand over-night. The contents were filtered through a filter Jena G 3, and nitrogen was determined on the clear filtrate.

Experiment 1. The casein-decomposing ability of normal nitrogen bacteria was examined 1. in casein solution *plus* bacteria, 2. in bacterial suspension without casein, and 3. in casein solution without bacteria. Only amino N was determined. The results appear from Fig. 2.

Experiment 2. Bacteria were grown in four different N-concentrations, and thus bacterial masses were obtained with the following N-contents.

	I	II	III	IV
$(NH_4)_2SO_4$, g/5 l	8	1	0.25	0.1
N % of bacteria dry matter	13.1	12.3	10.4	9.5

Decomposition of casein was observed with these bacterial masses by determining amino-N in the beginning of the experiment and after 5 and 20 days. The results are given in Table 1.

Experiment 3. Bacteria were grown in four different N-concentrations. The N-contents of the bacterial masses obtained were the following.

	I	II	III	IV
$(NH_4)_2SO_4$, g/5 l	8	1	0.25	0.1
N % of bacteria dry matter	13.2	12.6	10.4	10.4

Table 1. Decomposition of casein by E. coli with different N-content. NH_2-N determined by the Cu-method. In each experiment 40 mg bacteria (dry matter) in 120 ml 2 % casein solution, pH 6.5.

N of bacteria, % of dry matter	NH_2-N mg per ml		
	at start	after 5 days	after 20 days
13.1	0.100	0.284	0.478
12.3	0.101	0.277	0.464
10.4	0.100	0.271	0.441
9.5	0.100	0.270	0.413

The experiment comprised 8 test solutions each containing 40 mg bacteria dry matter in 120 ml 2 % casein solution. Four of these were examined after 6 days and four after 17 days. Decomposition of casein was observed with these bacterial masses by determining total N and amino N in the hydrolysate with the Cu-method. After that casein was precipitated and from the clear filtrate soluble N and amino N were determined both with the Cu and van Slyke methods. The results are seen from Table 2 and Fig. 3.

Table 2.

N of bacteria, % of dry matter	Total N of hydrolysate mg N/ml	NH_2-N of hydrolysate (Cu-method)		Soluble N % of total N	NH_2-N of the clear filtrate		
					(Cu-method)		(van Slyke)
		mg/ml	% of total N		mg/ml	% of sol. N	mg/ml
After 6 days							
13.2	2.53	0.33	13.0	64.2	0.28	17.3	
12.6	2.53	0.34	13.4	64.8	0.29	17.7	
10.4	2.40	0.31	12.9	61.7	0.28	18.9	
10.4	2.51	0.31	12.4	60.2	0.28	18.5	
Control *	2.50	0.11	—	8.3	0.04	—	
After 17 days							
13.2	2.50	0.50	20.0	78.8	0.42	21.3	0.42
12.6	2.51	0.51	20.3	78.1	0.42	21.2	0.45
10.4	2.39	0.45	18.8	79.9	0.38	20.0	0.39
10.4	2.50	0.46	18.4	77.6	0.39	20.1	0.40
Control *	2.50	0.15	—	17.8	0.06	—	0.06

* Without bacteria.

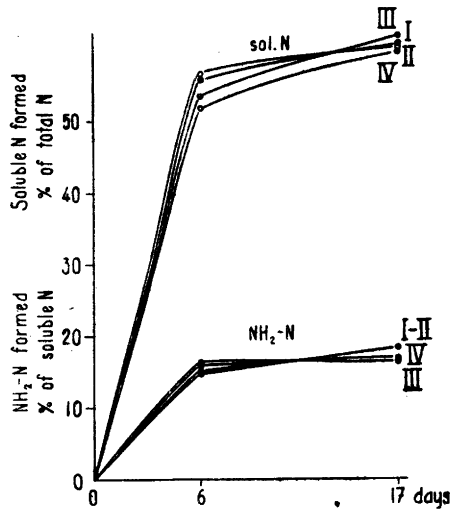

Fig. 3. Formation of soluble and amino nitrogen from casein by E. coli.

Curve I: 13.2 % N of dry bacteria
» *II: 12.6* » » » » »
» *III: 10.4* » » » » »
» *IV: 10.4* » » » » »

DISCUSSION

On the basis of the results obtained in the earlier works it is suggested that the decrease in the protein content of the cells is accompanied by a sharp weakening in the activity of the dispensable enzymes due to the fact that there will no longer be sufficient protein to build up the proteins of all the enzymes. A relatively slight lowering of the rate of respiration [4, 6] with a great decrease in the protein content of the cells is in agreement with this hypothesis since the indispensable enzyme system is then concerned. Good retention of the activity of catalase [1, 2] seems to imply that even this enzyme is indispensable for cells, though its role at the present is very unclear. The observation that the proteolytic enzymes maintain their activity fairly constant though the N-content of the cell varies from 13 to 9.5 % supports the idea that they are indispensable for the cell metabolism irrespective of the nature of nutrition. The proteolytic enzymes of *E. coli* are not adaptive.

The very distinct lessening of the activity of the disaccharide-hydrolyzing enzymes, saccharase [1, 2] and lactase [3], which takes place even when the nitrogen content of the cell lowers only 10—20 % of the optimum value, further confirms the idea that the dispensable enzymes are largely dependent on the protein content of the cells. The function of these enzymes is, according to the present knowledge, to enable the cells to use the particular disaccharides for their nutrition. If these enzymes disappear, the cells are no longer able to grow on the respective disaccharides though they are able to do so by means of other suitable carbon compounds, such as glucose. The said enzymes, arising

through adaptation, widen the living conditions of the bacteria but they are not indispensable for life. When the nitrogen nutrition is very poor the possibilities for the formation of adaptive enzymes seem to be lessened. So, for instance, our strain of *E. coli*, when transferred from lactose to lactose with only 0.1 g ammonium sulphate per 5 litres, starts to grow more slowly than when transferred from lactose to corresponding glucose nutrient solution. And yet the velocity of growth is almost the same in both solutions when the ammonium sulphate amount is high.

The enzyme decomposing formic acid disappears completely from our strain of *E. coli*, according to De Ley [4], when the N-content of the cell falls only 10 %. This enzyme is also adaptive and evidently not indispensable for life under all conditions. The rapid decrease in the anaerobic fermentation accompanying the drop in the N-content of the cell, and its total cessation when the N-content falls about 40 % is particularly noteworthy, as mentioned already at the beginning of this paper. According to our findings, the less the N-supply of the nutrient solution the more oxygen is needed by *E. coli* for its growth. These findings are in full accordance with those of De Ley on the disappearance of anaerobic fermentation.

The examination of the enzymatic activity of low-nitrogen bacteria and other organisms and the comparison with the respective normal nitrogen cells seems to open new lines for research in many directions.

SUMMARY

The proteolytic enzyme system of *E. coli* (strain K_3) retains its activity practically constant while the N-content of the cells falls from 13 % to 9.5 %. The dispensable enzymes, saccharase and lactase, decrease very sharply while the N-percentage lowers to 11.5 or more. The adaptive enzymes which are necessary only in definite nutritional conditions seem, as a rule, to decrease powerfully or to disappear entirely with the lowering of the N-content of the cells.

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Received March 14, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 279—296

Dimerization of α-Methyl-*p*-methylstyrene by Formic Acid and Structure of the Dimers Obtained

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Several reports have been published on the dimers of α-methyl-*p*-methylstyrene. Most of the papers deal with their production. Their constitution has attracted less notice. The dimers of the lower homolog, α-methylstyrene, have been studied more thoroughly. Because, however, the styrenes in question are analogous in regard to their polymerizing properties, there is good reason to consider also the reports on the dimers of α-methylstyrene when those of the higher homolog are being examined.

Errera [1] obtained an oily dimer distilling at 350° without decomposition by heating α-methyl-*p*-methylstyrene with hydrobromic acid (d. 1.59) at 190—200° in a sealed tube.

Klages [2] states that by heating dimethylphenylcarbinol with syrupy phosphoric acid to 120° an oily unsaturated dimer (that of α-methylstyrene), b. p. 302° was produced. For this he proposed the following structure:

$$C_6H_5C(CH_3) = CHCH_2CH(CH_3)C_6H_5$$

Tiffeneau [3] obtained a solid saturated dimer, m. p. 40°, by adding α-methyl-*p*-methylstyrene to cold concd. sulphuric acid. Similar reaction in which α-methylstyrene was employed yielded a dimer [4], m. p. 52°. For the latter product he proposed the structure of diphenyl-dimethylcyclobutane.

Staudinger and Breusch [5] reported in a paper on the polymerization of α-methylstyrene that the action of stannic chloride on α-methylstyrene yielded a solid dimer, m. p. 52°. They, too, assumed this product to be diphenyl-dimethylcyclobutane. On the other hand by using floridin, titanium tetrachloride or boron trichloride as catalysts, an oily unsaturated dimer was produced for which they proposed the structure:

$$C_6H_5C(CH_3) = CHCH_2(CH_3)CHC_6H_5$$
or $$C_6H_5CH(CH_3)CH = CH(CH_3)CHC_6H_5$$

The latest reports on the constitution of the two dimers of α-methylstyrene are due to Bergmann, Taubadel and Weiss [6]. The cyclic saturated dimer was prepared by them according to Staudinger *et al.*, and characterized as 1,1,3-trimethyl-3-phenylhydrindene. The oily unsaturated dimer produced by the Klages method was shown by the above authors on the basis of ozonization to be 2,4-diphenyl-4-methylpentene-2 in analogy with the unsaturated dimer of assym. diphenylethylene for which the corresponding structure was established already by Lebedew [7]. Furthermore, according to the authors the unsaturated dimer could be converted into the saturated one either with stannic chloride or aluminium chloride.

Puranen [8-10] has extensively studied the saturated dimer (dicymene) of α-methyl-*p*-methylstyrene. He obtained this dimer from cymene with nitrosyl sulphuric acid. According to his opinion cymene was hereby first oxidized to dimethyl-*p*-tolylcarbinol which then under the influence of concd. sulphuric acid was either directly condensed to the dimer or by elimination of water first converted to α-methyl-*p*-methylstyrene which subsequently dimerized. On the basis of experimental observations Puranen established for this dimer the structure 1,3,3,6-tetramethyl-1-*p*-tolylhydrindene in accord with the corresponding dimers of assym. diphenylethylene and α-methylstyrene.

In a treatise on the polymerization of α-methylstyrene Hersberger, Reid and Heiligmann [11], referring to the paper of Bergmann *et al.*, used the formula 2,4-diphenyl-4-methylpentene-1 for the unsaturated dimer of α-methylstyrene in a polymerization reaction between the dimer and maleic anhydride. This is incorrect in so far as the formula deduced by Bergmann *et al.*, is 2,4-diphenyl-4-methylpentene-2, not -1. The compound put forward by Hersberger *et al.*, is not reported in literature.

Recently a method [12] has been patented for producing unsaturated dimers of α-alkyl styrenes (including α-methyl-*p*-methylstyrene and α-methylstyrene). According to the patent report the unsaturated dimers were obtained substantially free of cyclic saturated dimers by intimately contacting an α-alkyl styrene with sulphuric acid of 30 % to 65 % concentration at temperatures between 65 and 105° C. In the case of α-methylstyrene, by reaction at 21 to 27° C for 8 hours with 60 % sulphuric acid and a 400 % ratio of acid the yield of the unsaturated dimer was 100 %. In addition, by using 80 % sulphuric acid at 24 to 38° C. α-methylstyrene could be quantitatively converted into its cyclic saturated dimer, m. p. 52—54°. The increase both in acid concentration and reaction temperature was stated to promote the formation of the cyclic saturated dimer.

A still later report [13] in the patent literature deals with the production of a solid dimer from α-methyl-p-methylstyrene. Accordingly a »new» dimer was prepared from α-methyl-p-methylstyrene in yields of 90—95 % by treating this at 200—250° C with an activated diatomaceous earth (2—10 %), *e. g.* fuller's earth or floridin. The dimer obtained was a white crystalline solid, m. p. 40—41°. Because Tiffeneau [3] reported the melting point of his dimer to be 40° and Puranen [8] that of his 37.5—38.5° there is every probability that the compounds in question in all three cases are identical. Moreover, the invention relates to a similar method for producing the solid dimer from the liquid one, which latter was formed in the process described above at temperatures not exceeding 200° C. By treating this at 200—250° in the same manner as above it was converted in yields of 90—100 % into the solid form.

A conclusion based on the reports mentioned above reveals that both α-methyl-p-methylstyrene and α-methylstyrene have been converted into their solid saturated and liquid unsaturated dimers by methods using inorganic compounds mostly of acidic character as dimerizing catalysts. The structure of the solid dimer of both styrenes can be regarded as established. As to the liquid form, in the case of α-methylstyrene one constituent has been fully characterized. This, however, does not by any means exclude the presence of some isomer.

The dimerizing action of formic acid on aromatic ethylene derivatives is not unknown. In spite of considerable time spent on the study of the literature, only one publication concerning this was found. The report of Glichitch [14] describes the action of formic acid on the propenylphenols and phenolethers. It stated that anethole, by boiling with an equal weight of 90 % formic acid for 1 hour, was dimerized to metanethole. Moreover, under the same conditions *iso*eugenol, methyl*iso*eugenol and *iso*safrole were converted to their dimers. On the basis of these experiments Glichitch deduced the conception that the dimerization under the influence of boiling formic acid would be characteristic for phenols and phenolethers possessing a propenyl side chain.

DIMERIZATION OF α-METHYL-p-METHYLSTYRENE BY FORMIC ACID

In the study described in this paper, the starting material used in majority of dimerization experiments was prepared by catalytic oxidation of cymene with air at 80—100° and by removal of the acids and higher boiling polymerization and condensation products as well as the bulk of the unchanged cymene from the oxidation product. The liquid thus obtained contained 62 % of dimethyl-p-tolylcarbinol and 35 % of p-methylacetophenone the rest being

primarily unchanged cymene still present. The intension was namely to study the possibilities for production of the dimers directly from the tertiary alcohol in presence of the ketone. At the same time some experiments were carried out by using for dimerization pure α-methyl-*p*-methylstyrene prepared by catalytic removal of water from the tertiary alcohol in the above mixture, followed by separating the α-methyl-*p*-methylstyrene formed from the ketone by fractional distillation.

In this connection it may be pointed out that the present study originated in experimenting with various methods for removing water from the tertiary alcohol in the above mixture. In these tests a method proposed by Eisenlohr and Schulz [15], too, was tried. They claimed that α-methyl-*p*-methylstyrene could be obtained by dissolving 20 g of dimethyl-*p*-tolylcarbinol in 50 g of 90 % formic acid and distilling the resulting mixture. However, the experiment carried out by using the mixture of the tertiary alcohol and the ketone instead of the pure alcohol gave not at all the styrene in question but its unsaturated dimer in a good yield.

After many trials it was found that the best way for producing α-methyl-*p*-methylstyrene from the above mixture was to distil this under diminished pressure through a catalyst tube filled with activated alumina and maintained at a proper temperature (see Experimental). The yield was 93 %. By using potassium bisulfate as dehydrating agent markedly more polymerization products were formed.

I. Dimerization experiments using the mixture of dimethyl-*p*-tolylcarbinol and *p*-methylacetophenone as starting material

A mixture of the starting material and formic acid was distilled first under atmospheric pressure until the acid was removed, then under diminished pressure for separating the dimer fraction from the ketone and higher polymers (see Experimental). In the dimer fraction the quantity of unsaturated dimer was determined by means of bromine titration.

Results from 9 experiments are reported in the following table (Table 1). For comparison a table (Table 2) of Hersberger *et al.*[12] concerning the dimerization of α-methylstyrene by sulphuric acid is included.

In the optimum cases (experiments 3 and 4 in Table 1) the formic acid method gave a yield of unsaturated dimer exceeding 80 %. The concentration of acid was 95 % and its quantity 20—33 % of that of the tertiary alcohol-ketone mixture used. Upon decreasing the quantity of the acid (experiments

Table 1. Dimerization experiments with the mixture of dimethyl-p-tolylcarbinol and p-methylacetophenone.

No.	Formic acid		Reaction products			
	Concentration	Quantity	Unconverted + monomer	Dimers		Trimers + polymers
				Unsat.	Sat.	
	%	%	%	%	%	%
1	95	133	4	26	47	23
2	95	67	7	66	8	19
3	95	33	6	84	0	10
4	95	20	12	81	0	7
5	95	13	59	37	0	4
6	95	7	80	18	0	2
7	70	29	34	61	0	5
8	95	133	0	5	73	22
9	95	67	8	79	1	12

Experiments 1—7 were done by distilling the reaction mixture directly.
Experiment 8 was done by refluxing the reaction mixture for 2 hours.
Experiment 9 was carried out at room temperature.
Unconverted among the reaction products was calculated as α-methyl-p-methylstyrene.

Table 2. Dimerization of α-methylstyrene by sulphuric acid. Hersberger, Hill and Heiligmann [12].

Sulphuric acid		Reaction		Per cent unconverted	Per cent unsat. dimer	Per cent sat. dimer
Concentration	Quantity	Temp. °F	Time h			
%	%					
30	400	170—190	4	60	40	0
40	400	170—190	4	57	43	0
50	400	122—180	6	0	99	1
60	400	122—180	6	0	98	2
60	3	150—180	6	88	12	0
60	6	150—180	6	80	20	0
60	100	150—180	6	23	75	2
70	400	180—190	6	0	10	90
50	400	70— 80	8	80	20	0
60	400	70— 80	8	0	100	0
70	400	75—100	8	0	90	10
80	400	75—100	5	0	0	100

5 and 6) plenty of undimerized α-methyl-*p*-methylstyrene was obtained. On the other hand when more acid was used, saturated dimer was formed in increasing quantity at the expense of the unsaturated one (see later the isomerization of the unsaturated dimer into the saturated one by formic acid). Prolongation of the reaction time had a similar effect (experiment 8). It is therefore possible to prepare also the saturated dimer directly from the tertiary alcohol-ketone mixture although in a considerably poorer yield owing to the formation of appreciable quantities of higher polymers as by-products.

If the results of the experiments 2 and 9 in Table 1 are compared with each other, it is seen that the increase in temperature had hardly any effect on the reactivity of the starting material but did have an effect on the ratio of the reaction products.

When the formic acid method is compared with the sulphuric acid method, it can be stated that for achieving the same yield of dimers an appreciably smaller amount of acid was sufficient in the former case. The reason, however, is not to be sought in the possibly different dimerizing actions of those acids. The main reason is that in the formic acid method the starting material for dimer (dimethyl-*p*-tolylcarbinol) forms with formic acid (also in the presence of *p*-methylacetophenone) a fully homogeneous mixture resulting in the best possible reaction conditions. Hereby the intimate contacting of the reactants, an important factor in the sulphuric acid method according to the patent report [12], becomes unnecessary.

From the technical standpoint the formic acid method is markedly simpler than the sulphuric acid method. In the former, distillation is the only operation and both the distillation of formic acid under atmospheric pressure and the fractionation of the reaction products under diminished pressure can be carried out in the same distillation apparatus. Formic acid can be recovered. It will be in diluted form owing to the cleavage of water from dimethyl-*p*-tolylcarbinol (the dilution in experiment 3 from 95 % to 78 %). In the sulphuric acid method according to the patent report [12] the following operations are to be taken into consideration:

1. Intimate contacting of the reactants at a defined temperature.
2. Separation of the dimer layer from the acid layer.
3. Neutralization of the dimer layer with a solution of a base.
4. Fractionation of the reaction products under diminished pressure.

The acid can be recovered in this method without dilution.

II. Dimerization experiments using α-methyl-p-methylstyrene as starting material

The α-methyl-*p*-methylstyrene employed was 98 % pure, prepared by catalytic dehydration of dimethyl-*p*-tolylcarbinol (see Experimental). Cymene was the principal impurity.

Because α-methyl-*p*-methylstyrene was insoluble in formic acid the situation in this respect was comparable with the dimerization of α-methylstyrene by sulphuric acid. Three experiments were carried out resulting in following data:

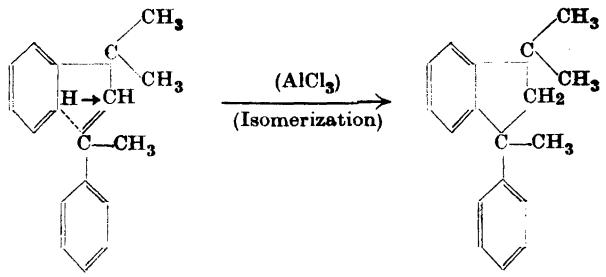
Table 3. Dimerization of α-methyl-p-methylstyrene by formic acid.

No.	Formic acid		Reaction products			
	Concentration	Quantity	Unconverted	Dimers		Trimers + polymers
				Unsat.	Sat.	
	%	%	%	%	%	%
1	95	133	0	4	63	33
2	95	18	5	40	37	18
3	82	51	7	83	6	4

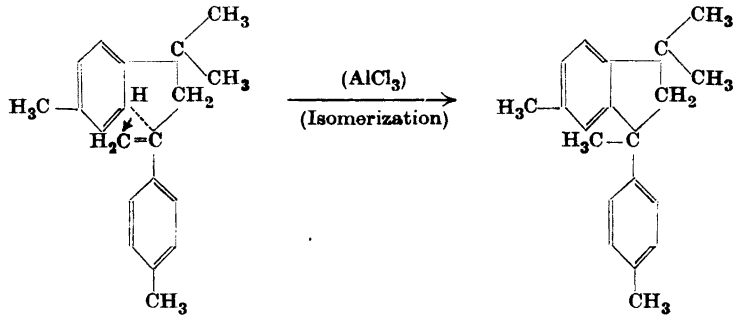
The results indicate that 95 % formic acid is too concentrated giving rise to a strong formation of polymers. In addition, because in this case no dilution of the acid takes place, the original concentration of acid can be taken smaller. The acid can be reused immediately after distillation. From the technical point of view this method is therefore simpler than the formic acid method using dimethyl-*p*-tolylcarbinol as starting material.

ISOMERIZATION OF THE UNSATURATED DIMER OF α-METHYL-p-METHYLSTYRENE INTO THE SATURATED DIMER

Trials were made with powdered aluminium chloride or formic acid as isomerizing agents. Both gave positive results. The latter, however, proved decisively simpler and better. Sulphuric acid was also used but only at a low temperature; the bulk of the dimer was then converted into a sulphonic acid. Aluminium chloride gave analogous results to those achieved by Bergmann *et al.* [6] in the case of the unsaturated dimer of α-methylstyrene. The reaction mechanism proposed by them was as follows:



An analogous scheme may be true in the case of the unsaturated dimer of *α*-methyl-*p*-methylstyrene. However, on the basis of the structures established for it in this investigation (see later) the following possibility may be considered just as probable, *viz.:*



ESTABLISHMENT OF THE STRUCTURE OF THE DIMERIC *α*-METHYL-*p*-METHYLSTYRENES OBTAINED BY FORMIC ACID

I. Solid, saturated dimer

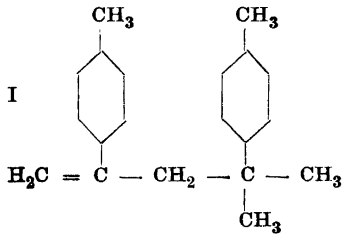
The solid dimer obtained by formic acid from three different starting materials, dimethyl-*p*-tolylcarbinol, *α*-methyl-*p*-methylstyrene and its liquid dimer, was found to be the same compound in every case and identical with the »dicymene» prepared from cymene according to the directions given by Puranen [6]. This being the case the solid dimer must have the structure 1,3,3,6-tetramethyl-1-*p*-tolylhydrindene.

II. Liquid, unsaturated dimer

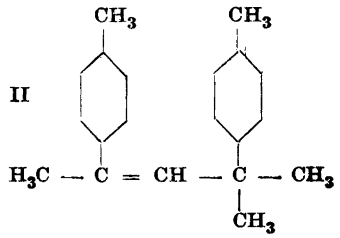
The structure of this dimer was established by ozonization. When the ozonide had been decomposed with water the following products could be identified:

1. Formaldehyde.
2. α-Oxo-γ-methyl-α,γ-di-p-tolyl-butane.
3. p-Methylacetophenone.
4. Dimethyl-p-tolylacetaldehyde.
5. Dimethyl-p-tolylacetic acid.
6. p-Toluic acid.

On the basis of these decomposition products the starting dimer must be a mixture of two isomeric hydrocarbons having the following formulas:



2,4—di—(p—tolyl)—
4—methyl—pentene—1



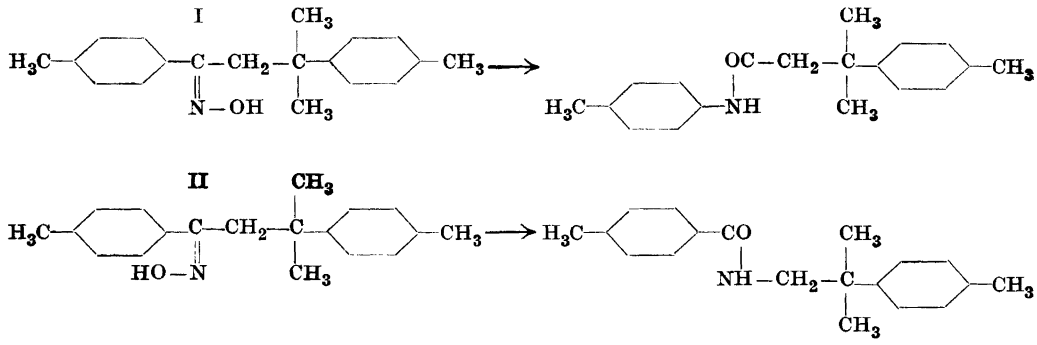
2,4—di—(p—tolyl)—
4—methyl—pentene—2

The fragments of the former are formaldehyde and α-oxo-γ-methyl-α,γ-di-p-tolyl-butane, those of the latter p-methylacetophenone and dimethyl-p-tolyl-acetaldehyde. p-Toluic acid and dimethyl-p-tolylacetic acid are secondary products.

On the basis of the amounts of the two ketones the quantities of both isomers in the starting mixture were calculated. Accordingly, it was found to contain 66 % of the isomer I and 13 % of the isomer II. These are, however, to be considered as minimum values and only as approximative ones, because the secondary products are not taken into account. Based on the figures obtained the ratio of the isomers I and II in the mixture will be 5 : 1. Neither of these isomers has been previously reported in the literature. They couldn't be separated by fractional distillation since their boiling points are very close to each other.

Because α-oxo-γ-methyl-α-γ-di-p-tolyl-butane has not been previously reported in the literature, the structure of the higher boiling ketone isolated from the ozonization products had to be established. To that purpose the ability of the ketoximes to undergo the Beckmann rearrangement was made use of. By hydrolyzing the acid amides formed and identifying the hydrolysis products it should be possible to provide evidence of the structure of the original ketone.

According to Meisenheimer [16] the Beckmann rearrangement of α-oxo-γ-methyl-α,γ-di-*p*-tolyl-butane oximes (two possible stereoisomeric forms) into acid amides would proceed as follows:



However, only one oxime of the ketone under investigation could be obtained and accordingly only one acid amide could be isolated from the rearrangement products. When the acid amide was hydrolyzed by a solution of potassium hydroxide in methanol, *p*-toluidine and an acid (m. p. 70.0—70.6°) were obtained as hydrolysis products. The latter was proved to be β-*p*-tolyl*iso*valeric acid (not previously reported) by synthesizing it in another way. Only minute amounts of *p*-toluic acid could be isolated from hydrolysis products giving, however, evidence for the existence of the other stereoisomeric oxime (II) expected.

At this point it may be mentioned that on carrying out the acid amide hydrolysis with 70 % sulphuric acid *p*-toluidine was certainly obtained in a good yield but the acid hardly at all. Instead of this, plenty of a neutral compound, m. p. 42—43°, was obtained. This was identified as 3,3,6-trimethylindanone-1 (not previously reported) afforded by the intermediate β-*p*-tolyl*iso*valeric acid through intramolecular water cleavage. The same compound could be prepared in a 80 % yield directly from β-*p*-tolyl*iso*valeric acid by means of concentrated sulphuric acid and also from the acid chloride by the usual method (Friedel-Crafts reaction).

The liquid dimer proved to correspond to its simplest aliphatic analogue, di*iso*butylene. Butlerow [17], Whitmore *et al.* [18–20] and Mc. Cubbin and Adkins [21] have shown that the dimer obtained from *iso*butylene by the sulphuric acid method consists of 2,4,4-trimethylpentene-1 and 2,4,4-trimethylpentene-2 in a ratio of 4 : 1.

It seems possible that the liquid dimer of α-methylstyrene investigated by Bergmann *et al.*[6] would also have a similar composition although they were

able to prove the existence of only one isomer, 2,4-diphenyl-4-methyl-pentene-2. The reason was that they isolated the ozonization products as the semicarbazones after a reaction time of 20 hours. In the present work, however, it turned out that the semicarbazone of α-oxo-γ-methyl-α,γ-di-p-tolyl-butane was formed very slowly. Only after two weeks did it begin to separate (see Experimental). This being the case it is to be thought that the corresponding ketone, α-oxo-γ-methyl-α,γ-diphenyl-butane, has escaped the notice of Bergmann *et al.*

DECOMPOSITION OF THE LIQUID DIMER OF α-METHYL-p-METHYLSTYRENE BY ATMOSPHERIC DISTILLATION

When the dimer was heated to its boiling point (about 330°) at atmospheric pressure, decomposition resulted. A light, colorless liquid, b. p. 176—189°, slowly distilled over. Its main constituents were proved to be p-cymene and α-methyl-p-methylstyrene. Accordingly the dimer had reconverted into its monomer. The dimer also decomposed in another way yielding p-cymene. The corresponding acetylenic fragment could not be identified. It was probably polymerized and remained in the tarry residue amounting to one half of the starting material.

EXPERIMENTAL

General dimerization method

75 g of the mixture of dimethyl-p-tolylcarbinol and p-methylacetophenone were placed in a 200 ml round-bottomed flask and formic acid was added (the acid used was a product of Schering-Kahlbaum, d. 1.22, about 95 %). At first a clear solution was formed. Depending on the acid quantity used, the solution got turbid either at room temperature or when heated to boiling, the resulting dimer separating as a distinct layer. The bulk of the formic acid was distilled off on an oil bath at atmospheric pressure by using a 40 cm high fractionating column filled with porcelain rings. The ketone and dimer fractions were distilled at diminished pressure. The residue consisted of trimers and higher polymers. p-Methylacetophenone remained unchanged throughout the process. The dimer fraction had a boiling point of 170—180° at 5 mm pressure; the boiling point of the saturated dimer was about 5° lower than that of the unsaturated one. The unsaturation of the dimer fraction was determined by means of bromine titration [22] and it was on this basis the quantities of the saturated and unsaturated dimers were calculated.

In experiments with α-methyl-p-methylstyrene the method employed was a similar one except that after the addition of formic acid, the mixture was refluxed for 1 hour owing to a smaller reaction velocity as a result of the insolubility of the hydrocarbon in the acid. Because the boiling acid layer was under the hydrocarbon layer, comparatively effective mixing was caused by refluxing.

Dimerization at room temperature

75 g of the tertiary alcohol-ketone mixture and 50 g of 95 % formic acid were mixed and placed immediately into a graduate. 50 minutes after the mixing, the solution became turbid and began to separate into two layers. Three hours from the beginning the boundary between the two layers had reached its lowest level. After the acid had been removed by neutralization, the layers were separated and analyzed. The dimer, ketone and acid were divided into the two layers as follows:

	Dimer layer	Acid layer
Dimer	95 %	5 %
Ketone	48 »	52 »
Acid	4 »	96 »

Production of α-methyl-*p*-methylstyrene from the tertiary alcohol-ketone mixture

435 g of the mixture (b. p. 96—111°/8 mm), containing 62 % of the tertiary alcohol, were vaporized *in vacuo* (13 mm). The vapors were passed through a Pyrex tube 60 cm long and 1 ½ cm in diameter, packed with coarse (6—8 mesh) granules of alumina and maintained at a temperature of 220—230°. The cooling system consisted of a spiral condenser followed by a suction flask kept in an ice-salt mixture. After the aqueous layer (28 g) had been removed, the distillate was dried with anhydrous sodium sulphate and fractionated at atmospheric pressure. 230 g (93 %) of α-methyl-*p*-methylstyrene, b. p. 189—191° and 128 g of *p*-methylacetophenone, b. p. 224—225°, were obtained.

Conversion of liquid dimer to solid dimer

1. Isomerization with aluminium chloride

9.5 g of the liquid dimer were dissolved in 10 ml of dichloroethane and 5 ml of a 5 % solution of aluminium chloride in dichloroethane were added with stirring. The mixture was allowed to stand for 2 hours. 3 g of a crystalline material, m. p. 38—39°, were isolated from the reaction product.

$C_{20}H_{24}$	Calc.	C 90.9	H 9.1	Mol. wt. 264
	Found	» 90.8	» 9.0	» » 257

It was shown by mixed melting point to be identical with authentic 1,3,3,6-tetramethyl-1-*p*-tolylhydrindene.

2. Isomerization with concentrated sulphuric acid

150 ml of concd. sulphuric acid (d. 1.84) were allowed to act on 25 g of the liquid dimer for 3 days at — 5° and then for 14 days at room temperature. Only 2.5 g (10 %) of the solid dimer, m. p. 38—39° were obtained. In addition, 2.5 g of an unsaturated (one double bond) hydrocarbon, b. p. 98—100°/8 mm, mol. wt. 167, was isolated from the reaction product. The remainder was found to be sulphonated. Upon diluting with water and neutralizing with ammonia 20 g of an ammonium salt of a sulphonic acid could be isolated.

3. Isomerization with formic acid

40 g of the liquid dimer were refluxed with 52 g (130 %) of 95 % formic acid for 2 hours. When the formic acid had been distilled off at atmospheric pressure and the remainder had been fractionated *in vacuo*, 37 g (92 %) of a dimer fraction containing 95 % of the saturated dimer and 5 % of the unsaturated one were obtained. 8 % of the starting material had been further polymerized.

Structure of the liquid unsaturated dimer

After the liquid dimer had been fractionally distilled in vacuo, it was a colorless viscous oil, b. p. 181—186°/7 mm. As freshly prepared it was odorless but on longer standing an intense smell of formaldehyde became noticeable.

$C_{20}H_{24}$	Calc.	C 90.9	H 9.1	Mol. wt. 264
	Found	» 91.0	» 8.9	» » 248

The double bond determination by perbenzoic acid method gave 1.01 double bonds for one dimer molecule.

Ozonization of the unsaturated dimer

20.0 g of the dimer (b. p. 186—191°/8 mm) were dissolved in 200 ml of glacial acetic acid. Ozonized air containing about 2 % of ozone was passed through the solution until the double bonds had disappeared. The reaction vessel was cooled in cold water.

The solvent was evaporated *in vacuo* at room temperature by using a mercury vacuum pump. The residue was a viscous, yellow liquid.

The ozonide was decomposed by dropping it with stirring into a boiling mixture consisting of 150 ml of water, 5 g of zinc dust, 26 mg of silver nitrate and 17 mg of hydroquinone. Steam was then passed through the mixture until the volatile substances had come over. The steam distillate had a strong smell of formaldehyde. The resorcinol and Schiff tests for formaldehyde were positive. By iodometric titration the distillate contained 0.98 g of formaldehyde.

The steam distillate was extracted with ether and the ethereal extract shaken with saturated sodium bicarbonate solution to separate the acids formed. From the neutral extract, after evaporation of the solvent, the carbonyl compounds were isolated as the semicarbazones. 1.88 g of a crystalline precipitate were separated during 24 hours. Once crystallized from ethanol it melted at 204—205° and was shown by mixed melting point to be identical with authentic *p*-methylacetophenone semicarbazone.

The alcoholic mother liquor of the semicarbazone was steam distilled in order to remove the volatile compounds. Dilute sulphuric acid was added to the remainder and a second steam distillation carried out. This time a pleasent smelling oil giving a positive Schiff test for aldehydes passed over. It afforded 0.35 g of a semicarbazone, m. p. 171.5° when once crystallized from methanol. It gave no depression when mixed with authentic dimethyl-*p*-tolylacetaldehyde semicarbazone.

From the ozonization product non-volatile with steam, zinc dust was filtered off and washed with ether. The ethereal filtrate was shaken with saturated sodium bicarbonate solution to separate the acids. These were combined with the acids obtained from the

volatile part. Thus a total of 0.92 g of acids were obtained. After a distillation *in vacuo* and a lengthy fractional crystallization the acid mixture could be divided into two parts:

I Part: 0.4 g, m. p. 179—180° when crystallized from benzene. It gave no depression when mixed with authentic *p*-toluic acid.

$C_8H_8O_2$	Calc.	C 70.6	H 5.9	Neut. equiv.	136
	Found	» 70.6	» 5.8	» »	136

II Part: 0.35 g, m. p. 80—81° after several recrystallizations from petrol ether. It was shown by mixed melting point to be identical with authentic dimethyl-*p*-tolylacetic acid.

After separation of the acids from the ozonization product non-volatile with steam, the remainder was distilled in vacuo. Thereby 13.3 g of a yellow oil, b. p. 201—205°/7 mm passed over. This oil was considerably more viscous than the original dimer. It had a pleasent although faint odor. It was scarcely volatile with steam. The Schiff test for aldehydes was negative.

$C_{19}H_{22}O$	Calc.	C 85.7	H 8.3	Mol. wt.	266
	Found	» 85.7	» 8.2	» »	253

α-Oxo-γ-methyl-α-γ-di-p-tolyl-butane semicarbazone

The semicarbazide acetate prepared by dry mixing of 0.84 g of semicarbazide hydrochloride and 0.74 g of potassium acetate was dissolved in 8 ml of ethanol. The potassium chloride precipitated was filtered off. 2.0 g of the above ketone were added and the mixture was allowed to stand at room temperature. It was only after 2 weeks that crystals began to separate from the solution. 1.06 g of the semicarbazone were obtained which on recrystallization from ethanol formed clusters of white needles, m. p. 173—174°.

Oximation of α-oxo-γ-methyl-α,γ-di-p-tolyl-butane

A mixture consisting of 6.44 g of the ketone, 1.86 g of hydroxylamine hydrochloride, 10 ml of abs. ethanol and 10 ml of pyridine was heated on a boiling water bath under reflux for 2 hours. The solvents were distilled off in vacuo. The remainder afforded on crystallization from ligroin 5.1 g of an oxime. On recrystallization from a mixture of petrol ether and ether the oxime formed white needles, m. p. 124—125°. The other stereoisomeric oxime could not be isolated by fractional crystallization.

$C_{19}H_{23}NO$	Calc.	C 81.1	H 8.2	N 4.97	Mol. wt.	281
	Found	» 81.2	» 8.0	» 4.92	» »	276

Beckmann rearrangement of the oxime

2.3 g of the oxime were dissolved in 23 ml of abs. ether. Powdered phosphorus pentachloride was added until it did not disappear. 2.0 g of an acid amide were obtained. When crystallized from ligroin it formed clusters of long, white needles, m. p. 128—129°.

$C_{19}H_{23}NO$	Calc.	C 81.1	H 8.2	N 4.97
	Found	» 80.8	» 8.0	» 5.02

By means of hydrolysis the acid amide was shown to be β-*p*-tolyl*iso*valeric acid *p*-toluidide.

Hydrolysis of the acid amide

1. With hydrochloric acid:

0.8 g of the acid amide and 16 ml of concentrated hydrochloric acid were refluxed for 2 hours. Only about 3 % of the amide were hydrolyzed.

2. With sulphuric acid:

A mixture of 0.77 g of the acid amide and 8 ml of 70 % sulphuric acid was refluxed for one-half hour. 0.04 g of an acid, m. p. about 70°, 0.24 g of an amine and 0.32 g of a neutral compound were obtained as reaction products.

The amine: On crystallization from petrol ether it formed large plates, m. p. 43—44°.

C_7H_9N	Calc.	C 78.5	H 8.4	N 13.03
	Found	» 78.5	» 8.3	» 13.10

It was shown by mixed melting point to be identical with authentic *p*-toluidine. The yield of *p*-toluidine on hydrolysis was 81 %.

The neutral compound: It crystallized from petrol ether in large plates, m. p. 42—43°.

$C_{12}H_{14}O$	Calc.	C 82.7	H 8.1	Mol. wt. 174
	Found	» 82.5	» 8.1	» » 167

It was shown by mixed melting point to be identical with 3,3,6-trimethylindanone-1 synthesized by the Friedel-Crafts reaction from β-*p*-tolyl*iso*valeric acid chloride. The compound formed a semicarbazone, m. p. 214—215° (with decomposition) when crystallized from ethanol. The yield of indanone on hydrolysis was 67 %.

3. With a solution of potassium hydroxide in methanol:

0.2 g of the acid amide and 10 ml of a 25 % solution of potassium hydroxide in pure methanol were kept in a sealed tube at 70° for 3 days. Only 10 % of the acid amide were hydrolyzed.

0.5 g of the acid amide (not recrystallized) and 20 ml of the potassium hydroxide solution mentioned above were kept in a sealed tube at 130° for 24 hours. 97 % of the amide were hydrolyzed. The yield of *p*-toluidine was 70 %. 0.32 g of an acid were obtained, m. p. 67—69° (crude product). On fractional crystallization minute amounts of *p*-toluic acid could be isolated. After three recrystallizations from petrol ether the main product was obtained as large, almost colorless crystals, m. p. 76.0—76.6°.

$C_{12}H_{16}O_2$	Calc.	C 75.0	H 8.4	Neut. equiv. 192
	Found	» 74.9	» 8.3	» » 192

The acid gave no depression in melting point when mixed with β-*p*-tolyl*iso*valeric acid. The yield of the acid on hydrolysis was 93 %.

Synthesis of β-p-tolylisovaleric acid

From the mixture of dimethyl-*p*-tolylcarbinol and *p*-methylacetophenone the latter was removed as the semicarbazone. The crude carbinol thus obtained was converted into dimethyl-*p*-tolylchloromethane by saturation with dry hydrogen chloride at a temperature below 0°. β-*p*-tolyl*iso*valeric acid was prepared from this by the malonic ester synthesis according to the directions given by Hoffman [23] for producing the corresponding phenyl derivative. A dicarbonic acid was formed as an intermediate. This was first obtained as an oil which on long standing solidified to a mass of oily crystals. After two recrystallizations from benzene (the separation of the crystals took place very slowly) small hemispherical crystals were obtained, m. p. 131.5—132.5° (with decomposition).

$C_{13}H_{16}O_4$	Calc.	C 66.0	H 6.8	Neut. equiv. 118
	Found	» 66.2	» 6.7	» » 120

On the basis of its synthesis and the analytical figures obtained the dicarbonic acid must have the structure β-methyl-β-*p*-tolylpropane — α,α-dicarbonic acid (not previously reported in the literature).

The dicarbonic acid when heated at 200° afforded a monocarbonic acid which on crystallization from petrol ether was obtained as large, colorless crystals, m. p. 75.5—76.0°.

$C_{12}H_{16}O_2$	Calc.	C 75.0	H 8.4	Neut. equiv. 192
	Found	» 75.2	» 8.3	» » 195

The yield of β-*p*-tolyl*iso*valeric acid calculated from the weight of dimethyl-*p*-tolylchloromethane was 15 %. The main product was α-methyl-*p*-methylstyrene produced by elimination of hydrogen chloride from dimethyl-*p*-tolylchloromethane.

Synthesis of 3,3,6-trimethylindanone-1 from β-p-tolylisovaleric acid

A mixture of 0.5 g of β-*p*-tolyl*iso*valeric acid and 2.5 g of concentrated sulphuric acid was kept on a boiling water bath for 3 hours. When cold, the reaction mixture was poured onto ice and extracted with ether. The ethereal extract was shaken with saturated sodium bicarbonate solution to remove the possibly unconverted acid. After evaporation of the ether the indanone was obtained in large, colorless plates, m. p. 42—43°, yield 80 %.

Synthesis of dimethyl-p-tolylacetaldehyde

The synthesis was carried out by known methods [24–26] by starting from *p*-methylacetophenone.

Synthesis of dimethyl-p-tolylacetic acid

The above aldehyde was oxidized to the corresponding acid by silver oxide according to Delépine and Bonnet [27]. On crystallization from petrol ether the acid was obtained as coarse crystals, m. p. 80.5—81.5°, yield 98 %.

$C_{11}H_{14}O_2$	Calc.	C 74.1	H 7.9	Neut. equiv. 178
	Found	» 74.0	» 7.8	» » 178

Rupe and Bürgin [28] reported the melting point of dimethyl-*p*-tolylacetic acid to be 70—71° »after previous sintering». Obviously the sample must have been impure because the melting point now found is 10° higher.

Decomposition of the unsaturated dimer by atmospheric distillation

The dimer (36 g) was heated to its boiling point in a distilling flask equipped with a 20 cm fractionating column. A light, colorless oil (17.8 g), b. p. 176—189°, slowly distilled over. According to bromine titration the unsaturation of the distillate was 33 % (calculated as α-methyl-*p*-methylstyrene).

Identification of the decomposition products

6.0 g of the above distillate were dissolved in 60 ml of chloroform and ozonized until the solution ceased to decolorise bromine.

The ozonide was decomposed by boiling with water. Formaldehyde and *p*-methylacetophenone (0.9 g) were found among decomposition products. The latter was isolated as the semicarbazone. Hereby the unsaturated part of the starting material was proved to be α-methyl-*p*-methylstyrene.

The mother liquor of the above semicarbazone afforded the saturated part of the starting material (3.0 g, b. p. 176—177°). On oxidation with $KMnO_4$ 1.1 g of the saturated compound yielded 0.4 g of hydroxycumic acid (m. p. alone and mixed with an authentic specimen 155—156°). Accordingly the saturated part was *p*-cymene.

SUMMARY

I. A method was worked out for production of the two dimers of α-methyl-*p*-methylstyrene either directly from the neutral liquid oxidation product of cymene (a mixture of dimethyl-*p*-tolylcarbinol and *p*-methylacetophenone) or from α-methyl-*p*-methylstyrene by using formic acid as a dimerizing agent. By this method it is possible in both cases to prepare a solid, saturated dimer and a liquid, unsaturated dimer separately. Further, by means of formic acid the liquid dimer can be isomerized into the solid one.

II. The structure of the liquid, unsaturated dimer of α-methyl-*p*-methylstyrene was established. In analogy with di*iso*butylene, the dimer was found a mixture of two isomeric hydrocarbons, 2,4-di-(*p*-tolyl)-4-methyl-pentene-1 and 2,4-di-(*p*-tolyl)-4-methyl-pentene-2 about in a 5 : 1 ratio.

The author wishes to thank Doctor G. A. Nyman for his kind advice in connection with this investigation.

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Received February 3, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 297—305

Short Communications

Molecular Structures of Dodecafluoro-Cyclohexane and Decafluoro-Cyclopentane

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The vapours of dodecafluoro-cyclohexane and decafluoro-cyclopentane have been investigated by the electron diffraction sector method. In Fig. 1 the $\frac{\sigma(r)}{r}$-curve obtained for totally fluorinated cyclohexane is reproduced. Below the distribution curve the r-values of internuclear distances which would occur in the molecule if it retained the »chair» form of the mother hydrocarbon and strictly tetrahedral angles are indicated by vertical lines. The C—C bond distance is assumed to be 1.54 Å and the C—F bond distance is that derived from the $\frac{\sigma(r)}{r}$-curve itself, 1.38 Å. It is easily recognized that the observed $\frac{\sigma(r)}{r}$-curve is explained in a satisfactory way by the assumptions just mentioned. A still better agreement seems to be obtained, however, if a minor distortion of the valency angles is assumed to take place leaving the C—C- and C—F-distances unchanged. This distortion consists in a bending of the ε—C—F-bonds away from the chief axis of the carbon ring, the bending taking place in one of the three planes of symmetry of the undistorted molecule. The symmetry of the model is thus left unchanged by the distortion. The described bending of the C—F bonds is just what might be expected, because the distance between ε—F-atoms in 1,3-position would be only 2.52 Å in the »ideal» structure, a distance smaller than we should expect to find between fluorine atoms not directly linked together.

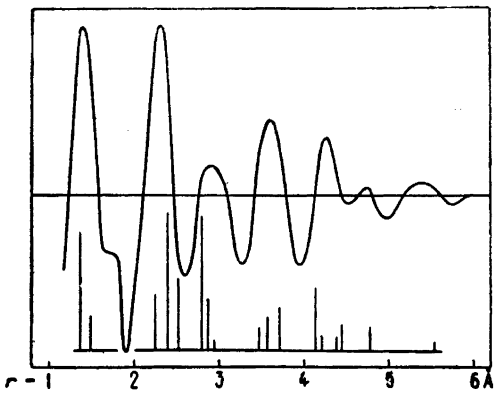
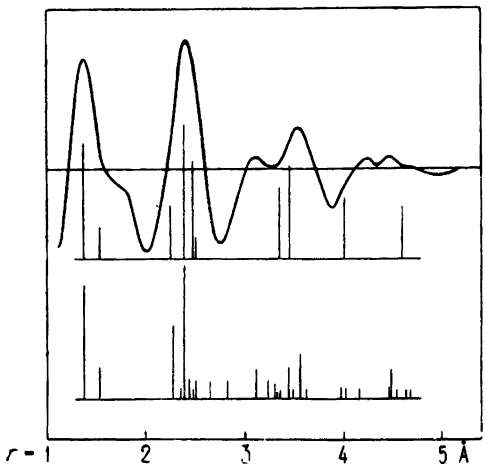


Fig. 1. $\frac{\sigma(r)}{r}$*-curve of dodecafluoro-cyclohexane.*

In Fig. 2 the observed $\frac{\sigma(r)}{r}$-curve for decafluorocyclopentane is reproduced. Here also the C—F bond distance is found equal to 1.38 Å. It seems legitimate to assume a C—C bond distance of 1.54 Å in this case also. If we further try to explain the $\frac{\sigma(r)}{r}$-curve assuming a planar carbon ring and fivefold symmetry of the molecule with valency angle F—C—F equal to 109.5°, the positions of the two first maxima are easily explained.

From Fig. 2, however, in which the r-values corresponding to this structure are



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indicated in the line diagram just below the curve, it is seen that the positions of the remaining peaks are not in accordance with the pentagonal model described above. A less symmetrical structure, however, will give a satisfactory agreement with the experimental curve. We have not been able to demonstrate that the five-membered ring must necessarily be non-planar, but it seems rather probable that a deviation from a planar carbon ring is present. Models having atomic distances in agreement with the experimental curve may be obtained in different ways, all leading to more favourable F—F distances than those of the highsymmetrical model. A definitive solution of the problem can not be given at present based on the electron diffraction method. The model for instance having the internuclear distances represented by the line diagram at the bottom of Fig. 2 seems to be rather satisfactory, but other models may be regarded to be just as probable. The line diagram in the lower part of Fig. 2 corresponds to a model in which the carbon ring is non-planar, the carbon atom 1 being situated below the plane containing the carbon atoms 2, 4, 5 and the carbon atom 3 above this plane. The directions of the C—F bonds are chosen in a way which makes the average value of the shortest F—F distances greater than in the symmetrical model.

The dodecafluoro-cyclohexane used in this investigation was purchased from the Chemistry Department of Purdue University. A sample of very pure decafluoro-cyclopentane was offered us by professor G. H. Cady, Seattle, and we wish to express our gratitude to him for his kindness.

Received April 4, 1949.

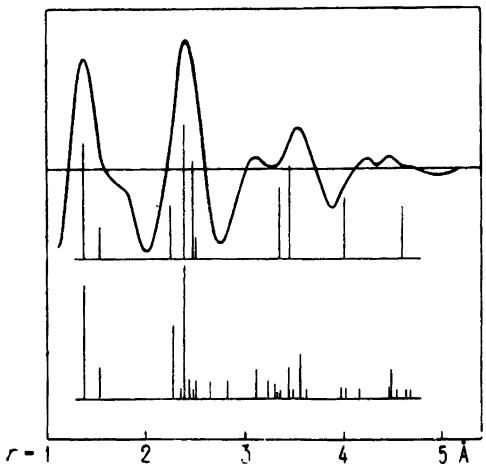
On the Influence of pH and Inhibitors on the Ammonia and Nitrate Assimilation by Azotobacter

TIHAMÉR Z. CSÁKY

Microbiological Institute, Agricultural College, Uppsala, Sweden

Virtanen, Csáky and Rautanen [1] found in low nitrogen *Torula* yeast a relatively more rapid protein formation on nitrate feeding than on ammonia feeding. In the case of nitrate assimilation small amounts of oxime N were found, whereas no formation of oxime N could be detected when ammonia was assimilated. It could therefore be assumed that the nitrate assimilation possibly can proceed in a different way *i. e.* that it is not absolutely necessary to assume that the only way of assimilation of nitrate is the reduction to ammonia and assimilation of the latter.

In order to get some idea of the ammonia and nitrate assimilation by azotobacter, the influence of pH and some inhibitors on the assimilation of nitrate and ammonia by azotobacter has been studied. The bacteria *(Azotobacter chroococcum)* were cultivated on a nutrient medium containing salts and 0.5 % glucose [2, 3]. The same amount of N (5 mg %) was administered



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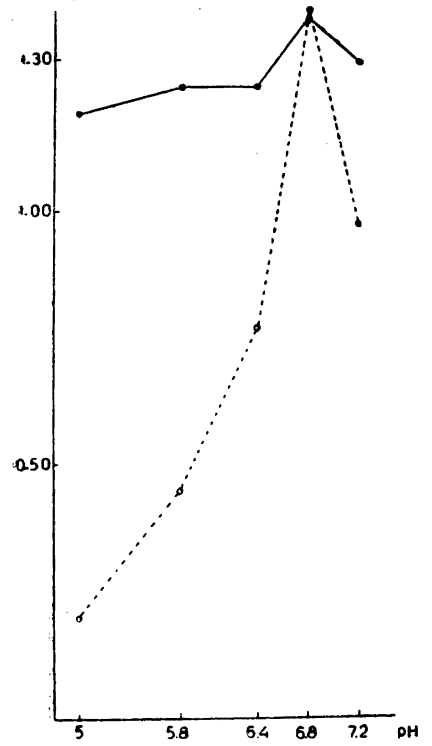

Fig. 1. Growth of azotobacter on ammonia (interrupted line) and nitrate (full line) within 5 days at different pH values. Ordinate: turbidity (extinction), abscissa: pH.

in one case as ammonium sulphate and in the other as potassium nitrate. The growth was registered by measuring the turbidity with an electric photometer.

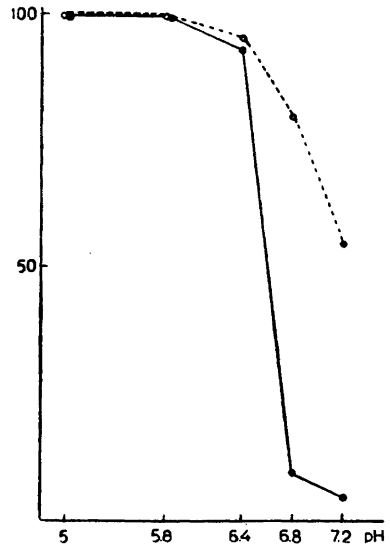

Fig. 2. Inhibitory effect of sodium fluoride (10^{-2} M) on the growth of azotobacter on ammonia (interrupted line) and nitrate (full line) in 5 days experiment at different pH values. Ordinate: inhibition in %; abscissa: pH.

The growth on ammonia is very much influenced by the initial pH of the solution while the growth on nitrate is nearly independent of it (Fig. 1).

Sodium fluoride (10^{-2} *M*) inhibits the growth on ammonia while the assimilation of nitrate is unaffected. Salicylaldehyde (10^{-3} *M*) inhibits the growth on ammonia to 62 %, on nitrate to 22 %, while 8-oxyquinoline (10^{-4} *M*) totally inhibits the nitrate uptake and up to about 59 % the

Table 1. Effect of inhibitors on the growth of azotobacter on ammonia or nitrate feeding within 5 days.

Inhibitors	Initial pH	Turbidity (Extinction)		Inhibition %	
		ammonia	nitrate	ammonia	nitrate
None	6.8	1.000	1.010	—	—
Sodium fluoride 10^{-2} *M*	6.8	0.215	1.020	78	0
Salicylaldehyde 10^{-3} *M*	6.8	0.376	0.775	62	22
8-Oxy-quinoline 10^{-4} *M*	6.8	0.410	0.002	59	100

„Bound" Chlorine in Casein and in Tissue Proteins

VIKTOR MUTT

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Lindahl [1] recently analyzed casein and found that it contained about one per cent chlorine in bound form. In the analytical method used by him the casein was treated with a boiling mixture of chromic and sulphuric acids and the gases evolved passed through a known quantity of silver nitrate containing As_2O_3. The consumption of silver nitrate, assumed to be due to the chlorine in the casein was determined by the Volhard method. As the presence of chlorine in casein does not agree with the generally accepted opinion on the composition of this protein it seemed necessary to check Lindahls results. At the suggestion of Professor E. Jorpes I undertook to control the analytical procedure. Dr. Ragnar Berg who performed the analyses for Lindahl kindly demonstrated his technique in our laboratory, thereby greatly facilitating our work.

Casein, prepared by isoelectric precipitation with acetic acid was analyzed for chlorine by the Carius method. No trace of chlorine could be detected. When the analysis was performed with the technique used by Lindahl a precipitate insoluble in nitric acid was actually formed and the Volhard titration showed a consumption of silver nitrate corresponding to 0.25 per cent chlorine in the casein. It was however observed that the precipitate differed from silver chloride in that it did not darken when exposed to sunlight. When fused with Na_2CO_3 no chloride was obtained in the alkaline filtrate whereas a similar quantity of silver chloride gave a quantitative precipitate with $AgNO_3$ on acidification with HNO_3. Evidently the precipitate formed in the trap with silver nitrate was not silver chloride. Of the different silver salts that could come in question the formiate, carbonate and acetate are readily soluble in dilute nitric acid. The oxalate darkens easily in sunlight. The silver cyanide, however, is practically insoluble in dilute and only slowly soluble in concentrated nitric acid. Moreover it does not darken when exposed to sunlight. Consequently the precipitate was assumed to be silver cyanide. This assumption was confirmed by analyses of the sample. A qualitative test for the cyanide ion with ammonium polysulphide and ferric chloride was positive. On ignition of 31.265 mg the silver residue weighed 25.065 mg or 80.17 per cent. The calculated amount of silver in silver cyanide is 80.57 per cent. In another sample the ignition residue was dissolved in nitric acid, precipitated with hydrochloric acid

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ammonia assimilation (Table 1). The selective inhibitory effect of sodium fluoride on ammonia assimilation largely depends on the initial pH of the solution (Fig. 2).

The experiments show that the pH and the inhibitors have a different influence on ammonia and nitrate assimilation. In some cases the assimilation of ammonia is inhibited in a very high degree while that of nitrate is not disturbed. It seems therefore that even in the case of azotobacter the assimilation of nitrate does not necessarily proceed through ammonia but it can have other ways too.

I am indebted to the *Swedish Natural Science Research Council* for a grant which has financed this work.

1. Virtanen, A. I., Csáky, T. Z., and Rautanen, N. *Acta Chem. Scand.* 2 (1948) 533, *Biochim. Biophys. Acta.* In press.
2. Burk, D., and Lineweaver, H. *J. Bact.* 19 (1930) 389.
3. Lee, S. B., and Burris, R. H. *Ind. Eng. Chem.* 35 (1943) 354.

Received April 8, 1949.

A Note on the Amino Acid Content of Bence-Jones Protein

GUNNAR ÅGREN

Department of Medical Chemistry, University of Uppsala, Uppsala, Sweden

Dent and Rose [1] recently published a chemical study of Bence-Jones protein with special reference to its methionine content. We have for some time been engaged in a study of the amino acid content of this protein and the following short report may be given.

From a patient with multiple myelomatosis Bence-Jones protein was isolated by means of the classical heat reaction. A comparison was made of the amino acid content of two samples of protein, one prepared from urine, the other from a tumor extirpated for diagnostic purposes. Analysis of the hydrolyzed materials were carried out by paper chromatography (Consden, Gordon and Martin [2]), with some slight modifications [3]. Typical photographs of the chromatograms are given in Figs. 1 and 2.

A comparison of the spots in Figs. 1 and 2 shows that the protein isolated from

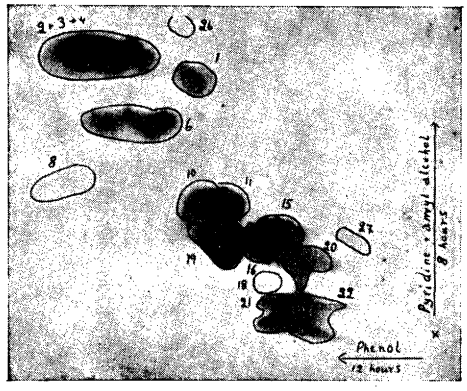

Fig. 1. Phenol-cupron (1 %)/pyridine-amyl alcohol chromatogram, showing the positions of the amino acids from 0.66 mg of hydrolyzed Bence-Jones protein prepared from a tumor.

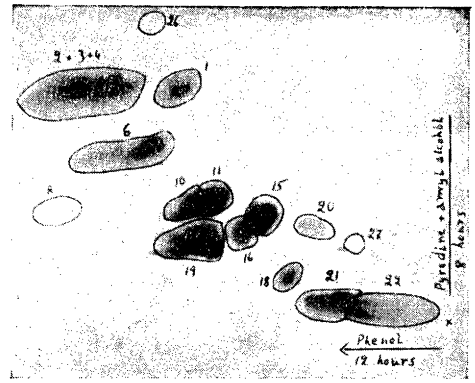

Fig. 2. Phenol-cupron (1 %)/pyridine-amyl alcohol chromatogram, showing the positions of the amino acids from 0.33 mg of hydrolyzed Bence-Jones protein prepared from urine.

1 = Tyr	*6 = Val*	*15 = Ser*	*20 = Asp*	*Abbreviations*
2 = Phe	*8 = Pro*	*16 = Gly*	*21 = Arg*	*according to*
3 = Ileu	*10 = Ala*	*18 = His*	*22 = Lys*	*Brand and Edsall* [7]
4 = Leu	*11 = Thr*	*19 = Glu*	*27 = (Cys-)$_2$*	

and the precipitate weighed. The silver content of the chloride corresponded to 80.2 per cent of the original sample.

Evidently this source of error in the chlorine determination escaped the attention of Lindahl. His conclusions about the protein bound chlorine in the body are consequently erroneous.

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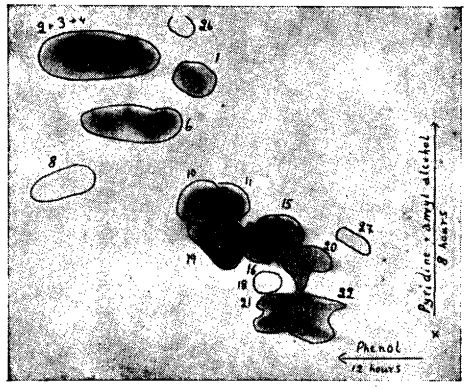

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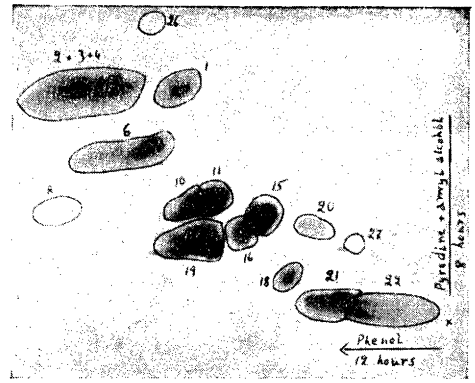

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No attempt has been made to compare the amino acid content of the two samples of protein from a quantitative point of view but it is obvious from an inspection of the two series of chromatograms with different amounts of total nitrogen applied to the paper that the sizes and colour intensities of the spots are very similar. Microbiological determinations of the amino acids are at present carried out and will definitively decide on this point.

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5. Block, R. J., and Bolling, D. *The amino acid composition of proteins and foods.* (1945).
6. Devine, J. *Biochem. J.* **35** (1941) 433.
7. Brand, E., and Edsall, J. T. *Ann. Rev. Biochem.* **16** (1947) 223.

Received May 4, 1949.

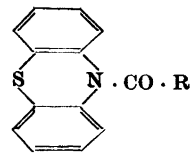
Some New Phenothiazine Derivatives of Pharmacological Interest

TORSTEN EKSTRAND

Centrallaboratoriet, Astra, Södertälje, Sweden

If phenothiazine (1 mole), dissolved in boiling benzene, is allowed to react with a halogeneacylhalogenide (1.5 mole), hydrogene halogenide is evolved and the resulting 10-halogeneacylphenothiazine separates from the cooled reaction mixture.

Table 1. Halogeneacylphenothiazines.



R	M. p. °C
$-CH_2 \cdot Cl$	115—116.5
$-\overset{CH_3}{\underset{H}{C}}-Br$	147.5—148.5
$-CH_2 \cdot CH_2 \cdot Cl$	142—143
$-\overset{C_2H_5}{\underset{H}{C}}-Br$	120—121

The halogene compounds react easily with primary, secundary and cyclic amines (cyclohexyl-, dimethyl-, diethylamine and piperidine) when heated with the amine (2.6 mole) to 70° in benzene solution (sealed tube). The reaction mixture is filtered and the filtrate evaporated. The residue is recrystallised or, when oily, transferred to hydrochloride.

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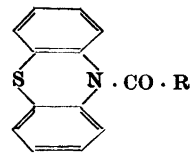

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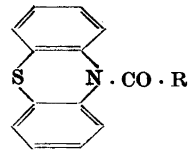


Compound no.	R	M. p. °C
I	$-CH_2 \cdot N(CH_3)_2$	114.5—116
II	$-CH_2 \cdot N(C_2H_5)_2$	58 —59
III	$-CH_2 \cdot$ N-ring	164—165
IV	$-CH_2 \cdot NH-$ ring	124—126
V	$-CH(CH_3) \cdot N(C_2H_5)_2$	99.5—100.5
VI	$-CH(CH_3) \cdot$ N-ring	110—111
VII	$-CH(C_2H_5) \cdot N(CH_3)_2$	98—99
VIII	$-CH(C_2H_5) \cdot N(C_2H_5)_2 \cdot HCl$	203 (dec.)
IX	$-CH(C_2H_5) \cdot$ N-ring $\cdot HCl$	216 (dec.)

An Improved Synthesis of Ethyl *Iso*propylidenecyanoacetate and the Construction of a New Water Separator

SIGVARD WIDEQVIST

Chemical Institute, University of Uppsala, Uppsala, Sweden

Ethyl *iso*propylidenecyanoacetate was first prepared by Komppa [1] by condensation of acetone and ethyl cyanoacetate by means of diethylamine. The reaction mixture was kept for one month at room temperature, and the yield of ethyl isopropylidenecyanoacetate was about 8 %.

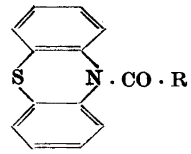
A better yield was obtained by Scheiber and Meisel [2], when a mixture of one mole of ethyl cyanoacetate, 3 moles of acetone, and 0.2 mole of zinc chloride and aniline was boiled for 8 hours, thus yielding 40 % of ethyl *iso*propylidenecyanoacetate.

These compounds have been subject to preliminary pharmacological tests (S. Wiedling). They all show a rather high local anesthetic power, for instance a 0.7 % solution of compound V has the same duration as a 2 % solution of Xylocaine-HCl (pH 5.6) but a longer time of onset when tested on rabbit cornea. The antihistaminic effect was rather weak, when tested on isolated guinea-pig intestine, for instance compound I excerted about 1/5 of the effect of Benadryl. The compounds had a good antispasmodic activity, compound V was twelve times more active than Benadryl when tested against acetylcholine on isolated guinea-pig intestine. Preliminary toxicity tests show that compound V has a Dl_{50} about 2 g/kg when injected subcutaneously on mice.

Complete chemical and pharmacological results will be reported later.

Received April 5, 1949.

Table 2. Alkylaminoacylphenothiazines.



Compound no.	R	M. p. °C
I	$-CH_2 \cdot N(CH_3)_2$	114.5—116
II	$-CH_2 \cdot N(C_2H_5)_2$	58 —59
III	$-CH_2 \cdot$ N-ring	164—165
IV	$-CH_2 \cdot NH-$ ring	124—126
V	$-CH(CH_3) \cdot N(C_2H_5)_2$	99.5—100.5
VI	$-CH(CH_3) \cdot$ N-ring	110—111
VII	$-CH(C_2H_5) \cdot N(CH_3)_2$	98—99
VIII	$-CH(C_2H_5) \cdot N(C_2H_5)_2 \cdot HCl$	203 (dec.)
IX	$-CH(C_2H_5) \cdot$ N-ring $\cdot HCl$	216 (dec.)

An Improved Synthesis of Ethyl *Iso*propylidenecyanoacetate and the Construction of a New Water Separator

SIGVARD WIDEQVIST

Chemical Institute, University of Uppsala, Uppsala, Sweden

Ethyl *iso*propylidenecyanoacetate was first prepared by Komppa [1] by condensation of acetone and ethyl cyanoacetate by means of diethylamine. The reaction mixture was kept for one month at room temperature, and the yield of ethyl isopropylidenecyanoacetate was about 8 %.

A better yield was obtained by Scheiber and Meisel [2], when a mixture of one mole of ethyl cyanoacetate, 3 moles of acetone, and 0.2 mole of zinc chloride and aniline was boiled for 8 hours, thus yielding 40 % of ethyl *iso*propylidenecyanoacetate.

These compounds have been subject to preliminary pharmacological tests (S. Wiedling). They all show a rather high local anesthetic power, for instance a 0.7 % solution of compound V has the same duration as a 2 % solution of Xylocaine-HCl (pH 5.6) but a longer time of onset when tested on rabbit cornea. The antihistaminic effect was rather weak, when tested on isolated guinea-pig intestine, for instance compound I excerted about 1/5 of the effect of Benadryl. The compounds had a good antispasmodic activity, compound V was twelve times more active than Benadryl when tested against acetylcholine on isolated guinea-pig intestine. Preliminary toxicity tests show that compound V has a Dl_{50} about 2 g/kg when injected subcutaneously on mice.

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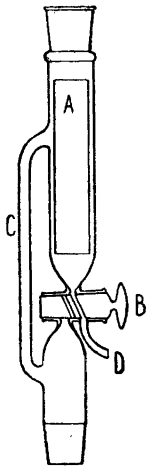

Fig. 1. The water separator.

At last Vogel [3] by using piperidine as condensing agent obtained a yield of 54 % of the ester. The reaction mixture was kept at room temperature for 60 hours, refluxed for 4 hours and again kept at room temperature for 60 hours.

Recently Cope, Hofmann, Wyckoff, and Hardenbergh [4] reported an excellent method for condensing ketones with cyanoacetic ester, in which the reactants were refluxed together with benzene, ammonium acetate, and acetic acid. The water produced during the condensation forms an azeotropic mixture with the benzene and is removed by means of a Dean and Stark constant water separator. This method is unfortunately useless for condensing ethyl cyanoacetate with acetone, as no azeotropic mixture is formed in this case. It is, however, possible to make the method useful also for condensations with acetone, when chloroform is used instead of benzene. In this way ethyl *iso*propylidenecyanoacetate was prepared in a yield of 75 %. When chloroform is substituted for the benzene, it is not possible to use the Dean and Stark separator. For this reason another separator was constructed. An ordinary simple Thielepape extractor (Fig. 1) was fitted with an inner open glass tube (A) and connected with the flask, containing the reaction mixture, and a reflux condenser. When the tap (B) is locked, an azeotropic mixture of acetone, chloroform and water distills up in the condenser and drops into the inner tube (A) in the separator. The water separates and forms the upper layer. The chloroform flows out from the bottom of the tube and up on the outside. When it reaches the side tube (C) of the separator, it flows down into the flask again. By means of the tap (B) the chloroform in the separator tube (A) may be tapped down into the reaction vessel, and the water is removed through the tap tube (D).

Experimental. A mixture of 85 g of ethyl cyanoacetate (0.75 mole), 56 g of acetone (0.97 mole), 9 g of glacial acetic acid, 6 g of ammonium acetate, and 75 ml of chloroform was boiled in a 500 ml round bottomed flask, fitted with a reflux condenser and a water separator, until no more water separated. The reaction was finished after about two hours. After cooling, the reaction mixture was washed several times with water and dried with anhydrous sodium sulfate. The chloroform was distilled off and the residue was fractionated *in vacuo* through a Widmer column. The distillate crystallized in the receiver, and it was necessary to feed the condenser with warm water in order to prevent crystallization in the condenser during the distillation. B. P. 107°/10 mm. Yield 86 g of ethyl *iso*propylidenecyanoacetate (75 %).

1. Komppa, G., *Ber.* **33** (1901) 3532.
2. Scheiber, J., and Meisel, F., *Ber.* **48** (1915) 254.
3. Vogel, I., *J. Chem. Soc.* (1928) 2019.
4. Cope, A., Hofmann, C., Wyckoff, C., and Hardenbergh, E., *J. Am. Chem. Soc.* **63** (1941) 3452.

Received May 10, 1949.

Isolation of *nor*-Adrenaline from the Adrenal Gland

SUNE BERGSTRÖM, U. S. v. EULER AND ULLA HAMBERG

Department of Physiological Chemistry, University of Lund, Lund, Sweden, and Department of Physiology, Karolinska Institutet, Stockholm, Sweden

In recent years it has been demonstrated that a factor with the physiological properties of synthetic L-*nor*-adrenaline [1] occurs as a regular constituent of adrenergic nerves [2] and apparently plays an important role as chemical nerve transmitter [3].

With biological and colorimetric tests it has been demonstrated to occur in various organs and tissues [2], adrenal medulla [4–6] and medullary tumors [7]. However, so far *nor*-adrenaline never seems to have been isolated and identified from natural sources [8].

We now wish to report the isolation of L-*nor*-adrenaline from cattle adrenals where it occurs together with L-adrenaline in the approx. proportions 1 : 4. The mixture of these bases was isolated from the crude protein free extract with the aid of ion exchangers [9].

The bases were then separated with counter-current distribution between 0.02 *N* HCl and phenol. After extraction of the phenol with ether pure L-*nor*-adrenaline was isolated as the crystalline base by addition of ammonia.

$C_8H_{11}O_3N$ (169.18)

Calc.	C 56.79	H 6.56	N 8.28
Found	» 56.37, 56.22	» 6.40 6.46	» 7.93

The ultraviolet absorption spectra and the x-ray powder diffraction patterns of the isolated product and of a synthetic specimen were indentical [10].

When compared with the colorimetric method of Euler and Hamberg [11] and in biological tests (cat's blood pressure, hen's rectal caecum) the samples were also found identical.

A full report will be published in Acta Physiol. Scand.

1. Tainter, M. L., Tullar, B. F., and Luduena F. P. *Science* **107** (1948) 39.
2. Euler, U. S. v. *Acta Physiol. Scand.* **16** (1948) 63.
3. Cannon, W. B., and Rosenblueth, A. *Am. J. Physiol.* **104** (1933) 557.
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8. In a private communication to one of us (U.S. v. E.) Dr. M. L. Tainter has informed us that Dr. B. F. Tullar has isolated L-*nor*-adrenaline from commercial adrenalin preparations.
9. Bergström, S. To be published.
10. We are indebted to Dr. E. Stenhagen for the x-ray diffractions measurements.
11. Euler, U. S. v., and Hamberg, U. *Acta Physiol. Scand.* In press.

Receiwed May 22, 1949.

New Books

Encyclopedia of Chemical Technology. Volume 1 : A — Anthrimides. Editors: Raymond E. Kirk and Donald F. Othmer. Assistent editors: Janet D. Scott and Anthony Standen. Interscience, New York 1947. 982 pp. $20 per volume.

The scope of this Encyclopedia is described in the preface: »It is neither a dictionary nor a handbook, nor is it a series of technological monographs ... for the benefit of advanced specialists ... Rather it is designed to present the entire field of chemical technology for profes-

sional chemists and chemical engineers who may wish to know the methods that are employed in a special field, often outside that of their immediate experience. It is intended both for those working in industry and for those in universities and other research institutions.»

The complete work will consist of 10 volumes, each of about 960 pages, and will appear at the rate of 2 or 3 volumes a year. Till now, two volumes have been completed, although only the first has as yet reached the office of this Journal.

In order to avoid excessive splitting up and repetition, an attempt has been made to collect the material under a few rather comprehensive headings, either representing groups of substances or processes; for this reason each volume contains only about 100 articles. The search for a given compound or process is facilitated by a large number of cross references.

In the first volume, the number of authors is almost as large as that of articles, and mots of them are active industrial chemists. The work of collecting and coordinating all these articles must have been quite impressive.

The majority of the articles refer to names of substances or of groups of substances, such as acetic acid, acetylene alcohol (s), alkali metals (where, by the way, the recent discovery of Cs minerals in Sweden is not mentioned), alkaloids, alloys, aluminum, amino, resins and plastics, and ammonia. Such articles generally begin with a short historical introduction and with the most important physical and chemical properties of the substance in question. The technical processes are often elucidated by drawings and flow sheets; I have not, however, been able to find a single photograph. The data on prices and production volumes naturally stress American conditions.

Among the »functional» groups of substances we find abrasives, adhesives, and anesthetics (what, no xylocaine? Don't let that happen in the next edition!); among the processes are absorption, adsorption (with theoretical introduction by P. H. Emmett of the BET team), alkylation, amination by reduction, and ammonolysis.

»Alkali and chlorine» is the heading of a long article treating in addition the production of sodium carbonate. Of stray articles we may mention »analytical chemistry» (where the section on quantitative analysis is written by E. B. Sandell); acid-base systems, acoustical building materials, air conditioning and allergens.

The editors admit without blushing that the nomenclature presents an interesting mixture of such names as are approved by the International Union of Chemistry and such as the IUC does its best to stamp out. This applies to both organic and inorganic names. When the Encyclopedia reaches P, I shall be quite curious to see whether KCl is called muriate of potash or potassium chloride.

This encyclopedia will certainly be found very useful by many chemists in the Scandinavian countries, too. Its usefulness is enhanced by numerous references following most articles.

Lars Gunnar Sillén.

Isolation of *nor*-Adrenaline from the Adrenal Gland

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ACTA CHEMICA SCANDINAVICA 3 (1949) 307—320

The Electric Mobilities of Glycine, Alanine, and Glycyl-glycine

HARRY SVENSSON, ALLAN BENJAMINSSON and INGER BRATTSTEN

Institutes of Physical Chemistry and Biochemistry, University of Uppsala, Uppsala, Sweden

Mobility measurements on weak electrolytes are very scarce in the literature. For amino acids, there are only a few investigations on transference numbers and conductivities, which data have been brought together and discussed by Schmidt [1] in his monograph on proteins and amino acids. Recently, ionophoresis has been used for the separation of amino acids from each other and from peptides (Consden, Gordon, and Martin [2]; Butler and Stephen [3]). An extensive use of this method, however, necessitates a thorough knowledge of the mobilities of different amino acids and peptides under different conditions, especially pH.

EXPERIMENTAL

The moving boundary systems used for the mobility studies are shown schematically in Fig. 1. The system 1 a gives a sharp descending boundary in the acid range, 1 b a similar boundary in the alkaline range. The amino acid or peptide radical is denoted by A^-, its zwitterionic form by $^+HA^-$, and its positive ion by H_2A^+. In some experiments, the formic acid was replaced by acetic acid.

This type of moving boundary systems for amino acids was proposed to one of us (H. S.) by Professor Tiselius in the year 1937. It was used by him and Eriksson-Quensel[4] in an electrophoretic study of the peptic digestion products of egg albumin. The sharpening of the amino acid boundary is due to a considerable pH change across the boundary and not, as is generally the case, to a great change in conductivity. The pH in phase (2), Fig. 1 a, is much lower than that in phase (3). Since the degree of dissociation of an

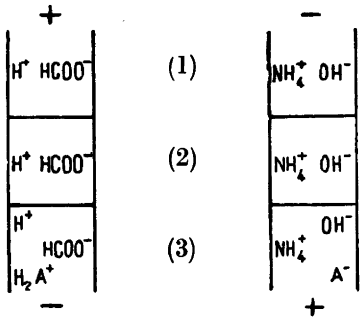


Fig. 1. The moving boundary system.

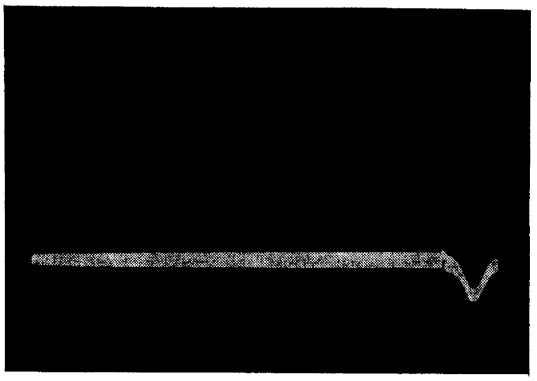


Fig. 2. Optical pattern of glycine in ammonia. Plus is to the left.

amino acid, and thus even its mobility, rises with an increasing acidity in the medium, amino acid radicals lagging behind the moving boundary by diffusion will find themselves in a medium where they possess a higher mobility, with the result that they will speed up and take over the boundary again very soon. In Fig. 1 b the situation is the same. Moving boundaries at which the sharpening effect is contributable to different states of dissociation of weak electrolytes were predicted by Miller [5]. In the system sodium acetate-acetic acid proposed by him, the sharpening of the Na^+/H^+ boundary is also a pH effect, the constituent mobility of the hydrogen radical being a function of the acetate ion concentration.

The concentration of the amino acid or peptide was in most cases 0.1 per cent. The formic acid and the ammonia concentrations in the bottom solutions (3) were varied to give different pH:es. Although rather low, the ionic strength was therefore not constant throughout the investigation. Estimated values of this function are included in the Tables 1—6. In the top solution (1), the concentration was chosen to secure gravitational stability at the stationary boundary between (1) and (2). Thus the formic acid concentration in (1) was constantly appreciably lower than that in (3), whereas the ammonia concentration in (1) was always higher than that in (3), due to the negative density increment of ammonia. The refractive index increment of the ammonical solutions seemed to parallel the concentration of ammonia, as is evident from Fig. 2.

The electrophoresis apparatus according to Tiselius was used, essentially in the form described by Svensson [6]. The electrodes were silver-silver chloride surrounded by 1 *M* sodium chloride solution. The positive limb was kept

closed during the runs, and no volume correction was carried out. This correction is well below the experimental errors since the current densities were very low in these experiments.

The conductivities of the amino acid solutions were measured in an ice bath, and the experiments were also run at a water-bath temperature of 0.0° C., ethyl alcohol being added to the water in order to make this possible.

The pH measurements were carried out at room temperature with a hydrogen electrode. Potassium biphthalate was used as a standard (pH 4.00); in order to avoid drifting readings due to catalytic reduction of the phthalate, the adjustment of the potentiometer was made while the hydrogen gas was bubbling through. The pH:es of the ammoniacal solutions were measured using hydrogen gas coming from a washing flask containing ammonia of the same concentration as in the sample in the pH cell. In this way systematic errors due to evaporation of ammonia were avoided; the readings were both constant and reproducible.

RESULTS

The experimental data have been collected in the Tables 1—6. The first column gives the computed ionic strength, the second gives the concentration of formic acid (acetic acid; ammonia) used in the bottom solutions, the third the concentration of amino acid or peptide. In the fourth to sixth columns, we have the observed pH:es, conductivities, and mobilities. In Figure 3, the experimental mobilities have been plotted against pH. Since the spreading of the points is fairly great, the following method of finding the most probable mobility curve has been adopted.

The dissociation of a monovalent amino acid in the acid range is governed by the equation:

$$(H^+)\ (^+HA^-) = K\ (H_2A^+) \tag{1}$$

where K is the thermodynamic dissociation constant and ( ) denotes activities. Passing from activities to concentrations except for the hydrogen ion, we have:

$$(H^+)\ [^+HA^-] = K\ f\ [H_2A^+] \tag{2}$$

where [ ] denotes concentrations and f is the activity coefficient for a monovalent ion. From this equation, we can derive the degree of dissociation

$$\alpha = \frac{(H^+)}{(H^+) + Kf} \tag{3}$$

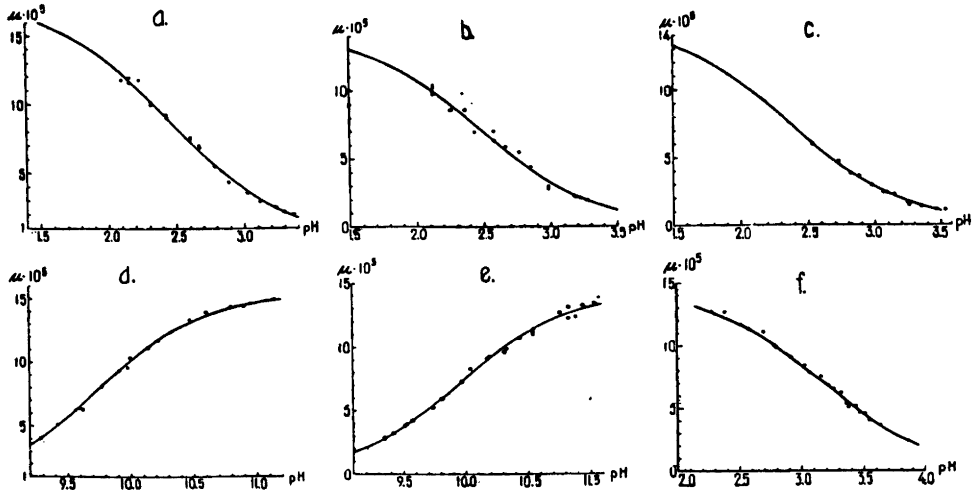


Fig. 3. The Mobilities of glycine, alanine, and glycyl-glycine at 0° C as functions of pH.

a. The mobility of glycine in formic acid solutions.
b. The mobility of alanine in formic acid solutions.
c. The mobility of alanine in acetic acid solutions.
d. The mobility of glycine in solutions of ammonia.
e. The mobility of alanine in solutions of ammonia.
f. The mobility of glycyl-glycine in formic acid solutions.

If the observed mobility is u and the mobility of the free ion H_2A^+ is u_o (in the same medium), we have the relation:

$$u = \alpha u_o \tag{4}$$

which gives with the aid of (3):

$$(H^+) = u_o \frac{(H^+)}{u} - Kf \tag{5}$$

Thus, if f and u_0 are constant throughout the series of experiments, it can be concluded that the hydrogen ion activity should be a linear function of the ratio $(H^+)/u$. This is of course not exactly true since both the ionic strength and the concentration of formic acid (acetic acid) varies. Thus the experimental material cannot be interpreted in this simple way if the plot of (H^+) versus $(H^+)/u$ shows a definite deviation from a straight line. If it does not,

Table 1. The mobility of glycine at 0° C in formic acid solutions.

$\mu \cdot 10^3$	mC HCOOH	per cent glycine	pH	$\varkappa \cdot 10^3$	$u \cdot 10^5$
2	6	0.1	3.36	0.200	1.98
	8	0.1	3.29	0.230	2.19
	10	0.1	3.23	0.239	2.53
	15	0.1	3.11	0.296	2.47
3.5	20	0.1	3.02	0.353	3.57
	33	0.1	2.88	0.461	4.35
5.5	50	0.1	2.78	0.578	5.53
	75	0.1	2.67	0.729	6.90
	75	0.1	2.67	0.729	6.93
7	100	0.1	2.60	0.849	7.53
	100	0.1	2.60	0.845	7.45
9	200	0.1	2.43	1.283	9.02
	200	0.1	2.43	1.273	9.17
10	300	0.1	2.315		10.01
	300	0.1	2.315	1.520	10.18
11.5	400	0.1	2.23	1.852	11.81
	500	0.1	2.16	2.092	11.60
	500	0.1	2.16	2.086	11.98
14.5	600	0.1	2.10	2.329	11.78

Table 2. The mobility of alanine at 0° C in formic acid solutions.

$\mu \cdot 10^3$	mC HCOOH	per cent alanine	pH	$\varkappa \cdot 10^3$	$u \cdot 10^5$
2	10	0.1	3.18	0.233	2.22
3	20	0.1	2.98	0.342	2.84
	20	0.1	2.98	0.351	2.88
	35	0.1	2.85	0.478	4.37
4.5	50	0.1	2.76	0.590	5.46
	75	0.1	2.66	0.748	5.85
6	100	0.1	2.575	0.862	6.29
	100	0.1	2.575	0.948	6.99
	160	0.1	2.43	1.111	6.94
8	200	0.1	2.36	1.283	8.57
8.5	285	0.1	2.245	1.535	8.53
	500	0.1	2.12	2.100	10.20
11.5	500	0.1	2.12	2.060	9.77
	500	0.1	2.12	2.082	9.83
	500	0.1	2.12		10.37

Table 3. The mobility of alanine at 0° C in acetic acid solutions.

mC HAc	per cent alanine	pH	$u \cdot 10^5$
20	0.25	3.35	1.40
100	0.25	3.26	1.57
130	0.25	3.25	1.66
150	0.25	3.14	2.24
200	0.25	3.08	2.37
300	38.7 mC	3.06	2.39
300	100 mC	3.26	1.62
500	100 mC	3.46	1.16
500	50 mC	2.975	2.94
500	0.25	2.88	3.60
500	25 mC	2.82	3.80
500	0.1	2.73	4.72
1000	0.1	2.54	6.00

Table 4. The mobility of glycine at 0° C in solutions of ammonia.

$\mu \cdot 10^3$	mC NH_3	per cent glycine	pH	$\varkappa \cdot 10^3$	$u \cdot 10^5$
3	6.35	0.1	9.28	0.207	— 4.05
	10.0	0.1	9.42	0.262	— 5.13
5	15.0	0.1	9.56		— 6.23
	16.5	0.1	9.59	0.330	— 6.39
	25.34	0.1	9.77	0.423	— 8.04
	36.2	0.1	9.92	0.489	— 9.28
	43.1	0.1	9.98	0.514	— 9.51
7	45.0	0.1	10.00	0.517	— 10.27
	70.0	0.1	10.14	0.573	— 11.05
	90.5	0.1	10.24	0.595	— 11.66
8.5	165.2	0.1	10.47	0.686	— 13.32
	226	0.1	10.60	0.751	— 13.93
9	362	0.1	10.80	0.775	— 14.32
10.5	452	0.1	10.91	0.819	— 14.39
12	905	0.1	11.14	0.896	— 14.94

Table 5. The mobility of alanine at 0° C in solutions of ammonia.

$\mu \cdot 10^3$	mC NH_3	per cent alanine	pH	$\varkappa \cdot 10^3$	$u \cdot 10^5$
2	5	0.1	9.35	0.1460	— 2.86
	5	0.1	9.415	0.1460	— 3.24
3.5	9	0.1	9.53	0.1936	— 3.81
3.5	10	0.1	9.52	0.220	— 3.87
	10.9	0.1	9.57	0.2091	— 4.20
	15	0.1	9.74	0.2830	— 5.24
4.5	20	0.1	9.81	0.3148	— 5.92
	30	0.1	9.97	0.3703	— 7.25
5.5	37.5	0.1	10.04	0.3804	— 8.31
	50	0.1	10.18	0.4438	— 9.04
	52	0.1	10.19	0.4459	— 9.15
	75	0.1	10.31	0.4831	— 9.56
6.5	80	0.1	10.33	0.4840	— 9.80
	105	0.1	10.43	0.514	— 10.69
	144.8	0.1	10.545	0.547	— 11.00
	144.8	0.1	10.545		— 11.15
7.5	150	0.1	10.555	0.561	— 11.40
	250	0.1	10.755	0.621	— 12.74
	300	0.1	10.83	0.638	— 12.27
8.0	300	0.1	10.83	0.641	— 13.18
	350	0.1	10.89	0.652	— 12.39
	400	0.1	10.95	0.667	— 13.29
	500	0.1	11.04	0.681	— 13.49
8.0	600	0.1	11.075	0.714	— 13.91

however, the interpretation outlined may be accepted as a means of levelling the experimental data.

In ammoniacal solutions, the same treatment leads to a somewhat different equation:

$$(H^+) = \frac{Ku_0}{f}\frac{1}{u} - \frac{K}{f} \tag{6}$$

In this case, therefore, (H^+) should be a linear function of $1/u$.

The plots of the hydrogen ion activity, taken as the negative antilogarithm of pH, against $(H^+)/u$ in acid and against $1/u$ in alkaline solutions are shown in Figure 4. It is evident that a definite curvature cannot be seen, which means that the assumption of a constant f and of a constant u_0 does not violate the

Table 6. The mobility of glycyl-glycine at 0° C in formic acid solutions.

$\mu \cdot 10^3$	mC HCOOH	per cent peptide	pH	$\varkappa \cdot 10^3$	$u \cdot 10^5$
4	10	0.2	3.545	0.260	4.05
	12	0.2	3.51	0.261	4.61
5.5	15	0.2	3.44	0.314	5.16
	18	0.2	3.38		5.12
	19	0.2	3.355	0.345	5.34
6	21	0.2	3.33	0.360	6.24
	24.7	0.2	3.26	0.401	6.50
	35	0.2	3.16	0.493	7.51
	98.9	0.5	3.08		7.81
8	49.5	0.2	3.03	0.575	8.41
	70	0.2	2.915	0.687	9.06
10	98.9	0.2	2.79	0.815	10.00
	98.9	0.2	2.79	0.816	9.94
11.5	180	0.2	2.58	1.098	11.30
	297	0.2	2.39	1.433	12.63
13	400	0.2	2.28	1.681	12.70

experimental results. The straight lines in these figures have been constructed with the aid of the method of least squares.

The two parameters of the straight lines give the limiting mobilities for complete dissociation and approximate figures for the pK:s. These data have been collected in Table 7. The pK' values given there are the negative logarithms of Kf in acid and of K/f in alkaline solutions. Their deviations from the thermodynamic pK:s, also given in the table (taken from Cohn and Edsall [7]), are due in part to the factor f, in part to slight variations in f and u_0 from experiment to experiment. Also, the thermodynamic pK values refer to room temperature, whereas this investigation was carried out at 0° C. The limiting mobilities given in Table 7 cannot be regarded as very accurate. Mobility values from experiments performed at the most extreme pH:es exert a great influence on the values of u_0. These experiments are the least reliable ones since the sharpening of the boundaries was not very pronounced in high concentrations of formic acid or ammonia.

The curves in Figure 3 are the theoretical curves derived from the u_0 and pK' values in Table 7. Numerical values taken from these curves for every tenth of a pH unit are presented in Table 8.

It was tried to extend the investigation to glycyl-glycine in ammonia, but certain difficulties arose in these experiments. The cause of the disturbances is not quite understood for the present.

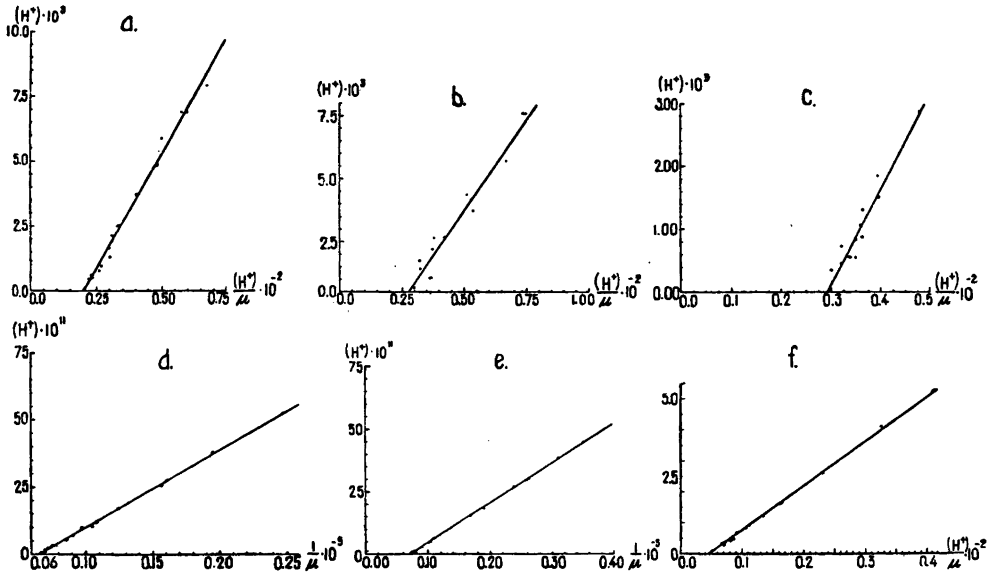


Fig. 4.

a. Plot of (H^+) versus $(H^+)/u$ for glycine in formic acid solutions.
b. Plot of (H^+) versus $(H^+)/u$ for alanine in formic acid solutions.
c. Plot of (H^+) versus $(H^+)/u$ for alanine in acetic acid solutions.
d. Plot of (H^+) versus $1/u$ for alanine in ammoniacal solutions.
e. Plot of (H^+) versus $1/u$ for alanine in ammoniacal solutions.
f. Plot of (H^+) versus $(H^+)/u$ for glycyl-glycine in formic acid solutions.

Table 7. The mobilities at 0° C of the completely ionized forms, and the apparent pK values, of glycine, alanine, and glycyl-glycine in media of aqueous formic acid, acetic acid, and ammonia.

	Glycine in		Alanine in			Glycyl-glycine in
	HCOOH	NH_3	HCOOH	CH_3COOH	NH_3	HCOOH
$u_0 \cdot 10^5$	17.8	− 15.4	14.5	15.1	− 14.3	14.3
pK'	2.44	9.73	2.46	2.36	9.95	3.18
pK	2.35	9.78	2.35	2.35	9.87	3.08

Table 8. The mobilities of glycine, alanine, and glycyl-glycine.

pH	Glycine in	Alanine in		Glycyl-glycine in
	HCOOH	HCOOH	CH_3COOH	HCOOH
2.2	11.3	9.35		12.9
2.3	10.35	8.6		12.6
2.4	9.3	7.7		12.2
2.5	8.3	6.9	6.3	11.8
2.6	7.3	6.1	5.5	11.25
2.7	6.35	5.3	4.7	10.7
2.8	5.4	4.5	4.0	10.0
2.9	4.6	3.85	3.35	9.3
3.0	3.9	3.2	2.8	8.6
3.1	3.2	2.7	2.3	7.8
3.2	2.65	2.2	1.9	6.95
3.3	2.2	1.8	1.55	6.1
3.4	1.75	1.5	1.25	5.3
3.5				4.55
3.6				3.9
3.7				3.3
3.8				2.8
3.9				2.3
4.0				1.9
pH	NH_3	NH_2		
9.2	− 3.5	− 2.1		
9.3	− 4.2	− 2.6		
9.4	− 4.9	− 3.1		
9.5	− 5.7	− 3.7		
9.6	− 6.6	− 4.4		
9.7	− 7.45	− 5.1		
9.8	− 8.3	− 5.9		
9.9	− 9.2	− 6.7		
10.0	− 10.0	− 7.5		
10.1	− 10.8	− 8.35		
10.2	− 11.5	− 9.1		
10.3	− 12.15	− 9.85		
10.4	− 12.7	− 10.5		
10.5	− 13.2	− 11.1		
10.6	− 13.6	− 11.65		
10.7	− 13.9	− 12.1		
10.8	− 14.2	− 12.5		
10.9	− 14.45	− 12.85		
11.0	− 14.6	− 13.1		

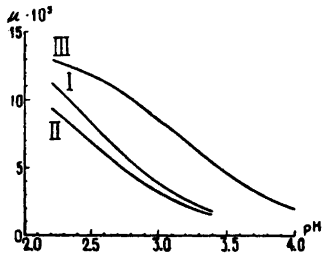


Fig. 5. Comparison between the mobilities of glycine (I), alanine (II), and glycyl-glycine (III) in formic acid solutions.

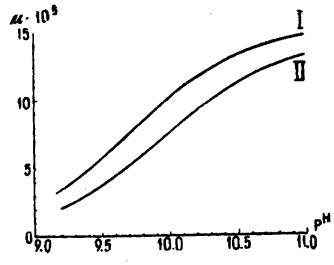
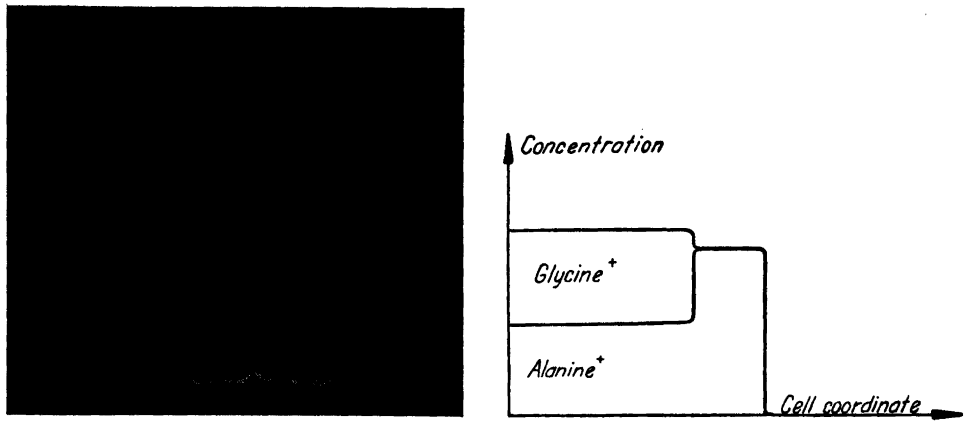


Fig. 6. Comparison between the mobilities of glycine (I) and alanine (II) in ammoniacal solutions.

DISCUSSION

In the Figures 5 and 6, the pH-mobility curves of the two amino acids and the peptide have been put together for comparison. It is realized that an electrophoretic separation of them should be possible. In the case of glycine and alanine, this is due to a difference in limiting mobility since their pK:s are practically the same. The separation of glycyl-glycine from anyone of the amino acids is still more favourable, but this depends upon the appreciable difference in pK.

In order to demonstrate the applicability of the experimental conditions prevailing in these experiments for analytical and for preparative purposes, two more experiments were performed. Fig. 7 a shows the electrophoretic pattern of a mixture of equal parts (0.5 %) of glycine and alanine in formic acid solution at a pH of 2.79. The big spike is the (slower) alanine boundary. The glycine boundary appears very small because it is superimposed by a considerable negative concentration increment of alanine, as illustrated in Fig. 7 b. The optical pattern, therefore, does not give direct information about the composition of the original solution. For separation purposes it is favourable that the concentration of alanine in the isolated fraction is higher than in the original solution, but it also implies the risk that, with other mixtures of amino acids and peptides, the density decrement given by the surrounding amino acids may be greater than the increment due to the leading one (the one that disappears in the boundary). Then a gravitationally unstable system would result, which constitutes one limitation in the method. The exposure in Fig. 7 a was taken after the passage of 114 coulombs; thus the separation speed was very low, and it was not possible to prolong the experiment until the adjusted solution between the moving boundaries could be removed in sufficient quantity for analysis. Integration of the pattern gave 8.74 as the ratio



a. Fig. 7. b.

a. Optical pattern of the system 0.5 % glycine and 0.5 % alanine in 0.3—C formic acid. Plus is to the right.

b. Schematic representation of the concentration changes of glycine and alanine across the boundaries in the same system.

between the areas, roughly corresponding to 0.90 % alanine in the adjusted solution.

With a mixture of glycine and glycyl-glycine in the same pH range, it is evident from Fig. 5 that the conditions for separation are much more favourable, and, in addition, their density increments (per equivalent) parallel their mobilities. Fig. 8 shows the pattern from an electrophoresis experiment where the initial bottom solution contained 0.5 % glycine and 0.5 % glycyl-glycine in a formic acid medium of pH 2.76. The exposure was taken after

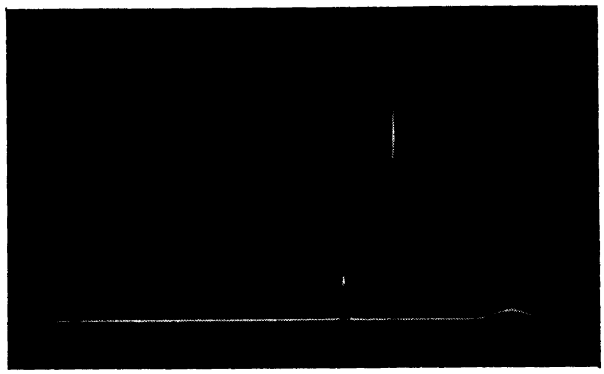


Fig. 8.

Optical pattern of the system 0.5 % glycine and 0.5 % glycyl-glycine in 0.3—C formic acid. Plus is to the right.

passage of 54 coulombs; thus the speed of separation was much greater than in the first experiment. The picture shows that the enclosed areas correspond more closely to the true composition of the original mixture than in the glycine-alanine experiment. Analysis of the adjusted solution containing glycine showed that pH was lowered to 2.69. At this pH glycine has a higher mobility than in the original solution of pH 2.76. The separation velocity between the boundaries is therefore somewhat smaller than what corresponds to the difference in mobility in the original solution. The glycine concentration in the isolated fraction was 0.77 %, as determined by Kjeldahl analysis. Integration on the plate gave an apparent concentration of 0.74 %.

In the moving boundary systems used in this investigation, we are very far from the »ideal» electrophoresis, where the migrations in the two limbs are mirror image processes. Deviations from this behaviour were called boundary anomalies by Tiselius [8]. The cause of this »anomalous» behaviour is twofold. First, the buffering action of amino acids and peptides, per unit weight, is much greater than in the case of proteins. Second, the concentrations of other ions are low. As a matter of fact, the amino acids themselves contribute with a considerable part to the ionic strength, and their effects on the pH and the conductivity of the solution are pronounced. In the experiments with two amino acids reported here, it was found that very great changes in these properties took place at the slower boundary, whereas at the faster boundary a considerable decrease in the concentration of the slowest component tended to counteract these changes. In agreement with theoretical expectations, the deviation from the state of »ideal» electrophoresis are greater the smaller the difference in mobility between the components. This is strikingly illustrated by the disproportion between the two peaks in each of the Figures 7 a and 8.

As in the case of the classical two-salt boundary systems for the measurement of transference numbers, advantage is here taken of these »anomalous» effects. For mobility measurements the sharp descending boundaries are very useful, and in separation runs the increased concentration of the slowest component should be advantageous. The limitations of the moving boundary method can be overcome by using powders or gels as stabilizers against convection. In this way it seems possible to isolate each component in a mixture in one run and with a high yield (for references, see Svensson [9]).

If a complete separation is required, the experimental conditions used here with only a weak acid or base in the medium is not suitable because the sharpening of the descending boundaries is accompanied by a corresponding blurring of the ascending boundaries. A more symmetrical migration can of course be obtained by the use of buffered solutions, although certain difficulties due to the high buffering capacity of the amino acids themselves will most

probably remain. In the acid range, it can be expected that formate buffers will be useful. In cases where the ionic strength is increased, the mobilities given here are of course not applicable unless they are supplemented with information about the influence of the medium. They can be expected to retain a qualitative significance, however, at least indicating the sequence of the different amino acids as to their mobilities at a given pH.

SUMMARY

The electric mobilities of glycine, alanine, and glycyl-glycine have been measured in solutions of formic acid, acetic acid, and ammonia as functions of pH. The results were used to discuss the possibilities for electrophoretic separation of amino acids and peptides. A couple of experiments with mixtures of these substances illustrated, in addition, pronounced and interesting »boundary anomalies» which are easily explained by the moving boundary equation.

The authors wish to express their sincere gratitude to Professor Arne Tiselius for his kind interest in this work and for critically reviewing the manuscript. We are also indepted to Professor The Svedberg for the privilege of working at this Institute. The investigation was financially supported by the Swedish Natural Science Research Council.

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Received February 28, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 321—334

Studies on Ionic Solutions in Diethyl Ether

II. Silver-Silver Ion Potentials and Solubility Products of Silver Halogenides in $LiClO_4$-Ether Medium

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Berglund and Sillén[1] have suggested that the method of measuring ionic concentrations by emf methods, which has proved so useful for aqueous solutions, can also be applied to ionic solutions in diethyl ether. If, for example, a high and constant concentration of $LiClO_4$ is maintained in the ether, the activity factors of other ions present in small amounts will be almost constant, and thus the potentials of reversible electrodes giving the ions in question should follow Nernst's formula. The validity of this assumption was proved by measurements of cells with Ag, AgBr electrodes in ether solutions containing 2 C $LiClO_4$ and small amounts of LiBr.

After this first success, we tried to measure the concentrations of Mg^{2+} and Zn^{2+}, dissolved as perchlorates in $LiClO_4$-ether, using electrodes of pure Mg or Zn metal, or of their liquid amalgams. However, certain difficulties were met with, which were finally traced to minute amounts of oxidizing substances such as oxygen still present in the ether. Steps are now being taken to remove the last traces of these impurities, after which the experiments on Mg and Zn compounds will be started again.

In the meantime, however, experiments have been made on the behaviour of the Ag^+/Ag electrode in ether solutions, since silver electrodes were assumed to be less sensible to oxidizing agents. It was thought desirable to prove that it is possible in principle to measure the concentration of positive ions in ether also. This end was achieved. Moreover a few equilibrium constants were obtained for our solvent, namely the solubility products of AgCl and AgBr, and approximate values of the solubility product of AgI and the complex product of AgI_2^-.

PRINCIPLES

The cells used were of the following types (X = Cl, Br or I):

$$-\text{Ag,AgX} \,|\, \text{S}_c(\text{LiX}) \,|\, \text{S}_b(1\text{C LiClO}_4) \,|\, 100 \text{ ml S(LiX)} + v \text{ ml T(AgClO}_4) \,|\, \text{Ag}+ \quad (1)$$

All the solutions —S_c, S_b and T—contained so much $LiClO_4$ that $[X^-] + [ClO_4^-] = 1$ C. During a titration the halide concentration of S_c at the reference electrode (left) was kept constant. Thus the electrode potential on an arbitrary scale, e_{ref}, was also constant. At the right electrode there is at first an excess of X^- ions. During the titration they are gradually precipitated as AgX. When the equivalence point is passed there is a large increase in $[Ag^+]$, and a decrease in $[X^-]$. If the reversible emf is measured, and if our assumption that the activity factors remain constant is valid, then the electrode potential e will follow the formula

$$e = e_{0AgX} - 58.86 \log [X^-] = e_{0Ag} + 58.86 \log [Ag^+] \quad (2)$$

where e_{0AgX} and e_{0Ag} are constants.

Of course, at least one of $[Ag^+]$ and $[X^-]$ is always very small.

From (2) it follows immediately

$$e_{0Ag} - e_{0AgX} = -58.86 \log [Ag^+][X^-] = -58.86 \log k_s \quad (3)$$

where k_s is the solubility product of AgX in the $LiClO_4$-ether solvent.

If the liquid junction potential can be neglected, the emf E of the cell is the difference between the electrode potentials:

$$E = e - e_{ref} \quad (4)$$

In the definition of the electrode potential there is always an arbitrary constant which, however, need not concern us here since it cancels out in (4).

From the measured data we can then calculate

$$e_{0Ag} - e_{ref} = E - 58.86 \log [Ag^+] \quad (5)$$

or

$$e_{0AgX} - e_{ref} = E + 58.86 \log [X^-] \quad (6)$$

according to which of $[Ag^+]$ or $[X^-]$ is in excess and thus known from the analytical data.

The constancy of the expressions (5) and (6) during each titration, and of $(e_{0Ag} - e_{0AgX})$ when several titrations are compared, should be a measure of the validity of our fundamental assumptions. With iodine certain complications were met with, which will be discussed below.

A number of the bromide titrations were made by a reverse process, with $AgClO_4$ in S, and LiX in T; the calculations are, of course, analogous.

REAGENTS

Diethyl ether, lithium perchlorate and *lithium bromide* were obtained as described by Berglund and Sillén [1].

Lithium chloride of Baker's make (BPC) was kept for 40 hours at 200° C in a current of dry air (apparatus as in Part I [1]). We tried to dissolve the product in ether, but the solubility was so low that Cl^- could not be detected with Ag^+. Since we had previously observed that LiBr is more soluble in $LiClO_4$-ether than in pure ether, the dry LiCl was digested with 1 C $LiClO_4$-ether, which proved to dissolve about 20 mC LiCl.

Silver perchlorate: A mixture of $AgNO_3$ and a slight excess of 70 % $HClO_4$ was evaporated to dryness. The $AgClO_4$ formed was dried for 40 hours at 160° C in a current of dry air and digested with ether; the solution contained about 100 mC $AgClO_4$.

Lithium iodide (Baker's BPC) was dried and dissolved in ether, which gradually became yellow because free iodine was formed. The colour was barely visible after one hour. LiI is more soluble in ether than LiCl and LiBr.

APPARATUS

The apparatus was mainly the same as that used by Berglund and Sillén[1].

The experiments were performed in a thermostat room at a temperature of 23.5 ± 0.2 °C. The cell was kept in an oil bath at the same temperature (23.5 ± 0.1 °C). The emfs were measured by means of a valve potentiometer (Radiometer PHM 3 g). The thermostat room was screened off from external electric fields by an earthed metallic network in the walls and ceiling.

The Ag,AgX electrodes were prepared by Brown's method [2]. Silver electrodes were prepared in four different ways:

1) A Ag,AgBr electrode was first prepared by Brown's method and then the AgBr washed away by treatment with concentrated $Na_2S_2O_3$ solution. The electrode was finally washed free from salts by boiling repeatedly with distilled water.

2) A Pt coil was silvered and then coated with a thin layer of Ag_2O, which was decomposed by keeping it at 475 °C for 7—8 hours, leaving a porous layer of metallic silver (Brester [3]).

3) Silver was deposited on a Pt coil by electrolysis of $KAg(CN)_2$ solution for 17—18 hours with 0.2—0.3 mA (Brown [2]).

4) A silver wire of 1 mm diameter was used without further treatment.

The preparation of electrodes by method 2) proved rather difficult, since the silver layer is brittle and liable to peel off. Moreover, when the composition of the solution was changed it took a long time — several hours — for the electrode to reach the new equilibrium potential; perhaps this inertia was due to the slowness of diffusion through the pores.

Electrodes of type 1), 3) and 4) gave steady emfs within 10—15 minutes. Since it was easier to prepare, type 3) was preferred to type 1), and was used in most of our experiments. Only one titration was performed with an electrode of type 4).

ANALYSES

Lithium perchlorate was determined by evaporating 5 ml of solution to dryness, dissolving in water, and passing the solution through an ion exchanger saturated with H^+. The content of H^+ liberated (equivalent to the Li^+ of the sample) was determined by titration with NaOH. — It was checked from time to time with Ag^+ that the stock solution (3.6 C $LiClO_4$) did not contain any Cl^-.

Silver perchlorate was determined by titration with 10 mC KSCN, and the *lithium halides* by Volhard titration.

The solutions S, T, etc. were prepared by transferring with burettes calculated amounts of the stock solutions of $LiClO_4$ and LiX or $AgClO_4$ to a measuring flask, and diluting to the mark with ether. The determination of $LiClO_4$ was made with the stock solution, the titrations for Ag^+ and X^- with the mixed solutions.

In an emf titration as described below the equivalence point usually occurred at the volume of T calculated from the analyses for Ag^+ and X^-. When a divergence occurred — usually only one or a few 0.1 % — the halide concentration was assumed to be accurate, and the concentration of the silver solution was corrected.

A larger error may have been caused by the high viscosity of the strong $LiClO_4$ solutions, which made them flow very slowly out of burettes and pipettes. (One reason for our choosing 1 C $LiClO_4$ instead of 2 C $LiClO_4$ (as in Part I [1]) was that the viscosity of the solutions is lower at lower concentrations). The $LiClO_4$ concentration may sometimes have differed from 1.00 C by as much as 0.01; as we shall see later, the corresponding error in the emf is however rather small.

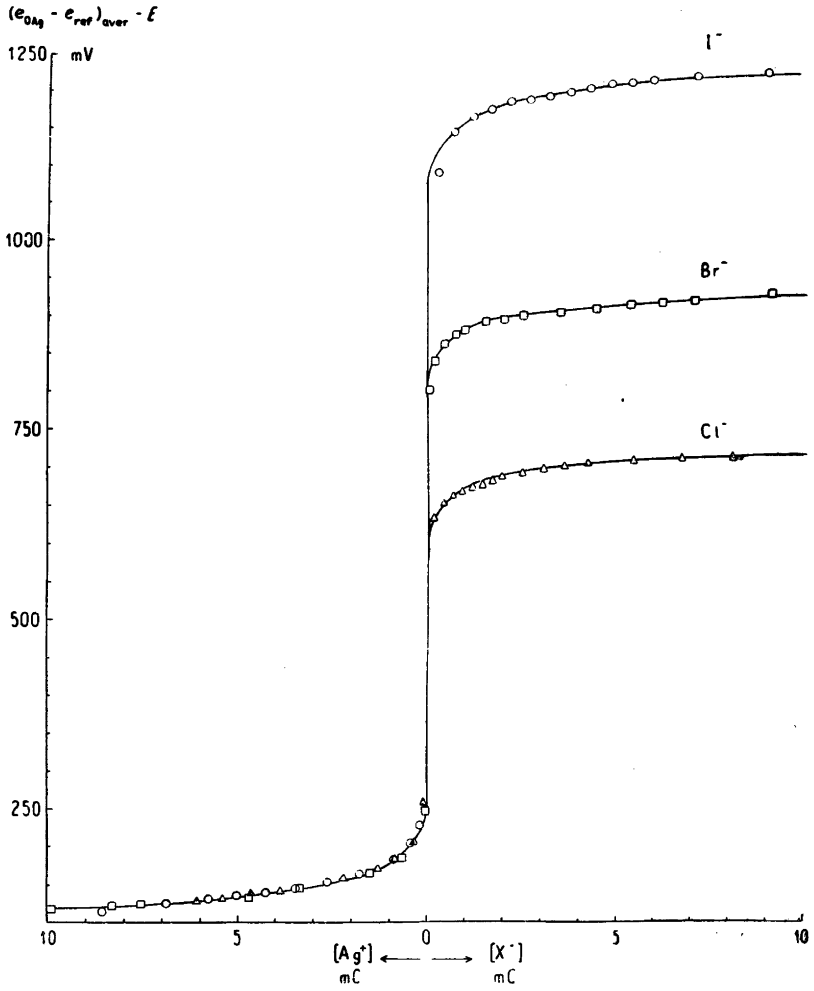

Fig. 1. Electrometric titration of Ag^+ with halide ions X^- in $LiClO_4$ — ether medium.

PROCEDURE AND EXPERIMENTS

The solutions were prepared and transferred to the electrode vessels in the same way as described by Berglund and Sillén [1]. Usually two Ag electrodes of different types were inserted in the same solution, and the emf measured between each of them and the reference electrode. Before the emf measurements began, a few ml of T [X^-] were added to S so that AgX precipitated. After one hour of stirring — in order to get temperature

equilibrium and allow the AgX to form larger grains — the measurements began. After each new addition of T, we waited till the emf had been constant for 5 minutes, which generally took 10—15 minutes. It was essential to have the stirrer working all the time, so that AgX was kept suspended in the solution.

On titrations of Ag^+ in S with Cl^-, Br^- or I^- in T, the titration curves *E versus v* were of the same type as obtained in aqueous solutions (Fig. 1); if desired the equivalence points could be determined very accurately. In Fig. 1 $[(e_{0Ag} - e_{ref})_{average} - E]$ has been plotted against the excess concentration of Ag^+ or X^-. If (5) is valid, this expression should be equal to -58.86 log $[Ag^+]$. In fact, the experimental points with excess of Ag^+ are seen to coincide with the theoretical curve (black line). With excess of X^-, the points for the different halides are widely separated. The highest values are obtained for I^-, which indicates that the solubility product increases in the order AgI, AgBr, AgCl, just as for aqueous solutions.

In Tables 1, 3, and 5 are given the figures for one titration with each of Cl^-, Br^-, and I^-. The values of $e_{0Ag} - e_{ref}$, and $e_{0AgX} - e_{ref}$, calculated by means of (5) and (6), spread slightly but show no definite trend. Thus, as far as each individual titration is concerned, Nernst's law seems to be valid for the concentrations of Ag^+, Cl^-, Br^- and I^-.

Tables 2, 4 and 6 give a survey of the results of different titrations; the largest number of experiments was made with Br^-, which was used in all the preliminary experiments. (In the average values, the experimental points with $[Ag^+]$ or $[X^-]$ less than 1 mC have not been taken into account.)

Although $e_{0Ag} - e_{ref}$ and $e_{0AgX} - e_{ref}$ are generally constant within $\pm$ 5 mV for each titration, the variations between different titrations are larger, sometimes amounting to $\pm$ 20 mV. On the other hand, the difference, $e_{0Ag} - e_{0AgX}$ varies less, by $\pm$ 6—8 mV. From the averages we calculated the following values for the solubility products in 1 C $LiClO_4$-ether at 23.5° C (maximal deviations are given):

$$\begin{aligned} &\text{AgCl}: e_{0Ag} - e_{0AgCl} = 841.6 \pm 6.5 \text{ mV} \quad \log k_s = -14.30 \pm 0.11 \\ &\text{AgBr}: e_{0Ag} - e_{0AgBr} = 1045.0 \pm 8.4 \text{ mV} \quad \log k_s = -17.75 \pm 0.14 \\ &(\text{AgI}: e_{0Ag} - e_{0AgI} = 1330.5 \pm 6.4 \text{ mV} \quad \log k_s' = -22.60 \pm 0.11)^* \end{aligned} \tag{7}$$

Since we always tried to keep the same concentration, 0.1 C X^-, in the reference electrode solution (S_c), one might expect $e_{0AgX} - e_{ref}$ to be constant, even if the Nernst equation value (— 58.86 mV) cannot be expected to apply at such high $[X^-]$ as 0.1 C. As causes of the variations one might suggest:

* See »Complications with I^-» below.

Table 1. Titration Cl^- + Ag^+.

S = 0.0161 C LiCl; 0.98_{39} C $LiClO_4$ (62.2 ml).
T = 0.0425 C $AgClO_4$; 0.95_{75} C $LiClO_4$ (v ml).
S_b = 1.00 C $LiClO_4$.
S_c = 0.010 C LiCl; 0.990 C $LiClO_4$.

Electrodes: Ag-electrode using method 3).
Ag; AgCl using Brown [2].

E mV	*v* ml	$10^3[Cl^-]$	log $[Cl^-]$	$e_{0AgCl} - e_{ref}$
112.6	16.1	4.032	— 2.3945	— 28.4
119.5	17.1	3.445	— 2.4628	— 25.5
124.0	18.1	2.873	— 2.5417	— 25.7
126.3	18.6	2.592	— 2.5864	— 26.0
130.8	19.1	2.314	— 2.6356	— 24.4
133.3	19.6	2.039	— 2.6906	— 25.1
139.0	20.1	1.768	— 2.7525	— 23.1
144.0	20.6	1.500	— 2.8239	— 22.3
149.4	21.1	1.235	— 2.9083	— 21.8
154.1	21.6	0.9737	— 3.0116	— 23.2
158.0	22.1	0.7153	— 3.1455	(— 27.2)
189.9	23.1	0.2075	— 3.6830	(— 27.0)

E mV	*v* ml	$10^3[Ag^+]$	log $[Ag^+]$	$e_{0Ag} - e_{ref}$
603.1	24.1	0.2885	— 3.5398	(811.5)
628.0	25.1	0.7732	— 3.1117	(811.2)
643.1	26.1	1.247	— 2.9041	(814.1)
656.0	27.1	1.471	— 2.8324	822.8
662.4	28.1	1.926	— 2.7153	822.3
667.2	29.1	2.370	— 2.6252	821.8
671.2	30.1	2.805	— 2.5521	821.5
679.2	32.1	3.645	— 2.4383	822.8
683.4	34.1	4.454	— 2.3512	821.8
687.0	36.1	5.227	— 2.2817	821.3
689.4	38.1	5.971	— 2.2239	820.3
692.0	40.1	6.685	— 2.1749	820.1

Average values:

$e_{0Ag} - e_{ref} = 820.9 \pm 1.3$
$e_{0AgCl} - e_{ref} = -24.7 \pm 3.3$

Table 2. Summary of results for $Ag^+ + Cl^-$.

Electrodes acc. to	$e_{0Ag} - e_{ref}$	$e_{0AgCl} - e_{ref}$	$e_{0Ag} - e_{0AgCl}$
Method 3)	814.9 ± 2.5	− 23.3 ± 5.3	838.2
»	820.9 ± 1.3	− 24.7 ± 3.3	845.6
»	814.4 ± 1.2	− 32.0 ± 2.6	846.4
»	806.8 ± 1.4	− 26.7 ± 2.7	833.5
»	808.5 ± 1.6	− 36.1 ± 3.9	844.6
Method 4)	792.4 ± 1.6	− 49.0 ± 5.2	841.4

Average: $e_{0Ag} - e_{0AgCl} = 841.6 \pm 6.5$

$$pk_s = \frac{e_{0Ag} - e_{0AgCl}}{58,\ 86} = 14.30 \pm 0.11$$

1) The liquid junction potential S_c/S_b varies for different experiments according to the way the junction has been formed.

2) The silver halides AgX precipitate in forms (*e. g.* very fine-grained) of varying activity, higher than that of the equilibrium form, and are transformed to the latter only very slowly.

3) Small variations in the concentration of $LiClO_4$ cause large variations in the emf (*e. g.* because of changes in the liquid junction potential or in the activity factors).

The third possibility, at least, could be ruled out by the experiments described in Tables 7 a and b. There the ionic strength has been varied in the titration vessel, whereas the concentration of Ag^+ (Table 7 a) or Br^- (Table 7 b) has been kept constant. The emf shifted by about — 8 mV in the first case, and about + 25 mV in the second when the ionic strength I was increased from 0.5 to 1.2 C. The changes are in both cases in the direction corresponding to a lowering of the activity factor with increasing ionic strength. The changes in the liquid junction potential between S + T and S_b cannot give the chief contribution, since in such case the shift would have had the same sign and approximately the same magnitude in the experiments with Ag^+ and Br^-.

COMPLICATIONS WITH I^-

The titrations with I^- differed in one respect from those with Br^- and Cl^-. According to (2) the quantity $(e_{0AgX} - e_{ref})$ should be equal to 58.86 log $[X^-]$ at the reference electrode; since the concentration of X^- was always 0.1 C, one would expect a negative value, though may be numerically somewhat less

Table 3. Titration Br^- + Ag^+.

S = 0.02 C $AgClO_4$; 0.98 C $LiClO_4$ (50 ml).
T = 0.0533 C LiBr; 0.94$_{67}$ C $LiClO_4$ (v ml).
S_b = 1.00 C $LiClO_4$.
S_c = 0.10 C LiBr; 0.90 C $LiClO_4$.

Electrodes: Ag-electrode using method 3);
Ag; AgBr using Brown [2].

E mV	*v* ml	10^3 [Ag^+]	log [Ag^+]	$e_{0Ag} - e_{ref}$
916.9	2.0	17.18	— 1.7650	1020.8
913.2	4.0	14.57	— 1.8365	1021.3
908.5	6.0	12.15	— 1.9154	1021.3
903.0	8.0	9.890	— 2.0048	1021.0
897.2	10.0	7.783	— 2.1088	1021.4
889.6	12.0	5.813	— 2.2356	1021.2
885.6	13.0	4.875	— 2.3120	1021.7
879.9	14.0	3.966	— 2.4016	1021.3
873.7	15.0	3.085	— 2.5107	1021.5
866.2	16.0	2.230	— 2.6517	1022.3
855.0	17.0	1.402	— 2.8532	(1023.0)
828.4	18.0	0.5956	— 3.2250	(1018.3)

E mV	*v* ml	10^3 [Br^-]	log [Br^-]	$e_{0AgBr} - e_{ref}$
174.8	19.0	0.1855	— 3.7317	(— 44.9)
148.6	20.0	0.9443	— 3.0249	(— 29.5)
132.3	21.0	1.682	— 2.7742	— 31.0
121.1	22.0	2.397	— 2.6203	— 33.2
114.8	23.0	3.096	— 2.5092	— 32.9
110.0	24.0	3.774	— 2.4232	— 32.7
107.0	25.0	4.435	— 2.3531	— 31.6
103.6	26.0	5.078	— 2.2943	— 31.5
101.9	27.0	5.704	— 2.2438	— 30.3
99.7	28.0	6.314	— 2.1997	— 29.8
97.3	29.0	6.909	— 2.1606	— 29.9
95.3	30.0	7.489	— 2.1256	— 29.9

Average values:

$e_{0Ag} - e_{ref} = 1021.4 \pm 0.7$
$e_{0AgBr} - e_{ref} = -31.3 \pm 1.2$

Table 4. Summary of results for $Ag^+ + Br^-$.

Electrodes acc. to	$e_{0Ag} - e_{ref}$	$e_{0AgBr} - e_{ref}$	$e_{0Ag} - e_{0AgBr}$
Method 3)	1007.1 ± 4.0	− 32.5 ± 1.6	1039.6
»	1021.4 ± 0.7	− 31.3 ± 1.2	1052.7
»	1035.0 ± 5.6	− 10.4 ± 3.0	1045.4
»	1034.0 ± 3.5	− 10.5 ± 3.2	1044.5
»	1048.1 ± 3.6	− 25.7 ± 0.6	(1073.8)
»	1012.2 ± 1.7	− 14.0 ± 3.0	(1026.2)
»	1020.3 ± 4.7	− 15.6 ± 3.6	1035.9
»	1033.9 ± 5.0	− 15.3 ± 3.5	1049.2
»	1043.2 ± 1.5	− 20.2 ± 4.5	(1063.4)
Method 1)	1008.8 ± 3.5	− 41.0 ± 0.9	1049.8
Method 2)	1015.2 ± 0.7	− 27.4 ± 8.4	1042.6

Average: $e_{0Ag} - e_{0AgBr} = 1045.0 \pm 8.4$

$$pk_s = \frac{e_{0Ag} - e_{0AgBr}}{58,\ 86} = 17.75 \pm 0.14$$

than — 58.86 mV, because the activity coefficients for X^- cannot be expected to be the same in S_c as in the solution (S + T). For Cl^- and Br^- values between — 20 and — 40 mV were in fact found.

For I^-, however, the quantity $(e_{0AgX} - e_{ref})$ proved to be *positive*. In order to find out the reasons for this unexpected behavior, we carried out a few titrations of Ag^+ with I^- where $[I^-]$ finally amounted to about 100 mC (0.1 C). In order to get about the same relative accuracy in different ranges of I^- we first added a weaker I^- solution and in the last part of the titration a stronger one.

When the solution (S + T) ought to have had the same composition as S_c, E was about 60 mV and not, as expected, in the neighbourhood of 0.

Part of the explanation of these puzzling results came when one of us happened to observe that at the end of a titration the ether solution was quite clear. Thus the precipitate of AgI which had previously been repeatedly observed at lower $[I^-]$ had dissolved in excess of I^-. At the reference electrode all AgI has then certainly dissolved in the 0.1 C LiI solution; the silver concentration at the reference electrode must depend on the amount of AgI originally deposited on the silver.

The most plausible hypothesis is that a complex ion AgI_2^- had been formed. In such case the excess of I^- over Ag^+, X_e, is no longer equal to the concentration of free I^- but

Table 5. Titration I^- + Ag^+.

S = 0.010 C LiI; 0.99_0 C $LiClO_4$ (100 ml).
T = 0.0550 C $AgClO_4$; 0.94_{50} C $LiClO_4$ (v ml).
S_b = 1.00 C $LiClO_4$.
S_c = 0.10 C LiI; 0.90 C $LiClO_4$.

Electrodes: Ag-electrode using method 3);
Ag, AgI using Sillén-Ekedahl [4].

E mV	*v* ml	10^3 [I^-]	log [I^-]	$e_{0AgI} - e_{ref}$
141.0	6.5	6.033	− 2.2195	10.3
148.5	7.5	5.465	− 2.2624	15.3
149.2	8.5	4.908	− 2.3091	13.2
155.3	9.5	4.361	− 2.3604	16.3
161.0	10.5	3.824	− 2.4175	18.7
165.5	11.5	3.296	− 2.4820	19.4
171.0	12.5	2.778	− 2.5563	20.5
172.7	13.5	2.269	− 2.6442	17.0
182.0	14.5	1.769	− 2.7523	19.9
191.4	15.5	1.277	− 2.8938	21.0
212.0	16.5	0.7940	− 3.1002	(29.5)
264.5	17.5	0.3192	− 3.4959	(58.7)

E mv	*v* ml	10^3 [Ag^+]	log [Ag^+]	$e_{0Ag} - e_{ref}$
1126.3	18.5	0.1477	− 3.8306	(1351.8)
1151.8	19.0	0.3782	− 3.4223	(1353.3)
1173.0	20.0	0.8333	− 3.0792	(1354.3)
1184.1	21.0	1.281	− 2.8924	1354.4
1190.4	22.0	1.721	− 2.7642	1353.2
1202.3	24.0	2.581	− 2.5882	1354.7
1210.2	26.0	3.413	− 2.4669	1355.5
1215.3	28.0	4.219	− 2.3748	1355.1
1219.3	30.0	5.000	− 2.3010	1354.8
1223.2	32.0	5.758	− 2.2397	1355.1
1229.3	35.0	6.852	− 2.1642	1356.7
1238.8	40.0	8.571	− 2.0670	1360.5

Average values: $e_{0Ag} - e_{ref} = 1355.6 \pm 3.6$

$e_{0AgI} - e_{ref} = 17.2 \pm 5.3$

Table 6. Summary of results for $Ag^+ + I^-$.

Electrodes acc. to	$e_{0Ag} - e_{ref}$	$e'_{0AgI} - e_{ref}$	$e_{0Ag} - e'_{0AgI}$
Method 3)	1355.6 ± 3.6	17.2 ± 5.3	1338.4
»	1355.7 ± 3.1	17.5 ± 5.2	1338.2
»	1345.6 ± 7.7	19.8 ± 5.3	1325.8
»	1347.0 ± 8.7	21.3 ± 5.6	1325.7
»	1330.5 ± 1.1	4.7 ± 8	1325.8
»	1329.4 ± 1.6	0.5 ± 10	1328.9

Average: $e_{0Ag} - e'_{0AgI} = 1330.5 \pm 6.4$

$$pk'_s = \frac{e_{0Ag} - e'_{0AgI}}{58,86} = 22.60 \pm 0.11$$

Table 7. Variation of E with concentration of $LiClO_4$.

Electrodes: Ag-electrodes using method 3);
Ag, AgBr using Brown [2].
(I is the ionic strength in the titration vessel.)

a)
S = 0.040 C $AgClO_4$; 0.50 C $LiClO_4$ (50 ml)
T = 0.040 C $AgClO_4$; 2.0 C $LiClO_4$ (v ml).
S_b = 1.0 C $LiClO_4$
S_c = 0.10 C LiBr; 0.90 C $LiClO_4$

E mV	v ml	I
951.6	0.0	0.540
951.6	1.0	0.569
950.4	6.0	0.701
948.1	11.0	0.810
946.5	16.0	0.904
945.6	21.0	0.984
945.0	26.0	1.053
944.5	31.0	1.114
943.9	36.0	1.168
943.3	41.0	1.216
942.8	45.5	1.255

b)
S = 0.020 C LiBr; 0.50 C $LiClO_4$ (50 ml)
T = 0.020 C LiBr; 2.0 C $LiClO_4$ (v ml)
S_b = 1.0 C $LiClO_4$
S_c = 0.10 C LiBr; 0.90 C $LiClO_4$

E mV	v ml	I
58.3	0.0	0.520
64.7	5.0	0.656
69.0	10.0	0.770
73.2	16.0	0.884
75.0	20.0	0.949
77.2	25.0	1.020
78.2	30.0	1.083
81.0	40.0	1.187
84.6	45.0	1.231

$$X_e = [Ag]_{tot} - [I]_{tot} = [I^-] + [AgI_2^-] \quad (8)$$

Introducing the complex product $\varkappa_2$, the equilibrium conditions are, as long as solid AgI is present

$$[Ag^+]\ [I^-] = k_s$$

$$[AgI_2^-] = \varkappa_2[Ag^+]\ [I^-]^2 = \varkappa_2 k_s[I^-] \quad (9)$$

and thus

$$X_e = [I^-]\ (1 + \varkappa_2 k_s) \quad (10)$$

As for the quantity $(e'_{0AgI} - e_{ref})$ and the »solubility product» k'_s for AgI, which were calculated in the same way as for AgCl and AgBr it is easily seen that

$$e'_{0AgI} - e_{ref} = E + 58.86\ \log\ X_e \quad (11)$$

$$\log\ k'_s = e'_{0AgI} - e_{0Ag} = \log\ [Ag^+]\ X_e \quad (12)$$

$$k'_s = [Ag^+]X_e = k_s\ (1 + \varkappa_2 k_s) \quad (13)$$

Thus k'_s and $(e'_{0AgI} - e_{ref})$ should have constant values as long as solid AgI is present. The value for $\varkappa_2$ can be estimated from measurements at higher X_e, where all AgI has dissolved, since in such cases

$$[AgI_2^-] = [Ag]_{tot}$$

$$[I^-] = [I]_{tot} - 2\ [Ag]_{tot} = X_e - [Ag]_{tot}$$

As usual, $[Ag^+]$ can be obtained from E,

$$[Ag^+] = \text{antilog}\left[[E - (e_{0Ag} - e_{ref})]\ 58.86^{-1}\right]$$

Thus all concentrations are known that are needed for calculating $\varkappa_2$. From the preliminary experiments we calculated values of $\varkappa_2 = (2 \pm 1)10^{22}$. (If it was instead assumed that AgI_3^{2-} is formed, the spread in the values for $\varkappa_3$ was still larger.) With values of $\varkappa_2$ of this order of magnitude one would calculate $\log\ k_s = -22.8 \pm 0.3$.

Our experiments with I^- are to be considered as preliminary, since at any rate the presence of small amounts of free iodine may have disturbed the measurements. We intend to repeat them with an ether that is much freer from oxidizing substances.

SUMMARY

The behavior of silver electrodes has been studied during emf titrations of $Ag^+ClO_4^-$ with Li^+Cl^-, Li^+Br^-, and Li^+I^-, using as solvent 1 C $LiClO_4$-diethylether. Titration curves were obtained of the same type as in aqueous solution. When the ionic strength was kept constant, the emfs were found to follow Nernst's law rather well, both with excess of Ag^+ and halide ions. The effect of varying the ionic strength between 0.5 and 1.2 C has been studied.

For the solubility product in 1 C $LiClO_4$-ether at 23.5° C we found: AgCl: log $k_s = -14.30 \pm 0.11$, AgBr: log $k_s = -17.75 \pm 0.14$.

With I^- the measurements were complicated by the formation of a soluble complex, probably AgI_2^-, and by the appearance of small amounts of free iodine. Preliminary values: AgI: log $k_s = -22.8 \pm 0.3$; $\varkappa_2 = [AgI_2^-]\,[Ag^+]^{-1}\,[I^-]^{-2} = (2 \pm 1)\ 10^{22}$.

The values for log k_s in water at 25° C are according to Latimer [5]: AgCl — 9.77, AgBr — 12.48, AgI — 16.07.

This work has been supported by a grant from *Statens Naturvetenskapliga Forskningsråd.*

We wish to thank Professor Karl Myrbäck for his helpful interest. As in the first part of this work, Mr. Lars Evers has been an invaluable aid in the preparative work. We are indebted to Mr. Erik Ekedahl and Miss Karin Solders for many valuable discussions.

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Received March 4, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 335—353

The Formation of Cyclic Compounds from Acetylpyruvic Esters

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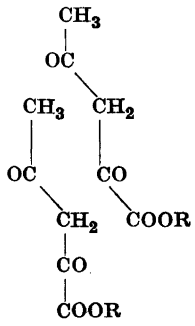
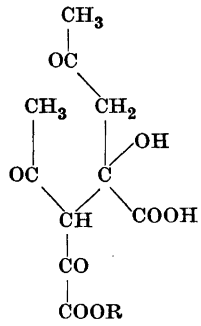
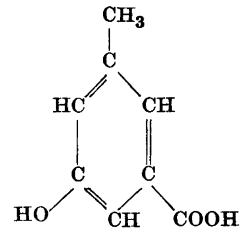
An intermolecular condensation of esters of acetylpyruvic acid was first described by Claisen [1]. On mixing the sodium compound of ethyl acetylpyruvate with glacial acetic acid and subsequently adding diluted sulphuric acid Claisen obtained a crystalline condensation product $C_{12}H_{16}O_8$ with m. p. 90—91°. The condensation product could be titrated as a monobasic acid, the titrated solution turning strongly yellow on the addition of an excess of alkali. When the product was heated with aqueous barium hydroxide oxalic acid and ethanol were split off and 3-methyl-5-hydroxybenzoic acid was formed. From these data Claisen concluded that the condensation product had the aliphatic structure I which is written so as to indicate the transformation into the aromatic acid II:



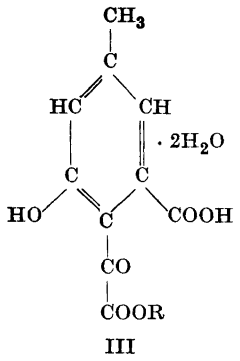
I



II



Claisen also found that the condensation took place in the same manner when the methyl ester of acetylpyruvic acid was used instead of the ethyl ester.



III

Heikel [2, 3], who had previously studied the condensation of acetylacetone in the presence of alkali into an aromatic compound, drew the conclusion that even the primary condensation product of the acetylpyruvic ester was an aromatic compound to which he assigned the structure III. In order to account for the composition found by analysis the compound should contain two molecules of water of crystallisation. He found, however, that the product on drying in a vacuum gave off one molecule of water only which was taken up again in a moist atmosphere. Heikel also discussed the possibility that the condensation product could have an aliphatic structure differing from the one postulated by Claisen only in the reversed positions of the free and the alkylated carboxyl group. He would then explain the loss of water on drying as being due to the formation of a lactone.

In connection with another investigation the present authors obtained the condensation product of the ethyl ester of acetylpyruvic acid, and finding it to be subject to several interesting reactions and transformations took up the question of its constitution. We have also investigated the analogous condensation product of the methyl ester of acetylpyruvic acid. The condensation product of the ethyl ester is shortly called the *ethyl product* and that of the methyl ester the *methyl product.*

We soon arrived at the conclusion that neither the formula of Claisen nor any of the two formulae put forward by Heikel could explain satisfactorily the experimental data. This will be evident from the following four points.

1. The ethyl product which is a monoethyl ester of a dibasic acid contains one molecule of crystal water. This is in accordance with Heikels drying experiments which we have verified. It has further been checked by the fact that the ethyl product, more correctly written $C_{12}H_{14}O_7 \cdot H_2O$, by treatment with diazomethane gave an ethyl methyl ester $C_{12}H_{16}O_7$ identical with the ester obtained when the anhydrous ethyl product reacted with diazomethane.

2. The ethyl product could be resolved into optically active components of high specific rotation (about 83°). The aromatic formula of Heikel is decisively excluded by this fact, but also the open-chained formula of Claisen and that of Heikel may be excluded for the reason that an aliphatic compound would hardly give such a high specific rotation.

3. The ethyl product gave a di-2,4-dinitrophenylhydrazone. From the aromatic formula one should expect a mono-hydrazone to be formed, and from the open-chained formulae tri-hydrazones.

4. By catalytic hydrogenation the methyl product took up two molecules of hydrogen giving a compound $C_{11}H_{16}O_7$ which did not contain water of crystallisation and which gave a mono-2,4-dinitrophenylhydrazone. On sublimation in a vacuum the hydrogenated product lost one molecule of water forming a lactone which on dissolving in alkali again took up water.

Other points which must be considered before postulating a new structural formula for the condensation product are:

5. The easy formation of 3-methyl-5-hydroxybenzoic acid in addition to the oxalic acid.

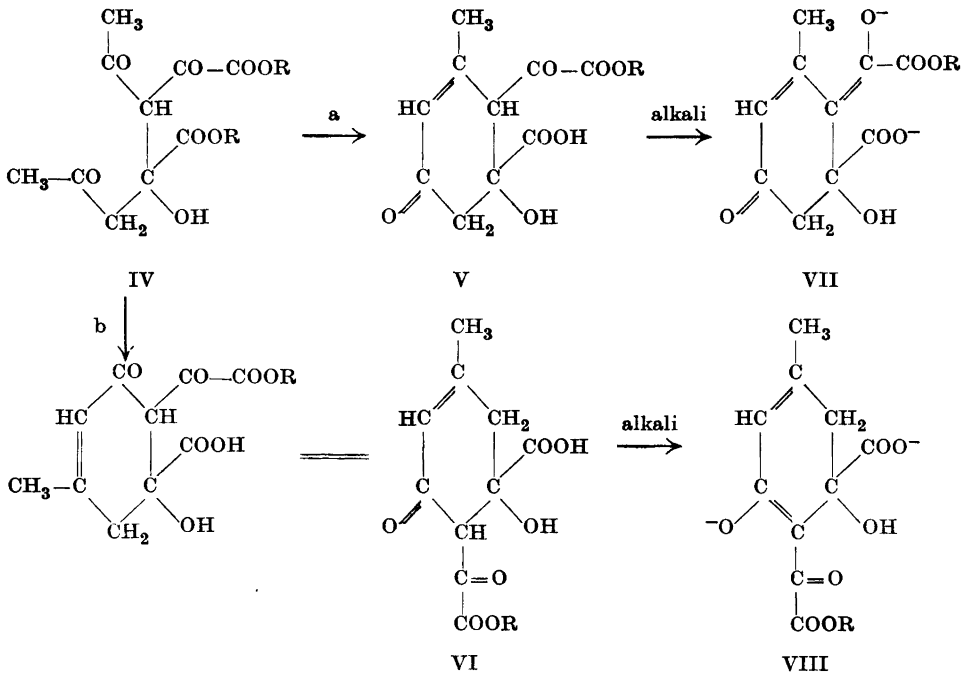
6. The yellow colouring of the solution of the condensation product caused by an excess of alkali. On acidification of the yellow solution shortly after the addition of the alkali most of the condensation product could be recovered unchanged. Even the fully alkylated substance obtained by treatment of the condensation product with diazomethane produced a yellow colour in alkaline medium, and it gave on prolonged treatment with diazomethane in the presence of methanol a yellow syrup, being evidently the methylated enol-form.

7. The methyl product gave a positive iodoform reaction with alkaline iodine solution.

8. a) The hydrogenated methyl product gave a negative iodoform reaction. b) It could be dissolved in one equivalent of alkali without coloration, but the solution turned yellow with an excess of alkali. On acidifying the colour disappeared and the unchanged hydrogenated product was easily recovered.

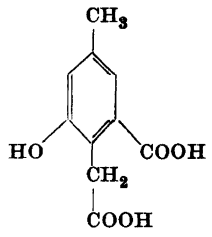
9. The hydrogenated condensation product could be transformed into two substances $C_{10}H_{10}O_5$ and $C_8H_{12}O_3$ together with oxalic acid. This point will be discussed in detail below.

The fact mentioned in point 2 would suggest that the ethyl product (as also the methyl product) must be a cyclic compound. The initial stage of the condensation leads most probably to an open-chain compound (IV). On further reaction in the weak alkaline medium the formation of a six-membered homocyclic ring takes place, during which process one molecule of water is split off. At the same time one of the ester groups is hydrolysed. The second stage of the condensation can be assumed to follow either of the two routes a and b leading to the two formulae V and VI. As the condensation product does not give carbon dioxide by oxidation with hydrogen peroxide it is most likely not a free α-ketoacid, and the carboxylic acid group bound directly to the ring must therefore be the free one. Both the formulae V and VI are in accordance with the experimental data set forth under points 1, 2, 3, 4, 5, 7 and 8 a. In order to decide between them it is necessary to consider also the points 6 and 9.



The yellow colouring of the condensation product or its methylated derivative mentioned in point 6 must be due to the formation of an enol-form. Formula V would give rise to an enol-form with the structure VII, whilst formula VI would give an enol-form with structure VIII. In the latter case there is also the possibility that the mobile hydrogen atom could migrate to the carbonyl in the side-chain. The arrangement of the double bonds in formula VII could be regarded as a semi-quinoid grouping and would probably represent a better chromophore than the system of conjugated double bonds in formula VIII. This might accordingly indicate that the condensation product has the structure given in formula V. In this connection it is noteworthy that compounds obtained by diene syntheses using p-benzoquinone as the dienophilic component contain a similar semi-quinoid grouping and that they are yellow in colour. See for example the recent results of Lora-Tamayo and Leon [4]. It must however be admitted that the experimental fact from point 6 can not alone be decisive in the question and therefore the facts from point 9 must be taken into account.

Assuming provisionally formula V for the ethyl product (R = C_2H_5) the hydrogenated product has been assigned formula X (see p. 341). By the hydrogenation, two molecules of hydrogen are added, and as the hydrogenated



IX

product forms a monohydrazone one molecule of hydrogen is taken up by the double bond and the other by one of the carbonyl groups. When the hydrogenated product was sublimed in a vacuum a lactone was obtained. From the formula V it will be seen that this result can be explained equally well in terms of the reduction of either one of the carbonyl groups. On the other hand the facts mentioned below concerning the degradation of the hydrogenated product can be explained in the simplest way by assuming that the carbonyl group in the side-chain is reduced to a secondary alcohol group as indicated in formula X.

On heating with aqueous barium hydroxide the hydrogenated product X was transformed into three substances: a) an aromatic compound $C_{10}H_{10}O_5$, b) a hydroaromatic compound $C_8H_{12}O_3$ and c) oxalic acid. Starting with one mole of X a little more than half a mole of oxalic acid could be isolated.

The compound $C_{10}H_{10}O_5$ is isomeric with the 5-hydroxy-3-carboxy-p-tolueneacetic acid (IX) previously described by one of us [5]. In some respects the new compound showed a great resemblance to the substance IX. Both compounds could be titrated as dibasic acids, and they gave with diazomethane, dimethyl esters. On treatment with dimethyl sulphate and alkali the phenolic hydroxyl group also was methylated giving trimethoxy compounds. When these were hydrolysed with alkali the corresponding methyl ethers of the free acids were obtained. The methyl ether of IX (m. p. 206°) could be degraded by oxidation. By using potassium permanganate the nuclear methyl group only was oxidised to a carboxyl group. When the product from this oxidation was treated with dilute nitric acid the side-chain was oxidised, resulting in the formation of methoxybenzene-2,3,5-tricarboxylic acid (l. c.). Recently we have found that the methyl ether (m. p. 206°) by direct oxidation with nitric acid gave 4-methyl-6-methoxy-phtalic acid which had the same m. p. (200°) as given for this substance in the literature. On the other hand we have not succeeded in degrading by oxidation either the new compound $C_{10}H_{10}O_5$ or its methyl ether. When oxidation did occur, complete destruction of the substance took place. It is evident therefore that the arrangement of the substituents in the new substance gives rise to marked steric hindrance. This is obvious also from the fact that it has not been possible to obtain a benzylidene derivative by treatment with benzaldehyde. Another outstanding difference between the two isomers lies in the fact that the old one (IX) easily gives a lactone, while the new one does not give one.

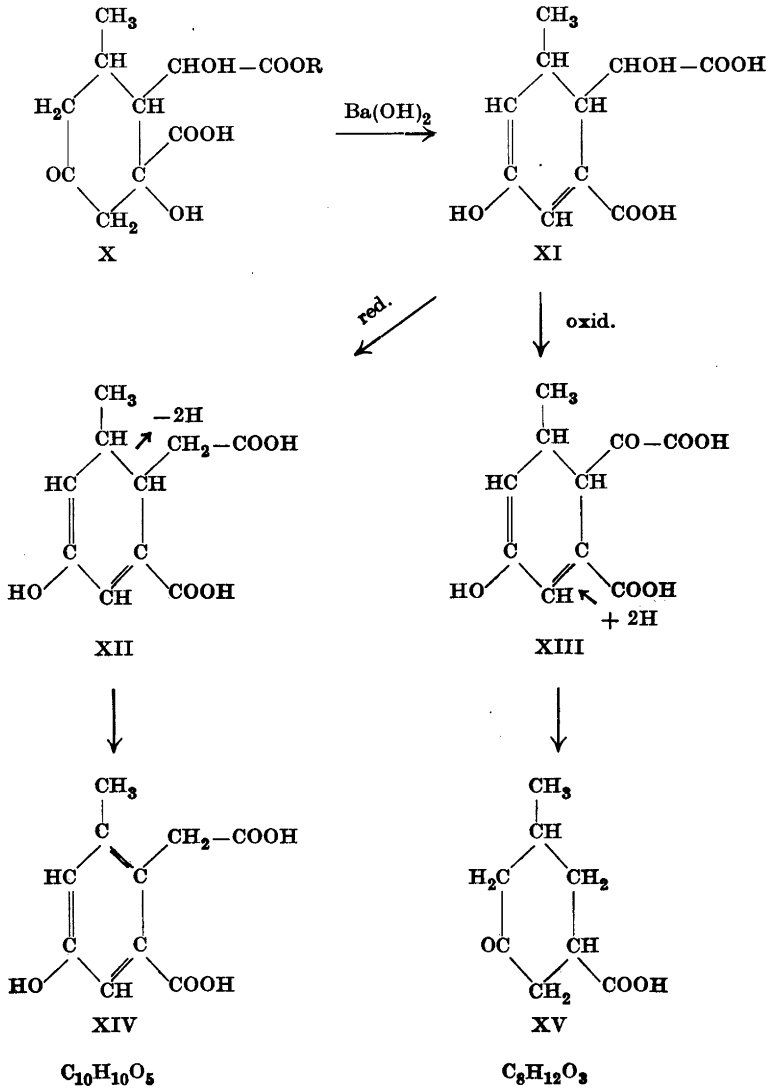
In agreement with formula IX the methyl ether of that substance on treatment with acetic anhydride gives an acid anhydride which dissolves in alkali

with the production of a yellow colour. It has previously been found by W. Dieckmann [6] that the anhydride of homophtalic acid owing to enolisation gives an intense yellow coloration with alkali. On the other hand the new isomeric acid does not give any intramolecular anhydride. Heating the acid itself with acetic anhydride results in the acetylation of the hydroxyl group, and when the methyl ether of the acid is used a mixed anhydride with acetic acid is formed. The fact that no intramolecular anhydride is obtained must be due to the steric hindrance mentioned above. The only reasonable explanation of the experimental data must therefore be that the new compound $C_{10}H_{10}O_5$ is 5-hydroxy-3-carboxy-*o*-tolueneacetic acid (XIV). This is also in accordance with the fact that the acid on treatment with dilute nitric acid gives a dinitro-compound in which the hydroxyl group is so strongly activated that the substance can be titrated as a tribasic acid and that it gives a trimethoxy derivative with diazomethane. It should be added that if the original condensation product had the formula VI given above this would have led to a compound identical with IX.

The compound $C_8H_{12}O_3$ is evidently a methylcyclohexanone-carboxylic acid. It forms a monomethyl ester and gives both a 2,4-dinitrophenylhydrazone and a semicarbazone. As the original condensation product can so easily be transformed into the 3-methyl-5-hydroxybenzoic acid the compound $C_8H_{12}O_3$ is most probably a 3-methylcyclohexanone-5-carboxylic acid (XV). Following the procedure of Meldrum and Perkin jun.[7] the 3-methyl-5-hydroxybenzoic acid by hydrogenation and subsequent oxidation has been transformed into a liquid mixture of stereoisomeric forms of 3-methylcyclohexanone-5-carboxylic acid. On treatment of this with 2,4-dinitrophenylhydrazone a mixture of two crystalline hydrazones was obtained which could be separated by fractional crystallisation. One of the hydrazones had the same m. p. as the hydrazone of $C_8H_{12}O_3$ and showed no depression when mixed with the latter. In accordance with the structural formula XV our methylcyclohexanone-carboxylic acid gave a dibenzylidene derivative.

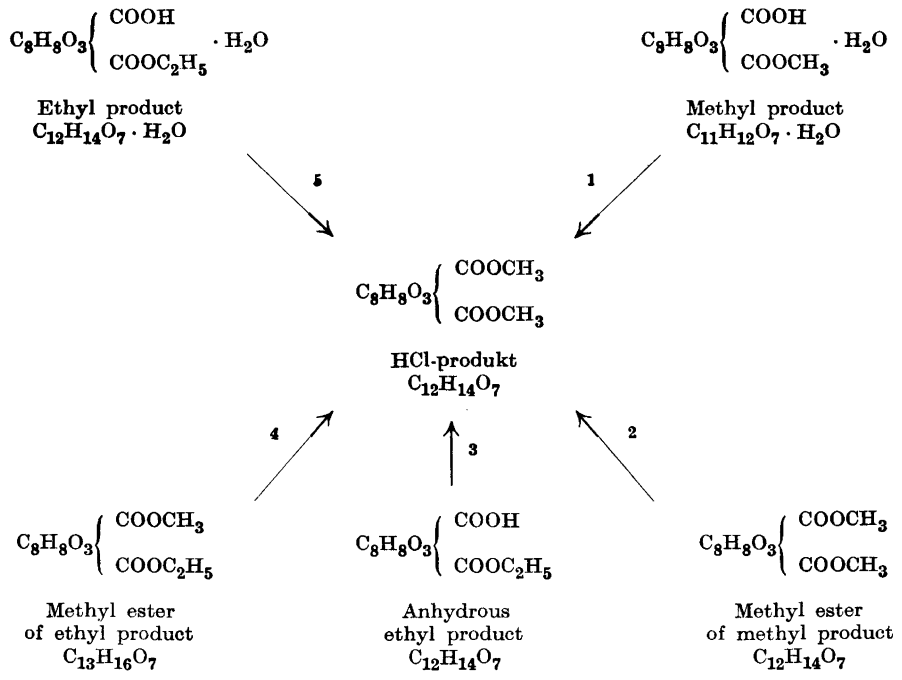
The following scheme explains the transformation of the hydrogenated product X into the compounds just described. On heating with aqueous barium hydroxide the ester group in X is hydrolysed, and at the same time one molecule of water is split off thereby forming a double bond in the ring. Through enolisation a second double bond is established in the ring, and the first intermediate product XI may therefore be regarded as a derivative of dihydrobenzene. Consequently it shows great reactivity, and the assumption can now be made that two kinds of reactions take place. By the first of these reactions pairs of molecules XI react in such a way that the side-chain —CHOH—COOH in one molecule is reduced to —CH_2—COOH whilst in the

other molecule it is oxidised to —CO—COOH giving the substances XII and XIII respectively. By the second reaction substance XII gives off two atoms of hydrogen which are taken up by substance XIII. Substance XII is thereby transformed into the aromatic compound XIV, while substance XIII owing to the simultaneous splitting off of oxalic acid gives the hydroaromatic compound XV. As will be seen this scheme also explains the formation of approximately half a molecule of oxalic acid from each molecule of the hydrogenated product.



From the preceeding experimental data it would appear most probably that the methyl and the ethyl product have the structure V (R = CH_3 and C_2H_5 respectively).

We now turn to some remarkable transformations which take place when the methyl or ethyl product or the corresponding esters, obtained by means of diazomethane, are treated with anhydrous methanol containing hydrogen chloride. In an attempt to esterify the free carboxylic group in the methyl product with methanolic hydrogen chloride a substance was obtained which was different from the dimethyl ester prepared by means of diazomethane. The new substance which is shortly designated the *HCl-product* contained two methoxyl groups as expected, and it was found to be isomeric with the ester prepared by means of diazomethane. Quite unexpectedly however it could still be titrated as a monobasic acid, and an excess of alkali produced a yellow coloration of the solution. The following scheme shows the alternative substances from which the same HCl-product can be obtained by treatment with methanolic hydrogen chloride.



As will be seen the transformations include in some cases an esterification, in some an alcoholytic exchange of ethyl with methyl also, and in all cases an intermolecular rearrangement. In transformation 2 the rearrangement is the

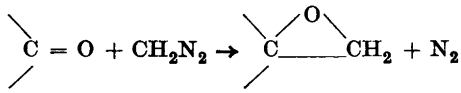
only process. It should be added that when ethanol was used instead of methanol an analogous HCl-product containing two carboxethyl groups was obtained.

A closer examination of the HCl-product gave the following results. On heating with aqueous barium hydroxide the HCl-product was decomposed into oxalic acid and the same methyl-hydroxybenzoic acid as obtained from the original condensation product. The acid group could not be a carboxyl group since the HCl-product was recovered unchanged even after boiling for one hour with thionyl chloride. Neither could a salt be obtained with bases such as strychnine. The HCl-product gave a di-2,4-dinitrophenylhydrazone. As the product could be prepared also from the methyl ester of the methyl product it must certainly contain two methylated carboxyl groups. Of the seven oxygen atoms four will thus belong to the two ester groups and two to the carbonyl groups. The one remaining oxygen atom must therefore belong to a hydroxyl group which is accordingly responsible for the acid character.

When the HCl-product was treated with diazomethane a reaction took place according to the equation:

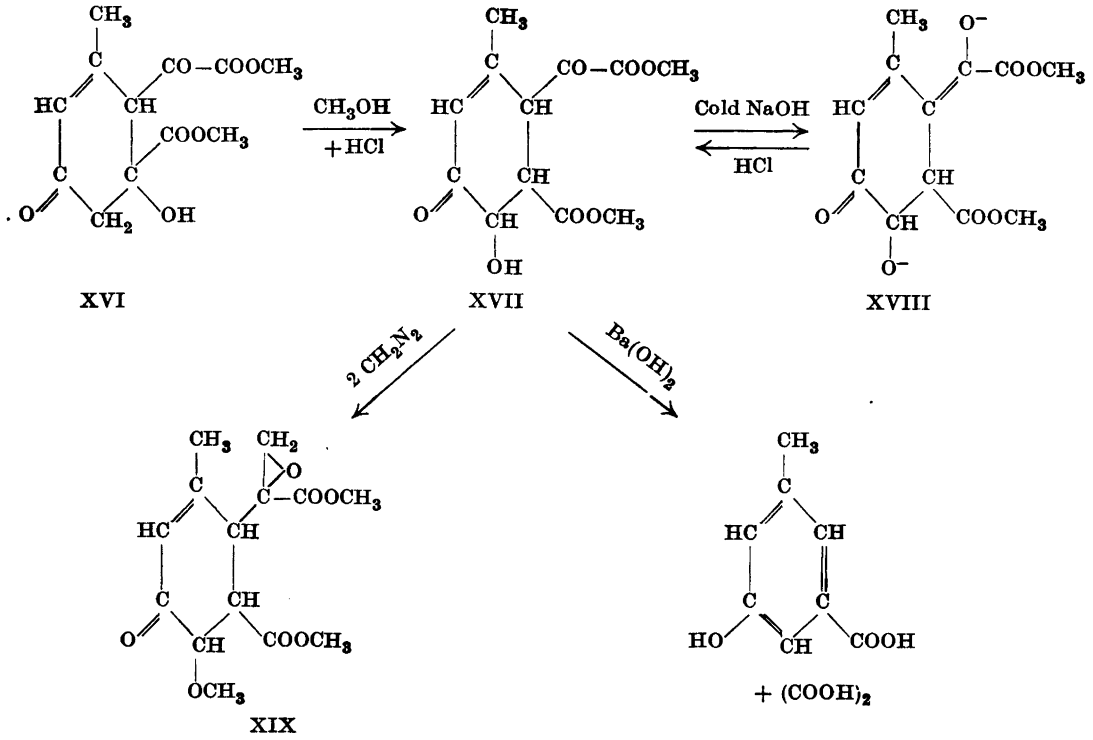
$$C_{12}H_{14}O_7 + 2\ CH_2N_2 \rightarrow C_{14}H_{18}O_7 + 2\ N_2$$

Consequently one would expect the new substance to contain four methoxyl groups. However by the Zeisel-Fanto determination three methoxyl groups only were found. A methylene group must therefore have been taken up in such a form that it is not split off as methyl iodide on boiling with hydroiodic acid. From the literature it is known that diazomethane can react with ketones forming ethylene oxides or homologous ketones [8]. Since the HCl-product as mentioned was a diketone it could be assumed that one of the ketone groups had reacted in a similar way. This seems to be the case when the product from the treatment with diazomethane gave only a mono-2,4-dinitrophenylhydrazone. Most probably one of the keto-groups has reacted with diazomethane in the following way:



The substance $C_{14}H_{18}O_7$ did not give a yellow coloration with alkali, and on heating with aqueous barium hydroxide it did not give rise to any oxalic acid.

The formula XVII which from these experimental facts has been postulated for the HCl-product will be found in the scheme below. In the same scheme the isomerisation of the methyl ester of the methyl product XVI has



XVI XVII XVIII XIX

been demonstrated, and as will be seen this isomerisation involves only the migration of the hydroxyl group from the meta to the para position in regard to the nuclear methyl group. Formula XVIII represents the structure responsible for the yellow colouring in alkaline solution. Formula XIX represents the substance $C_{14}H_{18}O_7$, and it is in accordance with the fact that this substance does not give oxalic acid or a yellow coloration with alkali.

Finally it should be mentioned that we have not been able to give a satisfactory explanation of the yellow coloration appearing when an excess of alkali is added to the hydrogenated product X.

EXPERIMENTAL PART

Condensation of ethyl acetylpyruvate

To ethyl acetylpyruvate (15.8 g) was added a solution of sodium acetate (6.8 g) in water (30 ml), and the mixture left at room temperature over night. Next day the ester had dissolved and the rather dark-coloured solution was acidified with 50 per cent sulphuric acid. The crystalline ethyl product which then separated (9 g = 70 per cent yield) was recrystallised from water and dried in the air to constant weight; m. p. 80—91°.

Owing to the yellow coloration produced with excess of alkali the titrations had to be carried out very carefully. About 200 mg of the substance were dissolved in 15 ml ethanol and 0.1 *N* sodium hydroxide added drop by drop.

0.233 and 0.204 g required 8.20 and 7.24 ml 0.1 *N* NaOH

$C_{12}H_{14}O_7 \cdot H_2O$	Calc.	C 50.00		H 5.55		C_2H_5O	15.65		*M* 288.3
	Found	» 49.74,	49.56	» 5.50,	5.63	»	15.25,	15.57	» 284, 282

The finely powdered ethyl product dispersed in a thin layer on a watch glass was dried at room temperature in a vacuum above phosphorous pentoxide. The substance first deliquesced and then crystallised. In two days 568 mg lost 33.5 mg water, calc. for one mole water 35.5 mg. The watch glass with the substance was then placed in a moist atmosphere and in the course of one day the weight increased with 33.5 mg. The analysis showed that the substance had regained the same composition as the original ethyl product.

Ethyl product and aqueous barium hydroxide

The ethyl product (1 g) was heated on the water-bath with barium oxide (1.5 g) and water (20 ml) for half an hour. The barium oxalate which separated was filtered off and the oxalic acid identified in the usual way. The filtrate was evaporated to a small volume and acidified with hydrochloric acid. The 3-methyl-5-hydroxybenzoic acid which separated was purified by sublimation in a vacuum; m. p. 207° (decomposition).

$C_8H_8O_3$	Calc.	C 63.15		H 5.26	
	Found	» 62.88,	62.94	» 5.11,	5.11

Methyl ester of ethyl product

Powdered ethyl product (15 g) was mixed with ether (50 ml) and an ethereal solution of diazomethane added until the yellow colour remained. After evaporation of the ether the ester was recrystallised twice from ethanol. Yield 12 g; m. p. 58—59°.

$C_{13}H_{16}O_7$ (284.3)	Calc.	C 54.93		H 5.63	
	Found	» 54.73,	54.67	» 5.90,	6.00

Alkoxyl determination after Zeisel-Fanto:
0.1630 and 0.1927 g subst. gave 0.2645 and 0.3152 g AgI. Calc. for one ethoxyl and one methoxyl group 0.2692 and 0.3180 g AgI.

Cryoscopic measurements in benzene:

0.4435 g subst.,	19.67 g benzene,	△ 0.425°	*M* 272
0.7755 »	»	19.67 »	»	» 0.73°	» 277

Anhydrous ethyl product and diazomethane

The ethyl product was dried as described above. It was then powdered again and dried for two days more. The completely dried powder was added to an ethereal solution of diazomethane which had been dried first with potassium hydroxide and then by placing

it for some hours above metallic sodium. After removing the ether the ester was recrystallised from ethanol. The m. p. 58—59° was not altered after mixing with ester prepared from the ethyl product containing crystal water.

$C_{13}H_{16}O_7$	Calc.	C 54.93	H 5.63
	Found	» 54.61, 55.31	» 5.54, 5.29

Optical resolution of ethyl product

a) In water: Ethyl product (2 g) and strychnine (2.34 g) were dissolved by heating in water (100 ml), and the solution filtered. After two days the strychnine salt which had separated was filtered off and recrystallised from water (40 ml). Yield 1.5 g. The salt was dissolved in 40 per cent sulphuric acid, and the ethyl product extracted with ether. After drying with sodium sulphate and removal of the ether 0.5 g ethyl product remained; m. p. 80—90°.

$$[\alpha]_D^{20} = -83.3° \ (\alpha \ -7.82°, \ c \ 9.38 \text{ in ethanol, 1 dm tube}).$$

b) In acetone: Ethyl product (2 g) and strychnine (2.34 g) were dissolved in acetone (40 ml). The strychnine salt crystallised much more slowly from acetone than from water. After two days 1.12 g salt had separated and the ethyl product (0.42 g) isolated as above; m. p. 81—92°.

$$[\alpha]_D^{20} = -82.7° \ (\alpha \ -7.75°, \ c \ 9.38 \text{ in ethanol, 1 dm tube}).$$

The isolation of the dextro-form was performed in the following way. The aqueous mother-liquor from the salt of the levo-acid was left for one week in an open beaker when some more strychnine salt separated. Having removed this the filtrate was concentrated in a vacuum desiccator to half volume. After acidifying with sulphuric acid 0.73 g of the dextro-acid was isolated by extraction with ether; m. p. 81—91°.

$$[\alpha]_D^{20} = +58.6° \ (\alpha + 5.50°, \ c \ 9.38 \text{ in ethanol, 1 dm tube}).$$

No attempts were made to obtain the optically pure dextro-form.

Condensation of methyl acetylpyruvate

The necessary methyl acetylpyruvate was most conveniently prepared by adding first ethyl oxalate (256 g) and then acetone (100 g) to anhydrous methanol (1200 ml) in which sodium (40 g) had been dissolved. Simultaneously with the condensation an alcoholysis took place, and the sodium salt of the methyl acetylpyruvate separated in the course of a day. This salt is much more easily filtered than the sodium salt of the ethyl ester. It was stirred up with ice-water and sufficient 40 per cent sulphuric acid added. The solid methyl acetylpyruvate (161 g = 67 per cent of theory) after filtration and washing with water was then sufficiently pure for our needs.

The intermolecular condensation of the methyl acetylpyruvate in the presence of sodium acetate was carried out as described for the ethyl ester. It could also be per-

formed without first isolating the methyl acetylpyruvate. In this case an equivalent quantity of acetic acid was added to the sodium salt of the methyl acetylpyruvate directly after filtration. The methyl product after recrystallisation from water had m. p. 68—75°.

0.181 and 0.161 g subst. required 6.83 and 6.04 ml 0.1 *N* NaOH

$C_{11}H_{12}O_7 \cdot H_2O$	Calc.	C 48.50	H 5.12	CH_3O 11.31		*M* 274.2	
	Found	» 48.22, 48.40	» 5.29, 5.20	» 11.34, 11.21		» 265, 267	

The methyl product gave a strongly positive iodoform test.

Methyl ester of methyl product

The methyl product was treated with diazomethane as described for the ethyl product. After recrystallisation three times from ethanol the methyl ester of the methyl product had m. p. 74—75°.

$C_{12}H_{14}O_7$	Calc.	C 53.33	H 5.18	CH_3O 22.97
	Found	» 53.67, 53.47	» 5.18, 5.00	» 22.80

When sodium ethylate was added to a solution of the methyl ester of the methyl product in ethanol the solution became intensely yellow in colour. To a solution of the methyl ester in anhydrous ether containing about 10 per cent methanol an ethereal solution of diazomethane was added and the whole left over night at room temperature. After the solvent had been evaporated under reduced pressure a yellow syrup remained which did not crystallise.

Preparation of the hydrazone: The ester was dissolved in methanol and heated for a few minutes with a sufficient quantity of 2,4-dinitrophenylhydrazine dissolved in 2 *N* hydrochloric acid. Next day an orange-yellow substance had separated which could be recrystallised by dissolving in glacial acetic acid and cautiously adding water; m. p. 212°. The substance was according to the analysis a dihydrate of the dihydrazone.

$C_{24}H_{22}O_{13}N_8 \cdot 2H_2O$ Calc. N 16.81, Found N 16.54, 16.65

Hydrogenation of methyl product

A solution of methyl product (10 g) in methanol (50 ml) to which platinum oxide (0.6 g) had been added took up 1280 ml hydrogen (1190 mm Hg) in 24 hours; calc. for 2 mole hydrogen 1220 ml. After filtering the methanol was evaporated on the water-bath and the hydrogenated product recrystallised from water. Yield 5 g; 164—165° (decomp.).

26.31 and 21.40 mg subst. required 1.054 and 0.833 ml 0.1 *N* NaOH

$C_{11}H_{16}O_7$	Calc.	C 50.77	H 6.15	CH_3O 11.90		*M* 260.2	
	Found	» 50.78, 50.89	» 6.39, 6.15	» 11.52		» 250, 257	

The hydrogenated methyl product could be sublimated in a vacuum, losing at the same time one molecule of water and giving a product with m. p. 162—163°.

0.1133 and 0.1286 g subst. required 4.75 and 5.30 ml 0.1 *N* NaOH

$C_{11}H_{14}O_6$	Calc.	C 54.51	H 5.78	CH_3O 12.81	*M* 242.2
	Found	» 54.68, 54.75	» 5.67, 5.69	» 12.80	» 239, 243

The product from the sublimation, which was a lactone, was dissolved in sodium hydroxide and the solution acidified. The substance which then separated was identical with the hydrogenated methyl product. M. p. 164—165°.

Found C 51.05, 51.28 H 6.39, 6.10

A mono-2,4-dinitrophenylhydrazone of the hydrogenated product was prepared in the usual way. The orange-yellow hydrazone was recrystallised from ethanol; m. p. 186—187°.

$C_{17}H_{20}O_{10}N_4$ Calc. N 12.73 Found N 12.69, 12.97

Degradation of hydrogenated product

The hydrogenated product (12.5 g) was heated with barium oxide (16.4 g) and water (250 ml) for half an hour on a water-bath. The colour of the solution which was at first intensely yellow changed to orange and finally became rather dark. During the heating a salt separated which was found to be barium oxalate. It was dissolved in dilute hydrochloric acid and again precipitated by adding ammonia until p_H 6. The dry salt then weighed 6.82 g which is 59 per cent of the amount (10.82) calculated on the assumption that one mole of oxalic acid had been split off from each mole of the hydrogenated product. The oxalic acid was isolated by extracting the solution of the barium salt in hydrochloric acid with ether. From the ethereal solution a crystalline substance was obtained which had m. p. 101° and gave a crystalline precipitate with calcium ions.

The filtrate from the barium oxalate was acidified with hydrochloric acid and extracted five times with 200 ml portions of benzene. Evaporation of the combined and dried extracts gave 3.4 g of a crystalline substance. This on recrystallising twice from benzene gave colourless needles with m. p. 94—95°. The substance was a monobasic acid.

94.0 and 82.1 mg subst. required 6.04 and 5.35 ml 0.1 *N* NaOH

$C_8H_{12}O_3$	Calc.	C 61.55	H 7.69	*M* 156.2
	Found	» 61.63, 61.58	» 7.72, 7.56	» 156, 153

Molecular weight by ebulliometric method:

0.1808 g subst. in 8.18 g ether, Δ 0.33° *M* 141

The solution which had been extracted with benzene was now extracted five times with ether in 300 ml portions. After drying with sodium sulphate the ether was distilled off. A crystalline substance (3.5 g) remained which possessed a fatty acid odour. It was recrystallised from water containing a few per cent of hydrochloric acid and was then obtained in glossy shells without any smell; m. p. 268° (decomp.). The substance was a dibasic acid.

25.53 and 30.20 mg subst. required 2.36 and 2.80 ml 0.1 *N* NaOH

$C_{10}H_{10}O_5$	Calc.	C 57.14	H 4.76	*M* 210.2
	Found	» 57.14, 57.20	» 4.70, 4.81	» 216, 216

5.42 mg subst., 46.7 mg camphor, △ 22.8° *M* 204

Investigation of $C_8H_{12}O_3$

The substance was easily soluble in ether, benzene, chloroform and glacial acetic acid. It was sparingly soluble in cold water but dissolved on heating. The iodoform reaction was negative.

A mono-2,4-dinitrophenylhydrazone was prepared in the usual way. It crystallised from 70 per cent ethanol in yellow needles, m. p. 165—166°.

$C_{14}H_{18}O_7N_4$	Calc.	C 50.00	H 5.36	N 16.82
	Found	» 50.00	» 5.11	» 17.00

Semicarbazone: On mixing 50 mg of the substance with an appropriate quantity of semicarbazide a mono-semicarbazone was immediately formed. It was recrystallised from water. Yield 50 mg, m. p. 200°.

$C_9H_{15}O_3N_3$	Calc.	C 50.70	H 7.03
	Found	» 50.59, 50.59	» 6.91, 6.77

The methyl ester of $C_8H_{12}O_3$ was prepared by means of diazomethane. The ester was liquid. It gave a semicarbazone which after crystallisation from water had m. p. 162—164°.

$C_{10}H_{17}O_3N_3$	Calc.	C 52.86	H 7.49
	Found	» 52.72, 52.70	» 7.40, 7.42

No oxime could be obtained from $C_8H_{12}O_3$.

A dibenzylidene derivative was prepared in the following way. A solution of the substance (1.56 g) and benzaldehyde (2.12 g) in ethanol (10 ml) and 2 *N* sodium hydroxide (8 ml) was left for two days at room temperature. Addition of 2 *N* hydrochloric acid (10 ml) caused the separation of a syrup which crystallised when stirred with a glass rod. After recrystallising several times from diluted ethanol the dibenzylidene compound was obtained as light yellow crystals, m. p. 170°.

$C_{22}H_{20}O_8$	Calc.	C 79.49	H 6.07
	Found	» 79.75, 79.60	» 6.32, 6.18

Investigation of $C_{10}H_{10}O_5$

The substance did not lose water on sublimation in a vacuum. The sublimated product had m. p. 268° (decomp.) and the composition was unchanged.

$C_{10}H_{10}O_5$	Calc.	C 57.14	H 4.76
	Found	» 57.35, 57.35	» 4.62, 4.71

On heating with acetic anhydride an acetyl derivative was formed which had m. p. 173° and from which the original substance with m. p. 268° could be recovered by treatment with alkali.

The dimethyl ester of $C_{10}H_{10}O_5$ was prepared by means of diazomethane. Recrystallised from diluted ethanol, m. p. 109—110°.

$C_{12}H_{14}O_5$	Calc.	C 60.50	H 5.88	CH_3O 26.05
	Found	» 60.68, 60.73	» 6.09, 6.15	» 25.80

Nitration: By the oxidation of $C_{10}H_{10}O_5$ with potassium permanganate no definite oxidation products could be isolated. In an attempt to oxidise with dilute nitric acid (about 20 per cent) it was found that a dinitro compound was formed. The nitration was carried out as follows. The substance (200 mg) was heated for ten minutes on the water-bath with the nitric acid (5 ml). Subsequent extraction with ether gave 90 mg of a crystalline substance. After recrystallisation from hydrochloric acid diluted with one volume of water the nitro compound was obtained in yellow needles; m. p. 225° (decomp.). Unlike the original substance the nitro derivative was easily soluble in cold water. It also dissolved easily in ether, acetone and ethyl acetate, but only slightly in benzene. On titration it required three equivalents of alkali for neutralisation.

24.48 and 17.38 mg subst. required 2.47 and 1.72 ml 0.1 *N* NaOH

$C_{10}H_8O_9N_2$	Calc.	C 40.01	H 2.66	N 9.35	*M* 300.2
	Found	» 39.70	» 2.85	» 9.33, 9.20	» 297, 300

On treatment with diazomethane the dinitro compound was transformed into a trimethoxy derivative which was colourless and had m. p. 86—87°.

$C_{13}H_{14}O_9N_2$	Calc.	C 45.61	H 4.09
	Found	» 45.94, 45.70	» 3.86, 3.90

Methyl ether of $C_{10}H_{10}O_5$

On treatment of $C_{10}H_{10}O_5$ with dimethyl sulphate in the presence of sodium hydroxide the carboxylic groups as well as the phenolic hydroxyl were methylated. The ester was not however isolated, but the ester groups were hydrolysed by boiling with an excess of alkali. The methyl ether was precipitated by acidifying the alkaline solution. Yield about 90 per cent of theory. The methyl ether which could be recrystallised from diluted ethanol was dimorphic, the modifications having m. p. 187° and 215°. Each of the forms could be transformed into the other by dissolving and inocculating the solution with crystals of the other form. The ether was a dibasic acid.

26.3 mg subst. required 2.28 ml 0.1 *N* NaOH

$C_{11}H_{12}O_5$	Calc.	C 58.94	H 5.35	CH_3O 13.84	*M* 224.2
	Found	» 58.56, 58.56	» 5.33, 5.17	» 14.00	» 231

On heating with acetic anhydride the methyl ether formed a mixed acid anhydride with acetic acid. The anhydride which was a liquid reacted with ethanol giving ethyl acetate. After treatment with alkali the unchanged methyl ether could be recovered.

HCl-Product from ethyl product

The ethyl product (15 g) was dissolved in methanol (110 ml) containing hydrogen chloride (1.1 *N*) and the solution left at room temperature for two days. A substance had then separated in large rhombic crystals. From the mother-liquor more of the same substance was obtained after dilution with water. The HCl-product (9 g) was recrystallised from methanol and had m. p. 156°. It could be titrated as a monobasic acid, and the solution became strongly yellow coloured when an excess of alkali was added. On acidification of the yellow solution a few minutes later, the HCl-product could be recovered unchanged in good yield. It contained two methoxyl groups.

0.3705 and 0.3090 g subst. required 13.91 and 11.53 ml 0.1 *N* NaOH

$C_{12}H_{14}O_7$	Calc.	C 53.33	H 5.18	CH_3O 22.97	*M* 270.2
	Found	» 53.16, 53.18	» 5.40, 5.41	» 22.45, 22.65	» 266, 268

Molecular weight in acetone:

0.4692, 0.5942 g subst., 5.73, 10.33 g acetone, △ 0.50, 0.35° *M* 273,274

The HCl-product dissolved easily in acetone but was only slightly soluble in ether and water.

An identical substance was prepared by treating the anhydrous ethyl product (obtained by drying the hydrate in a vacuum above phosphorous pentoxide) with methanolic hydrogen chloride. M. p. 156°, alone and in admixture.

Found C 53.09, 53.20 H 5.34, 5.15 CH_3O 22.72, 22.67

HCl-Product from other substances

By treatment of the methyl product, the methyl ester of the ethyl product and the methyl ester of the methyl product with 1.1 *N* hydrogen chloride in methanol three substances, A, B and C respectively were obtained. These were all identical with the HCl-product prepared from the ethyl product. M. p. in all cases 156°, alone and in admixture.

$C_{12}H_{14}O_7$	Calc.	C 53.33	H 5.18	CH_3O 22.97
A	Found	» 53.37, 53.20	» 5.24, 5.20	» 22.83, 22.73
B	»	» 53.21, 53.30	» 5.25, 5.20	
C	»	» 53.31, 53.25	» 5.13, 5.15	

Investigation of the HCl-product

A solution of the HCl-product (1 g) and barium oxide (1.5 g) in water (20 ml) was heated for half an hour on the water-bath. Barium oxalate separate and was filtered off. On acidifying the filtrate an acid was isolated which was found to be identical with the 3-methyl-5-hydroxybenzoic acid obtained from the ethyl product. M. p. 207°, no depression.

$C_8H_8O_3$	Calc.	C 63.15	H 5.26
	Found	» 63.10, 63.16	» 5.30, 5.33

A 2.4-dinitrophenylhydrazone of the HCl-product was prepared in the usual way. The hydrazone was orange-yellow. Recrystallisation could not be satisfactorily accomplished so the raw hydrazone had to be used for analysis.

Monohydrazone	Calc.	N 12.40	
Dihydrazone	»	» 17.78	Found N 16.45, 16.35

Reaction with diazomethane: Finely powdered HCl-product (5 g) was added to an ethereal solution of diazomethane which was cooled with ice-water. Before all the product had reacted a new crystalline substance began to separate. To complete the reaction the substance was left for 5—6 hours at room temperature with an excess of diazomethane. After distilling off the ether the residue was recrystallised several times from ethanol; colourless needles, m. p. 135—137°.

$C_{14}H_{18}O_7$	Calc.	C 56.36	H 6.06	CH_3O 31.21
	Found	» 56.46, 56.53	» 5.93, 5.97	» 30.84, 31.02

When alkali was added to a solution of the new substance in ethanol no yellow coloration was produced, and on heating for half an hour with aqueous barium hydroxide no barium oxalate was formed.

A mono-2,4-dinitrophenylhydrazone was prepared in the usual way. After recrystallisation from diluted ethanol the orange-yellow hydrazone had m. p. 172—173°.

$C_{20}H_{22}O_{10}N_4$ Calc. N 11.71 Found N 11.92, 12.11

Ethyl product with ethanolic hydrogen chloride

The ethyl product (3 g) was dissolved in anhydrous ethanol (22 ml) containing hydrogen chloride (1.1 *N*). After three days at room temperature 1 g of a crystalline substance had separated which was recrystallised from ethanol. M. p. 122—123°. The substance contained two ethoxyl groups, and it could be titrated as a monobasic acid. Excess of alkali gave a yellow coloration.

27.4 and 26.5 mg subst. required 0.932 and 0.912 ml 0.1 *N* NaOH

$C_{14}H_{18}O_7$	Calc.	C 56.36	H 6.06	C_2H_5O 30.22	*M* 298.3
	Found	» 56.37, 56.40	» 5.71, 6.14	» 29.48, 30.26	» 294, 297

An identical substance was obtained by treating the methyl product with ethanolic hydrogen chloride. M. p. 122—123° alone and in admixture.

Found C 56.46, 56.30 H 6.10, 6.00 C_2H_5O 29.93

On heating this ethyl HCl-product with aqueous barium hydroxide 3-methyl-5-hydroxybenzoic acid and oxalic acid were formed.

SUMMARY

It has been found that the intermolecular condensation of an ester of acetylpyruvic acid led to a substance with cyclic structure. The condensation product could be resolved into optically active components, it could be esterified by means of diazomethane, and it gave a dihydrazone. By catalytic hydrogenation the condensation product took up two molecules of hydrogen, and on heating with aqueous barium hydroxide the hydrogenated product could be degraded to an aromatic hydroxy-dicarboxylic acid, a hydroaromatic keto-carboxylic acid and oxalic acid. On treatment of the condensation product with methanolic hydrogen chloride an interesting isomerisation took place alongside of the esterification, resulting in the formation of a substance which could be titrated as a monobasic acid.

We are indebted to cand. real. Ingvald Augestad for his assistance in some of the preparations.

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Received March 14, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 354—358

Investigations on Plasmin

I. On the Proteolytic Activity of Plasmin

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As the nomenclature of the fibrinolytic enzyme in blood plasma has been very unclear, a short survey of the nomenclature may be appropriate. The enzyme has been called plasma proteas, plasma trypsin (Schmitz[1]), lysin factor (Milstone[2]), tryptas (Ferguson[3]), fibrinolas (Dyckerhoff[4]), plasma proteolytic enzyme (Ratnoff[5]), fibrinolysin (Kaplan *et al.*[6]; Loomis *et al.*[7]), and finally plasmin suggested by Christensen and MacLeod[8]. The name plasmin is to prefer to fibrinolysin as the latter has been used also for the activator from hemolytic Streptococci, the streptokinase (Christensen[9]). The precursor of plasmin is consequently called plasminogen[8] and the inhibitor present in the albumin fraction of plasma is called antiplasmin by Macfarlane and Pilling[10].

The proteolytic activity of plasmin has been determined in different ways. Christensen[11] chose as the unit of activity that amount plasmin in a volume of 0.2 ml that will lyse a standard fibrin clot in 30 minutes at pH 7.2—7.3 and a temperature of 40° C. Later Christensen[9] determined the activity viscosimetrically on gelatin according to the formula: $\frac{(V_0 - V_{10}) \cdot 100}{V_0 \cdot 10}$, where V_0 is the initial specific viscosity and V_{10} is the viscosity at 10 minutes, the temperature being 37.0° C and at pH 7.4. Kaplan *et al.*[6] measured the decrease in viscosity of a 4 per cent gelatin solution after one hour at 37.5° C at different pH values in phosphate- and imidazol buffers. Loomis *et al.*[7] defined one unit of plasmin activity as »that amount which will dissolve one ml of 0.3 per cent fibrin clot in 120 seconds at pH 7.2 and 45° C in an isotonic saline system buffered with imidazol».

Recently Hultin[12] has introduced an expression for enzymic activity, first experimentally tested on the degradation of starch by α-amylase[13]. Later the

proteolytic activity of pepsin, gelatinase, and trypsin upon gelatin [14] was determined by means of this formula.

The expression is:

$$A^{t^\circ}_{e/s} = C_s^2 \cdot \frac{\mathrm{d}\frac{1}{\eta_{sp}}}{\mathrm{d}t}$$

where

A enzymic activity in units per gram of solution at t° C
$^e/_s$ abbreviation for enzyme and substrat
c_s concentration of substrate in grams per gram of solution
η_{sp} specific viscosity and
t time in seconds

As the results seem to confirm the formula and as the reproducibility is good, it seems appropriate to use the formula for measuring the proteolytic activity of plasmin. In some viscosity measurements it is necessary to use an extended formula taking the ionic factor in account. That has recently been done by Ingelman and Malmgren [15] in calculating enzymic breakdown of polymetaphosphate. In this investigation however no ionic factor has been introduced as all the experiments have been performed under the same conditions.

The plasmin was prepared from ox blood, the fibrin was removed by stirring and subsequent straining, the erythrocytes were removed by centrifugation, and the serum was treated according to Loomis *et al.*[7]. The plasmin, which is in the euglobulin fraction, was precipitated between 25 and 29 per

Table 1. Activity of different plasma fractions.

No.	Batch	Fraction	Per cent N	Activity: $A \cdot 10^6$/g N	Storage
1	I	25—29	3.54	63.2	—
2	I	25—29	3.54	49.9	60 days, 18°, dry
3	II	0—25	10.3	41.1	1 ½ hours, 18°, sol.
4	II	0—25	10.3	32.8	7 » 18°, »
5	II	0—25	10.3	21.7	11 » 18°, »
6	II	0—25	10.3	23.9	90 days, 18°, dry
7	II	25—29	8.76	62.6	—
8	III	15—25	3.81	29.8	—
9	III	25—29	8.34	58.2	—
10	III	29—33	9.11	20.9	—
11	III	15—25	3.81	21.7	1 year, 2°, dry
12	III	25—29	8.34	16.1	1 » 2°, »
13	III	29—33	9.11	5.8	1 » 2°, »

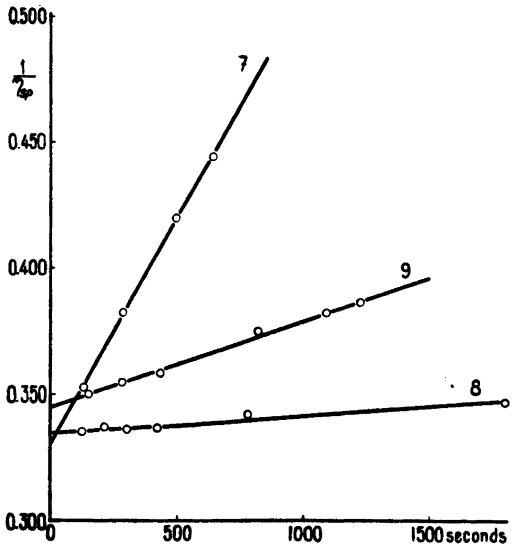

Fig. 1. The enzymic activity calculated from the slope of the line.

cent ammonium sulfate saturation. The antiplasmin was abolished by shaking with chloroform for half an hour. For comparison the fractions obtained before 25 and after 29 percent saturation with ammonium sulfate were also tested. The samples were made up of 2.00 ml plasmin solution (5.0 per cent dry weight) and 5.00 ml of a 3.00 per cent gelatin solution at 35.5° C and pH 7.2. The outflow times were measured in Ostwald viscosimeters.

As can be seen in Table 1 there is also enzymic activity in the fractions before 25 and after 29 per cent saturation with ammonium sulfate. The experiments no 2 and 6 show that there is a certain loss of activity in plasmin even when kept in dry state.

The experiments nos. 7, 8 and 9 from Table 1 are shown in Fig. 1. As can be seen the inverse values of the specific viscosity are proportional to the time in accordance with the formula.

After storing one year the loss of activity is less in the profraction (15—25 per cent). The reason for this might be due to a different content of plasminogen. Dyckerhoff [4] found that after a plasmin solution had stood for 48 hours at room temperature, no activity remained in the solution. Experiments nos. 3, 4 and 5 show a decrease in activity of 4—5 per cent per hour.

In order to find out something about the stability of a plasmin solution the following experiments were carried out.

A one per cent (dry weight) solution of plasmin (batch III, fraction 25—29, one year old) was used, the nitrogen content in the solution being 0.042 per

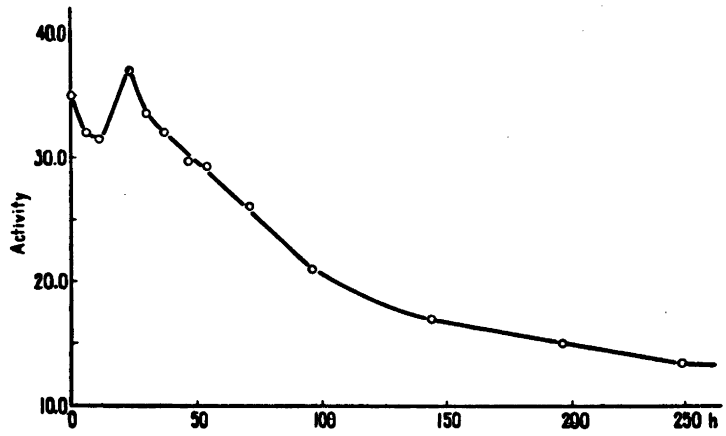

Fig. 2. Influence of time upon the proteolytic activity of a plasmin solution.

cent. From this solution stored at 18° C one ml samples were taken at different times. Each assay sample consisted of 1.00 ml plasmin and 3.00 ml of a 4.00 per cent gelatin solution and each sample was run at 35.5° C for about 75 hours. The pH was 7.3 chosen in the range of maximum stability — pH 7.2—7.4 — which for plasmin is the same as the range of maximum activity according to Christensen and MacLeod [8]. Controls with inactivated plasmin (30 min boiling) were constant.

Table 2. Activity of a plasmin solution.

Age of solution in hours	Comparative activity
0	5.21
8	3.96
24	3.86
50	5.28
78	4.03
170	3.47

Table 2 shows that after 170 hours there is still about 50 per cent of the original activity in the solution. A very strange fact is that after 50 hours the activity is increasing again and then decreasing. This gave reason for a repeated investigation of the influence of time upon a plasmin solution. Therefore a new series was started, each sample with a control of inactivated plasmin, and the

result can be seen in Fig. 2. The increase in activity after a certain time occurs also in this series. The explanation of this phenomenon may be due to the presence of the proenzyme plasminogen in the beginning, the plasminogen being activated in some way. Therefore besides the decreasing activity of the plasmin present from the beginning there is also during a certain time an increased activity due to new formed plasmin. When all plasminogen is converted into plasmin there is only the decreasing activity. The resulting activity when measured thus gives a course like that in Fig. 2.

A discussion of this phenomenon in connection with the mathematical treatment of the problem will appear in another paper in this journal.

SUMMARY

The activity and stability of some plasmin preparations are calculated according to a recently introduced expression.

A strange phenomenon in the influence of time upon the proteolytic activity of a plasmin solution is reported.

I wish to express my thanks to Prof. J. Runnström for the previlege of carrying out this work in his laboratory. I thank Mr. H. Löw for valuable help in preparing the plasmin.

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Received March 31, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 359—373

The Behaviour of Weak Electrolytes in Moving Boundary Systems. II. Methodological Investigation of Acetic Acid in Simple Moving Boundary Systems

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Right from the time of Kohlrausch there seems to have been a general agreement among the workers in the field of moving boundary electrophoresi- that the electropositive and electronegative radicals (ion constituents) should be regarded as the units of the system. The principles involved in this treatment of a moving boundary system were explicitly discussed by Hartley and Moilliet [1]. One consequence is that the mobility of a radical will be a function of the state of equilibrium in which the species is occurring in a certain medium, and it will thus show a variability due to shifts in the ionic equilibria. From the papers of Kohlrausch it is evident that this consequence was realized [2, 3]. The condition of constant mobilities was introduced only as a device to simplify the mathematical treatment, as is also the case when later theories were proposed on the assumption of constant relative mobilities (Longsworth [4], Svensson [5, 6], Dole [7]). The methodological experimental research, which is now mainly serving the purpose to improve and to elucidate the Tiselius' electrophoresis technique of analyzing colloidal mixtures, has been concentrated to the problem of correlating the experimental findings with the available theories, and, in fact, this seems to be the main reason why the restriction of constant relative mobilities is generally adhered to. This condition can be expected to be valid when monovalent strong electrolyte ions are concerned and also in many cases of weak electrolyte systems, when the changes in the state of equilibrium across boundaries are sufficiently depressed.

The accessory constituents in a solution subjected to electrophoresis, the buffer substances, are not yet sufficiently investigated as to their behaviour in moving boundary systems, however, and the purpose of the present investigation

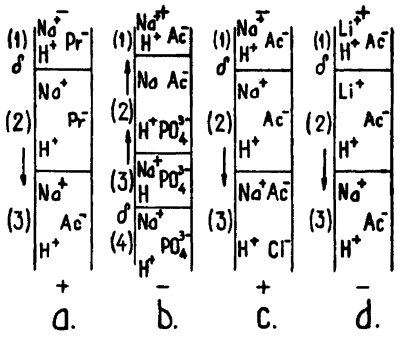

Fig. 1. Schematic representation of the systems investigated. — The stationary boundary is denoted by δ.

is to deal with some typical species of this kind. With this aim, no *a priori* assumptions as to the constancy of the mobilities are needed, or even possible; the variation of this function is instead being investigated. It is then immediately evident that the extended theories are not necessarily applicable, but the moving boundary equation, which does not involve any approximation and no assumption of constant mobilities, should be adequate to describe the mass transport of the constituents across boundaries. This equation is used here in the symmetrical form given by Svensson [6]:

$$\frac{c_{ij}\,(u_{ij} - U_j')}{\varkappa_j} = \frac{c_{i,\,j+1}\,(u_{i,\,j+1} - U_j'')}{\varkappa_{j+1}} \tag{1}$$

where c is the concentration of the constituent, and u its mobility. U' and U'' are the velocities of the boundaries divided by the field strengths above and below it, respectively; they have the dimensions of a mobility and may be called the mobilities of the boundary. $\varkappa$ is the conductivity. The first subscript, i, denotes the number of the constituent, the second, j, the number of the boundary and the number of the inter-boundary layer. The quantities U' and U'' are related to the velocity of the boundary, V, in cm per sec, by the equations

$$V_j = \frac{U_j' I}{\varkappa_j} = \frac{U_j'' I}{\varkappa_{j+1}} \tag{2}$$

where I is the current density.

The concentration unit inserted in equation (1) was millimol per liter in this investigation.

Fig. 2. Optical pattern of an acetate-propionate experiment arranged according to Fig. 1 a. The current direction is from right to left.

The mass transport of acetic acid was investigated in some simple moving boundary systems. With the aid of experimentally determined quantities it was tested if the transfer took place according to the moving boundary equation under the experimental conditions. Three different types of experiments were performed.

System 1

The original boundary was formed between a solution (1) of sodium propionate and propionic acid and a solution (3) containing sodium acetate and acetic acid. On passage of current, the system indicated in Fig. 1 a developed in the negative limb. Analyses showed that the acetate radical disappeared completely in the boundary and the original acetate solution was followed by an adjusted propionate solution. Thus both the negative radicals disappeared in the moving boundary, and the moving boundary equation, applied to each of them, will give the mobilities of these constituents, for the propionate radical in solution (2), and for the acetate radical in solution (3). Just as we are accustomed to find in the case of colloids in electrophoresis, the mobility of the acetate radical depends on the pH of the solution, attaining low values when there is much free acid in excess, and approximating to that of the free acetate ion when the degree of dissociation is increased. It is evident that this type of experiment is useful to obtain the mobility of a radical in a certain medium, and it was used for this purpose in the further work. The conditions influencing the properties of the adjusted solution were investigated in some detail, and it was found sufficient to keep solution (1) enough dilute to ensure a gravitationally stable stationary boundary. The composition of solution (2) was determined by the properties of solution (3) exclusively, to within the experimental errors. Propionic acid was chosen as the indicator radical because it was expected to be slower than the acetate radical, which was confirmed by the experiment. Although gravitationally stable, however,

the resulting system showed an inverted gradient curve, indicating that the propionate solution (2) was optically denser than the acetate solution (3), Fig. 2.

System 2

Another type of system is obtained if the experiment is arranged as indicated in Fig. 1 b. The top solution (1) consisted of an acetate buffer of pH 4.90, the bottom solution (4) was a phosphate buffer of pH 6.80. Sodium ions made the positive constituent. On passage of current, two ascending boundaries developed in the positive limb; they remained sharp during the experiment. Analyses of the liquid layers gave the distribution of the constituents shown in Fig. 1 b. Thus solution (2) contained both the acetate and the phosphate radicals. From the velocity of the boundary (1) we can calculate the mobility of the phosphate radical, and from the velocity of the boundary (2) we obtain the mobility of the acetate radical. Both these mobilities refer to the state of solution (2), which is developed during the experiment and thus of unknown composition. Therefore, from this kind of experiment no mobility can be obtained referring to a solution of initially known composition. For the purpose of mobility determinations, this type of experiment is generally not as useful as that previously described.

The acetic acid is present on both sides of the boundary (1). In solution (1), the composition is known, and the mobility of the acetate radical can easily be determined experimentally; in solution (2) the composition can be determined analytically on an isolated sample, and the mobility of the acetate radical in this solution is that obtained from the boundary (2). If the conductivity of both solutions are measured, enough data are known to allow the transport of acetic acid across the phosphate boundary (1) to be calculated and checked with the requirements of the moving boundary equation.

It is obvious that the difference in behaviour of the acetate radical in the systems 1 and 2 is caused by the difference in mobility and acidity of the two indicator substances, propionic acid and phosphoric acid. The same phenomenon was observed and investigated by Alberty and Nichol [8] in independent research.

System 3

With the experiments arranged according to Fig. 1 c, a system was obtained which also contained the acetate radical in all liquid layers. Here the descending boundary in the negative limb was a chloride boundary, or, in some instances, a perchlorate boundary. The initial boundary was

formed between a solution (1) containing sodium acetate and acetic acid, and a solution (3) containing these substances and, in addition, sodium chloride (sodium perchlorate). The transfer of substance across the stationary boundary was not investigated; the absolute composition of solution (1) is therefore not of interest for the work. It was kept at the same relative concentration as the adjusted solution (2), but somewhat more dilute, to ensure gravitational stability. The adjusted solution (2) was isolated and analyzed after the experiment. After the conductivities of the solutions (2) and (3) had been measured, the quantities U' and U'' were computed from the boundary velocity. The mobilities of the acetate radical in the solutions (2) and (3) were determined in separate experiments, and since the composition of solution (3) was known, the transport of the acetate radical across the boundary (2) could be calculated.

EXPERIMENTAL

The electrophoresis apparatus used was of the type delivered by LKB-Produkter Fabriks-AB, Stockholm. Observations were made with the cylindrical lens method (Svensson [6]). The current was read on a calibrated precision milliammeter. As the peaks were of constant sharpness throughout the runs, direct reading of the cell coordinate and plotting against time proved to give satisfactory results. The electrodes were silver plates coated with silver chloride and surrounded by one-molar sodium chloride. The volume correction on the mobilities (MacInnes and Longsworth [9]) was not carried out, thus the mobilities refer to the apparatus as the plane of reference. All experiments were carried out at 0° C.

In the course of the investigation it turned out that certain disturbances, such as anomalous conductivity values of the adjusted solutions, the presence of foreign ions in the U-tube after the experiment, steadily incresing current, *etc.*, were caused by convections in the electrode tubes. These convections may have different origin, heat convection, electro-osmotic streaming at the glass wall, and convections due to the formation of gravitationally unstable moving boundaries. These effects were avoided by using packings of some kind, such as cotton or paper pulp, in the electrode vessels. In order to prevent a net transport of liquid through the U-tube due to the increased electro-osmosis, the apparatus was kept closed during the run.

The chemicals used were of analytical grade. The distilled water usually showed a conductivity of 2.10^{-6} ohm^{-1} cm^{-1} and was not further purified. The sodium chloride was recrystallized from water. The solutions used in the experiments were prepared from stock solutions kept in alkali-resistant glass bottles with equipment to exclude the carbon dioxide in the air. Common analytical methods were used in the standardizations; sodium chloride was determined by weighing, the acids and the bases acidimetrically.

In the experiments of system 2, the analyses of the adjusted solutions (2) involved the determination of phosphoric acid and acetic acid. The former acid was determined by the volumetric molybdate method, the later by steam distillation of the samples after addition of concentrated sulfuric acid and $MgSO_4$. The distillate was then titrated with standard base. Usually the values obtained by this method were a little low, and the concentration of acetic acid was therefore also computed indirectly from the analytical

data for phosphoric acid and total acidity, after the concentration of the sodium ion constituent was calculated by means of the moving boundary equation.

The adjusted solutions isolated in the experiments belonging to system 3 were analyzed as follows. First, the conductivity was measured. Second, neutralization by standard base gave the amount of free acid. The neutralized solution was evaporated with sulfuric acid, ignited, and weighed. A small correction had to be applied for impurities, mainly from the standard base used in the neutralization. It was therefore necessary to run a blank; with these corrections applied, the analyses were generally reproducible to within 0.5 %.

All concentrations were determined in mols per liter at room temperature. The concentrations at 0° C were calculated from these data, and are given in the tables, where mC means milli-molar.

THE MOBILITY MEASUREMENTS

The main purpose of this work was to investigate the transfer of acetic acid, but in the course of the work it turned out necessary to make systematical determinations of the mobilities of the remaining constituents also. Due to a comparatively great spreading of the experimental material, it was advantageous to collect and to compare the results from similar experiments in order to level the data. This spreading was in many instances caused by imperfections in the experimental outfit. The special top cell with enclosed electrode tubes designed by Longsworth [10] for precision mobility measurements was not available to us in this investigation.

Thus the mobility values of the chloride ion were collected from the experiments according to system 3, where this constitutent disappeared in the boundary.

The mobilities of the acetate radical at different pH:es and ionic strengths were derived from experiments according to system 1.

The mobility of the sodium ion was determined in experiments arranged according to Fig. 1 d. A moving boundary system was formed between solution (1) containing a mixture of lithium acetate and acetic acid and solution (3) containing a mixture of sodium acetate and acetic acid of the desired pH and ionic strength. A moving boundary between Li^+ and Na^+ was formed, which remained sharp on the descending (positive) side.

The experiments were performed with rather dilute solutions and within a limited concentration range, 30—60 mC. Evidence from the conductivity measurements in comparison with the experimental mobilities of the constitutents showed that the degree of interaction could be approximately treated as an unspecific ionic strength effect, in addition to the specific interaction between the H^+ and the Ac^- radicals. Therefore, the mobilities were determined as functions of the ionic strength, and additivity was assumed.

Thus the mobilities of the Na^+ ion and of the free acetate ions were regarded as independent of the presence or absence of chloride provided that the ionic strength was the same. A direct determination of the mobility of Ac^- in solution (3) of system 3 could not easily be done due to the fact that the chloride ion is faster than the acetate radical. If Cl^- were present in the system, it would therefore be expected to give rise to a boundary migrating in front of the acetate boundary, and the mobility to be obtained for the acetate radical would refer to another medium than the original solution.

The mobilities of the free acetate ions were obtained from the experimental measurements of the mobilities of the radical. If we denote the mobility of the radical by u and the mobility of the corresponding free ion by v, we have for a monovalent weak acid or base:

$$u = \alpha \cdot v \tag{3}$$

where α is the degree of dissociation. In this procedure, the influence on the mobilities of varying concentration of free acetic acid was neglected. This was allowed since the absolute differences in the amounts of free acid were only slight. The conclusion was confirmed by conductivity measurements on the mixtures.

The ionic mobilities were plotted against the square root of the ionic strength, and straight lines were drawn originating from the known values of the limiting mobilities. The extension of the square root law to these concentrations involves a certain error, but the procedure was found to give satisfactory results for the present purpose.

The mobility values to be inserted in the moving boundary equation were taken from the plots, except in the case of experiment 7 of Table 8, which contained phosphoric acid. For this radical no extensive mobility determinations were available, and the invariant of the equation was calculated with the aid of the primary experimental data.

RESULTS

The experimental material is collected in the Tables 1—8. The values of the limiting mobilities of the ions were obtained from Landolt-Börnstein, Tabellen, or from International Critical Tables.

The experimental mobilities of Cl^- are given in Table 1. They were plotted against the square root of the ionic strength, and a straight line originating from the known limiting mobility, $-42.8 \cdot 10^{-5}$, was drawn. The leveled data obtained from this line are given in the last column of Table 1.

Table 1. The mobility of Cl^- in acetate solutions at 0° C.

Exp.	Concentration, mC			Mobility · 10^5	
	Na^+	Cl^-	Ac^-	exp.	from Diagram
1	35.10	14.98	30.50	— 38.82	— 38.68
2	35.00	14.98	30.50	— 39.06	
3	35.00	14.98	30.50	— 39.03	
4	40.20	15.20	30.52	— 38.33	— 38.40
5	40.20	15.20	30.52	— 38.51	
6	40.20	15.20	30.52	— 38.31	
7	40.20	15.20	30.52	— 38.31	
8	40.20	15.20	30.52	— 38.39	
9	40.20	15.20	30.52	— 38.34	
10	45.4	29.97	40.38	— 38.66	— 38.14
11	45.4	29.95	40.38	— 38.78	
12	45.4	29.95	40.38	— 37.27	
13	45.4	29.95	40.38	— 37.90	
14	45.4	30.00	40.38	— 37.48	
15	61.7	30.04	40.00	— 37.24	— 37.33
16	64.5	50.0	15.25	— 37.42	— 37.25
17	64.9	50.0	15.25	— 37.43	— 37.25
18	64.9	50.0	15.25	— 36.95	
19	64.9	50.0	15.25	— 37.32	

The mobilities of Na^+ are given in Table 2. Its limiting mobility was put $= 26.42 \cdot 10^{-5}$.

Table 3 gives the mobility data obtained for the acetate radical. The v values obtained according to equation (3) were plotted against the square root of the ionic strength, and the leveled mobilities are introduced into Table 3, column 9. The limiting mobility of the acetate ion was put $= -21.04 \cdot 10^{-5}$.

Table 2. The mobility of Na^+ in acetate solutions at 0° C.

Exp.	Concentration, mC		Mobility · 10^5	
	Na^+	Ac^-	exp.	from Diagram
1	30.20	35.46	24.08	24.02
2	30.20	40.36	24.05	
3	62.7	78.4	22.96	22.97
4	62.7	73.0	23.00	

Table 3. The mobility of the acetate radical at 0° C.

Exp.	Concentration, mC		$a \cdot 10^2$	Mobility · 10^5		
	Na^+	Ac^-		u	v	v from Diagram
1	30.18	40.36	74.8	− 14.16	− 18.94	− 18.65
2	30.18	35.46	85.1	− 16.12	− 18.95	
3	30.18	35.46	85.1	− 15.80	− 18.51	
4	35.20	49.9	70.5	− 13.10	− 18.58	− 18.43
5	40.20	68.5	58.9	− 10.61	− 18.00	− 18.26
6	40.25	47.0	85.6	− 15.62	− 18.24	
7	40.20	47.1	85.3	− 15.43	− 18.08	
8	50.3	71.0	71.0	− 12.54	− 17.67	− 17.91
9	54.8	111.3	49.2	− 8.80	− 17.78	− 17.75
10	54.8	68.3	80.2	− 14.30	− 17.82	
11	54.8	60.7	90.4	− 16.06	− 17.80	
12	63.0	75.8	83.0	− 14.37	− 17.31	− 17.49
13	62.9	78.4	80.1	− 14.30	− 17.84	
14	63.0	126.9	49.6	− 8.91	− 17.95	
15	63.0	86.1	73.1	− 12.81	− 17.51	

Table 4. The mobility of ClO_4^- from moving boundary experiments.

Exp.	Concentration, mC			Mobility · 10^5	
	Na^+	ClO_4^-	Ac^-	exp.	from Diagram
1	53.8	21.56	40.45	− 33.84	− 33.74
2	53.8	21.56	40.45	− 34.14	
3	53.8	21.56	40.45	− 33.86	

Table 5. The mobility of ClO_4^- in water solutions of $NaClO_4$ from conductivity measurements.

$\mu \cdot 10^3$	$\varkappa \cdot 10^3$	$(\varkappa/Fc) \cdot 10^5$	$(\varkappa/Fc) \cdot 10^5$ from Diagram	$u_{Na^+} \cdot 10^5$ from Table 2	$u_{ClO_4^-} \cdot 10^5$
27.12	1.541	58.90	59.19	24.15	− 35.04
43.33	2.407	57.55	57.65	23.53	− 34.12
54.3	2.983	56.93	56.84	23.18	− 33.66
54.4	2.996	57.11		23.18	

Table 6. The conductivity of sodium acetate-acetic acid solutions at 0° C.

a. Experimental.

Concentration, mC		$\varkappa \cdot 10^3$
Na^+	Ac^-	
20.12	23.05	0.851
30.20	35.45	1.247
30.20	40.57	1.242
30.20	31.23	1.245
35.32	50.0	1.441
40.25	80.0	1.620
40.25	47.1	1.620
40.25	68.2	1.625
40.30	47.0	1.624
40.30	42.09	1.629
45.2	60.8	1.828
50.4	70.9	2.010
50.3	58.6	1.955
50.3	51.0	1.917
54.8	68.2	2.169
55.1	60.9	2.181
62.9	78.1	2.468
63.0	126.8	2.458
63.0	86.1	2.468
70.2	80.9	2.702

b. Calculated from experimentally determined ionic mobilities.

$\varkappa \cdot 10^3$	$u_{Na^+} \cdot 10^5$	$u_{Ac^-} \cdot 10^5$	$(u_{Na^+} - u_{Ac^-}) \times 10^5$	$\varkappa \cdot 10^3$	
				calc.	from Table 6 a
30	24.04	— 18.64	42.68	1.236	1.237
40	23.65	— 18.26	41.91	1.617	1.620
50	23.32	— 17.90	41.22	1.989	1.990
60	22.96	— 17.58	40.54	2.347	2.350

For the perchlorate ion, only a few experimental mobilities were available, Table 4, but they were supplemented by mobilities computed from the conductivities of sodium perchlorate solutions. The latter data are collected in Table 5. In the third column, we have the observed conductivities, in the

Table 7. The conductivity of NaCl.

Comparison between values obtained in different ways.

Column 1: Mobility of Na^+ from Table 2.
Column 2: Mobility of Cl^- from Table 1.
Column 3: The sum of 1 and 2.
Column 4: The same sum calculated from experimentally determined conductivities.
Column 5: The same sum from International Critical Tables (equivalent conductivities).

$\mu \cdot 10^3$	1	2	3	4	5
30	24.0	39.0	63.0	63.0	63.0
40	23.65	38.4	62.05	62.05	62.3
50	23.32	37.85	61.2	61.2	61.7
60	22.99	37.4	60.4	60.5	61.2

fourth they have been divided by the Faraday constant. These values were plotted against the square root of the ionic strength, the limiting value of $u_{Na^+} + |u_{ClO_4^-}|$ being put $= 64.6 \cdot 10^{-5}$. The levelled data from the line are introduced into column 5. Corresponding values for Na^+ are found in column 6, and in column 7 the mobilities of the perchlorate ion have been computed by difference.

A valuable guide in drawing the straight lines was obtained from the requirement that the added mobilities of any pair of cation and anion must check reasonably with the conductivity of the corresponding salt. The straight lines were adjusted until this condition was fulfilled. Primary conductivity data for the adjusted acetate solutions are given in Table 6 a, and those calculated from the mobilities of Na^+ and Ac^- in Table 6 b. The experimental data were plotted against the ionic strength, and in the last column of Table 6 b interpolated conductivity values taken from this plot are given for comparison with the calculated values. Table 7 gives a comparison between values of $\varkappa/Fc$ for NaCl solutions obtained from the sum of u_{Na^+} and $|u_{Cl^-}|$, from direct conductivity measurements, and from *International Critical Tables.*

The tables now commented upon give us all data necessary for testing the moving boundary equation for both Na^+ and Ac^-, which constituents are present on both sides of the moving boundary. The final calculation is carried out in Table 8.

The first column is the number of the experiment. Each figure denotes an average of at least three determinations. It was generally found that more reproducible results were obtained for the concentrations than for the mobil-

Table 8. The validity of the moving boundary equation for Na^+ and Ac^-.

1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Exp.	j	pH	$\mu \cdot 10^3$	Concentration, mC			$\alpha \cdot 10^2$	c_{HAc}	Mobilities · 10^5			$\varkappa \cdot 10^3$		$-U \cdot 10^5$	$c\ (u - U)/\varkappa$	
				NaCl	HAc	NaOH			$-v_{Ac^-}$	$-u_{Ac^-}$	u_{Na^+}	exp.	calc.		Na^+	Ac^-
1	2		30.0	0.00	40.85	30.02	73.5	10.82	18.64	13.70	23.98	1.247	1.247	28.31	1.258	4.79
	3		35.0	14.98	30.50	19.94	65.4	10.55	18.45	12.06	23.78	1.704	1.709	38.68	1.284	4.77
2	2		35.8	0.00	41.07	35.78	87.1	5.30	18.40	16.03	23.76	1.450	1.462	29.20	1.304	3.73
	3		40.2	15.00	30.52	25.21	82.6	5.31	18.25	15.08	23.60	1.907	1.904	38.40	1.307	3.73
3	2	4.82	35.25	0.00	61.2	35.25	57.6	24.95	18.47	10.64	23.78	1.444	1.445	23.06	1.144	5.26
	3	4.41	45.5	29.97	40.38	15.51	38.4	24.87	18.07	6.94	23.43	2.388	2.384	38.14	1.170	5.28
4	2		51.0	0.00	60.9	51.0	84.5	9.43	17.80	15.00	23.25	1.995	1.962	24.82	1.230	3.05
	3		61.8	30.04	39.95	31.74	79.5	8.19	17.54	13.95	23.02	2.990	2.994	37.33	1.247	3.12
5	2	6.18	49.4	0.00	50.9	49.4	96.5	1.78	17.91	17.30	23.30	1.924	1.924	20.71	1.131	9.02
	3	6.09	64.5	50.0	15.25	14.54	95.3	0.72	17.45	16.63	22.98	3.461	3.402	37.25	1.120	9.06
				$NaClO_4$												
6	2		47.9	0.00	55.9	47.9	85.7	7.99	17.95	15.38	23.45	1.915	1.914	26.39	1.248	3.22
	3		53.8	21.56	40.45	32.28	79.8	8.17	17.79	14.20	23.17	2.448	2.460	33.74	1.252	3.23
				NaH_2PO_4												
7	1	4.90	35.0	0.00	55.0	35.0	63.6	20.0	18.45	11.63	23.82	1.423	1.427	15.94		1.627
	2	4.43	31.4	20.5	29.0	10.9	37.6	18.1	18.6	6.99	24.0	1.223		13.77		1.607

ities. In the experiments recorded here, the maximum deviation between the individual determinations never exceeded 1 %. The experiments 1—6 belong to the type of system 3; experiment no. 7 belongs to the type of system 2.

The second column of Table 8 gives the number of the liquid layer, $j = 1$ for the top solution, $j = 2$ for the adjusted solution, *etc.*, according to Fig. 1. Column 3 gives the pH. The columns 4—7 give the ionic strength and the concentrations of the components in the solutions; for greater convenience, we have given these concentrations rather than those of the electrical constituents, which are of course Na^+, H^+, Ac^-, Cl^-, ClO_4^-, and PO_4^{3-}. Column 8 gives the degree of dissociation; for this acid it can be put equal to the ratio between the concentration of sodium acetate and the added concentrations of acetate and free acid. Column 9 gives the concentration of free acetic acid present. In the columns 10—12, we find the mobilities of Na^+, derived from Table 2, that of the acetate ion derived from Table 3, and that of the acetate radical computed from equation (3). The experimentally found and the computed conductivities are given in the columns 13 and 14, and the quantities U' and U'' defined by equation (2) in column 15. The last two columns give the values of $c\ (u—U)/\varkappa$, claimed to be an invariant by the moving boundary equation.

The constancy of this function is well within the experimental errors in all instances. In the case of ion species of the sodium type, the validity of the moving boundary equation was shown by Longsworth (1945 [11], and previously), and the experiments reported here show that also the transport of a typical weak acid such as acetic acid is adequately described by the equation.

DISCUSSION

Column 8 of Table 8 is interesting because it shows that the concentration of free acid is very nearly unchanged across the moving boundary in the experiments 1—3 belonging to system 3. In a recent investigation by Longsworth [12], where the electrophoretical behaviour of some typical protein systems was computed with the aid of Dole's theory and compared with experiments, this condition was assumed to prevail in the system discussed. It was pointed out that it is possible in such a case to regard the undissociated portion of the acid as part of a mixed solvent, and to treat the ionic form as an independent constituent of the strong electrolyte type. Such a mode of treatment would allow the assumption of constant relative mobilities to be extended to this constituent. With the aid of the concentration change of the free acetate ions, calculated on these assumptions, the pH change across the boundary was computed, and a correction applied for the corresponding change in the mobility of the protein, which is a pH function. Thus the procedure was used

to improve the results obtained with the aid of the theory by taking into account deviations from the relative constancy of the mobilities in different ways for the simple acid and for the protein. It is evident, however, that this mode of treatment of a monovalent acid must be regarded as a special case, although it may be frequently met with in electrophoresis. Only a sufficient knowledge of the course of the equilibrium reactions in the boundary makes it possible to decide whether it is permissible or not. In the remaining experiments of the same kind (system 3) in Table 8, and in experiment 7, where the concentration distribution across an ascending phosphate boundary is investigated, the assumption of constant concentration of free HAc across the boundary is still a rather good approximation, but there are certain deviations that may be greater than the experimental errors. If the invariant of the moving boundary equation is computed for the free acid in these cases, the agreement is not satisfactory.

In the type of experiments described as system 1, all the free acetic acid is transferred into the ionic form in the boundary. In this case, it is therefore not possible to regard the two forms of acetic acid independently, and nor is this a possible way when more complicated substances are concerned. This was expressed in a generalized form by Hartley and Moilliet[1] who made the statement that it is important to distinguish between quantities pertaining to the radical, which are open to direct measurements, and quantities pertaining to the ionized forms of a radical, which depend upon a knowledge of the degree and manner of the dissociation. Only if we regard the mass transport of the radical from one homogeneous liquid layer to the adjacent one is it possible to avoid a consideration of the particular paths of the reactions in the boundary. But if we adopt this treatment, no possibilities are left to make any general assumptions as to the constancy of the mobilities. In general, these quantities must be determined experimentally as functions of the state of the solutions under investigation. In the experiments reported here, the laws of dilute solutions could be used to simplify the evaluation of the mobilities. In the type of systems generally met with in practice, however, the state of the interaction between the constituents is rather complex. Some further experiments, which stress this feature, will be reported in a following paper.

SUMMARY

The behaviour of acetic acid in some 3-component moving boundary systems was studied. With an acetate buffer placed below a propionate buffer, a sharp descending boundary was obtained in which both radicals disappeared. These boundaries can be used for measuring the mobilities of the radicals

under widely varying conditions (pH, ionic strength, *etc.*). — With a phosphate buffer placed below an acetate buffer, two sharp ascending boundaries appeared, the solution between them being a mixed zone containing both the acetate and the phosphate radicals. — In experiments where acetic acid was present on both sides of a moving boundary, the concentration distribution of the acetate radical was found to be in accordance with the moving boundary equation.

We wish to thank Professor Arne Tiselius for interesting and valuable discussions, and for critically reviewing the manuscript, and Professor The Svedberg for laboratory facilities put at our disposal. The work was financially supported by the Swedish Natural Science Research Council.

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Received February 28, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 374—400

On the Complex Chemistry of the Uranyl Ion

I. The Hydrolysis of the Six-valent Uranium in Aqueous Solutions

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The six-valent uranium ion U^{6+} does not exist at all in aqueous solution. Even in the most acid solutions, the uranyl ion UO_2^{2+}, which we may regard as a hydrolysed state of U^{6+}, is exclusively formed. Thus the only compound ever prepared that may be considered as a salt of U^{6+}, the uranium fluoride UF_6, is immediately and completely transformed into UO_2F_2 by water (Ruff and Heinzelmann [1]). The uranyl ion is then the only form of existence for six-valent uranium over a wide pH-range up to pH $\approx$ 2.5. If pH is raised over that value base is consumed, however, and further hydrolysis occurs (see *e. g.* Britton [2]). From such solutions »basic uranyl salts» have been prepared (see *e. g.* Colani [3]). Finally, at sufficiently high pH, insoluble uranate precipitates are formed.

Thus the uranyl group UO_2^{2+} forms a stable atom constellation, which behaves in a manner completely analogous to other metal ions: it keeps unchanged within wide acid pH-limits, and as pH further increases, it gradually is subjected to a hydrolysis more and more pronounced.

These are the current views as to the range of existence and hydrolysis of the uranyl ion. As an introduction to the extensive investigation of the complex chemistry of the ion, which is in progress, an independent test of their truth seemed to be highly desirable. This was performed in extinctiometric way, as follows. In such a test, as in all investigations where the properties of just the uranyl ion is to be determined, it is, of course, necessary to use a non-complex salt in the measurements. As such, the perchlorate has been selected.

Determination of the [H^+]-range where the six-valent uranium is found as uranyl ions only

The following extinction curves have been obtained photographically by two Hilger Medium Spectrographs one of which, with quartz prism, was used in the ultra violet, and the other, with glass prism, in the visible range where quartz gives a bad dispersion. They have been combined with a Hilger Spekker Photometer, graduated directly in extinctions E. About the measuring method, see Twyman and Allsopp [4] or Kortüm [5].

According to Beer, we have for a solution of a light absorbing substance

$$\log J_0/J = E = \varepsilon_0 \cdot C_0 \cdot d \tag{1}$$

where

J_0 = light intensity entering, and
J = light intensity leaving the absorbing layer
d = thickness of layer
C_0 = concentration of absorbing substance, and
ε_0 = the molar extinction, a constant characteristic of the substance at a given wave-length.

If several light absorbing substances are present in the same solution, E is put together additively:

$$E/d = \varepsilon_0 \cdot C_0 + \varepsilon_1 \cdot C_1 + \varepsilon_2 \cdot C_2 + \ldots . \tag{2}$$

This is the case in a light absorbing complex system; ε_0, ε_1, $\varepsilon_2 \ldots$ are then the molar extinctions and C_0, C_1, $C_2 \ldots$ the concentrations of the different components of the complex equilibrium.

In such cases, we know the total concentration of central atom, C_M, free as well as complexly bound. We may quite formally attribute E to this total concentration; *i. e.* we define a quantity ε_M according to

$$E/d = \varepsilon_M \cdot C_M \tag{3}$$

This quantity is always experimentally determinable.

ε_M so defined becomes identical to ε_0 in the limiting case where only one component exists, but in all other cases it is a formal quantity of calculation; t forms a sort of mean between the ε values of the different components, and ts value depends obviously upon the mutual proportions of the component

concentrations. How much it varies as these proportions are altered to a certain extent, depends, of course, on the differences between the ε values. At a separate wave-length these differences may by chance be small, and so no appreciable changes in ε_M occur by changes in the composition of the system. But in all probability this cannot be the case at all wavelengths; thus, if a displacement of an equilibrium occurs, ε_M is to change at least at one part of an extinction curve. On the other hand, we may state, that no appreciable equilibrium displacement has happened in such a case where a whole extinction curve has remained unchanged. This method to prove complex formation in a solution is in its first origin due to Bjerrum [6].

In the present case, the aim is to settle at which $[H^+]$ displacements of hydrolytic equilibria take place in uranyl solutions. So extinction curves have been determined for uranyl perchlorate at different $p[H^+]$* values: 0.1, 0.6, 1.0, 1.55, 2.7 and 4.1. The lower $p[H^+]$ values have been obtained by adding $HClO_4$, the higher by adding NaOH. The former have been calculated from the acid added, the latter by quinhydrone electrode measurements according to the method described below. The $p[H^+]$ values need not to be known but roughly.

As in all following measurements, a high and approximately constant ionic strength $I = 1$ has been maintained in the solutions, to secure constant activity conditions as far as possible. $NaClO_4$ has been used as ionic medium. All measurements have been made at 20° C. The chemicals used are the same as in the main series (see below).

The extinction curves found are given in Fig. 1. From $p[H^+]$ 0.1 (the lowest investigated one where the whole ionic medium is $HClO_4$) up to $p[H^+]$ 1.55, the courses are completely identical within the limits of the experimental error $\pm$ 2—3 %**. Thus no appreciable equilibrium displacement has occurred between these $p[H^+]$ values. According to the law of mass action, this result does not allow any other interpretation than that one hydrolysed state predominates over the whole of this $p[H^+]$ range. This hydrolysed state must be the uranyl ion, UO_2^{2+}. At $p[H^+]$ 2.7, however, a distinct deviation is found. Hence UO_2^{2+} begins to be perceptibly hydrolysed. At still higher $p[H^+]$ (4.1), in the neighbourhood of beginning precipitation, this deviation is very strongly pronounced.

One may, however, also imagine a non-hydrolytic complex reaction to take place in uranyl solutions: a polymerisation, forming $(UO_2)_2$, $(UO_2)_3$ ... $(UO_2)_n$. As such a formation is unaffected by $[H^+]$, its existence does not be-

* $p[H^+] = -\log [H^+]$.

** Corresponding to $\pm$ 0.01 log units.

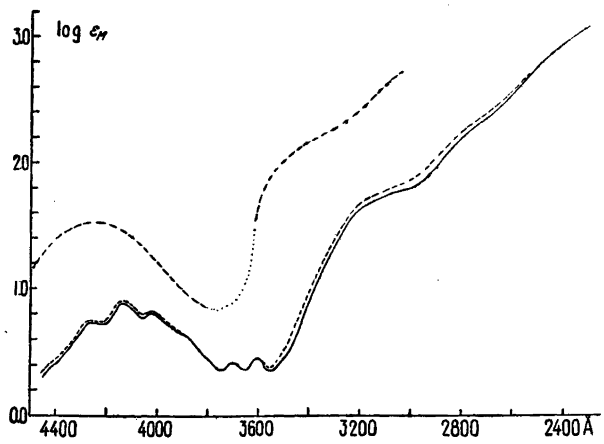

Fig. 1. Extinction curves of uranyl perchlorate solutions. — Fulldrawn curve: p $[H^+]$ = 0.1 to 1.55 (= extinction curve of the uranyl ion, UO_2^{2+}). — Dashed curves: p $[H^+]$ = 2.7 (lower curve) and p $[H^+]$ = 4.1 (upper curve).

come evident by pH variations. On the other hand, according to the law of mass action, displacements should take place in this case, when C_M is varied. Now identical curves are obtained at p$[H^+]$ 0.6 when $C_M \approx 40$ mC while d varies, and when $d = 2$ cm while C_M varies between 2.5 mC and 176 mC. From this the conclusion is to be drawn, that no polymers are formed.

So it is unambiguously shown that the monomer uranyl ion is the only ionic specie of uranium existing in perchlorate solutions in the p$[H^+]$ range 0.1—1.55. At higher p$[H^+]$ values further hydrolysis occurs. Thus the current views have been well confirmed.

Then it is evident, that the fulldrawn curve may be identified as the extinction curve of the uranyl ion, that is for this curve $\varepsilon_M = \varepsilon_0$. As far as I know this is the first carefully verified absolute determination of this subject.

In a great number of old investigations the absorption of several more or less complex uranyl salts was qualitatively determined. Quantitative extinction curves of uranyl perchlorate have, on the other hand, only been measured by v. Kiss *et al.*[7, 8] and by Sutton [9]. v. Kiss *et al.* have published two highly different versions, none of them in accord with mine. Sutton has only measured the range down to 3400 Å. In this, he finds curves of the same shape and pH dependence as I, but no absolute values of extinction were communicated.

The existing possibilities of complex formation in solutions of uranyl salts

After having fully proved that UO_2^{2+} is to be regarded as a stable complex forming central group, we may treat its complex chemistry in complete agreement with that of other metal ions. Thus in a solution of uranyl salt we have to count with the following possible complexes between the central group M ($= UO_2^{2+}$) and a ligand A, if the polymers M_2 etc are excluded, according to the above:

Mononuclear:	MA, MA_2, MA_3, ...
Dinuclear:	M_2A, M_2A_2, M_2A_3, ...
m-nuclear:	M_mA, M_mA_2, M_mA_3 .....

We know the total concentrations of M and A:

$$C_M = [M] + [MA] + [MA_2] + ... + 2([M_2A] + [M_2A_2] + ...) + ... + m([M_mA] + [M_mA_2] + ...) + ... \quad (4)$$

$$C_A = [A] + [MA] + 2[MA_2] + ... + [M_2A] + 2[M_2A_2] + ... + [M_mA] + 2[M_mA_2] + ... \quad (5)$$

As all solutions have an approximately constant ionic strength = 1, by $NaClO_4$, the law of mass action is valid for the connection of the complex concentrations with [M] and [A]. Generally we find for a complex M_mA_n:

$$[M_mA_n] = \beta_n^{(m)} \cdot [M]^m \cdot [A]^n \quad (6)$$

where $\beta_n^{(m)}$ is the n:th complex constant in the m-nuclear series. — Substituting these expressions in (4) and (5) we thus obtain C_M and C_A as functions of [M] and [A] only.

Of these two variables, one cannot measure [M] in an uranyl system; no practicable method is known. On the other hand, it is possible to find [A] in different ways. In such cases, the separation of the complex constants is done via the so-called complex formation curve (Bjerrum [10, pp. 21, 36], Olerup [11, p. 10], Fronaeus [12, p. 13]. Therefore the first step is to deduce its equation at the quite general complex formation, which is set forth here.

Deduction of the complex formation function

First we define a new quantity, the ligand number $\bar{n}$, according to

$$\bar{n} = \frac{C_A - [A]}{C_M} \quad (7)$$

that is the average number of complexly bound ligands per central atom; $\bar{n}$ is known as soon as [A] has been experimentally measured. $\bar{n}$, taken as a function of [A], is called the complex formation curve of the system in question. Its equation is obtained from (4) and (5) by substituting the expressions from (6) for the complex concentrations:

$$\bar{n} = \frac{\beta_1'[A] + 2\beta_2'[A]^2 + 3\beta_3'[A]^3 + \ldots + [M](\beta_1''[A] + 2\beta_2''[A]^2 + 3\beta_3''[A]^3 + \ldots) + \ldots + [M]^{m-1}(\beta_1^{(m)}[A] + 2\beta_2^{(m)}[A]^2 + 3\beta_3^{(m)}[A]^3 + \ldots) + \ldots}{1 + \beta_1'[A] + \beta_2'[A]^2 + \beta_3'[A]^3 + \ldots + 2[M](\beta_1''[A] + \beta_2''[A]^2 + \beta_3''[A]^3 + \ldots) + \ldots + m[M]^{m-1}(\beta_1^{(m)}[A] + \beta_2^{(m)}[A]^2 + \beta_3^{(m)}[A]^3 + \ldots) + \ldots} \quad (8)$$

If only mononuclear complexes exist in the solution, that is β'', β''', ..., $\beta^{(m)}, \ldots = 0$, it is seem from (8), that $\bar{n}$ is a function of [A] only, and independent of [M], On the other hand, if polynuclear complexes are formed, $\bar{n}$ is a function of both [A] and [M], that is C_M. Thus, by determining experimentally $\bar{n}$ as a function of [A] at different C_M, it is possible to decide between these two principal cases *.

This very important decision is the first thing to be done in every system to be investigated, since the treatment of (8) in order to separate the constants will depend on whether or not the terms from polynuclear complexes may be cut out. In these investigations, both cases have been met with and they will be treated as they become evident.

The hydrolysis as the first complex uranyl system investigated here

The further hydrolysis of UO_2^{2+} at p[H^+] exceeding ≈ 2.5 was selected as the first UO_2^{2+} system to be investigated, because the hydrolysis is to be considered at all other complex investigations of an ion in aqueous solution. For if hydrolysis complexes are formed to a considerable extent in addition to others in a solution of a salt, the conditions become so complicated that a quantitative interpretation is impossible. So it is, as a rule, quite necessary to reduce the concentrations of hydrolysis complexes to negligible amounts by performing

* This is true as a rule. But as pointed out by Leden [13, p. 23], it may happen, that the criterion does not function; the polynuclear complexes then remain unknown. The very fact that $\bar{n}$ in such cases is independent of [M] allows us nevertheless to determine the correct mononuclear constants of the system in the usual way.

measurements in sufficiently acid solutions. That means, however, a limitation of the pH range available for complex measurements which for easily hydrolysed ions, as UO_2^{2+}, may be very considerable. Especially in such cases, the course of the hydrolysis is the central problem of the complex chemistry of an ion.

Previous work at the hydrolysis of UO_2^{2+}

Several investigations have been made in this field; the most prominent of the recent ones by Jolibois and Bossuet [14], Britton [2], Flatt and Hess [15], Sutton [9]. With the possible exception of Sutton's work, hardly any is of a quantitative character. The greatest interest has as a rule been devoted to the composition of the precipitates obtained by a large addition of base. As these are slimy and filter very badly and have a great tendency to adsorb and occlude, it is not surprising that the authors have got widely different results; especially as the composition certainly depends on the base quantity used.

Sutton measures pH (with glass electrode?) and light absorption (see above) of UO_2^{2+} solutions and finds that the hydrolysis includes formation of polynuclear complexes. However, the result is uncertain, as the author has not taken into account all the possibilities of complex formation; instead he has selected some complexes, the formation of which he finds plausible. A judgment of the work is also difficult for the reason that no information is given as to how the constants have been calculated.

The complexes and the complex formation function of the hydrolysed uranyl system

In the measurements, base is consumed, while UO_2^{2+} is transformed in a hydrolysed state. We may, therefore, quite formally regard the hydrolytic reactions as a complex formation between UO_2^{2+} as central group and OH^- as ligand. To this complex formation we may apply the scheme above, which covers all cases.

To what extent this view also corresponds to the actual facts cannot be decided. The measurements give, of course, no information at all about what really happens just in the moment of reaction, as only the resulting products is determined. Moreover, the degree of hydratation of these still remains unknown. So a complex, which according to the scheme is written $(UO_2)_2(OH)_2$ may equally well have a formula $(UO_2)_2O$, which gives the same equilibrium equation in this diluted aqueous solution.

It is possible to introduce a limitation in the scheme of complex formation, when used in this case. The co-ordination number of oxygen cannot exceed four, so a OH^--group cannot co-ordinate more than three UO_2^{2+}. Hence the m-nuclear serie begins with a complex $M_m(OH)_p$, where p depends on m.

By construction of formulas, we find $p = (m—1)/2$ if m is odd, and $p = m/2$ if m is even. Besides MOH; M_2OH and M_3OH thus are the only possible complexes with one OH^--group.

In the following experiments it is not our free ligand concentration $[OH^-]$, but $[H^+]$ which is the directly measured quantity. They are, however, connected according to $[OH^-] = K_W/[H^+]$. As the exact value of the constant K_W is not known for the ionic medium used, the values of $[OH^-]$ then become uncertain with a constant factor. Therefore, the direct measured $[H^-]$ is preferred as a variable in the complex formation function. By substituting $[OH^-] = K_W/[H^+]$ and conjoining the constants β and K_W to new constants $\varkappa$, which may be regarded as dissociation constants of UO_2^{2+}, we obtain from (6):

$$[M_mA_n] = [M_m(OH)_n] = \beta_n^{(m)}[M]^m[OH^-]^n = \beta_n^{(m)} \cdot K_W^n \cdot \frac{[M]^m}{[H^+]^n} = \varkappa_n^{(m)} \cdot \frac{[M]^m}{[H^+]^n} \quad (9)$$

and hence we obtain the complex formation equation (from (8)) as:

$$\bar{n} = \frac{1}{[H^+]} \cdot \frac{\left(\varkappa_1' + \frac{2\varkappa_2'}{[H^+]} + \frac{3\varkappa_3'}{[H^+]^2} + \ldots\right) + [M]\left(\varkappa_1'' + \frac{2\varkappa_2''}{[H^+]} + \frac{3\varkappa_3''}{[H^+]^2} + \ldots\right) + \ldots + [M]^{m-1}\left(\frac{p\varkappa_p^{(m)}}{[H^+]^{p-1}} + \frac{(p+1)\varkappa_{p+1}^{(m)}}{[H^+]^p} + \frac{(p+2)\varkappa_{p+2}^{(m)}}{[H^+]^{p+1}} + \ldots\right) + \ldots}{\left(1 + \frac{\varkappa_1'}{[H^+]} + \frac{\varkappa_2'}{[H^+]^2} + \ldots\right) + 2[M]\left(\frac{\varkappa_1''}{[H^+]} + \frac{\varkappa_2''}{[H^+]^2} + \frac{\varkappa_3''}{[H^+]^3} + \ldots\right) + \ldots + m[M]^{m-1}\left(\frac{\varkappa_p^{(m)}}{[H^+]^p} + \frac{\varkappa_{p+1}^{(m)}}{[H^+]^{p+1}} + \frac{\varkappa_{p+2}^{(m)}}{[H^+]^{p+2}} + \ldots\right) + \ldots} \quad (10)$$

where $\bar{n}$ means the average number of OH^-, used up per UO_2^{2+}, whatever the actual mechanism of complex formation may be. — We observe, that in the equation so modified, $\bar{n}$ is a function of $[H^+]$ only, if merely mononuclear complexes exist, but of both $[H^+]$ and $[M]$ (C_M) if polynuclear complexes exist, too.

The following measurements fully prove, that the hydrolysis doubtless involves polynuclear complex formation. So (10) is valid in the general form given above, and we will see how far this complicated function allows a separation of the constants.

Separation of constants from the complex formation curve of the hydrolysis system

Certainly it is quite impossible to completely determine the composition of the system for two weighty reasons:

1. The great number of possible complexes; and

2. the impossibility of measuring [M] (=$[UO_2^{2+}]$), present in (10). No known electrode measures [M] with the exception of the redox electrode UO_2^{2+}/U^{4+}, but according to Titlestad [16] this electrode is very slow to attain equilibrium; it is also light-sensitive. Moreover, one has to know the U^{4+}-complexity in the solution to be able to calculate [M]; but we do not.

We have to be content with a limited aim, and it proves to be possible to calculate the first mononuclear constant $\varkappa_1'$, and also the first dinuclear $\varkappa_1''$ defined according to (9). — We multiply both members of (10) by $[H^+]$. If we then allow $[H^+]\to\infty$ (that is $1/[H^+]\to 0$), simultaneously $\bar{n}\to 0$. The left member $\bar{n}\cdot[H^+]$ then tends toward a limit, which is easily obtained from the right member, if we bear in mind, that simultaneously also $[M]\to C_M$:

$$\lim_{1/[H^+]\to 0} \bar{n}\cdot[H^+] = \varkappa_1' + \varkappa_1''\cdot C_M + \varkappa_1'''\cdot C_M^2 \qquad (11)$$

Thus, if the products $\bar{n}\cdot[H^+]$ are calculated from the experimentally found $\bar{n} = f([H^+])$ curves of different C_M so the products for increasing $[H^+]$ will tend towards the limits (11). If then the graphically estimated limits are plotted as a function of C_M, a curve ought to be obtained, the interception of which on the Y-axis is $= \varkappa_1'$; further the derivative in the point of section is $= \varkappa_1''$. At decreasing C_M, the curve better and better ought to be approximated by the straight line $\lim \bar{n}\cdot[H^+] = \varkappa_1' + \varkappa_1''\cdot C_M$, which exactly means the equation of the tangent in the point of section.

Chemicals used

The *uranyl perchlorate* has been prepared according to Salvadori [17], with the modification, that the perchlorate solution first obtained has been reprecipitated and then dissolved again, to avoid traces of NO_3^-, which will disturb the analysis. The preparation contains some potassium perchlorate, which, of course, is of no importance here, since sodium perchlorate is used as ionic medium. — The stock solutions of uranyl perchlorate have been analysed volumetrically according to Treadwell [18]. The method works very well if no NO_3^- is present.

Sodium perchlorate. Preparations of the quality »purum» have been recrystallised twice from water of c:a 90° C, according to Fronaeus [12, p. 31]. Then the salt is obtained without water of crystallisation. Dried at 110°. The Cl^- and ClO_3^- contents are $< 1/10$ ‰

Perchloric acid, Mercks *pro analysi*. The stock solutions have been analysed by alkalimetric titration.

Sodium hydroxide. Stock solutions were prepared from oily alkali and thus free from CO_3^{2-}. The solutions used for titrations were analysed with potassium hydrogen phthalate as a standard.

Quinhydrone was prepared according to Biilman and Lund [19].

EXPERIMENTAL DETERMINATION OF THE COMPLEX FORMATION FUNCTION AT DIFFERENT C_M

The measurements for determination of $[H^+]$ have been carried out by

1. Quinhydrone electrode and
2. Glass electrode

The latter has been used as an independent control of the former.

The procedure has been the potentiometric titration used by Leden [13, p. 34]: known amounts of sodium hydroxide were added from a burette to uranyl perchlorate solutions of known concentration in the electrode vessel; all solutions used had the same ionic strength (= 1). The mixing of the measuring solutions was brought about by a stream of nitrogen gas. Every titration was repeated at least once.

The nitrogen gas had, to be released from the oxygen present, passed over copper of 200° C, finely divided on infusorial earth. After that, the gas was bubbled through a wash-bottle containing 1 C $NaClO_4$ to get the right pressure of aqueous vapour.

As a reference electrode (RE) in all measurements, a quinhydrone electrode of the composition

$$\mathrm{Au} \;\Big|\; \begin{matrix} 10.10 \text{ mC } HClO_4 \\ 990 \text{ mC } NaClO_4 \\ \text{quinhydrone (s.)} \end{matrix}$$

was used, connected with the other half-cell by a salt bridge (written ||), containing 1000 mC $NaClO_4$ (*cf.* Leden [13, p. 33]). — The experiments were made at 20.0° C in an electrically regulated thermostate.

As it is a very difficult task, if realizable at all, to obtain the extremely soluble uranyl perchlorate in the proper stoicheiometric proportions, one cannot get a defined starting condition simply by dissolving the salt in $NaClO_4$-solution. According to the accidental excess or deficit of acid in the preparations one finds different $[H^+]$ in such solutions and in no case that $[H^+]$, which corresponds to the spontaneous hydrolysis, *i. e.* the $[H^+]$ of the point of equivalence, $[H^+]_{eq}$:

$$[H^+]_{eq} = [MOH] + 2\,[M(OH)_2] + \ldots + [M_2OH] + 2[M_2(OH)_2] + \ldots. + p[M_m(OH)_p] + (p+1)[M_m(OH)_{p+1}] + \ldots. \quad (12)$$

In the following way, however, it was possible to determine this point experimentally.

To the uranyl perchlorate was added an excess of perchloric acid. A base titration then indicated, that UO_2^{2+}, though acting as a rather strong acid, nevertheless was weak in comparison with $HClO_4$, and so the titration curve showed a well determinable point of inflection, indicating the point of equivalence (12), which thus was certainly found. The amount of base added to this point is equivalent with the amount of »free» acid present in the solution at the outset; this corresponds to an acid concentration C_H. This experimentally known quantity is the total $[H^+]$ at the outset, C_H^0, except that which might have been formed by an even in this solution possibly existing hydrolysis, $[H^+]_M$:

$$C_H = C_H^0 - [H^+]_M \quad (13)$$

The »free» acid C_H, in its turn, combines from the extra perchloric acid added, 25.3 mC (30.1 mC at the glass electrode measurements)*, and the acid excess or deficit of the uranyl salt. The latter is proportional to the C_M at the start, C_M'. Thus we have

$$C_H = 25.3 + l \cdot C_M' \quad (14)$$

where l ought to be a constant, if C_H is properly determined by the point of inflection. This proved to be the case, and so it was shown, that the points of equivalence searched for really coincide with the points of inflection found.

The hydrolytic reactions occur at $p[H^+]$ 2.5 to 5, so $[OH^-]$ of the solutions may always be quite neglected in comparison with the base amount added. The ligand number, defined according to (7), therefore in this system adopts the form $\bar{n} = C_{OH}/C_M$, where C_{OH} is the base taking part in UO_2^{2+} complex formation, that is the quantity used up by UO_2^{2+}. At a certain point of titration, this is

$$C_{OH} = B - (C_H^0 - [H^+]) + [H^+]_M \quad (15\,a)$$

* With this rather large acid addition, the solutions at the outset gave $p[H^+]$ 1.5—1.6. According to the extinctiometric measurements above, the hydrolysis of such solutions rather certainly is negligible. However this need not to be utilized in the present formulas or in the quinhydrone electrode measurements, but is confirmed there and will then be used in the glass electrode measurements.

where B = the total concentration of base added. — Introducing (13) we find

$$C_{OH} = B - C_H + [H^+] \qquad (15\ b)$$

As C_M of a solution is always known from C'_M, corrected for the dilution, now we are finally able to calculate $\bar{n}$ from experimental data, according to

$$\bar{n} = \frac{C_{OH}}{C_M} = \frac{B - C_H + [H^+]}{C_M} \qquad (16)$$

It should be pointed out, that also the C_H originally determined has to be corrected for the dilution at the titration, when inserted in (15) and (16).

Now we plot $\bar{n}$ as a function of $p[H^+]$ for the different C'_M used, and so we obtain the complex formation curves searched for.

The determinations have been extended over as wide a pH range as possible by continuing the titrations until a precipitate was definitively formed. This happened when $\bar{n}$ reached a value about 1.30.

Also the C'_M range was made as wide as possible, 2 to 60 mC (at the glass electrode 5 to 60 mC), to secure a good test of polynuclear complex formation. $C'_M < 2$ would give too small a base consumption; $C'_M > 60$ would certainly cause too large medium changes.

Measurements with the quinhydrone electrode

The electrode has once before been used in uranyl solutions by Singh and Ahmad [20].

The emf:s were measured with a Leeds-Northrup »Student's Potentiometer». The standard cell was a Weston cell of saturated type. As a zero instrument, a mirror galvanometer was used.

First, the behaviour of the electrode in uranyl solutions was controlled. It is true that any reduction of UO_2^{2+} by hydroquinone is not to be feared, as a calculation with the normal potentials shows, but on the other hand, one may imagine that a complex formation is possible between UO_2^{2+} and some quinhydrone component, preferably the hydroquinone.

This control had to be undertaken at so high a $[H^+]$, that the hydrolysis could not disturb, not even in the solution richest in $[UO_2^{2+}]$. I therefore chose pH ≈ 1, and prepared a cell

$$+\ \text{Au} \;\Big|\; \begin{matrix} 100\ \text{mC}\ HClO_4 \\ 900\ \text{mC}\ NaClO_4 \\ \text{quinhydrone (s)} \end{matrix} \;\Big\|\; \text{RE} -$$

To the left half-cell a solution 200 mC $UO_2(ClO_4)_2$, 100 mC $HClO_4$, 300 mC $NaClO_4$ was added. Correction was introduced for the acid content of the uranyl salt, known from the main titrations (equ. (14)).

In this control, a deviation ΔE_q, increasing with C_M, was found between the observed and calculated emf:s:

Table 1. ΔE_q as a function of C_M

C_M mC	5	10	15	30	60	100
ΔE_q mV	0.1	0.2	0.3	0.7	1.4	2.6

The direction of ΔE_q was such that $[H^+]$ became higher than the proper value. This is just to be expected if a complex formation UO_2^{2+}-hydroquinone occurs. The effect cannot to any great extent be attributed to influences on the activity coefficient f_{H^+} by medium changes, as the analoguous control of the glass electrode (see below) gives a much smaller deviation. Nor is the effect to be ascribed to a salt error in the quinhydrone electrode. Such an error ought to appear in much the same degree at all divalent metal ions, but Leden [13, p. 93] has shown for Cd^{2+} and Fronaeus [12, p. 37] for Cu^{2+} that only a very small quinhydrone effect, if any, is to be found. A slight complex formation UO_2^{2+}-hydroquinone therefore seems to be the only reasonable explanation for the main part of ΔE_q.

ΔE_q causes an error in the measured $[H^+]$: s, the relative magnitude of which is determined by C_M or, rather, by $[UO_2^{2+}]$, as it may be assumed that the hydrolytic complexes show a very slight affinity for hydroquinone, having their places of co-ordination occupied by firmly bound OH^-:s. In the very beginning of the hydrolysis, $[UO_2^{2+}]$ is high and, moreover, $[H^+]$ is a considerable part of C_{OH}. So $\bar{n}$ is affected in a serious manner; at very small C_{OH} the calculation of $\bar{n}$ becomes quite illusory. As the hydrolysis proceeds, however, $[UO_2^{2+}]$ grows less and $[H^+]$ becomes smaller in comparison with C_{OH}: so the influence of ΔE_q rapidly decreases with increasing pH.

As a whole, however, the UO_2^{2+}-quinhydrone effect involves an uncertainity which among other things has lead to the control by the glass electrode.

By the hydrolysis titrations, cells of the following type are measured:

$$+ \text{RE} \;\Big\|\; \begin{matrix}[H^+] \\ C_M \\ \text{quinhydrone (s)} \\ I = 1\ (NaClO_4)\end{matrix} \;\Big|\; \text{Au} -$$

The emf in mV is given by

$$E_q = 58{,}2 \log \frac{10{,}10}{[H^+]} \tag{17}$$

that is, if $-\log [H^+] = p[H^+]$ (18)

$$p[H^+] = \frac{E_q}{58.2} + 1{,}996 \tag{19}$$

which gives $[H^+]$ * from the measured E_q, if we may neglect the hydroquinone complex formation, the effect of medium changes and the diffusion potentials.

The influence of hydroquinone complexes, and the medium effect and diffusion potential caused by UO_2^{2+} are included in the quinhydrone effect discussed above. Other medium changes occur through the diminishing of the ionic strength by the hydrolysis complex formation. By a separate experiment with lower ionic strength, it has been established, however, that the effect of this changes is to be neglected. The diffusion potentials depending on $[H^+]$, finally, may be estimated to ≈ 0.5 mV, at most, for those $[H^+]$ values which were used for $\bar{n}$-calculation; this error may also be neglected. So we find that (19) ought to be valid with great accuracy, if only $[H^+]$ is not too high (*i. e.* $\bar{n}$ too low). In the tables below, $\bar{n}$ is calculated only when the total systematic and experimental error is considered not to exceed ≈ 15 % of the value.

The potentials measured were very steady, and reproducible within ± 0.1 mV; they attained their proper value almost at once. No sign of any proceeding reaction in the solution could be detected. When precipitation occurs, the potential becomes disturbed and slips; this indicates the end-point of the titration.

The E_q measured at different C'_M are found in Table 2 A—F; according to (19) the $p[H^+]$ values have been calculated from these, and especially the $p[H^+]$ at the point of equivalence, $p[H^+]_{eq}$. This point is readily determined from E_q and the volume of base added, v_B, as the point of minimum buffer capacity. From v_{Beq} and v_H, the volume of the solution at the outset, C_H has been calculated, and so one is able to calculate C_{OH} and $\bar{n}$ according to (15 b) and (16). Then all quantities are known to form the complex formation function $\bar{n} = f(p[H^+])$, Fig. 2; and the function $\bar{n} \cdot [H^+] = f(1/[H^+])$, Fig. 3; used in the calculation of the constants.

From Fig. 2, it will at once be noted that $\bar{n} \approx 0$, when $p[H^+] < 2$—2.5, as predicted in the extinctiometric measurements.

* Note, that the measurement gives $[H^+]$, not the activity a_H.

Table 2. Determination, by quinhydrone electrode, of $\bar{n}$ as a function of $p[H^+]$ and $\bar{n} \cdot [H^+]$ as a function of $1/[H^+]$, at different C_M.

Table 2 A: $C'_M = 2.02$ mC ×*

$p[H^+]_{eq} = 3.71$
$v_{B_{eq}} = 5.00$ ml 94.8 mC + 1.85 ml 18.96 mC
$v_H = 20.0$ ml
$C_H = 25.5$ mC

v_B ml 18.96 mC **	E_q mV	(19) $p[H^+]$	(15 b) $1/[H^+]$ mC^{-1}	(15 b) C_{OH} mC	C_M mC	(16) $\bar{n}$	$\bar{n} \cdot [H^+]$ $10^{-5} \cdot C$
	−22.1						
0	47.9	2.82					
1.40	78.0	3.34					
1.60	86.5	3.48					
1.80	96.0	3.65					
2.00	107.3	3.84	7.1	0.25	1.50	0.17	2.4
2.20	116.3	4.00	10.0	0.34	1.49	0.23	2.3
2.40	123.4	4.12	13.2	0.46	1.48	0.31	2.4
2.60	129.3	4.22	16.7	0.58	1.47	0.39	2.4
2.80	134.5	4.31	20.4	0.70	1.45	0.48	2.4
3.00	139.2	4.39	24	0.82	1.44	0.57	2.3
3.40	147.3	4.53	33	1.07	1.42	0.75	2.3
3.80	155.3	4.67	48	1.30	1.40	0.93	2.0
4.00	159.4	4.74	56	1.43	1.39	1.03	1.9
4.20	164.1	4.82	66	1.54	1.38	1.12	1.7
4.40	169.3	4.91	81	1.66	1.37	1.21	1.5
4.60	175.3	5.01	102	1.77	1.37	1.29	1.25
4.80	181.7	5.12	132	1.89	1.36	1.39	1.05

* These signs are used to indicate the respective C'_M in the Figs 2, 3 and 4.

** First, 5.00 ml 94.8 mC NaOH is added to the starting solution ($E_q = -22.1$ mV); then the titration is continued with 18.96 mC NaOH (amounts in the first column).

Measurements with the glass electrode

The glass electrode, of the bulb type, was provided with a Ag-AgCl-electrode, dipping in 0.1 C HCl. It was manufactured by Radiometer, Copenhagen and used in connection with a Radiometer valve potentiometer. To avoid stray currents the stem of the electrode was covered with paraffine, and the measurements were made in a room of low moisture, arranged as an air thermostate.

To determine the slope S, the electrode was checked by three different kinds of buffer solutions, together covering the pH-range 1 to 6. They were prepared thus, that the pH-step between two solutions of the same kind had to be exactly one unit (*cf.* Fronaeus [12, p. 42]):

Table 2 B: $C'_M = 5.06$ *mC* ◆

$p[H^+]_{eq} = 3.40$
$v_{B_{eq}} = 5.44$ ml 94.8 mC
$v_H = 20.0$ ml
$C_H = 25.8$ mC

v_B ml 94.8 mC	E_q mV	(19) $p[H^+]$	(15 b) $[1/H^+]$ mC^{-1}	C_{OH} mC	C_M mC	(16) $\bar{n}$	$\bar{n} \cdot [H^+]$ 10^{-5}. C
0	−22.5						
5.20	54.7	2.94					
5.30	63.6	3.09					
5.40	76.4	3.31					
5.50	90.0	3.54	3.5	0.51	3.97	0.13	3.7
5.60	101.4	3.74	5.5	0.77	3.95	0.195	3.5
5.70	109.2	3.87	7.4	1.10	3.93	0.28	3.8
5.80	115.2	3.99	9.8	1.42	3.92	0.36	3.7
5.90	121.2	4.08	12.0	1.77	3.90	0.455	3.8
6.00	126.2	4.17	14.7	2.11	3.89	0.54	3.7
6.10	130.7	4.24	17.2	2.46	3.87	0.635	3.7
6.20	135.1	4.32	20.8	2.80	3.86	0.73	3.5
6.30	139.2	4.39	24.5	3.14	3.84	0.82	3.4
6.40	142.3	4.44	28	3.49	3.83	0.91	3.3
6.50	147.4	4.53	33	3.82	3.81	1.00	3.0
6.60	151.8	4.60	40	4.16	3.80	1.09	2.7
6.70	156.7	4.69	49	4.50	3.79	1.19	2.4
6.80	162.3	4.79	62	4.84	3.78	1.28	2.1
6.90	168.8	4.90	79	5.16	3.77	1.37	1.7

Table 2 C: $C'_M = 10.11$ *mC* ●

$p[H^+]_{eq} = 3.17$
$v_{B_{eq}} = 5.52$ ml 94.8 mC
$v_H = 20.0$ ml
$C_H = 26.2$ mC

0	−23.3						
5.20	43.9	2.75					
5.30	50.2	2.86					
5.40	57.5	2.98					
5.50	66.3	3.14					
5.60	75.3	3.29	1.96	0.81	7.90	0.105	5.2
5.70	82.8	3.42	2.63	1.04	7.87	0.13	5.0
5.80	88.8	3.53	3.3	1.33	7.84	0.17	5.1
6.00	98.8	3.70	5.0	1.95	7.78	0.25	5.0
6.20	105.6	3.81	6.4	2.61	7.72	0.34	5.3
6.40	111.4	3.91	8.1	3.28	7.66	0.43	5.3
6.60	116.6	4.00	10.0	3.95	7.61	0.52	5.2
6.80	121.1	4.08	12.0	4.61	7.56	0.61	5.1
7.20	130.8	4.24	17.2	5.91	7.44	0.79	4.6
7.60	140.1	4.40	25.0	7.19	7.33	0.98	3.9
8.00	149.1	4.56	36	8.43	7.22	1.16	3.2

Table 2 D: $C'_M = 15.17$ *mC* ▲

$p[H^+]_{eq} = 3.02$
$v_{B_{eq}} = 5.65$ ml 94.8 mC
$v_H = 20.0$ ml
$C_H = 26.8$ mC

v_B ml 94.8 mC	E_q mV	(19) $p[H^+]$	(15 b) $1/[H^+]$ mC^{-1}	C_{OH} mC	C_M mC	(16) $\bar{n}$	$\bar{n} \cdot [H^+]$ $10^{-5} \cdot C$
0	−24.1						
5.50	49.5	2.85					
5.60	55.9	2.96					
5.70	62.7	3.08					
5.80	69.3	3.19	1.54	1.20	11.8	0.10	6.6
5.90	75.1	3.29	1.96	1.43	11.7	0.12	6.3
6.00	79.5	3.36	2.27	1.72	11.7	0.145	6.4
6.50	96.0	3.64	4.4	3.27	11.4	0.285	6.6
7.00	106.5	3.83	6.8	4.89	11.2	0.435	6.5
7.40	113.6	3.95	8.9	6.16	11.1	0.555	6.2
7.80	120.1	4.06	11.5	7.41	10.9	0.68	5.9
8.20	126.1	4.16	14.5	8.64	10.8	0.80	5.5
8.60	132.1	4.26	18.2	9.84	10.6	0.93	5.1
9.00	138.2	4.37	23.2	11.0	10.5	1.05	4.5
9.40	144.7	4.48	30	12.1	10.3	1.17	3.9
9.80	152.1	4.60	40	13.2	10.2	1.30	3.2

Table 2 E: $C'_M = 30.34$ *mC* ▼

$p[H^+]_{eq} = 2.83$
$v_{B_{eq}} = 6.00$ ml 94.8 mC
$v_H = 20.0$ ml
$C_H = 28.4$ mC

0	−26.0						
5.70	36.1	2.62					
5.80	40.0	2.69					
5.90	44.0	2.76					
6.00	48.4	2.83					
6.10	52.6	2.90					
6.20	56.7	2.97	0.93	1.79	23.2	0.077	8.2
6.50	67.0	3.15	1.41	2.50	22.9	0.11	7.8
7.00	78.5	3.35	2.22	3.96	22.5	0.175	7.9
7.60	88.1	3.51	3.2	5.81	22.0	0.265	8.2
8.40	97.5	3.67	4.7	8.22	21.4	0.385	8.2
9.20	105.2	3.80	6.3	10.6	20.8	0.51	8.1
10.00	112.1	3.92	8.3	12.8	20.2	0.635	7.6
10.80	118.6	4.03	10.6	14.9	19.7	0.755	7.1
11.60	124.7	4.14	13.7	16.9	19.2	0.88	6.4
12.10	128.6	4.20	15.9	18.1	18.9	0.96	6.0
12.60	132.5	4.27	18.5	19.3	18.6	1.04	5.6
13.10	136.1	4.33	21.3	20.4	18.4	1.11	5.2
13.60	139.8	4.40	25	21.4	18.2	1.18	4.7

Table 2 F: $C'_M = 60.7$ *mC* ■

$p[H^+]_{eq} = 2.63$
$v_{B_{eq}} = 3.40$ ml 94.8 mC
$v_H = 10.0$ ml
$C_H = 32.2$ mC

v_B ml 94.8 mC	E_q mV	(19) $p[H^+]$	(15 b) $1/[H^+]$ mC^{-1}	(15 b) C_{OH} mC	C_M mC	(16) $\bar{n}$	$\bar{n} \cdot [H^+]$ $10^{-5} \cdot C$
0	−29.9						
3.20	26.4	2.45					
3.30	31.4	2.54					
3.40	36.9	2.63					
3.50	42.3	2.72					
3.60	47.4	2.81	0.64	2.94	44.6	0.066	10.2
3.70	51.8	2.89	0.77	3.38	44.3	0.076	9.9
3.80	55.6	2.95	0.89	3.87	44.0	0.088	9.9
4.00	61.8	3.06	1.15	4.94	43.4	0.115	9.9
4.20	66.9	3.15	1.41	6.05	42.8	0.14	10.0
4.60	74.5	3.28	1.92	8.30	41.6	0.20	10.4
5.00	80.5	3.38	2.38	10.5	40.5	0.26	10.9
5.40	85.5	3.47	2.94	12.6	39.4	0.32	10.9
5.80	90.3	3.55	3.6	14.7	38.4	0.38	10.6
6.20	94.5	3.62	4.2	16.6	37.5	0.445	10.6
6.60	98.7	3.69	4.9	18.5	36.6	0.505	10.3
7.00	102.3	3.76	5.8	20.3	35.7	0.57	9.9
7.40	106.0	3.82	6.6	22.0	34.9	0.63	9.5
8.20	112.8	3.94	8.7	25.1	33.4	0.75	8.6
9.00	119.6	4.06	11.5	28.0	32.0	0.875	7.6
9.80	126.4	4.18	15.1	30.7	30.6	1.00	6.6
10.60	133.4	4.29	19.6	33.2	29.5	1.13	5.8
11.40	140.8	4.42	26.3	35.5	28.4	1.25	4.8
12.20	148.3	4.54	34.5	37.6	27.4	1.37	4.0

1. 100 mC $HClO_4$ and 900 mC $NaClO_4$ — 10 mC $HClO_4$ 990 mC $NaClO_4$ — for the step 1 → 2;
2. 1000 mC NaClAc * 500 mC HClAc — 1000 mC NaClAc 50 mC HClAc — and 1000 mC NaClAc 5 mC HClAc — for the steps 3 → 4 and 4 → 5;
3. 1000 mC NaAc ** 500 mC H Ac — and 1000 mC Na Ac 50 mC H Ac — for the step 5 → 6.

At pH 1 → 2, $[H^+]$ is so high that one must take the diffusion potential into consideration. It has been measured by quinhydrone electrode to reach

* $ClAc^-$ = chloroacetate ion.

** Ac^- = acetate ion.

5.5 mV *. At the higher pH:s, the diffusion potentials are negligible. — With this correction, a constant value $S = 57.5 \pm 0.5$ mV is found over the whole pH-range and used in the following calculations.

The behaviour of the electrode in UO_2^{2+}-solutions was then controlled in a manner analogous to the quinhydrone electrode, Tab. 3:

Table 3. ΔE_g as a function of C_M

C_M mC	5	10	15	30	60	100
ΔE_q mV	0.1	0.1	0	0.2	0.3	0.8

The deviations found, ΔE_g, were very slight even at high C_M. They are to be considered as pure effects of medium change. So the glass electrode ought to give $[H^+]$ free from such systematic errors as ΔE_q causes at the quinhydrone electrode.

By the hydrolysis titrations, cells of the following type were measured:

$$+ \text{RE} \;\Big\|\; \begin{matrix} [H^+] \\ C_M \\ I = 1 \ (NaClO_4) \end{matrix} \;\Big|\; \begin{matrix} \text{glass} \\ \text{electrode} \end{matrix} -$$

The direct measured emf E_g in mV is given by

$$E_g = E_{RE} - E_{AgCl} - S \log \frac{a_{H+}}{a_{HCl}} - A \qquad (20)$$

where

E_{RE} and E_{AgCl} are the potential jumps of the RE and Ag, AgCl-electrode; a_{H+} and a_{HCl} the hydrogen ion activities of the measuring solution and of 0.1 C HCl; A is the asymmetry potential of the glass electrode.

E_{RE}, E_{AgCl} and $S \log a_{HCl}$ are constants, their sum is a constant E_k. Hence

$$E_g = E_k - A - S \log a_{H+} \qquad (21\ a)$$

Only over a short period of time A is a constant; by measuring a standard solution of known $[H^+] = [H^+]_0$ on the same occasion we may, however, write:

$$E_g^0 = E_k - A - S \log a_{H_0+} \qquad (21\ b)$$

* The formula of Henderson gives 5.3 mV.

Further, as the same ionic medium is used in both cases, $a_{H^+}/a_{H^+_0} = [H^+]/[H^+]_0$ and so we obtain

$$E_g - E_g^0 = S \cdot \log \frac{[H^+]_0}{[H^+]} \qquad (22\ a)$$

Table 4. Determination, by glass electrode, of $\bar{n}$ as a function of p $[H^+]$ and $\bar{n} \cdot [H^+]$ as a function of $1/[H^+]$, at different C_M.

Table 4 A: $C'_M = 5.03$ mC ◆

$p[H^+]_{eq} = 3.41$; $v_{B_{eq}} = 5.87$ ml 100.9 mC; $v_H = 20.0$ ml; $C_H = 29{,}6$ mC and $E_g^{(10)} - E_g^{(C_H)} = 26.4$ mV

v_B ml 100.9 mC	$E_g - E_g^{(10)}$ mV	(23) p$[H^+]$	(15 b) $1/[H^+]$ mC^{-1}	(15 b) C_{OH} mC	C_M mC	(16) $\bar{n}$	$\bar{n} \cdot [H^+]$ $10^{-5} \cdot$ C
0	−26.4						
5.60	53.3	2.93					
5.70	61.5	3.07					
5.80	72.2	3.25					
5.90	84.9	3.48	3.0	0.45	3.89	0.115	3.8
6.00	96.5	3.68	4.8	0.72	3.87	0.185	3.9
6.20	112.2	3.95	8.9	1.38	3.84	0.36	4.0
6.40	123.2	4.14	13.7	2.09	3.81	0.55	4.0
6.60	132.1	4.30	20.0	2.82	3.78	0.745	3.8
6.80	140.4	4.44	28	3.54	3.75	0.945	3.4
7.00	148.7	4.59	39	4.25	3.73	1.14	2.9
7.20	158.2	4.75	56	4.96	3.70	1.34	2.4

Table 4 B: $C'_M = 15.08$ mC △

$p[H^+]_{eq} = 3.07$; $v_{B_{eq}} = 4.33$ ml 100.9 mC; $v_H = 15.0$ ml; $C_H = 29.1$ mC and $E_g^{(10)} - E_g^{(C_H)} = 26.0$ mV

0	−26.0						
4.10	42.1	2.73					
4.20	49.6	2.86					
4.30	58.6	3.02					
4.40	68.4	3.19	1.54	1.01	11.7	0.087	5.7
4.50	76.1	3.32	2.08	1.36	11.6	0.115	5.6
4.60	82.4	3.43	2.7	1.76	11.5	0.155	5.6
4.80	91.8	3.60	4.0	2.65	11.4	0.23	5.7
5.20	104.8	3.82	6.6	4.50	11.2	0.395	6.0
5.60	114.6	3.99	9.8	6.33	11.0	0.575	5.9
6.00	123.0	4.14	13.9	8.10	10.8	0.75	5.4
6.40	131.2	4.28	18.9	9.82	10.55	0.93	4.9
6.80	139.3	4.42	26	11.5	10.4	1.11	4.2
7.20	148.1	4.57	37	13.1	10.2	1.29	3.5

Table 4 C: $C'_M = 60.1$ *mC* □

$p[H^+]_{eq} = 2.66$
$v_{B_{eq}} = 2.73$ ml 100.9 mC
$v_H = 10.0$ ml
$C_H = 27.5$ mC and $E_g^{(10)} - E_g^{(C_H)} = 24.6$ mV

v_B ml 100.9 mC	$E_g - E_g^{(10)}$ mV	(23) $p[H^+]$	(15 b) $1/[H^+]$ mC^{-1}	C_{OH} mC	C_M mC	(16) $\bar{n}$	$\bar{n} \cdot [H^+]$ $10^{-5} \cdot C$
0	−24.6						
2.50	25.4	2.44					
2.60	30.6	2.53					
2.70	36.3	2.63					
2.80	42.1	2.73					
2.90	47.4	2.82	0.66	2.8	46.6	0.060	9.1
3.00	52.3	2.91	0.81	3.3	46.2	0.071	8.7
3.40	65.8	3.15	1.41	5.7	44.8	0.125	9.0
4.00	78.5	3.37	2.32	9.6	42.8	0.225	9.7
4.60	86.6	3.51	3.2	13.2	41.2	0.32	9.9
5.40	96.0	3.67	4.7	17.7	39.0	0.455	9.7
6.20	104.2	3.81	6.5	21.8	37.1	0.59	9.1
7.00	111.8	3.94	8.7	25.5	35.3	0.72	8.3
8.00	120.6	4.10	12.5	29.6	33.3	0.89	7.1
9.00	129.3	4.25	17.8	33.4	31.6	1.06	5.9
10.00	138.2	4.40	25	36.7	30.0	1.22	4.9

from which $[H^+]$, the only unknown quantity, may be calculated. The assumption made, that A is a constant during the time of a titration series gives as a consequence that $E_g - E_g^0$ at a certain point ought to be a constant if the titration is repeated. This proves to be the case, and so the assumption must be true.

As a solution of known $[H^+]$ to be measured at the same occasion as the unknown $[H^+]$, it is suitable to choose the solution before titration. As stated above, the hydrolysis of a solution of such a high acidity as 30 mC may be neglected. $\therefore [H^+]_M = 0$, $C_H^0 = C_H = [H^+]_0$ (13), where C_H is experimentally determined.

On the other hand, a considerable diffusion potential arises at this high acidity, as it has been stated at the determination of S above. This must be corrected for, if the determined constant value $S = 57.5$ is to be used. To avoid this correction, the solution with $[H^+] = 10$ mC was choosen as a standard instead of the solution at the outset; for from $p[H^+]$ 2 upwards the diffusion potentials might be neglected. Moreover, this $p[H^+]$ was the standard of the quinhydrone electrode measurements * (see (17)), which thus become directly comparable with those of the glass electrode.

* Or, exactly, $p[H^+] = 1.996$.

But for the use of $[H^+] = 10$ mC as a standard solution, it was necessary to know its E_g difference to the directly measured solution at the outset. This was determined by base titrations without UO_2^{2+} : it was found that the difference in E_g between $[H^+] = 30.1$ mC and $[H^+] = 8.2$ mC is 31.6 mV; hence the apparent slope without diffusion potential correction was 56.0 in this range. With this figure, the E_g difference between the C_H of the measuring series and $[H^+] = 10$ mC was calculated (Table 4).

With the choosen $[H^+]_0 = 10.0$ mC and $S = 57.5$, (22 a) becomes

$$E_g - E_g^{(10)} = 57.5 \log \frac{10.0}{[H^+]} \qquad (22\ b)$$

and hence

$$p[H^+] = \frac{E_g - E_g^{(10)}}{57.5} + 2.00 \qquad (23)$$

The reproducibility of E_g increases with the buffering capacity of the solution; at low C_M in the proximity of the point of equivalence it was as poor as $\pm$ 0.5 mV, but in most cases one may calculate to $\pm$ 0.2 mV.

E_g attained as a rule its final value within a few minutes; at low $p[H^+]$ or when a great $p[H^+]$ jump had just occurred, a longer time was needed. The attained values were steady.

As a whole, the accidental errors were severely larger than for the quinhydrone electrode; on the other hand the systematic errors were considerable smaller, as was stated above. Here too, $\bar{n}$ was not calculated from the measured potentials, if the error might exceed $\approx$ 15 %.

The measurements are given in Tables 4 A—C and in Figs. 2 and 3 the curves $\bar{n} = f\ (p[H^+])$ and $\bar{n} \cdot [H^+] = f\ (1/[H^+])$ are found.

Conclusions from the measurements

As seen from Figs. 2 and 3, both the $[H^+]$ measuring methods used give essentially the same result. The deviations between the curves when C'_M is 60 mC and 15 mC are to be expected on account of the UO_2^{2+}-hydroquinone complex formation, and the deviation at $C'_M = 5$ mC is most likely to be explained by the accidental error, which is large at low C_M especially for the glass electrode.

The $\bar{n} = f\ (p[H^+])$ curves do not coincide, not even for the lowest C'_M. So the hydrolysis reaction involves a polynuclear complex formation, and so strongly pronounced that it manifests itself very distinctly even at so low a

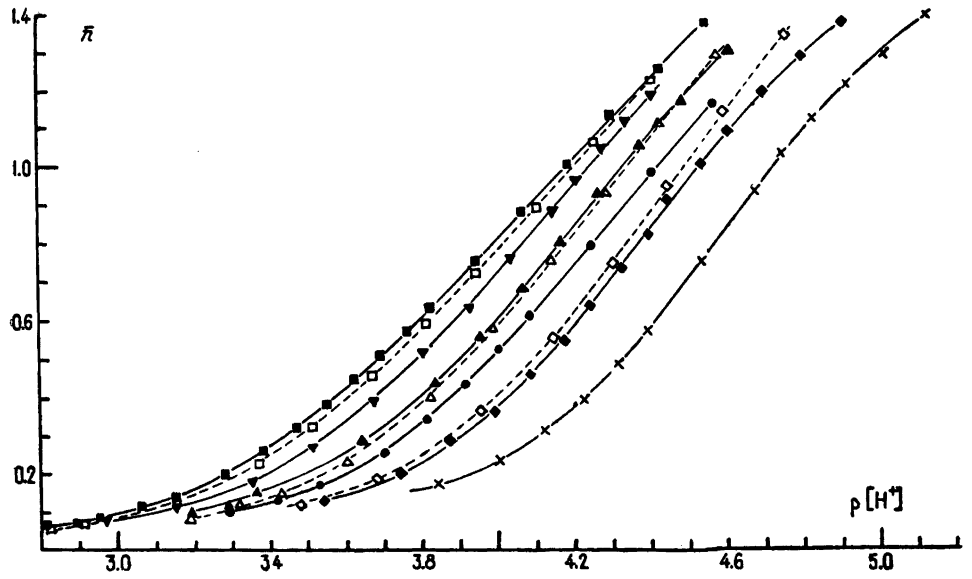

Fig. 2. Complex formation curves of the hydrolysed uranyl system at different C'_M, indicating the existence of polynuclear complexes. — The different signs relate to C'_M-values according to Tables 2 and 4. Filled signs and fulldrawn curves relate to the quinhydrone electrode measurements; open signs and dashed curves to the glass electrode measurements.

C_M as $\approx$ 5 mC. As it has been proved above, it is quite impossible that diffusion potentials, medium changes or electrode effects are the causes of the deviations.

This tendency of polynuclear complex formation puts the conditions in the uranyl solutions in a striking analogy to those existing in chromate, molybdate, and tungstate solutions (see *e. g.* Jander and Jahr [21]). Thus all metals of the group VI a in their six-valent state appear as complex ions, containing oxygen. These are in the cases CrO_4^{2-}, MoO_4^{2-} and WO_4^{2-} bases (in the sense of Brønsted) with increasing strength in the order mentioned, while UO_2^{2+} is an acid. When the ionic charge is forced to diminish, which happens by increasing $[H^+]$ at the ions of Cr, Mo, and W, but at decreasing $[H^+]$ at the U-ion, then all these metal-oxygen ions show a very strong tendency to aggregate, a tendency which before was compensated by strong Coulomb forces *.

* Curiously enough, Jander and Jahr [22] have come to the result, that the aggregation they have proved in solutions of chromate, molybdate, and tungstate would not occur in uranyl solutions. The reverse is to be expected, and the present results proves that this is the case.

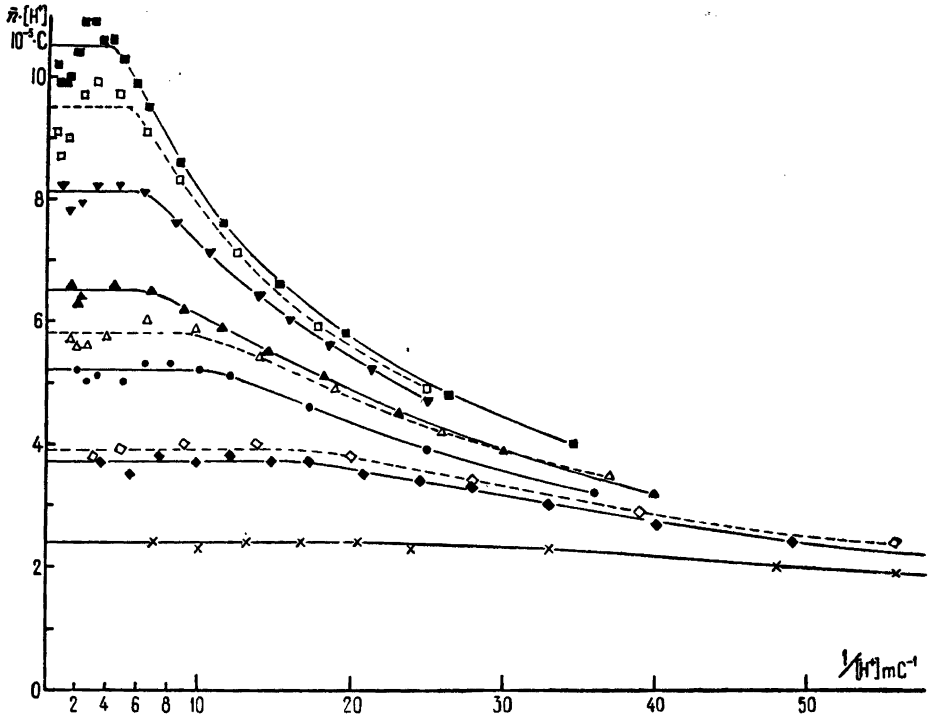

Fig. 3. $\bar{n} \cdot [H^+]$ *as a function of* $1/[H^+]$, *to determine lim* $\bar{n} \cdot [H^+]$, *when* $1/[H^+] \to 0$. — *About the signs, see Fig. 2.*

By other hydrolysis reactions, too, polynuclear complexes exist. This has been proved qualitatively for numerous metal ions (especially by Jander and Jahr [22]) and, more recently, also quantitatively for Bi^{3+} (Granér and Sillén [23]) and for Cu^{2+} (Pedersen [24]). So a polynuclear complex formation in such reactions seems to be a rule rather than an exception.

For the calculation of the constants $\varkappa_1'$ and $\varkappa_1''$, the limits $\bar{n}\,[H^+]$ when $1/[H^+] \to 0$ were extrapolated from Fig. 3. The limits found are in Table 5, together with the values of C_M at the lowest determined $\bar{n}$ (at these C_M the extrapolations of Fig. 3 are made, and so they are to be inserted in (11)). In Fig. 4, finally, the limits are plotted as a function of C_M. As predicted, the found curve is with good approximation a straight line in its first part. From its axis interception and slope we get, (11):

$$\varkappa_1' = (2.0 \pm 0.4) \cdot 10^{-5} \text{ C}; \qquad \varkappa_1'' = (4 \pm 1) \cdot 10^{-3}$$

Table 5. lim $\bar{n}$. $[H^+]$ as a function of C_M. $1/[H^+] \to 0$

C'_M mC	quinhydrone		glass	
	lim $\bar{n}$ · $[H^+]$ 10^{-5} · C	C_M mC	lim $\bar{n}$ · $[H^+]$ 10^{-5} . C	C_M mC
2	2.4	1.5	—	—
5	3.7	4.0	3.9	3.9
10	5.2	7.9	—	—
15	6.4	11.8	5.8	11.7
30	8.1	23	—	—
60	10.5	45	9.5	47

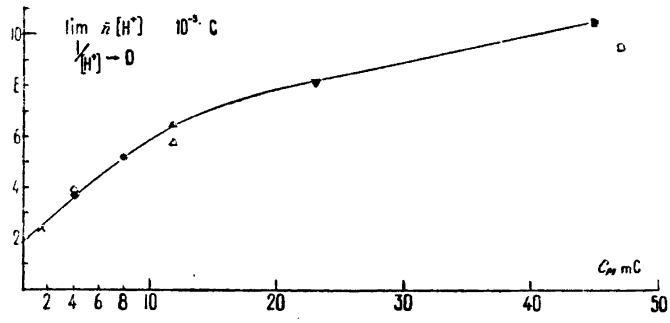

Fig. 4. Lim $\bar{n}$ · $[H^+]$ as a function of C_M, to determine $\varkappa'_1$ and $\varkappa''_1$ · $1/[H^+] \to 0$. — About the signs, see Fig. 2. — The curve is drawn in accordance with the points of the quinhydrone electrode measurements.

If we assume $K_W = 10^{-14}$, this corresponds to β values, (9) : $\beta'_1 = 2 \cdot 10^9$ C^{-1}; $\beta''_1 = 4 \cdot 10^{11}$ C^{-2}.

At higher C_M, the curve, according to (11) ought to bend upwards, if it bends at all. Unfortunately, the contrary is the case. The only reasonable explanation of this seems to be that the limits searched for are not attained at higher C_M, and, further, the curves here have such a course that the extrapolation gives an entirely misleading result. That the proper limits really are attained at low C_M is just proved by the fact that (11) in these cases really has the predicted course.

SUMMARY

It has been shown by extinctiometric measurements that the uranyl ion UO_2^{2+} is the only existing complex of six-valent uranium in aqueous solution from $p[H^+] \approx 0.1$ (the lowest investigated) up to $p[H^+] \approx 2$. Over that

p[H^+] further hydrolysis complexes are gradually formed. So UO_2^{2+} may be regarded as a complex forming central group, and formulas for its complex formation have been developed in a manner analogous to other metal ions.

The extinction curve of UO_2^{2+} has been determined; by this the data of the literature have been found to be erroneous.

As the first complex uranyl system to be investigated, just the further hydrolysis of UO_2^{2+} has been selected. It has been studied by [H^+] measurements when $UO_2(ClO_4)_2$ solutions were titrated with NaOH. The total uranium concentration ranged from 2 mC to 60 mC. The ionic strength of the solutions was kept constant = 1 by the aid of $NaClO_4$.

The determinations were done, firstly with quinhydrone electrode, secondly with glass electrode. Both electrodes gave essentially the same image of the hydrolysis complex formation, which thus with certainity may be described as follows:

The hydrolysis becomes appreciable at p[H^+] ≈ 3. Precipitation does not occur until, on the average, 1.3 OH^- has been used per UO_2^{2+}; p[H^+] is then 4.5-5.

The complex formation curves found at different C_M prove without any doubt that a polynuclear complex formation plays a very important role in the hydrolysis, even at the smallest C_M. This fact places UO_2^{2+} in an analogous position to other metallic ions, containing oxygen, in the same group of the periodic system: CrO_4^{2-}, MO_4^{2-}, WO_4^{2-}.

The first mononuclear dissociation constant $\varkappa_1$ and the first dinuclear $\varkappa_1''$, defined above, have been determined. At 20° C they were found to be, for the ionic medium used,

$$\varkappa_1' = (2.0 \pm 0.4) \cdot 10^{-5} \text{ C}; \qquad \varkappa_1'' = (4 \pm 1) \cdot 10^{-3}$$

These uranyl investigations have been suggested to me by my teacher in chemistry, Professor Sven Bodforss, to whom I am very much indebted for the valuable aid he has given in different respects.

With my friend Docent Sture Fronaeus I have had many fruitful discussions. They have given me views and information, which have strongly promoted my work.

Further I wish to thank *Försvarets Forskningsanstalt (FOA)*, Stockholm, for a liberal financial support.

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Received February 5, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 401—407

Some Remarks on Chromatography

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The chromatography has been promoted by Tiselius [1] introducing refined methods for measuring changes in refractive power, *e. i.* variations in concentration, by means of an interferometer apparatus. Claesson [2] has designed a selfrecording apparatus in which changes in refractive power of a solution leaving the filter with the adsorbent v e r s u s the amount of solution are given as a curve on a photographic paper in an incontrovertible documentation. Such a selfrecording apparatus is of course very valuable especially when running analyses are performed. However, to use the apparatus for such purposes the inconveniences due to certain characteristics of the apparatus must be reduced to a minimum. But, unfortunately difficulties in interpreting experimental results have arisen during our work, and also in other laboratories. Therefore we think some of our experiences with the f i r s t specimen put on the market by Fabriks AB LKB-Produkter, Stockholm, could be of some interest to workers in this field.

There are evidently three principally different sources from which the difficulties in reading curve may originate: imperfectness in apparatus design, unsuitable handling of the machine and faulty performing of the actual experiment, if of course they are not due to the problems under investigation. Therefore the investigator must be aware of from which source the difficulty may originate to be able to eliminate undesired complications.

As for the details concerning the construction of the apparatus (Fig. 1) and instructions how to perform different kind of experiments we refer to the description given by Claesson [2]. In following lines we have restricted our communications only to some factors which now and then bring us more or less troubles and which at times are difficult to eliminate. As the reading of the curve is important part of the work with the apparatus we illustrate our examples with the original curves.

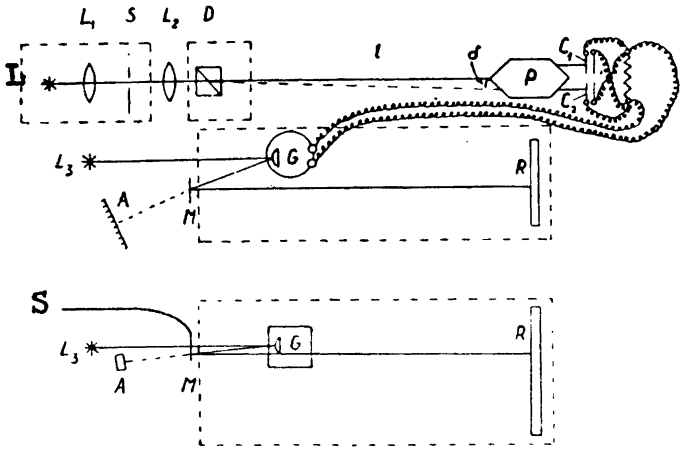

Fig. 1. Sketch of apparatus, S. Claesson (2).

L: *Tungsten ribbon lamp.*
L_1 and L_2: *Lenses.*
S: *Slit.*
D: *Thermostat with cuvette doubble prism.*
δ: *Deviation of light beam due to changes in refractive power of the liquid in one of the halves of the doubble prism.*
P: *Hexagonal prism.*
C_1 and C_2: *Photo element, selenium cells.*
G: *Galvanometer.*
S: *Spring carrying the reciever for liquid from the cuvette.*
M: *Mirror attached to the spring S.*
R: *Screen or photografic paper.*

The apparatus is very sensitive to outer disturbances like vibrating of the floor, draught, deviations of the room temperature in relation to that of the thermostat. To check the absence of any disturbances in the apparatus it is not advisable to carry out a full experiment but to observe the light spot directly on the screen for a longer time with the cuvette in the thermostat or — and better — without the cuvette but with a suitable aperture reducing the intensity of the light from the tungsten ribbon lamp, as too high intensity of the light spoils the selenium cells. It is advisable to provide the room with constant temperature adjusted to the thermostat temperature. This favourable condition enables to start an experiment almost immediately after the cuvette has been plunged into the thermostat.

It is important to control that the deflections of the light spot caused by changes in refractive power of the liquid in the cuvette, or by turning the

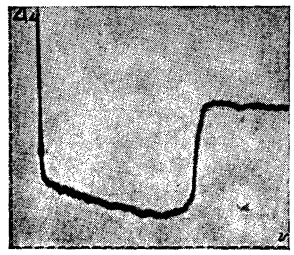

Fig. 2. Frontal analysis. Inperfect washing. Note the inclined volume line befor the step.

micrometer screw operating the hexagonal prism (P in Fig. 1), are at (an almost) right angle to the deviations caused by loading the spring (M in Fig. 1) carrying the receiver for liquid leaving the cuvette. For correction in this respect the level of the table supporting the apparatus may be altered and the position of the mirror (M in Fig. 1) attached to the spring.

Further, there is no stirring in the inner thermostat (D in Fig. 1) and a slight decrease or increase in temperature during especially long experiments is more or less unavoidable. It may cause a change of the refractive power as if in concentration of the solute, which is of course apparent. For instance, if there is a long retention volume period the volume line on the curve is not longer perpendicular to the concentration axis (Fig. 2). Such an systematic error is rather difficult to distinguish from an imperfect washing of the adsorbent.

There are some factors which have great influence upon the sensitivity of the apparatus. If a sensitivity is desired to a certain value a good rheostat should be substituted for the small radio potentiometer in the selenium cell-galvanometer curcuit. Air bubbles in the cuvette chambers (Fig. 3) and its window pockets are to be thoroughly eliminated. In order to avoid air bubbles in the pockets the deeper one is to be filled with the liquid from the inner

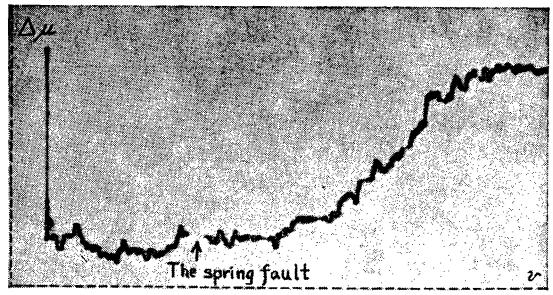

Fig. 3. Frontal analysis. Air-bubbles inside the cuvette. (On spring troubles cf. Fig. 3.)

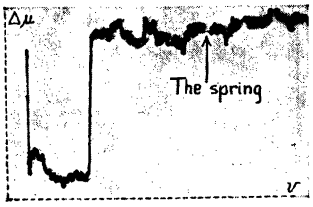

Fig. 4. Frontal analysis. Tungsten ribbon lamp troubles. (On spring troubles cf. Fig. 7.)

thermostat and the cuvette immersed in the position a little inclined to prevent loosing the liquid. If the latter precautions failed and the air bubbles are detected in the window pockets, they may be removed by means of a special syringe provided with a long tip which is bent at the end at a right angle. In order to keep windows in the cuvette and the thermostat clean, the liquid in the inner thermostat is replaced rather often. We have found it better to have water than 50 % alcohol-water mixture in the inner thermostat.

To get a convenient commodity for inspection the cuvette while in thermostat, the tube between the hexagonal prism (P in Fig. 1) and the thermostat (D in Fig. 1) is provided with a hole through which the examination may be made by means of a mirror. It may be possible to use a half reflecting mirror for inspection, which enables examination during the whole experiment, in which case, however, the sensitivity must be determined with the mirror in the tube.

We perform the determination of the sensitivity twice, firstly before the photografic paper is put on place, secondly when it is on place by turning the micrometer screw a certain angle. In the latter case we automatically get the refractive power, e. i. the concentration axis on the paper (Fig. 2).

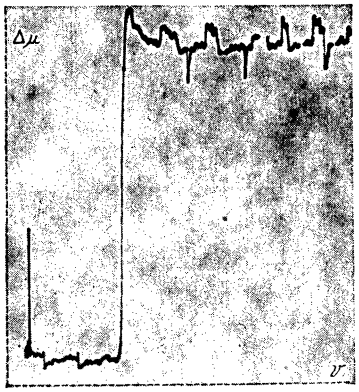

Fig. 5. Frontal analysis. Tungsten ribbon lamp troubles or bad contacts in selenium cell — galvanometer curcuit.

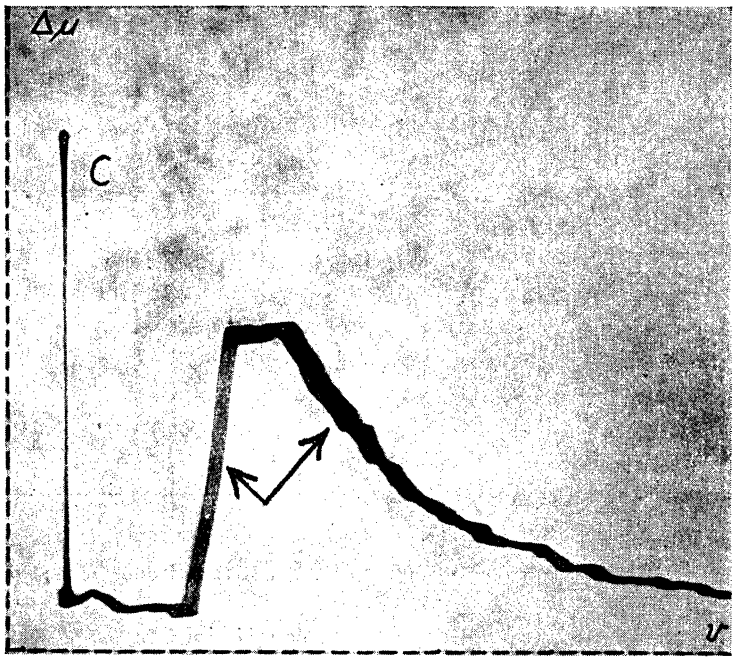

Fig. 6. Eluation analysis. Without aluminium foil. Note the swingings and broad lines in parts parallell with the concentration axis c.

The tungsten ribbon lamp (L in Fig. 1) causes at times difficulties. Originally a lamp holder was provided, which is however objectionable, and we recommend to have the lamp soldered to the wires. But even with this arrangement now and then difficulties arise from the lamp. As far as we have found they occur a longer or shorter time before the lamp is worn out and are due to

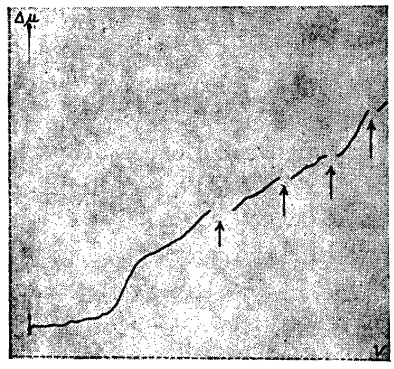

Fig. 7. Frontal analysis. Reciever holder hanging not frictionless. Note intervals in the curve.

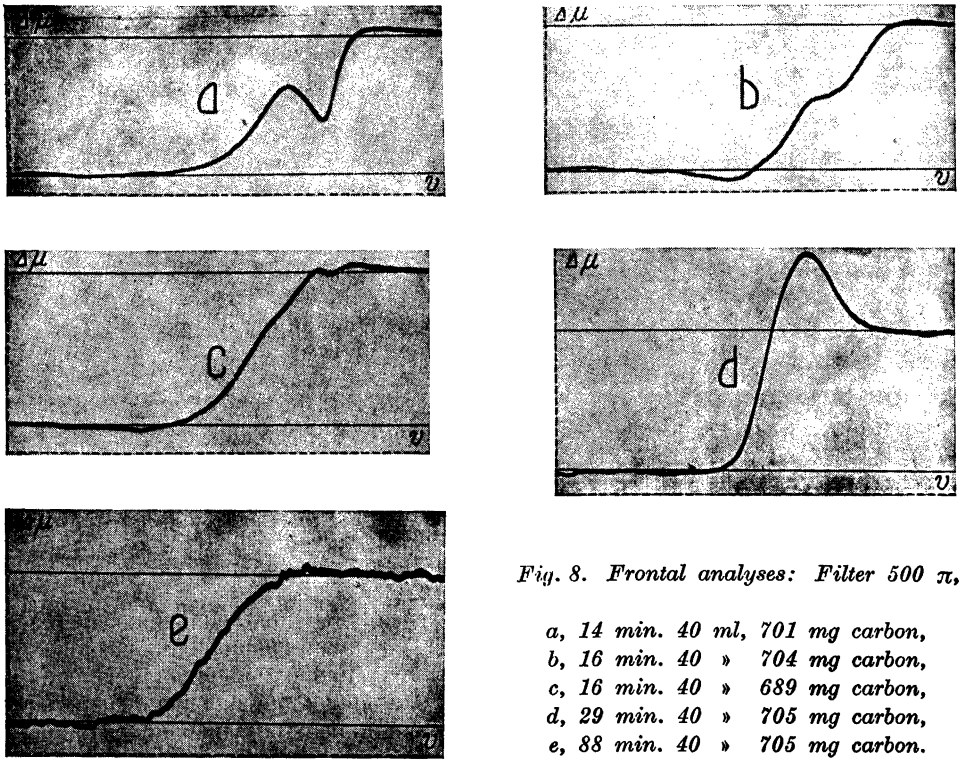

Fig. 8. Frontal analyses: Filter 500 π,

a, 14 min. 40 ml, 701 mg carbon,
b, 16 min. 40 » 704 mg carbon,
c, 16 min. 40 » 689 mg carbon,
d, 29 min. 40 » 705 mg carbon,
e, 88 min. 40 » 705 mg carbon.

small otherwise undetectable damages of the tungsten ribbon near the ribbon holder in the lamp (Figs. 4 and 5).

The galvanometer once put in order is very reliable. It may happen, that it must be removed for some reason and then, when put back again, the mirror is not working properly. The apparatus is then hypersensitive to slight knocking and vibrations, what indicates that the mirror is not hanging free.

The balance and the receiver for the liquid from the cuvette have caused a lot of troubles. We have replaced the glass receiver which has proved to be too heavy by a plastic bag put on a glass ring and hanged on the original holder attached to the spring. It is recommended to repress the oscillations of the balance caused by falling drops by a metal strip dipping into paraffin oil and what has proved to be also very advantageous in this respect a strip of aluminium foil attached to the receiver in such a manner that the falling drops slide down along this strip (Fig. 6). Much attention must be given to the way in which the receiver holder is attached to the spring. If the holder is not hanging quite free there may appear intervals in the curve (Fig. 7). In order to make

the movements quite uniform and to exclude friction we have attached the receiver to the spring with rubber strings.

In the literature it is said chromatography should be performed at a low rate. As far as we are informed only Claesson [2] has stated an upper limit, in case of filter 10 mm inside diameter. (Sillén and coworker [3] have amongst others treated the rate-problem in case of ion exchange.) It is of course an advantage if the time of an analysis can be shortened, therefore we looked for rapid working carbons in order to learn a little moore about this question. Thus we met some deviations from a normal curve which proved to be reproducible — at least in case so far investigated — if the rate of flow of the solution through the filter was taken in account. From Fig. 8 a—e it is clear how the shape changes with the rate. It is also obvious, quite a »good curve» (Fig. 8 c) may be obtained, but the result may nevertheless be in a way illusive, as the »good shape» disappears when the rate of flow is increased *or* decreased. Therefore, if difficulties in reading a curve like those in Fig. 8 arise they may be due to unsuitable rate of flow. We will on another occasion give some more details on this question, but we have mentioned this here as we at first thought the bad shape was due to unproper handling of the apparatus.

SUMMARY

Some experiences from work with a selfrecording apparatus designed by S. Claesson are given. Difficulties in proper understanding of experimental adsorption curves have arisen but ways to eliminate the difficulties are shown. Further, there is stated that the rate at which the solution passes through the filter, under at least certain circumstances, has a great influence upon the shape of the adsorption curve for frontal analysis so far investigated.

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Received March 19, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 408—414

The Molecular Structure of Biphenyl and some of its Derivatives

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It has been known for a long time that atrop-isomerism will occur when two or more of the ortho hydrogen atoms in biphenyl are replaced by larger atoms or groups. The phenomenon is easily explained as due to steric hindrance which prevents rotation about the central C—C′-bond in the biphenyl derivative. For instance Searle and Adams [1] have separated 2,2′-diiodo-4,4′-dicarboxybiphenyl and the corresponding bromocompound in *d*- and *l*-components. These molecules must consequently have a non-planar configuration. On the other hand X-ray crystallographic investigations have shown that meta- and para-substituted biphenyls as well as biphenyl itself have a completely planar configuration [2–5]. This fact has been explained by Sutton [6] and others [7] as a result of the partial double-bond character of the central C—C′-bond.

The ultraviolet spectroscopists [8] have studied many biphenyl derivatives and have found a marked difference in the ultraviolet absorption of the ortho-substituted and the non-ortho-substituted derivatives. Their view is that the ortho-substituted derivatives have a non-planar configuration and the others a completely planar configuration. They expect this to be found true not only in the crystalline state but also in liquids and gases.

The dipole moment measurements do not seem to be able to give information about the angle between the ring planes in the biphenyl derivatives. As an example of the numerous dipole moment measurements of these derivatives we may mention the series 4,4′-, 3,3′-, and 2,2′-dichlorobiphenyl [12, 13] which have been measured in solution. As was to be expected, the first of these has zero dipole moment. This shows that the atoms Cl_4, C_4, C_1, C_1', C_4' and Cl_4' lie on a straight line. The other two compounds have dipole moments 1,68D and 1,77D respectively. The plane *trans* form can therefore be excluded in

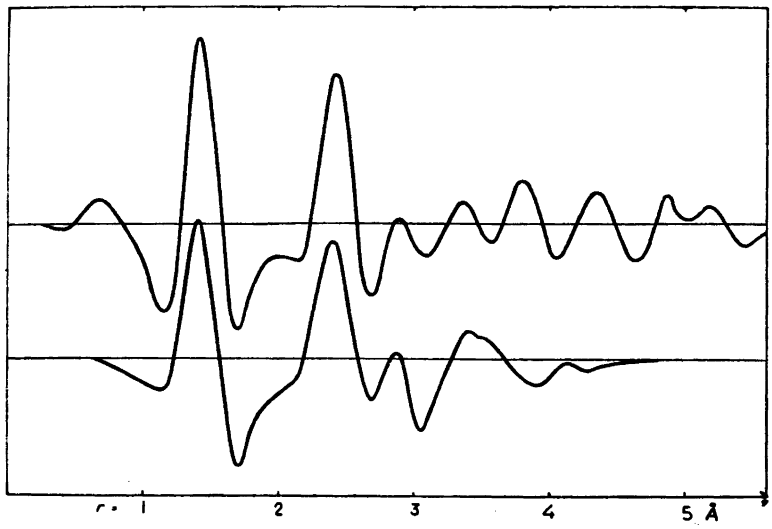

Fig. 1. $\sigma(r)$-curve for biphenyl and benzene.

both these cases, but we have to make a choice between three possibilities: 1) An equilibrium between *cis* and *trans* forms. 2) A free or restricted rotation about the central C—C'-bond. 3) A rigid non-planar configuration.

Karle and Brockway [14] have investigated gaseous biphenyl by the visual electron diffraction method. Their results do not seem to be entirely free from ambiguity, but they think they have found indications of a non-planar model.

We have by the electron diffraction sector method studied the structure of biphenyl, 3,3'-dichlorobenzidine, and 3,3'-dibromobiphenyl.

RESULTS AND DISCUSSION

Biphenyl. In Fig. 1 the upper curve is the $\sigma(r)$-curve for biphenyl. The lower curve is the $\sigma(r)$-curve for benzene multiplied by 2. The peak corresponding to the C—C-bond distances in the rings occurs at 1.40 Å. If there exist different C—C-bond distances in the phenyl rings, this can not be detected from the $\sigma(r)$-curve. The two curves are for the interval $0 > r > 3$ Å similar in their broad features. This is in accordance with the assumption that the phenyl rings in biphenyl to the first approximation are indentical with the benzene ring. The differential curve should therefore for the most part contain peaks corresponding to atomic distances *between* the two rings in biphenyl. The differential $\sigma(r)$-curve is given as the upper curve, A, in Fig. 2. The curves B, C, and D are theoretical differential curves calcu-

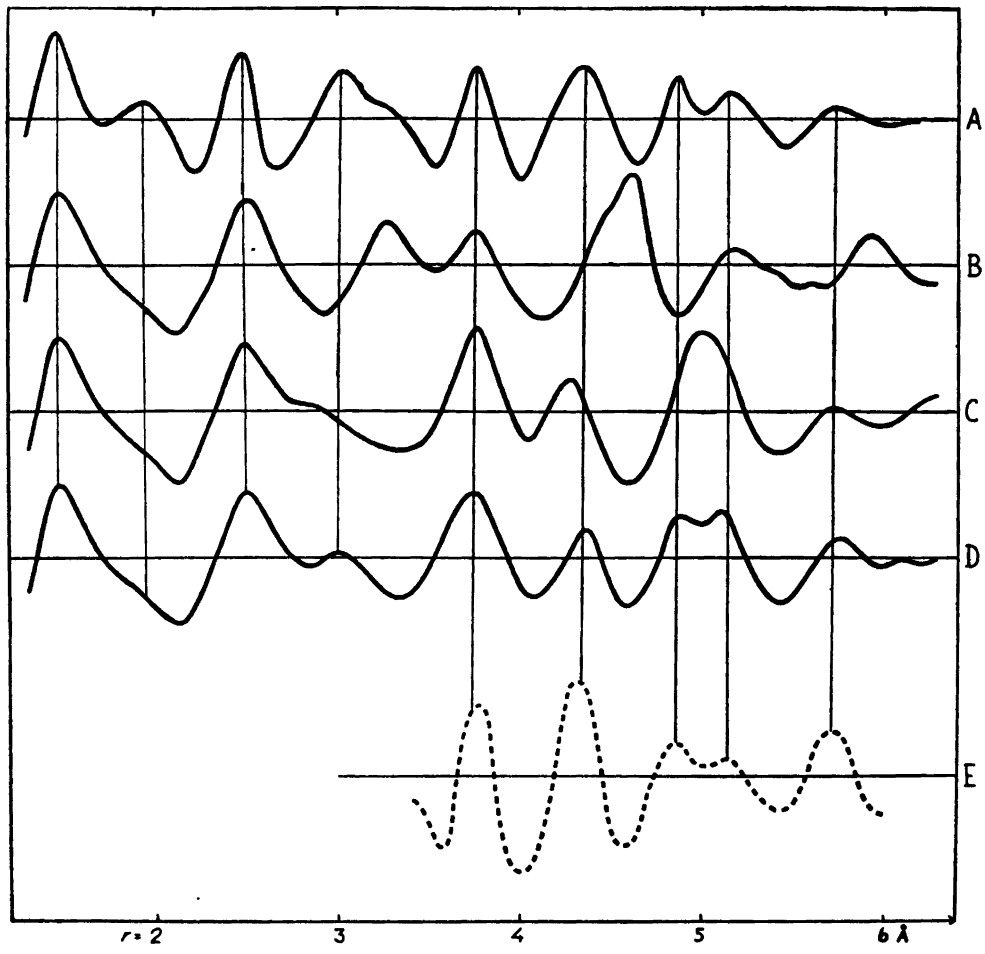

Fig. 2. Experimental and theoretical differential $\sigma(r)$-curves.
($\sigma(r)_{biphenyl} \div 2 \cdot \sigma(r)_{benzene}$).

lated on different assumptions as regards the angle φ between the ring planes. The φ-values are 90°, 0°, and 45° for the curves B, C, and D respectively. Some of the peaks in the experimental curve are found in all the theoretical curves. The corresponding distances are those which do not vary when the two rings are rotated relatively to each other. These peaks will indirectly give informations of the length of the central C—C′-bond which is found to be 1.48 Å.

The theoretical curve which is in best accord with the experimental one is undoubtedly curve D. Though the similarity of the two curves A and D is striking, especially with regard to the position of the maxima, a real deviation

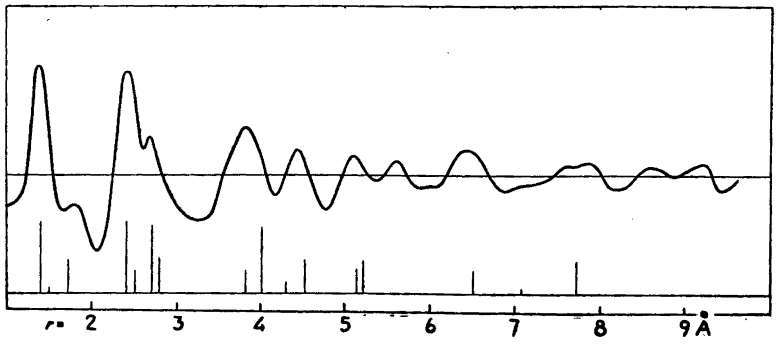

Fig. 3. $\sigma(r)$-curve for 3,3'-dichlorobenzidine.

is observable, particularly for the interval $1 > r > 3$ Å. The $\sigma(r)$-curves for biphenyl and benzene have in that interval the highest maxima and the deepest minima, and errors will therefore influence the differential curve to a relatively greater extent. The lower curve E in Fig. 2 is an experimental differential curve calculated from a new series of diagrams. This reinvestigation was done about a year after the calculation of the curves A, B, C, and D. It was carried out in order to demonstrate the reproducibility of the method in this particular case. The curve E was calculated only for the interval which is important for our studies.

From these considerations it seems evident that we should be able to exclude both the model which has the two ring planes perpendicular to each other and the strictly planar model found in the crystalline state. An intermediate configuration must therefore be assumed, but in spite of the rather good agreement between experiments and theory for the 45° model, it is very difficult by a systematic variation of the angle to settle the limits of the error. A rough estimation suggests, however, that the deviation from the 45° value is less than $\pm$ 10°. — In this connection we may mention that an exclusion of minor oscillations about the equilibrium state is impossible from our $\sigma(r)$-curves.

3,3'-Dichlorobenzidine. Fig. 3 shows the experimental $\sigma(r)$-curve for 3,3'-dichlorobenzidine. Most of the peaks occurring in the $\sigma(r)$-curve can be explained by distances which do not vary by a rotation about the central C—C'-bond. This is demonstrated in a simple manner by the line diagram below the $\sigma(r)$-curve. (The C—C- and the C—Cl-bond distances are found to be 1.40 Å and 1.73 Å respectively). Of the remaining peaks the most pronounced one occurs at 5.6 Å. It is readily interpreted as a C_2—Cl'-distance, if the angle between the two ring planes is assumed to be approximately 52°. The rest of

the $\sigma(r)$-curve does not seem to give further reliable information concerning the value of the angle. On the other hand a planar model can not by any means be compatible with the $\sigma(r)$-curve.

3,3'-Dibromobiphenyl. This compound must be expected to be very well suited for the study of our effects. The bromine substituted hydrocarbons have in many cases proved to be better fitted for electron diffraction than the derivatives containing other halogens. The 3,3'-dibromobiphenyl possesses further the advantage over the 3,3'-dichlorobenzidine of the absence of the amino groups.

The $\sigma(r)$-curve for 3,3'-dibromobiphenyl is given in Fig. 4. All the maxima for $r < 5.5$ Å may easily be explained by distances which do not vary when the two ring planes are rotated relatively to each other. These distances are indicated by the solid line diagram below the $\sigma(r)$-curve. The bond distances used in the calculation of the line diagram are: C—C = 1.40 Å, C—C' = 1.49 Å and C—Br = 1.88 Å. The maximum at 3.4 Å which does not correspond to any internuclear distance may easily be explained as a diffraction error maximum caused by the neighbourhood of the peaks at 2.86 Å and 4.18 Å. Besides the peaks mentioned which are easily interpretable a further six peaks, which must be explained as internuclear distances, have been observed.

Table 1. Values characterizing the magnitude of the deviation from coplanarity of 3,3'-dibromobiphenyl.

The r-values of the observed maxima	Distances	φ	
5.80 Å	Br_3-C_6'	56°	
6.49 »	Br_3-C_2'	48°	
7.14 »	Br_3-C_5'	62°	mean value
7.65 »	Br_3-C_3'	52°	54°
8.07 »	$Br-Br_c$	54°	
9.20 »	$Br-Br_t$	52°	

The position of these peaks (dashed line in the line diagram) is shown in the first column of Table 1. They correspond to distances occurring in a non-planar structure (Second column of Table 1). These distances belong to such as vary when the two ring planes are rotated relatively to each other. The other distances of this type will be unable to influence the $\sigma(r)$-curve to any appreciable extent. The distances Br—Br_c and Br—Br_t from second column in Table 1 are the two different Br—Br-distances which may occur in a molecule when the two ring planes are not at right angles to each other. The values

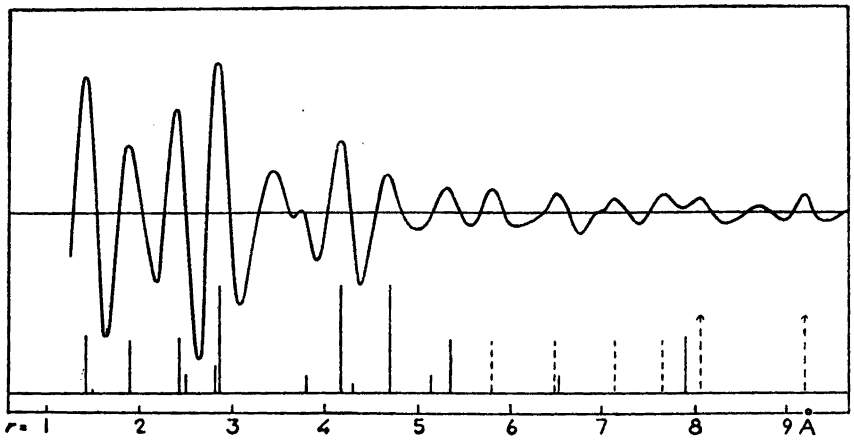

Fig. 4. $a(r)$-curve for 3,3'-dibromobiphenyl.

in the first column of Table 1 enable us to calculate six independent values for the angle φ between the ring planes. These values are given in the third column of Table 1. Though there exist some fluctuations in the φ-values thus obtained, the effect of the deviation from coplanarity seems to be demonstrated in a conclusive manner. The error in the determination of the angle φ is presumabely less than 5°, and in this case therefore we can give a real quantitative determination of the effect.

The deviation in the values of the angle φ for the three compounds under consideration is within the limits of the error. The non-planar configuration must in all the three cases be explained by the repulsive forces between adjacent hydrogen atoms. Dipole effects in 3,3'-dichlorobenzidine and 3,3'-dibromobiphenyl, might have been expected to contribute somewhat to the formation of the molecule configuration. The fact that these molecules have a very similar configuration to the biphenyl molecule shows that the intramolecular van der Waals forces certainly must have a predominant signification. The shortest H_2—H_2'-distances in a molecule with a φ-value of 54° is about 2.65 Å, corresponding to a van der Waals distance somewhat greater than that usually observed.

The usual assumption that resonance phenomena alone are responsible for the coplanarity of the biphenyl molecule and many of its derivatives in the crystalline state, is from our considerations obviously wrong. Of course resonance phenomena might contribute to the stabilization of the planar configuration in the crystalline state, but additional effects must be present. In a crystal not only *intra*-molecular forces will determine the shape of the

molecule, the *inter*-molecular forces must also be expected to play a certain part. Though the energy of an isolated molecule may have a minimum for the non-planar configuration, it may well be that a planar molecule is better suited as a unit for the construction of a stable lattice.

Finally we may mention that Merkel and Wiegand [15] in a recent work have independently come to results in good agreement with those given above. They find by ultraviolet spectroscopy that the molecules of biphenyl are planar in the crystalline state, but not in the liquid or gaseous state.

SUMMARY

The molecular structure of gaseous biphenyl, 3,3′-dichlorobenzidine, and 3,3′-dibromobiphenyl has been studied by the electron diffraction sector method. A non-planar configuration is found. The angle between the ring planes is found to be $45° \pm 10°$, $52° \pm 10°$, $54° \pm 5°$ for the biphenyl, 3,3′-dichlorobenzidine, and 3,3′-dibromobiphenyl respectively. The significance of the result is discussed.

I wish to express my gratitude to professor Dr. Roger Adams, University of Illinois for having placed a sample of 3,3′-dibromobiphenyl at my disposal. I also wish to thank *Fridtjof Nansens Fond* and *Det Vitenskapelige Forskningsfond av 1919* for economical aid without which this work could not have been accomplished.

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Received March 14, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 415—421

Intra-Molecular Hydrogen Bonds in Ethylene Glycol, Glycerol, and Ethylene Chlorohydrin

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The molecular structure of ethylene glycol, glycerol, and ethylene chlorohydrin has already been studied by various investigators and from various points of view. Kohlrausch and Köppel [1] have found some anomalies in the Raman spectrum of glycol, but have not been able to determine the structure. Saksena [2] has studied the Raman spectrum of glycerol and found that no symmetry element is present in the molecule. The spectrum of ethylene chlorohydrin studied by Kohlrausch and Ypsilanti [3] gives evidence of the existence of at least *two* different space forms of this molecule. Other spectroscopic investigations have been carried out, but none of them has given decisive information about the molecular structure of the compounds in question.

From the dipole moment measurements, Zahn [4] thinks there is support for *three* different theories concerning the structure of ethylene glycol:

1) Free rotation about all the single bonds.
2) Free rotation about the carbon-oxygen bonds, but not about the carbon-carbon bond. *Trans* form.
3) Same conditions, but *cis* form.

For the ethylene chlorohydrin *two* possibilities are given:

1) Free rotation about the carbon-oxygen bond, but not about the carbon-carbon bond. *Trans* form.
2) Rigid molecule. *Cis* form.

The results of our electron diffraction investigations are not in accordance with any of these assumptions.

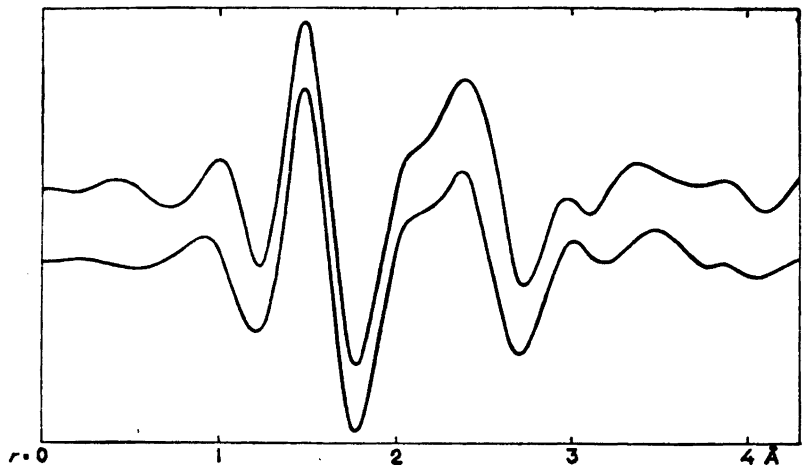

Fig. 1. Two $\sigma(r)$-curves for ethylene glycol calculated from different diagrams.

RESULTS

Ethylene glycol

In the study of the $\sigma(r)$-curve of ethylene glycol the peak corresponding to the oxygen-oxygen distance will obviously be the most interesting one. Unfortunately the contribution of this distance to the entire $\sigma(r)$-curve will be relatively small. As an additional unfavourable circumstance the peak corresponding to the oxygen-oxygen distance occurs very near a minimum caused by the neighbouring peaks. It is therefore of great importance in this case to know if the minor maxima occurring in the $\sigma(r)$-curve are real or may be explained as random errors. In Fig. 1 two $\sigma(r)$-curves for ethylene glycol are given. The lower curve has been calculated three years later than the other and from a new series of diagrams. The agreement between the two curves is a satisfactory demonstration of the reproducibility of the method. — The upper curve in Fig. 2 is the mean experimental $\sigma(r)$-curve. The dotted curve is the theoretical $\sigma(r)$-curve calculated from the Viervoll normal curves. The theoretical curve contains only such distances as are not altered by a rotation about the carbon-carbon bond. The calculation is made on the assumption of normal bond distances and valency angles (C—C = 1.54 Å, C—O = 1.43 Å, C—H = 1.08 Å, $<$C—C—O = 109.5°). The lower curve in Fig. 2 represents the difference between the experimental and the theoretical $\sigma(r)$-curve for the interval which has interest in the present connection. The highest peak in the differential curve occurring at 2.97 Å must be ascribed to the oxygen-oxygen

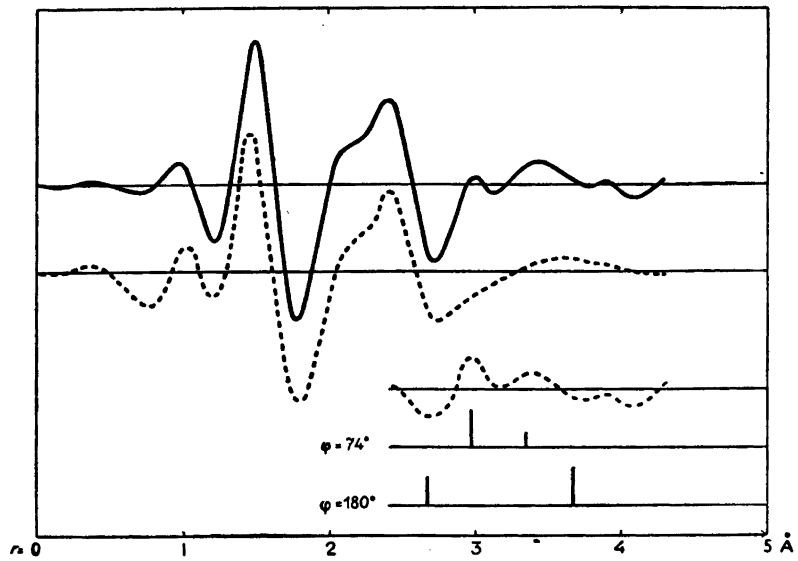

Fig. 2. Experimental, theoretical, and differential $\sigma(r)$-curves for ethylene glycol.

distance. The position of this peak corresponds to an angle φ between the two oxygen-carbon-carbon planes of 74°. The diagrams below the differential curve correspond to φ-values of 74° and 180° respectively. The exclusion of a rigid *trans*-form seems to be evident.

Glycerol

The curves given in Fig. 3 are the experimental, the theoretical, and the differential $\sigma(r)$-curves for glycerol. The curves correspond to those of ethylene glycol given in Fig. 2. The most pronounced peak in the differential curve occurs at 2.94 Å, *i. e.* — within the limits of the errors — at the same position as for ethylene glycol. The φ-value is accordingly in this case 71°. The angle φ does not, — owing to the more complex structure of the glycerol molecule, — suffice to describe the configuration unambiguously. The relative position of the two groups $H_2(OH)C$- and CH(OH)C — may be characterized by the letters α, β and γ. In the α-position the angle φ is equal to 71° and the oxygen atom in the $H_2(OH)C$-group is nearly in trans-position to the carbon atom in the —CH(OH)C-group. In the β-position the oxygen atoms are in trans-position. In the γ-position the angle φ is equal to 71° and the oxygen atom in the $H_2(OH)C$-group is nearly in trans-position to the hydrogen atom in the

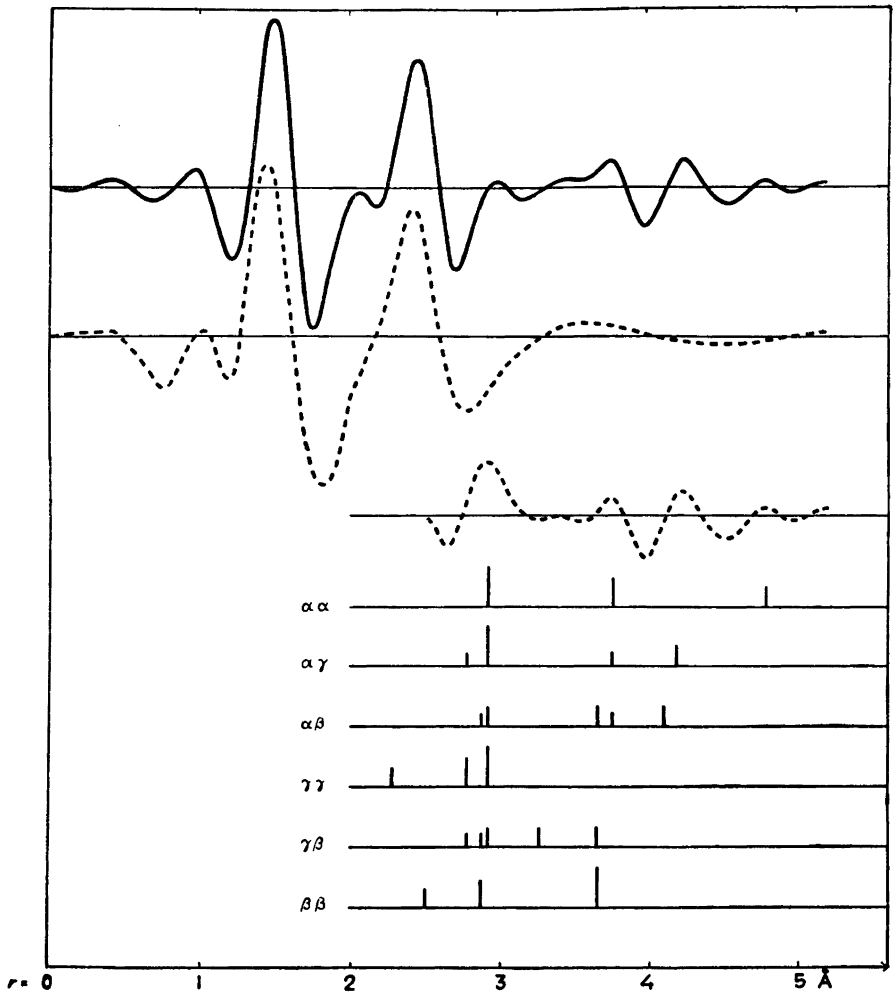

Fig. 3. Experimental, theoretical, and differential $\sigma(r)$-curves for glycerol.

—CH(OH)C-group. Though the β-position is, according to our experience concerning the glycol molecule, improbable, we shall provisionally include it. The possible configurations of the glycerol molecule are then, $\alpha\alpha$, $\alpha\gamma$, $\alpha\beta$, $\gamma\gamma$, $\gamma\beta$, and $\beta\beta$. The carbon-carbon, carbon-oxygen and oxygen-oxygen distances which might be expected in the differential curve are for all the configurations mentioned indicated in Fig. 3 by the line diagrams below the curves. From the diagrams it will be seen that all maxima in the differential curve may be

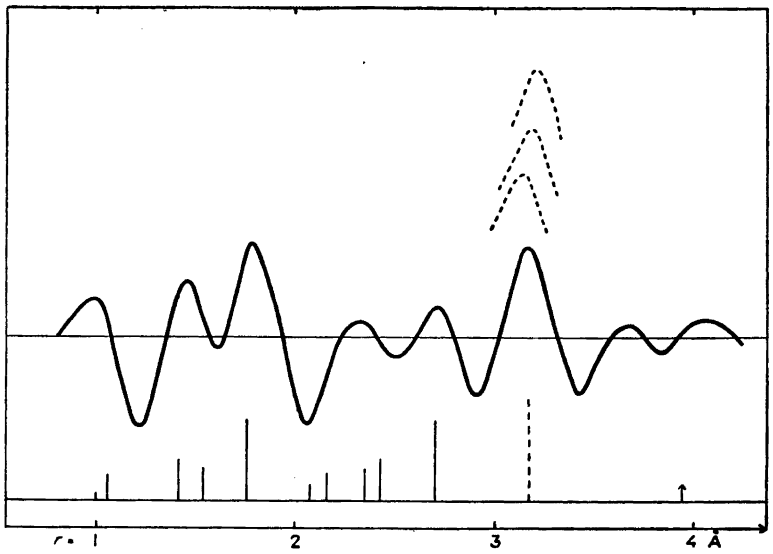

Fig. 4. $\sigma(r)$-curve for ethylene chlorohydrin.

explained assuming the two configurations $\alpha\alpha$ and $\alpha\gamma$ only. The trans-form seems therefore in this case also to be infrequent. The $\gamma\gamma$-position is improbable, because of the short 0_1—0_3-distance (2.28 Å).

Ethylene chlorohydrin

The $\sigma(r)$-curve of this compound is given in Fig. 4. In this case the most interesting distance, the chlorine-oxygen distance, must give a considerable contribution to the $\sigma(r)$-curve. The differential method is therefore unnecessary. The distances which are independent of the rotation about the carbon-carbon bond are indicated in the line diagram (solid lines). The carbon-chlorine bond distance is 1.76 Å. The other bond distances are the same as for the previous compounds. The pronounced peak at 3.17 Å must be explained as due to the oxygen-chlorine distance corresponding to a φ-value of 74°. The dotted peaks are parts of three different curves calculated from different photometer records. The φ-value varies in these three cases between 72° and 76°.

DISCUSSION

The results given above are readily explained by the assumption of intramolecular hydrogen bonds. The value of the hydrogen bond distance in ethylene glycol and glycerol of 2.96 Å $\pm$ 0.02 Å is somewhat greater than that

usually observed which vary between 2.5 Å and 2.95 Å. On the other hand, the intra-molecular hydrogen bonds assumed in our cases are not directly comparable with the common inter-molecular hydrogen bonds. In the latter the hydrogen atom is usually considered as lying on the straight line connecting the oxygen atoms. This is impossible in our case, because of steric relations. — The hydrogen bond distance between an oxygen and a chlorine atom in ethylene chlorohydrin of 3.17 Å seems fairly reasonable.

Another interpretation of the $\sigma(r)$-curve of ethylene glycol and glycerol may be mentioned: An oscillation about the carbon-carbon bonds with an amplitude of approximately 75°, and with the *trans*-position as the equilibrium position, would have resulted in a peak at 2.94—2.97 Å in the $\sigma(r)$-curve. The angular velocity is in the equilibrium position much greater than in the neighbourhood of the positions having maximal amplitudes. In the case of ethylene chlorohydrin, however, this interpretation is impossible, because of the great height of the peak at 3.17 Å. An oscillation would have decreased the height of this peak, while the actual observed height is even greater than what might have been expected from a rigid model. The assumption of an oscillation of the type mentioned must therefore, in the case of the other compounds under investigation also, be regarded as less probable than the assumption of hydrogen bonds, especially when the results of the study of ethane [5] and ethane derivatives are considered [6, 7].

A further argument based on calorimetric data is in favour of the theory of intra-molecular hydrogen bonds: In Table 1 we have listed calculated and observed values of the heats of combustion for five alcohols in the gaseous state. The calculated values are obtained in a straight forward way explained, for instance, by Wheland [8]. The experimental values are taken partly from Whelands book and partly from a work of Gallaugher and Hibbert [9]. It has been corrected for the heats of vaporization. In the fourth column the difference between calculated and observed values is given.

Table 1. Heats of combustion for some alcohols.

Compounds	Calculated	Observed	Calculated ÷ Observed
Ethyl alcohol	336.8 kcal/mol	336.8 kcal/mol	0.0 kcal/mol
n-Propyl alcohol	494.2 »	493.3 »	0.9 »
n-Butyl alcohol	651.6 »	650.0 »	1.6 »
Ethylene glycol	303.0 »	297.6 »	5.4 »
Glycerol	426.6 »	416.3 »	10.3 »

With respect to the three first compounds the difference must be regarded as zero, the deviations of the calculated values from the observed ones being

0.0 %, 0.18 % and 0.25 % respectively. For ethylene glycol and glycerol the deviations are 1.78 % and 2.42 %. This corresponds to *one* hydrogen bond within the ethylene glycol molecule and *two* within the glycerol molecule, if the hydrogen bond energy is equaled to about 5.0 kcal. This value is in good agreement with earlier observed hydrogen bond energies [10–12]. Such considerations, based on calorimetric data, must of course be treated with some caution, because of the well-known uncertainty in the determination of both calculated and observed heats of combustion and vaporization, and because of the anomalies which occur in the heats of combustion in branched hydrocarbons and non-primary alcohols.

Finally it may be mentioned that investigations on liquid ethylene glycol and glycerol by the aid of monochromatic X-rays have been carried out. These seem to lend support to our theory of intra-molecular hydrogen bonds.

SUMMARY

The structures of ethylene glycol, glycerol and ethylene chlorohydrin have been investigated by the electron diffraction sector method. Arguments in favour of the assumption of intra-molecular hydrogen bonds are given.

I should like to express my gratitude to Professor Dr. O. Hassel for his kind interest in my work and for many suggestive discussions. Without the fruitful scientific atmosphere created by him, this work would not have been accomplished. — I must also acknowledge my indebtedness to the *Fridtjof Nansens Fond* and *Det Vitenskapelige Forskningsfond av 1919* for financial aid.

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Received April 2, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 422—432

On the Turnover of Purines and Pyrimidines from Polynucleotides in the Rat Determined with N^{15}

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The study of the metabolic pathways of nucleic acids with the aid of isotopes has been seriously handicapped by the lack of convenient methods for the isolation of the different compounds of the nucleic acids. With the object of obviating this, especially with a view to isotope work, methods have been worked out at this laboratory for the preparation of the two nucleic acids (desoxyribonucleic acid = DNA, ribonucleic acid = PNA) from gram amounts of tissues [1], and for the preparation and separation of the purines from DNA, and the purines and pyrimidines from PNA [2-4] in mg amounts. Furthermore, a method has been elaborated for the degradation of adenine and guanine to the corresponding hydroxypurines [5].

By the employment of these methods, we were recently able to show that orotic acid containing N^{15} could be used by the rat for the synthesis of the PNA-pyrimidines in the liver [6]. In this experiment the N^{15} content of PNA-uridine was higher than that of PNA-cytidine. On the assumption, however, that the amino group of cytidine contained no isotope, the pyrimidine ring of cytidine would have had a higher isotope content than uridine. This was thought to be the case, but no evidence could be advanced, as at that time no method was available for the deamination of the small amounts of cytidine obtained in the experiment. The lack of a method was also the reason why the pyrimidines of DNA were not investigated.

The present investigation was carried out in order to decide these questions. For this purpose a method has been elaborated for the deamination of small amounts of cytidine. The separation of the resulting uridine from the non-deaminated cytidine was carried out by partition chromatography on starch. For the preparation of the pyrimidines from DNA the method of Vischer and Chargaff [7], hydrolysis with concentrated formic acid at 175° for 2 hours, was

employed. The experiments were carried out on nucleic acids obtained from the pooled kidneys, spleens and small intestines of the rats from the investigation of Arvidsson *et al.*[6]. The animals had received subcutanously six doses of 12.5 mg orotic acid/100 g of bodyweight (excess $N^{15} = 6.06$ %) at 12-hour intervals and had been killed 12 hours after the last injection.

Table 1. Purines and pyrimidines from polynucleotides from the mixed intestines, spleens and kidneys of two rats. These had received a total dose of 75 mg of orotic acid / 100 g of body weigth (excess $N^{15} = 6.06$ %) during 3 days. The value $\frac{E_{max.}}{\gamma N/ml}$ is a test for purity with respect to foreign nitrogen [2]. *For standard values see Reichard* [3] *and Table 4.*

	Excess N^{15}	$\frac{E_{max.}}{\gamma N/ml}$
PNA		
Cytidine	0.401	0.296
Cytidine ring	0.592	0.312
Amino group (calculated)	0.029	
Uridine	0.324	0.313
Adenine	0.004	0.179
Guanine	0.006	0.165
DNA		
Cytosine	0.091	0.227
Thymine	0.062	0.278
Adenine	0.005	0.182
Guanine	0.003	0.173

As can be seen from the table, the amino group of cytidine did not contain any significant amount of N^{15}. The N^{15} from orotic acid, furthermore, had entered the pyrimidines in DNA, though to a much smaller extent than in PNA. The purines contained no isotope.

The results of these experiments made it desirable to investigate the turnover rates of the nucleic acid pyrimidines with another precursor. Because of that, organs (kidney, spleen and small intestine) from a previous experiment of Hammarsten and co-workers [8], in which N^{15}-glycine had been used for the administration of isotope, were worked up for the pyrimidines from PNA and partly DNA. In this experiment the rats had received two subcutanous injections of 50 mg glycine/100 g of bodyweight (excess $N^{15} = 32$ %) at six-hour interval and had been killed six hours after the last injection.

In this case the isotope content of the deaminated cytidine is lower than that of the aminopyrimidine, indicating a relatively high turnover rate of the

Table 2. Pyrimidines from polynucleotides from the different organs of 28 rats. Each rat had received a total dose of 100 mg of glycine / 100 g of body weight during 12 hours.

	Spleen		Intestine		Kidney	
	Excess N^{15}	$\frac{E_{max.}}{\gamma N/ml}$	Excess N^{15}	$\frac{E_{max.}}{\gamma N/ml}$	Excess N^{15}	$\frac{E_{max.}}{\gamma N/ml}$
PNA						
Cytidine	0.145	0.275	0.285	0.279	0.087	0.290
Cytidine-ring	0.099	0.315	0.214	0.324	0.067	0.333
Amino group (calc.)	0.237		0.427		0.127	
Uridine	0.164	0.311	0.293	0.340	0.117	0.308
DNA						
Cytosine	0.060	0.219			0.028	0.209
Cytosine-ring	0.056	0.285				
Amino-group (calc.)	0.068					
Thymine	0.038	0.262			0.018	0.192 (?)
TCA insoluble	0.132				0.251	

amino group in cytidine. Uridine has the same (intestine) or a higher N^{15} content than the cytidine ring.

The purines from the PNA and DNA from intestine with N^{15} glycine as isotope precursor have been isolated previously and degraded to the corresponding hydroxypurines [5]. In order to get a more complete picture of some questions connected with the synthesis of purines in nucleic acids, adenine and guanine were now isolated from nucleic acids of spleen and kidney, too. The purines from the spleen were deaminated, but not those from the kidney, because of their very low N^{15} content. The results may be seen in Table 3, where the values for the intestine and regenerating liver from the previous experiment [5] are also given for comparison.

In the spleen the isotope content of the PNA-guanine is somewhat higher than that of adenine, though the relations are reversed for the corresponding hydroxypurines. This gives a high turnover rate for the aminogroup in guanine and a low for that of adenine. In DNA both hydroxypurines had a higher isotope content than the aminopurines. The results are in good agreement with those obtained from intestine. As for the kidney the isotope content there was rather low, but the difference between adenine and guanine for both PNA and DNA was significant. In both cases adenine had a higher turnover rate than guanine.

Table 3. Aminopurines and corresponding hydroxypurines from different organs from polynucleotides of the rat with N^{15}-glycine as a precursor. The experimental conditions were the same as in Table 3. The values for intestine and regenerating liver are taken from a previous publication [5] and are included for comparison.

	Spleen		*Kidney*		*Intestine*		*Regenerating liver*	
	Excess N^{15}	$\frac{E_{max.}}{\gamma N/ml}$	Excess N^{15}	$\frac{E_{max.}}{\gamma N/ml}$	Excess N^{15}	$\frac{E_{max.}}{\gamma N/ml}$	Excess N^{15}	$\frac{E_{max.}}{\gamma N/ml}$
PNA								
Adenine	0.285	0.181	0.065	0.162	0.46	0.171	0.43	0.178
Hypoxanthine	0.356	0.195			0.54	0.191	0.51	0.190
Amino group (calc.)	0.001				0.12		0.11	
Guanine	0.336	0.161	0.043	0.151	0.51	0.165	0.97	0.158
Xanthine	0.301	0.159			0.43	0.165	0.97	0.167
Amino group (calc.)	0.476				0.83		0.97	
DNA								
Adenine	0.165	0.187	0.054	0.178	0.27	0.169		
Hypoxanthine	0.205	0.192			0.33	0.192		
Amino group (calc.)	0.005				0.06			
Guanine	0.159	0.159	0.031	0.152	0.48	0.161		
Xanthine	0.196	0.158			0.59	0.169		
Amino group (calc.)	0.011				0.04			

EXPERIMENTAL

Preparation of purines and pyrimidine nucleosides from PNA. The preparation and separation of PNA and DNA were carried out according to Hammarsten [1]. The ribomononucleotides were isolated by precipitation with mercuric nitrate and decomposition with hydrogen sulfide as described previously[4]. If the precipitation with mercury was carried out at sufficiently low pH (not above 2), it was found that electrodialysis for the purification of the mononucleotides could usually be omitted.

It was found advantagous to separate the purines and pyrimidines before subjecting them to chromatography. This could be affected by splitting off the purines with acid and precipitating them with silver nitrate, according to Kerr and Seraidarian [9].

To the solution of the four mononucleotides sulphuric acid was added to 0.1 *N*. The solution was kept at 100° for one hour in a water-bath. After

cooling and neutralisation with *N* NaOH to pH 2, the purines were precipitated with one-fifth by volume of *N* silver nitrate. The silver purines were washed, decomposed with HCl, and subjected to chromatography on a starch column, as described previously [2,5].

The mother liquor, after the precipitation of the silver purines, contained the pyrimidine nucleotides, ribose phosphoric acid, ribose and phosphoric acid. The nucleotides, and probably most of the ribose phosphoric acid and phosphoric acid, were precipitated as silver salts by silver nitrate at neutral pH. This procedure has been used by Kerr and Seraidarian [9] for the precipitation of the purine mononucleotides.

The supernatant from the silver purines was continuously neutralized with 0.1 *N* NaOH. A white to yellowish precipitate of the silver nucleotides was formed, which, after a further addition of alkali, turned brownish owing to the formation of silver oxide. The addition of alkali was stopped when the solution with the suspended precipitate had taken on a distinctly light-brown colour.

The silver salts were allowed to precipitate in the ice box for 48 hours, centrifuged, washed twice with 0.05 *N* silver nitrate, twice with alcohol, and twice with ether. The dry precipitate was suspended in water and decomposed with hydrogen sulphide.

The silver sulphide was centrifuged off, washed twice with 5 ml of hot water, and the washings were added to the supernatant. The resulting solution was neutralized with *N* NaOH to pH 4, the nucleotides were converted into the nucleosides with the aid of prostate phosphatase, and the nucleosides separated by starch chromatography. The details of these procedures have been described elsewhere [3,4].

Preparation of purines and pyrimidines from DNA. The DNA in Hammarsten's procedure [1] is precipitated with lanthanum. This must be removed before any further steps can be taken. For this purpose the lanthanum precipitate was finely suspended in 1 ml of molar potassium carbonate. 9 ml water were added, the solution was heated 5 minutes in a water-bath and centrifuged. The whole procedure was repeated twice with 0.5 ml of potassium carbonate and 4.5 ml of water. The combined supernatants, which contained the DNA, were neutralized with concentrated acetic acid to a slightly acid reaction and precipitated with 4 volumes of alcohol. The precipitate was centrifuged off and dried with alcohol and ether.

For the further preparation of the bases from DNA, two alternatives were tried out.

The first was the complete method of Vischer and Chargaff [7], in which the purines were precipitated as the hydrochlorides by dry HCl in methanol. After removal of the excess HCl the purines were then separated by chromato-

graphy on starch. The pyrimidines were obtained from the supernatant from the cristallisation of the purines by hydrolysis with concentrated formic acid at 175° for two hours, as described by Vischer and Chargaff. The dark-brown solution after the hydrolysis was diluted to 10 ml with water and centrifuged. The supernatant was evaporated several times *in vacuo*. Before the last evaporation, the solution was neutralized with 0.1 *N* NaOH. The residue after the last evaporation was dissolved in butanol water and run on a starch column.

By this method a fairly good yield (70—80 %) for the pyrimidines was obtained, as compared with the values of Vischer and Chargaff. As in their case, the yield of the purines however, was not very satisfactory. Higher yields of these could be obtained by a method following the same principles as for the isolation of the bases from PNA.

In that case 30—50 mg of the dry DNA, free from lanthanum, were dissolved in 10 ml 0.1 *N* sulphuric acid and hydrolyzed for 1 hour at 100°. In experiments which were not carried out on specially pure samples of DNA, some matter usually remained undissolved, probably from protein in the DNA. This was centrifuged off at the end of the hydrolysis. After neutralisation to pH 2, the purines were precipitated and treated as described for PNA. The solution left after the precipitation of the purines contained the pyrimidines in some indefinite form (»thymic acid»). They were precipitated with silver at neutral reaction, and the silver was removed by hydrogen sulfide, as in the case of the PNA pyrimidine mononucleotides.

The pyrimidines were then split off with formic acid and, after the removal of the acid, separated by chromatography.

The latter procedure gave very good yields for the purines but considerably lower yields for the pyrimidines. The cause of this was that part of the pyrimidines was precipitated together with the purines with silver at acid pH. These pyrimidines, in the form of »thymic acid», were carried together with the purines to the purine chromatography. They showed, however, very slight solubility in alkaline butanol-water (the chromatographic medium for the purines). Therefore they were left as an insoluble residue when the purines were dissolved for chromatography. This residue may be dried, and hydrolyzed with formic acid, and the pyrimidines separated by chromatography, which procedure increased the yield.

Chromatographic separation of pyrimidines. Fig. 1 shows a chromatogram of the pyrimidine fraction from DNA. In order to ascertain the identity of the different compounds, a mixture of the three pyrimidines, cytosine, uracil and thymine, was subjected to chromatography under identical conditions. The result is shown by Fig. 2. By calculating the *R*-values [10], determining the

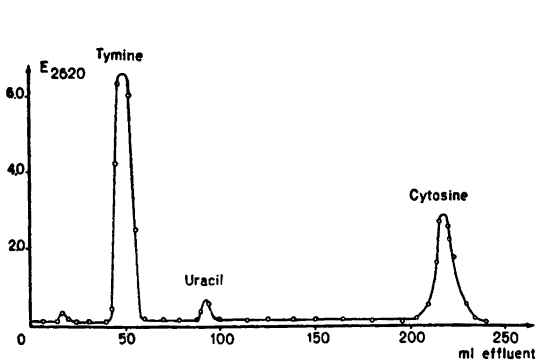

Fig. 1. Chromatographic separation of the DNA-pyrimidine fraction from 30 mg of DNA. Length of column: 205 mm, diameter: 22 mm. The peak in front of the thymine is an artefact and does not show the typical pyrimidine light absorption.

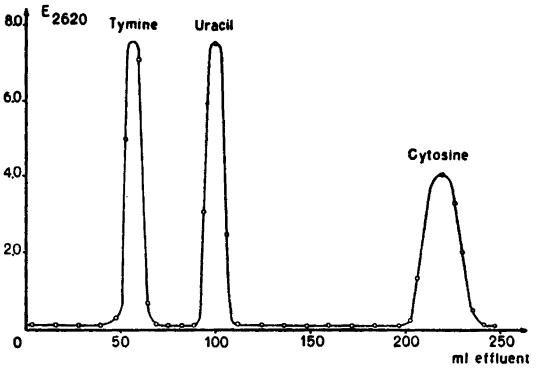

Fig. 2. Chromatographic separation of 2.2 mg thymine, 2.5 mg uracil and 2.3 mg cytosine. Length of column: 220 mm, diameter: 22 mm. Yield for each pyrimidine 90—100 %.

typical absorption curve in the ultra violet, and the constant $\frac{E_{\text{max.}}}{\gamma\text{N/ml}}$ [2], the identity of the pyrimidines was established. The results are summarized in Table 4.

Table 4. Characteristic values for the compounds obtained after chromatography of the hydrolysis products from DNA, as compared with those for standard pyrimidines. All light absorption values are determined in N HCl.

	R-value	$E_{\text{max.}}$ (Å)	$\frac{E_{\text{max.}}}{\gamma\text{N/ml}}$	$\frac{E_{\text{max.}}}{E2480}$
Products from DNA				
First compound	1.55	2650	0.265	1.83
Second compound	0.84	2590		1.30
Third compound	0.36	2750	0.215	3.72
Standard pyrimidines				
Thymine	1.48	2650	0.270	1.73
Uracil	0.83	2590	0.283	1.33
Cytosine	0.38	2750	0.229	3.48

The finding of uracil, even if in rather small amounts, seems to be rather surprising. Uracil is not supposed to be present in DNA. The presence of PNA in the DNA was highly improbable, as the experiment was carried out with a pure specimen of DNA, prepared according to Hammarsten [1]. The

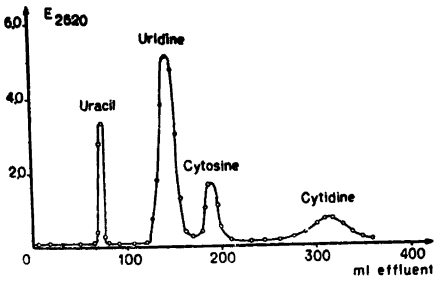

Fig. 3. Chromatographic separation of the products obtained after 48 hours hydrolysis at 100° with 0.4 N HCl of 8.3 mg of cytidine. Length of column: 185 mm, diameter: 22 mm. The peaks correspond to 0.7 mg of uracil, 4.1 mg of uridine, 0.5 mg of cytosine and 1.1 mg of cytidine.

explanation seemed to be that the uracil was formed during the hydrolysis from cytosine. This became still more probable when a model experiment with pure cytidylic acid, after hydrolysis with formic acid at 175° for two hours, gave rise to some uracil (5—10 % uracil of the cytosine formed).

Deamination of cytidine and cytosine. Various possibilities exist for the splitting off of the amino group of cytidine. One method outlined by Loring and Ploeser [11], consists in refluxing cytidine with 0.4 *N* sulphuric acid for 48 hours. This method has been tried out, though hydrochloric acid was substituted for sulphuric acid.

8.3 mg cytidine were dissolved in 5 ml 0.4 *N* HCl and refluxed on a sand-bath for 48 hours. The HCl was removed by repeated evaporation in vacuo. The residue was dissolved in 2 + 1 + 1 ml butanol-water and put on top of a starch column 22 mm in diameter and 185 mm in length. The result of the experiment is shown in Fig. 3.

Very little of the cytidine was left unaltered, but most of it had been converted into uridine. A considerable part of the nucleosides, however, had been converted into the free pyrimidines, and the method seemed to be unsuitable for the deamination of cytidine. The deamination of the free cytosine to uracil, however, has been carried out in this way.

Cytidine has been converted into uridine by treatment with an aqueous solution of pyridine (1 part pyridine + 3 parts water). No free pyrimidines

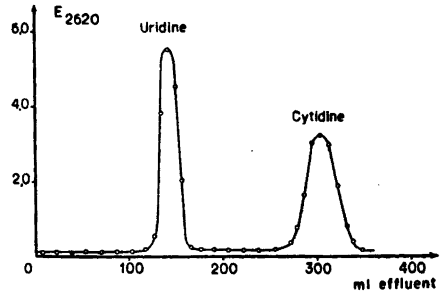

Fig. 4. Chromatographic separation of the products obtained after 5 hours hydrolysis at 160° with aqueous pyridine of 10.1 mg of cytidine. Length of column: 185 mm, diameter 22 mm. The peaks correspond to 4.5 mg of uridine and 5.1 mg of cytidine.

were formed in this way. 10.1 mg cytidine were treated with 1 ml of pyridine-water in a sealed tube at 160° for 5 hours. The pyridine was removed by four evaporations *in vacuo*; the residue was dissolved in butanol-water and subjected to chromatography (fig. 4).

As can be seen, about half the amount of cytidine has been deaminated and no free pyrimidines have been formed.

Deamination of aminopurines. This was carried out as described in a previous publication [5].

DISCUSSION

The experiment with *orotic acid* as a precursor for the pyrimidines of nucleic acids in the rat clearly indicates two things. Firstly, as might have been expected, the N^{15} of orotic acid only enters the ring of PNA-cytosine, and the amino nitrogen of this base is derived elsewhere. Secondly orotic acid may act, not only as a precursor for the pyrimidines of PNA, but also for the pyrimidines of DNA.

As regards the first finding, the results show that, with orotic acid as a precursor, the cytosine ring is rebuilt at a quicker rate than the uracil in PNA. This is very obvious in the present investigation, in which the PNA was derived from the combined intestines, spleens and kidneys. The cytosine ring contained about double the amount of N^{15} as compared with the uracil. This holds true also for the previous experiment with liver [6], if one assumes that liver does not differ from the other organs and thus does not use orotic acid nitrogen for the amino group of cytosine. On that assumption, the difference between the calculated cytosine ring (1.308 % excess N^{15}) and uracil (1.133 %) for liver is not as large as for the organs in the present experiment. The obvious conclusion from these data is that the synthesis of cytosine in these experiments was not preceded by a synthesis of uracil.

In DNA, too, cytosine has the highest turnover rate of the two pyrimidines. In this case the material was not sufficient for deamination, but there is no reason to assume that the amino group contains any isotope. On the assumption that all of the N^{15} is located in the cytosine ring, this would have about double the isotope content of thymine. When comparing the pyrimidines of DNA with those of PNA, one notes that the latter contain about five times as much N^{15} as the DNA pyrimidines. This finding is somewhat surprising, in view of the results obtained with glycine-N^{15} as a precursor. There the turnover ratio PNA/DNA varies between 1 and 2 for the three organs, intestine, spleen and kidney. The same results [12] were obtained with P^{32}. At first sight it would seem that the rat is able to use orotic acid to a lesser degree for the

synthesis of DNA pyrimidines than glycine as compared with the synthesis of PNA pyrimidines.

With glycine as a precursor, the turnover rates for the cytosine ring and uracil from PNA are reversed as compared with orotic acid. Now uridine has a significant higher isotope content than deaminated cytidine. The results show, furthermore, that cytidine, conformably with guanine, has an »active» amino group. The calculated isotope content of this is invariably higher than that of the ring. In DNA the low N^{15} content makes it impossible to interpret the results with certainty. At least in the case of spleen it seems certain that the turnover rate of cytosine is higher than that of thymine.

Another question which can be answered from the results of the present investigation is that of the existence or non-existence of uracil in PNA. It has at times been questioned whether uracil exists at all as a natural building stone of PNA or whether it has not its origin from deamination of cytosine during the preparation of the nucleic acid or during the preparation of the pyrimidines from PNA. The results with both N^{15}-orotic acid and N^{15}-glycine clearly indicate a difference in turnover rates for deaminated cytosine and uracil from PNA. This should afford fairly definite proof of the primary existence of uracil in PNA.

The results obtained in respect of the purines from spleen and kidney confirm the earlier finding for intestine [5]. In the spleen the higher isotope content of PNA-guanine as compared with PNA-adenine is reversed after deamination. In the kidney PNA-adenine has a higher isotope content than guanine. These findings can be easily fitted into the theory of Brown *et al.* [13] that adenine is a precursor for PNA-guanine.

In DNA the difference between the two purine rings is not significant for the spleen, while the turnover rate of adenine is higher than that of guanine for the kidney.

SUMMARY

A method is described for the preparation of cytosine and thymine from 30—50 mg desoxyribo nucleic acid by the use of hydrolysis with formic acid according to Vischer and Chargaff [7] and partition chromatography on starch.

The conversion of cytidine into uridine with the help of 0.4 *N* acid and aqueous pyridine is investigated, and the products are separated by chromatography on starch.

It is shown that orotic acid can act as a precursor, not only for PNA pyrimidines, but also for DNA pyrimidines. In the pyrimidines from PNA the cytosine ring is shown to have a higher turnover rate than uracil.

The results obtained with orotic acid as a precursor for pyrimidines are compared with those obtained with glycine as a precursor.

Some supplementary experiments are made with the purines from the spleen and kidney of the rat with N^{15}-glycine, with the object of investigating the possibility of using adenine as a precursor for guanine.

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Received March 20, 1949.

Short Communications

Pyrimidine Nucleosides as Precursors of Ribonucleic Acid (PNA) Pyrimidines

E. HAMMARSTEN, P. REICHARD and E. SALUSTE

Biochemical Department, Karolinska Institutet, Stockholm, and Wenner-Gren Institute, University of Stockholm, Stockholm, Sweden

It has been shown with the aid of N^{15} that the free pyrimidines uracil, thymine [1] and cytosine [2] cannot be utilized as precursors for polynucleotides by the rat. This does, however, not necessarily mean that these bases when bound as nucleosides or nucleotides cannot enter polynucleotides. The finding of Loring and Pierce [3] that pyrimidine ribonucleosides and nucleotides are from 10 to 60 times as active as free pyrimidines for a pyrimidine deficient strain of *Neurospora* strongly suggests the necessity of trying tracer marked nucleosides or nucleotides as precursors for polynucleotides.

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the yeast contained N^{15}. The biologically marked PNA was isolated from the yeast according to the method of Hammarsten [4] Cytidine and uridine were prepared from the PNA according to the method of Reichard [5]. The N^{15}-nucleosides after some dilution with non isotopic material were injected into rats at a level of 80 mg per kilo of body weight per day for 3 days. After that time the rats were killed, the polynucleotides from the pooled internal organs prepared and separated [4], the ribonucleosides isolated [5] from the PNA and analyzed for N^{15}. The results may be seen from Table 1.

Obviously the injected cytidine-N enters the PNA in a comparatively low dilution while uridine is active to a much lesser degree. The injected cytidine is not only a precursor for the cytidine of the PNA but also for uridine. A detailed report will be published later.

Table 1.

	Cytidine injection		Uridine injection	
Nucleoside injected	Atom % excess N^{15} 6.43	Calc. on basis of 100 % N^{15} in cytidine injected	Atom % excess N^{15} 11.20	Calc. on basis of 100 % N^{15} in uridine injected
Isolated:				
Mixed polynucleotides	0.102	1.59	0.024	0.21
Uridine	0.385	5.99	0.047	0.42
Cytidine	0.463	7.20	0.053	0.47
Protein	0.006	0.1	0.012	0.1

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Received June 3, 1949.

New Books

Niels Bjerrum. *Selected papers.* Edited by friends and coworkers on the occasion of his 70th birthday the 11th of March 1949. Einar Munksgaard, Copenhagen, 1949. 295 pp. 18 Danish Crowns.

A review of this tribute to one of the most outstanding chemists of this century can most adequately be commenced by quoting the book's introduction, written by Niels Bohr, acting chairman of the editorial committee.

»The scientific publications of Niels Bjerrum have initiated great advances of our knowledge and understanding in many fields of chemistry and physics and bear throughout witness of that same openness of outlook and balance of judgment, which his friends and colleagues admire so highly and which together with his straight forwardness and loyalty has secured him the confidence of the whole Danish community. Many important tasks have been entrusted upon him, and his fertile activities and the encouragement he has given to wide circles will be remembered with deep gratitude from most different sides on his 70th birthday. Deliberating how his colleagues best could contribute on this occasion, the committee has thought that an edition of a selection of the papers of Niels Bjerrum by which he has erected himself a lasting monument in science, would be the most fitting way to express the indebtness we owe to him, and we feel assured one which will be warmly welcomed by chemists and physicists all over the world.«

A reviewer must agree most emphatically with this statement. More especially

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Niels Bjerrum. *Selected papers.* Edited by friends and coworkers on the occasion of his 70th birthday the 11th of March 1949. Einar Munksgaard, Copenhagen, 1949. 295 pp. 18 Danish Crowns.

A review of this tribute to one of the most outstanding chemists of this century can most adequately be commenced by quoting the book's introduction, written by Niels Bohr, acting chairman of the editorial committee.

»The scientific publications of Niels Bjerrum have initiated great advances of our knowledge and understanding in many fields of chemistry and physics and bear throughout witness of that same openness of outlook and balance of judgment, which his friends and colleagues admire so highly and which together with his straight forwardness and loyalty has secured him the confidence of the whole Danish community. Many important tasks have been entrusted upon him, and his fertile activities and the encouragement he has given to wide circles will be remembered with deep gratitude from most different sides on his 70th birthday. Deliberating how his colleagues best could contribute on this occasion, the committee has thought that an edition of a selection of the papers of Niels Bjerrum by which he has erected himself a lasting monument in science, would be the most fitting way to express the indebtness we owe to him, and we feel assured one which will be warmly welcomed by chemists and physicists all over the world.«

A reviewer must agree most emphatically with this statement. More especially

he must express great satisfaction at seeing the most prominent of Niels Bjerrum's paper made accessible through this book. Many of them were originally published in journals, which are not easily available. Most of them were also written in Danish, French or German. In the present volume they have all been translated into English.

In the beginning of the book J. A. Christiansen gives a survey of the scientific papers of Niels Bjerrum and this chapter is followed by a bibliography of his publications. Then a total of 27 selected papers are reproduced. It has certainly not been an easy task to make the selection from the more than one hundred original publications (including books) but it seems as if the choice has been sound. In some cases only the summaries of the longer papers have been presented.

The collection makes an imposing impression, in no slight degree caused by the width of the author's sphere of activity. The first paper treats the development of chemistry in the nineteenth century. Then come three papers on chemical physics dealing with specific heat and infrared spectra of gases. The section on physical chemistry begins with seven papers illustrating Bjerrum's famous contribution to the electrolytic dissociation theory. It may be good to recall the fact that Bjerrum assumed the complete dissociation of the strong electrolytes already in 1909 and that he found that their behaviour could be explained by means of the interionic forces. One certainly must agree with Professor Christiansen when he says that »it may safely be stated that Bjerrum has contributed more to the victory of these ideas than any other single person».

The physico-chemical section is continued with seven papers which mainly deal with acid-base equilibria. Mention should be made of the theories and nomenclatures for acids, bases, and salts, the very important work on the constitution of ampholytes and the dissociation constants of multibasic acids and their relation to the molecular dimensions. Further the electrometric determinations of dissociation constants carried out together with Miss Unmack and investigations on the solubility of calcium phosphates.

The collection terminates with nine papers which deal with inorganic problems. Seven of them illustrate Bjerrum's fundamental work on the chromium complexes while two deal with gold complexes and free thiocyanogen.

In the first paper of the book Bjerrum states that the laboratories of Europe today are behind those of the United States of America, but he advises the former to seek consolation and hope in remembering that progress in scientific work »largely springs from the underlying spiritual force and mental discipline». Niels Bjerrum's own achievement by which he has contributed so much to the high esteem of his native country in science is the best possible illustration of this statement.

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ACTA CHEMICA SCANDINAVICA 3 (1949) 435—444

The Interrelationship between Monoseleno Polythionates *

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The polythionate series, trithionate, tetrathionate, pentathionate and hexathionate, has no selenium counterpart, containing oxygen and selenium, only, in the anions. This article is concerned with trithionate and pentathionate, in which only one of the sulphur atoms is substituted by selenium. Sodium selenopentathionate, or sodium selenium di(thiosulphate), $Na_2Se(S_2O_3)_2 \cdot 3H_2O$, and also the potassium salt, $K_2Se(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$, have been isolated in a pure state, as the first salts of selenopentathionic acid.

There is a striking difference between the polythionates and the monoseleno polythionates as regards their behaviour towards sulphite and thiosulphate. Tetrathionate, pentathionate and hexathionate react rapidly and quantitatively with sulphite, to give trithionate and thiosulphate. *E. g.*:

$$S(S_2O_3)_2^{--} + 2\overset{*}{S}O_3^{--} = \overset{*}{S}(SO_3)_2^{--} + 2S_2O_3^{--}$$

These reactions probably are ionic displacement reactions [2], as indicated through the stars. In the case of the monoseleno polythionates, an equilibrium exists:

$$\overset{*}{Se}(SO_3)_2^{--} + 2S_2O_3^{--} \rightleftharpoons Se(S_2O_3)_2^{--} + 2\overset{*}{S}O_3^{--}$$

In presence of formaldehyde, as a sulphite acceptor, in buffered solutions, the equilibrium goes quantitatively to the right. Thus, sulphite is displaced by thiosulphate, whereas in the case of the polythionates, thiosulphate is displaced by sulphite.

Trithionate, tetrathionate and pentathionate are derivatives[2] of divalent electropositive sulphur, S^{++}. In reactions with nucleophilic reagents, pentathionate acts as a monosulphur di(thiosulphate), $S(S_2O_3)_2^{--}$. The compounds

* This is the second in a series of papers on selenium sulphur compounds. First paper: Ref. 1.

discussed in this article are derivatives of divalent electropositive selenium, Se^{++}. Selenopentathionate is a selenium di(thiosulphate), $Se(S_2O_3)_2^{--}$, its thiosulphate groups being displaceable by diethyldithiocarbamate. Selenotrithionate, in the equilibrium with thiosulphate, behaves as a selenium disulphite, $Se(SO_3)_2^{--}$.

PREVIOUS WORK

Rathke [3] discovered selenotrithionic acid, $Se(SO_3H)_2$. He prepared the potassium salt, $K_2Se(SO_3)_2$, by adding aqueous selenious acid to a concentrated mixture of potassium sulphite and potassium selenosulphate. Schulze [4] reported the formation of selenotrithionic acid in a mixture of aqueous selenious acid and excess sulphurous acid. Foerster, Lange, Drossbach and Seidel [5] prepared the salt by means of Rathke's method, and so did Heuer [6]. According to Rathke, selenotrithionate is also formed, in small yields, when selenium is treated with aqueous potassium sulphite or hydrogen sulphite.

Morgan and Smith [7] found that selenium acetylacetonate reacts with hydrogen sulphites, to give selenotrithionates in quantitative yields. In this way, they prepared the lithium, sodium, rubidium, cesium, ammonium and barium salts. The free acid, formed from selenium acetylacetonate and sulphurous acid, could not be obtained in an anhydrous state, since selenium was liberated as soon as the concentration of the aqueous solutions reached about 50 %.

The existence of selenopentathionic acid, in aqueous solutions, was first ascertained by Norris and Fay [8]. They found that selenious acid, in acid solutions, reacts with sodium thiosulphate, to give a mixture of tetrathionate and selenopentathionate:

$$H_2SeO_3 + 4S_2O_3^{--} + 4H^+ = S_4O_6^{--} + Se(S_2O_3)_2^{--} + 3H_2O$$

The acid reaction mixtures are first clear, but, gradually, red selenium is liberated. If alkalies are added, the same process takes place immediately. Norris and Fay pointed out the analogy with pentathionate, which liberates sulphur when acted upon by small amounts of alkalies. The observations of Norris and Fay were confirmed by Foerster, Lange, Drossbach and Seidel [5].

The above-mentioned reaction forms the basis for the so-called Norris and Fay method [8,9] for the iodometric analysis of selenious acid, and has been used as such by various workers [10–13]. Excess of thiosulphate is back-titrated with iodine. According to the critical study by Coleman and McCrosky [14], the accuracy of the method is between 1 and 2 parts per 1000.

Norris and Fay [8] reported that »An effort was made to isolate the selenopentathionate, but without success, as selenium was always precipitated

when the solutions were concentrated by heat or in a vacuum». Unsuccessful attempts to isolate the selenopentathionate were also made by Heuer [6].

SODIUM AND POTASSIUM SELENOPENTATHIONATE

These salts have been prepared by means of the Norris and Fay reaction between selenious acid and thiosulphate. Their isolation, without any liberation of selenium, has been achieved by use of (a) an excess of selenious acid at every stage of the process (b) concentrated acetic acid as a solvent for the selenious acid. The excess of selenious acid is necessary, because thiosulphate acts as a catalyst in the decomposition of selenopentathionate, as already observed by Norris and Fay [8].

Sodium selenopentathionate forms small, shiny, pale yellowish green leaves (thin plates), the mass of crystals being fairly voluminous. It is very soluble in water, and is also appreciably soluble in methanol, insoluble in ethanol. It crystallizes with three moles of water.

Potassium selenopentathionate is in most cases obtained as needles or prisms, which may be more than 5 mm in length and 1 mm in thickness. The colour is yellowish green. It is less soluble in water than is the sodium salt, and insoluble in methanol. It crystallizes with one and a half mole of water, as does potassium pentathionate. The crystal water is kept very firmly, and is not given off *in vacuo* over sulphuric acid.

The salts are stable, though in the case of the potassium salt, spots of selenium have a tendency to develop inside some of the larger crystals after two or three days.

Aqueous solutions of the selenopentathionates are yellowish green. Even in 0.02 *M* solutions a pale green colour is observeable. In neutral and acid solutions, selenopentathionate seems to be at least as stable as are corresponding solutions of pentathionate. The solutions are very sensitive to alkalies, as are pentathionate solutions. According to Foerster and Hornig [15, 16] 1 millimole of pentathionate dissolved in 1 liter of water can be detected, when 1 drop of 2 *N* sodium hydroxide is added to 10 ml of the pentathionate. The corresponding reaction of selenopentathionate is at least 10 times as sensitive. 10 ml of a 10^{-4} *M* solution of selenopentathionate, to which is added 1 drop of 2 *N* sodium hydroxide, gives immediately a distinct brownish red colour (selenium).

Selenopentathionate reacts rapidly and quantitatively with excess diethyldithiocarbamate, to give selenium bis(diethyldithiocarbamate), and thiosulphate:

$$Se(S_2O_3)_2^{--} + 2(C_2H_5)_2NCS_2^- = Se(S_2CN(C_2H_5)_2)_2 + 2S_2O_3^{--}$$

Since the diethyldithiocarbamate, with little doubt, acts as a nucleophilic reagent, the reaction is an ionic displacement on Se^{++}. The thiosulphate groups are displaced by diethyldithiocarbamate. Selenopentathionate is thereby characterized as being a selenium di(thiosulphate).

It contains two thiosulphate groups bonded to a selenium atom. Since the thio sulphur atom of the thiosulphate group has a higher polarizability and hence a higher reactivity towards electrophilic centres than have the oxygen atoms, it is the thio sulphur atom of thiosulphate which is linked to the selenium, and not any of the oxygen atoms.

The selenium-sulphur bonds are covalent; though, presumably they possess a definite amount of ionic character, with excess electropositivity on the selenium. In reactions with nucleophilic reagents, the bonding electron pairs of the selenium-sulphur bonds are released to the thiosulphate groups, which thereby become liberated as thiosulphate anions.

In pentathionate, a monosulphur di(thiosulphate), two thiosulphate groups are bonded to a sulphur atom, through the thio sulphur atoms of the thiosulphate groups [2]. The polarity of the selenium-sulphur bonds in selenopentathionate is, presumably, more pronounced than the polarity of the corresponding sulphur-sulphur bonds in pentathionate, because of the slightly higher electropositivity of selenium as compared with sulphur.

EXPERIMENTAL

Sodium selenopentathionate, $Na_2Se(S_2O_3)_2 \cdot 3H_2O$. To 20 g of selenious acid dissolved in 20 ml of water and 100 ml of glacial acetic acid are added dropwise, in the course of about 20 minutes, under mechanical stirring and cooling with ice-sodium chloride freezing mixture, 130 g of sodium thiosulphate pentahydrate in 40 ml of water (dissolved by heating, and cooled to room temperature). The temperature of the reaction mixture should be kept at about 0° C. To the clear, viscous, yellowish green solution of sodium tetrathionate and sodium selenopentathionate, containing an excess of selenious acid, are then added 150 ml of ethanol and, after the crystallization has begun, 50 ml of ether. The cooling and stirring are continued for 15 minutes, the product is then filtered off, drained well, washed with ethanol and with ether, and dried for a short time *in vacuo* over sulphuric acid.

The product, a yellowish green, voluminous mass of crystals, contains about 40 g $Na_2Se(S_2O_3)_2 \cdot 3H_2O$ with about 4 mole % of tetrathionate.

It is dissolved in 50 ml of 0.2 *N* hydrochloric acid at about 30° C, and the solution filtered with suction through a fine sintered glass filter. 100 ml of methanol are added, and the mixture cooled in ice-sodium chloride freezing mixture. The product, consisting of about 25 g pure sodium selenopentathionate trihydrate, is washed with ethanol and dried *in vacuo* over sulphuric acid.

For analysis, 0.5 millimole is dissolved in 25 ml of water. 10 ml of 6 *N* hydrochloric acid and 10 ml of a saturated solution of potassium bromide are added, and the solution is titrated with approx. 0.45 *N* potassium bromate. Toward the end of the titration the temperature of the solution is kept at about 40 or 50° C. The end point is observed by the appearance of a stable bromine colour, or by the use of 1 drop of a 0.2 per cent solution of methyl red as an indicator. The selenopentathionate is oxidized to selenious acid, and sulphate:

$$Se(S_2O_3)_2^{--} + 9Br_2 + 13H_2O = H_2SeO_3 + 4HSO_4^- + 18Br^- + 20\ H^+$$

0.5 millimole of selenopentathionate corresponds to 20 ml of 0.45 *N* potassium bromate. The high acidity and the large amount of potassium bromide serve to prevent [14] the further oxidation of selenious acid:

$$H_2SeO_3 + Br_2 + H_2O = HSeO_4^- + 2Br^- + 3H^+$$

The selenious acid formed may be determined by means of the Norris and Fay method. A few drops of a saturated alcoholic solution of acetanilide are added [14] to the titrated solution in order to discharge the slight excess of bromine. The solution is covered and heated just to boiling, cooled to below 20° C, and diluted to a volume of 200 ml. Then 0.1 *N* sodium thiosulphate is added, and the excess of thiosulphate is back-titrated with 0.1 *N* iodine. 0.5 millimole of selenious acid (or selenopentathionate) corresponds to 20 ml of 0.1 *N* thiosulphate (or iodine).

If tetrathionate is present, the amount of 0.45 *N* potassium bromate consumed is higher than the amount of 0.1 *N* thiosulphate. Tetrathionate is oxidized by bromine to sulphate[16]:

$$S_4O_6^{--} + 7Br_2 + 10H_2O = 4HSO_4^- + 14Br^- + 16H^+$$

0.1927 g substance: 21.50 ml of 0.4012 *N* potassium bromate (corresponding to 19.16 ml of 0.45 *N* bromate). 19.88 ml − 0.86 ml = 19.02 ml of 0.1006 *N* iodine (corresponding to 19.14 ml of 0.1 N iodine).

$Na_2Se(S_2O_3)_2 \cdot 3H_2O$ (403.3) Calc. Se 19.58 Found Se 19.60

Potassium selenopentathionate, $K_2Se(S_2O_3)_2 \cdot 1\frac{1}{2}H_2O$. This salt is best prepared from the sodium salt by metathesis with potassium acetate. To the filtered solution of the crude sodium selenopentathionate in 50 ml 0.2 *N* hydrochloric acid (p. 438) is added in portions, under mechanical stirring and cooling with ice-sodium chloride freezing mixture, a suspension of potassium acetate prepared as follows: 25 g of potassium acetate are dissolved by heating in 50 ml of ethanol, 25 ml of glacial acetic acid are added, and the mixture is cooled to room temperature. The crystals of potassium selenopentathionate are filtered off, and washed with ethanol and with ether. Yield about 34 g of almost pure product (containing 1 mole %, or less, of tetrathionate). It is dissolved in a double amount of 0.2 *N* hydrochloric acid at 45—50° C, the solution is filtered with suction through a fine sintered glass filter, and the filtrate is cooled in an ice-sodium chloride freezing mixture. Yield, about 25 g of pure product.

0.1995 g substance: 21.88 ml of 0.4012 *N* potassium bromate (corresponding to 19.51 ml of 0.45 *N* bromate). 19.88 ml − 0.48 ml = 19.40 ml of 0.1006 *N* iodine (corresponding to 19.52 ml of 0.1 *N* iodine).

$K_2Se(S_2O_3)_2 \cdot 1\,½H_2O$ (408.4) Calc. Se 19.33 Found Se 19.31

Potassium selenopentathionate may also be prepared directly from potassium thiosulphate. If 20 g of selenious acid dissolved in 40 ml of water and 80 ml of glacial acetic acid are reacted with 100 g of potassium thiosulphate (anhydrous) in 80 ml of water, under the same conditions as in the case of sodium thiosulphate, crystals begin to separate after about one third of the thiosulphate solution has been added. When the addition of thiosulphate is complete, the product is filtered off without any addition of ethanol or ether. In this way about 90 g of a yellowish green product is obtained, which, however, contains about 48 mole % of tetrathionate, as compared with only about 4 mole % in the case of the sodium salts. To remove the tetrathionate, the product is dissolved in 150 ml of 0.2 *N* hydrochloric acid at 45—50° C, the solution is filtered with suction through a fine sintered glass filter, and the filtrate is allowed to cool slowly to about 15° C. If the mixture is cooled further down, white tetrathionate crystals begin to separate out rather suddenly. The liquid is decanted from the crystals, and the crystals are brought on to the filter by means of a little water. Yield, about 18 g of a product which contains about 2 mole % of tetrathionate. It is finally recrystallized from a double amount of 0.2 *N* hydrochloric acid as described p. 439. The yield of the pure salt is thus about onehalf of that obtained by use of sodium thiosulphate.

Selenopentathionate and diethyldithiocarbamate. To 50 ml of 0.3 *M* sodium diethyldithiocarbamate were added, under stirring, 2.051 g $K_2Se(S_2O_3)_2 \cdot 1^1/_2\,H_2O$ dissolved in 50 ml of water. A light brown product immediately separated out. After 10 minutes stirring, the product was filtered off, washed with water, and dried *in vacuo* over sulphuric acid: 1.89 g (theoretically, 1.88 g $Se(S_2CN(C_2H_5)_2)_2$). To the filtrate was added, in order to remove the excess of diethyldithiocarbamate, a suspension of cadmium carbonate, freshly prepared by mixing 20 ml of 10 % Na_2CO_3 with an equal volume of 15 % $CdSO_4$ solution. The mixture was made up to 250 ml in a volumetric flask, and filtered through a dry filter. 50 ml of the filtrate, to which had been added 20 ml of 10 % acetic acid, consumed 20.29 ml of 0.09917 *N* iodine (theoretically, 20.25 ml).

The selenium *bis*(diethyldithiocarbamate) was recrystallized from carbon disulphide -ether, and thus obtained as yellowish green crystals, m. p. 116° C. It may also be recrystallized from carbon tetrachloride. When potassium hydroxide is added to its alcoholic solutions, red selenium is deposited.

For analysis, the compound was oxidized with nitric acid-sulphuric acid, the nitric acid was destroyed by means of urea, and the selenious acid was determined volumetrically by means of the Norris and Fay method.

0.2074 g substance: 24.85 ml — 2.75 ml = 22.10 ml 0.1006 *N* iodine.

$Se(S_2CN(C_2H_5)_2)_2$ (375.5) Calc. Se 21.03 Found Se 21.15

The optical transmittance of selenopentathionate solutions was measured by means of a Beckman quartz spectrophotometer, model DU (1 cm cells). 0.01 *N* hydrochloric acid was used as a solvent and as a blank. The results, for wave lengths from 360 mμ to 460 mμ, are listed in Table 1. A slow change of transmittance took place during the measurements. Beer's law is seen to hold.

Table 1. Molar extinction coefficient, ε, for potassium selenopentathionate dissolved in 0.01 N hydrochloric acid, as a function of wave length.

mμ	Molarity				Average
	0.02	0.05	0.1	0.2	
360	41.2	40.9			41.1
370	24.2	24.3			24.3
380	14.2	14.4			14.3
390	8.56	8.50			8.5
400	5.01	5.06			5.0
410	2.75	2.68	2.73	2.71	2.7
420	1.55	1.52	1.51	1.51	1.5
430	0.86	0.83	0.80	0.82	0.83
440	0.48	0.44	0.44	0.45	0.45
450		0.26	0.23	0.25	0.25
460		0.14	0.12	0.14	0.13

THE EQUILIBRIUM OF SELENOTRITHIONATE AND SELENOPENTATHIONATE WITH THIOSULPHATE AND SULPHITE

Selenotrithionate gives the following equilibrium with thiosulphate:

$$\overset{*}{Se}(SO_3)_2^{--} + 2S_2O_3^{--} \rightleftharpoons Se(S_2O_3)_2^{--} + 2\overset{*}{S}O_3^{--}$$

The equilibrium probably involves ionic displacements of sulphite by thiosulphate, and vice versa, as indicated through the stars, and not transfers of sulphur from thiosulphate to selenotrithionate, or from selenopentathionate to sulphite.

In buffered solutions, in presence of formaldehyde and excess thiosulphate, the equilibrium is displaced quantitatively to the right. Formaldehyde ties up sulphite as hydroxymethanesulphonate:

$$SO_3^{--} + CH_2(OH)_2 = HOCH_2SO_3^- + OH^-$$

The buffer serves to keep the solutions slightly acid and thus to prevent the liberation of selenium from selenopentathionate.

Under such conditions, the reaction may be employed for the iodometric analysis of selenotrithionate (see the experimental part). Excess of thiosulphate is back-titrated with iodine, hydroxymethanesulphonate [16,17] and also selenopentathionate being indifferent to iodine in acid solutions.

The equilibrium may be demonstrated from both sides, *i. e.*, also from selenopentathionate and sulphite.

In the case of trithionate and pentathionate, no such measurable equilibrium exists. Pentathionate reacts quantitatively with sulphite, to give trithionate, and thiosulphate *:

$$S(S_2O_3)_2^{--} + 2\overset{*}{S}O_3^{--} = S(\overset{*}{S}O_3)_2^{--} + 2S_2O_3^{--}$$

The reaction of pentathionate with sulphite proceeds in two steps: First one thiosulphate group is displaced, to give tetrathionate. Analogously, the displacement of sulphite in selenotrithionate by thiosulphate probably involves two steps, with a selenium sulphite-thiosulphate, *i. e.*, selenotetrathionate, $SeS_3O_6^{--}$, as an intermediate.

EXPERIMENTAL

Potassium selenotrithionate was prepared by means of Rathke's method [3]. The procedure differs in some details from that used by Foerster, Lange, Drossbach and Seidel [5] and by Heuer [6].

8 g of selenium (red or grey) are dissolved by heating in 20 ml of water containing 12 g of potassium hydroxide. To the dark red solution is added, cautiously, a hot solution of 72 g of potassium hydrogen sulphite in 100 ml water. The resulting solution is filtered, if necessary, and cooled to 30—40° C. Then 13 g selenious acid dissolved in 20 ml water are added. The mixture becomes hot, and crystals of potassium selenotrithionate begin to separate out. After cooling in water to 20° C, the crystals are filtered off, and washed with 50 % ethanol. Yield, about 52 g, being about 94 % pure. It is dissolved in 150 ml water at 45—50° C, the solution is filtered, if necessary, and cooled in ice-sodium chloride freezing mixture. Yield, about 36 g of pure potassium selenotrithionate, $K_2Se(SO_3)_2$.

It may be analyzed iodometrically as follows:

To 25 ml of 0.01 *M* selenotrithionate are added 2 g of sodium hydrogen carbonate and then 20 ml of 0.1 *N* iodine. After standing for 5 minutes in stoppered flask, 20 ml 10 % acetic acid are added, and the excess of iodine is back-titrated with 0.1 *N* thiosulphate.

The selenotrithionate is oxidized to selenite, and sulphate: $Se(SO_3)_2^{--} + 3I_2 + 10\ OH^- = SeO_3^{--} + 2SO_4^{--} + 6I^- + 5H_2O$. Selenite is indifferent to iodide in acetate buffers, therefore acetic acid can be used for back-titration of the excess iodine. The method is analogous to that worked out for the analysis of di-0-alkylmonoselenophosphates [18].

The following experiment shows the merit of the method:

0.7929 g of $K_2Se(SO_3)_2$ was dissolved to 250 ml, and 25 ml samples were pipetted out. Time of standing between the addition of iodine and acetic acid: 3, 5, 15, 20 minutes. Amounts of 0.1073 *N* thiosulphate consumed: 18.86 ml (by the 20 ml iodine employed) — 5.08 ml, 4.92 ml, 4.92 ml, 4.92 ml, respectively. 10 times 13.94 ml of 0.1073 *N* thiosulphate corresponds to 0.7911 g of $K_2Se(SO_3)_2$, *i. e.*, 99.77 %.

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* For literature references, see Ref. 2.

In neutral and acid solutions, selenotrithionate is oxidized by iodine to selenium and sulphate, as stated by Heuer[6]. Selenopentathionate, however, is indifferent to iodine in acid solutions, as shown by the accuracy and consistency of the Norris and Fay method.

Selenotrithionate and thiosulphate. If thiosulphate and formaldehyde are added to a solution of selenotrithionate, the mixture immediately becomes alkaline (to phenolphtalein). If an excess of thiosulphate is present, selenium is rapidly liberated.

In buffered solutions, the pale green colour of selenopentathionate gradually develops.

Thus, selenotrithionate may be analyzed iodometrically as follows:

1 millimole selenotrithionate is dissolved in 100—150 ml of water, and 25 ml of a buffer is added, being 0.5 *M* with respect to dihydrogen phosphate and 0.1 *M* with respect to monohydrogen phosphate (68.07 g KH_2PO_4 and 35.82 g $Na_2HPO_4 \cdot 12H_2O$ dissolved to 1 liter). Next 1 ml of 40 % formaldehyde and 25 ml of 0.1 *N* thiosulphate are added. After standing for 5 minutes, 20 ml of 10 % acetic acid are added, and the excess of thiosulphate is back-titrated with 0.1 *N* iodine.

The following experiment illustrates the method:

3.986 g of $K_2Se(SO_3)_2$ (the same specimen that was analyzed by means of the iodine-sodium hydrogen carbonate method) was dissolved to 250 ml, and 20 ml samples were pipetted out, and diluted to 120 ml. Time of standing between the addition of formaldehyde-thiosulphate and acetic acid: 5, 10, 15, 15 minutes. Amounts of 0.1013 *N* iodine consumed: 26.51 ml (by the 25 ml thiosulphate employed) — 6.70 ml, 6.70 ml, 6.70 ml, 6.71 ml, respectively. 12.5 times 19.81 ml of 0.1013 *N* iodine corresponds to 3.980 g of $K_2Se(SO_3)_2$, *i. e.*, 99.84 %.

In presence of 10 ml of 0.05 *M* tetrathionate: 6.69 ml of iodine. Thus, tetrathionate does not affect the results. If, however, the thiosulphate is added before the formaldehyde is added, too low results are obtained. This is because the sulphite formed reacts with tetrathionate to give thiosulphate, which thus becomes regenerated.

The buffer employed has a pH = 6.5. After the formation of 20 ml of 0.1 *M* hydroxymethanesulphonate the solutions will have approx. pH = 6.9. The solutions should not stand for more than 15 minutes before they are titrated, since after about 20 minutes a selenium colour begins to appear. The titrated solutions, the catalyzing thiosulphate having been removed, are stable for several hours. On addition of an appropriate amount of potassium hydroxide, selenium is liberated.

Selenotrithionate solutions are not very stable. In the approx. 0.05 *M* solution employed above (3.986 g of $K_2Se(SO_3)_2$ in 250 ml), the content of selenotrithionate, 14 hours after the preparation of the solution, had decreased by 2 % (determined by means of the formaldehyde-thiosulphate method).

Selenopentathionate and sulphite. To 50 ml of 0.02 *M* potassium selenopentathionate were added 10 ml of buffer solution (0.5 *M* $H_2PO_4^-$ and 0.1 *M* HPO_4^{--}) and 5 ml of 0.1 *M* sodium sulphite (in 20 % ethanol, as a stabilizer). The sulphite, in blind runs, consumed 9.92 ml of 0.1 *N* iodine. After standing, the mixture was titrated with 0.1 *N* iodine. Time of standing: 1, 2, 5 minutes. Amounts of 0.1 *N* iodine consumed: 6.42 ml, 6.31 ml, 6.30 ml, respectively. Thus selenopentathionate reacts with sulphite to give thiosulphate, though not quantitatively (would require a consumption of only 4.96 ml of iodine).

To a mixture of 50 ml of 0.02 *M* selenopentathionate, 10 ml buffer solution, and 5 ml 0.1 *M* sulphite, after standing, were added 100 ml water, 1 ml of 40 % formaldehyde, and 5 ml of 0.1 *N* thiosulphate (to displace the equilibrium more quantitatively towards selenopentathionate). The thiosulphate consumed, in blind runs, 5.02 ml of 0.1 *N* iodine.

After standing for 5 minutes, 20 ml of 10 % acetic acid were added, and the solution titrated with 0.1 N iodine. Time of standing between the addition of sulphite and formaldehyde-thiosulphate: 1, 2, 5 minutes. Amounts of 0.1 *N* iodine consumed: 5.06 ml, 5.10 ml, 5.16 ml, respectively. The experiment shows that although selenopentathionate reacts partly with sulphite, to give thiosulphate, the reaction is reversed on addition of formaldehyde and thiosulphate. Theoretical consumption of 0.1 *N* iodine in the last titrations, for quantitative displacement, would be 5.02 ml. The small discrepancy, increasing on standing, is probably due to hydrolysis of lower selenopolythionates formed by the action of sulphite.

No selenium was liberated in the solutions at any stage of the experiments.

SUMMARY

Sodium and potassium selenopentathionate have been prepared in a pure state, as the first salts of selenopentathionic acid. Selenopentathionate is a selenium di(thiosulphate).

It is shown that an equilibrium exists between selenotrithionate and thiosulphate, on one side, and selenopentathionate and sulphite, on the other side. This equilibrium has been utilized for the iodometric analysis of selenotrithionate.

The author wishes to express his thanks to Prof. E. Berner for the use of his Beckman spectrophotometer.

The work on selenopolythionates and analogous selenium sulphur compounds is being continued. Also the corresponding tellurium sulphur compounds are being investigated.

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Received April 4, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 445—458

The Diffusion Potential between Dilute Solutions and Concentrated Solutions of Potassium Chloride plus Potassium Nitrate

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When used as a salt bridge or a reference electrode solution for emf-measurements, a saturated solution of potassium chloride does not meet the demands of equitransference * to as great an extent as originaly supposed.

According to the classic investigations of Hittorf the potassium ion has the transference number 0.498 in potassium chloride. Since then several authors [2,3], having used both the classic method and the more accurate moving boundary method, have agreed that the transference number, which by the way is not fully independent of the concentration, lies near the value 0.490. As it is known, it is hardly possible to determine the diffusion potential directly and therefore we must generally calculate it. For such a calculation the transference number of potassium chloride is of great importance; this pertains particulary to very dilute solutions, as a consequence results obtained with cells with liquid-liquid junction aiming at an extrapolation to infinite dilution are greatly compromised by the uncertainty of the diffusion potential. This is to a great extent the case because calculation of the diffusion potential is based on the theoretically derived Henderson formula in connection with transference numbers that have been determined by methods which have nothing to do with emf-measurements. The aim of the present work has been:

1) to demonstrate that in accordance with the Henderson formula the diffusion potential increases from a certain limit with increasing dilution when the bridge solution is not equitransferent.

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* The term equitransferent has previously been defined by the author [1] as: "having the same effective cation- and anion-transference".

2) from experiments to determine the order of magnitude of the ratio KCl/KNO_3 which leads to an equitransferent solution; we presume that equitransference is present when the ratio is such that the measurements are in agreement with the activity laws, even in extreme dilution.

3) to give certain indications for the application of such a bridge solution.

Murray and Acree [4] (1931) and Kline, Meacham and Acree [5] (1932) have suggested the use of a concentrated solution of potassium chloride plus potassium nitrate in the mole ratio 3 : 1. A more detailed investigation of the problem was made by Manov, DeLollis and Acree[6] (1944). They found from theoretical viewpoints that the said solution is not completely equitransferent and that equitransference may be obtained by the addition of a small quantity of hydrochloric acid. The present author does not consider this solution of the problem suitable, as the hydrochloric acid may cause protolytic chances in the boundary and if some of the bridge solution enters the test solution the measurement is often totally compromised.

Instead it seams reasonable to try to increase the amount of potassium nitrate in proportion to potassium chloride. The reason that the above authors have not suggested this procedure is possibly that they wanted to keep up a total concentration that was above 4 normal and this is hardly possible at room temperature if the concentration of potassium nitrate is further increased.

CALCULATIONS BASED ON KNOWN TRANSFERENCE NUMBERS

If the cation transference t is known for potassium chloride and potassium nitrate, it is possible to calculate the ratio KCl/KNO_3 which leads to an equitransferent solution.

If u and v denote the mobilities of cations and anions, respectively, we obtain by applying the formula

$$t = \frac{u}{u + v} \tag{1}$$

$$v_{Cl} = \frac{u\ (1-t_{KCl})}{t_{KCl}} \text{ and } v_{NO_3} = \frac{u\ (1-t_{KNO_3})}{t_{KNO_3}} \tag{2}$$

If we put the total concentration $= c$ we have the following condition for equitransference

$$uc = v_{Cl}c_{KCl} + v_{NO_3}c_{NKO_3} \tag{3}$$

Equations (2) and (3) lead to

$$\frac{c_{KCl}}{c_{KNO_3}} = \frac{(2t_{KNO_3}-1)\ t_{KCl}}{(1-2t_{KCl})\ t_{KNO_3}} \tag{4}$$

We here meet the difficulty that the transference numbers depend to a certain extent on the concentration. Table 1 gives the mole ratio KCl/KNO_3 for some concentrations calculated from equation (4).

Table 1. The ratio c_{KCl}/c_{KNO_3} leading to equitransferent solution.

c	Cation transference *		Ratio c_{KCl}/c_{KNO_3}
	t_{KCl}	t_{KNO_3}	
0	0.4906	0.5072	0.749
0.01	0.4902	0.5084	0.828
0.05	0.4899	0.5093	0.885
0.10	0.4898	0.5103	0.969
0.20	0.4894	0.5120	1.082
1.0	0.4871	unknown	
3.0	0.4858	unknown	

* Values collected by Mc. Innes [7].

When dealing with a process of this kind which takes place in a boundary between the concentrated bridge solution and a dilute solution, *i. e.* through an interval with varying concentration of the bridge electrolyte, it is not possible to calculate the ratio KCl/KNO_3 according to equation (4). Instead of demanding absolute equitransference in the bridge solution we must claim that the mixture of this solution is such that the boundary between it and a dilute solution on average becomes equitransferent with regard to the ions originally present in the bridge solution. It will hardly be possible to calculate this ratio from known transference numbers.

EXPERIMENTS

Measurements have been made in cells of the type

$$\underset{a}{Ag}|AgCl,HCl|\underset{b}{glass}|\underset{c}{0.001\ N\ HCl + y\ N\ KCl}|\underset{d}{x\ N\ KCl + (3-x)\ N\ KNO_3}|\underset{e}{KCl\ (s)}, HgCl|\underset{f}{Hg} \tag{5}$$

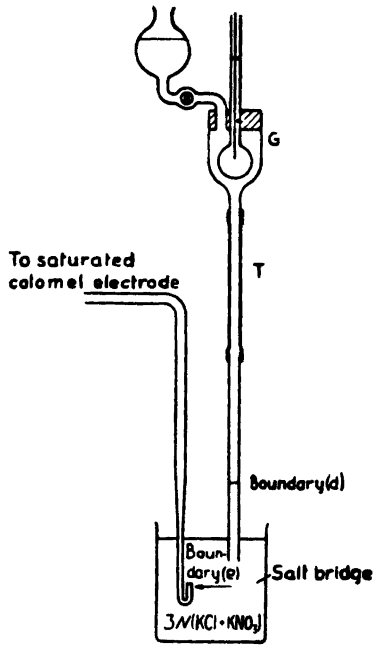

Fig. 1. Experimental arrangement. G glass electrode vessel, T rubber tubing.

The experiments were carried out in a room where the temperature was kept at 22° C ± 0.3° C. Fig. 1 shows the apparatus. A fortnight old Haber electrode with wery thin walls was used; it was made by melting 8 cg of Corning 015 glass to the end of a tube of common glass and blowing to a diameter of 2 cm. Measurements have been made with a vacuum tube electrometer (Radiometer, type P.H.M.3).

The test solution (0.001 N HCl + y N KCl) was placed in the electrode vessel (G) and measurements were first made with 3 N KCl as bridge solution, and then, after washing the vertical tube with the test solution, measurements were made with a mixture of 4 parts of 3 N KCl + 1 part 3 N KNO_3; then with a mixture of 3 parts 3 N KCl + 2 parts of 3 N KNO_3 etc., ending with a pure 3 N KNO_3 solution. And further, for sake of control, a 3 N KCl was again used as bridge solution. A series of such measurements corresponds to a column in Table 2. The values presented are averages of five readings taken just after the boundary has been prepared.

By careful suction (after compression of the rubber tubing (T) it is allowed to expand again) the boundary (d) between the test solution and the bridge solution has been lifted a few cms into the vertical tube. The object of making the boundary in this manner is to obtain cylindrical symmetry, which is of

Table 2. Potential differences in millivolts.

Molar ion concentration c			0.001	0.003	0.01	0.03	0.10	0.3	1.0	3.0
Composition of the test solution		c_{HCl}	0.001	0.001	0.001	0.001	0.001	0.001	0.001	0.001
		c_{KCl}	0	0.002	0.009	0.029	0.099	0.3	1.0	3.0
System no.	Parts 3 N KCl + parts 3 N KNO_3 in bridge solution									
1		0 + 5	64.9	65.1	65.6	66.1	68.1	68.0	64.6	51.0
2		1 + 4	62.6	63.3	64.0	64.8	66.7	67.0	64.2	
3		2 + 3	60.6	61.2	62.1	63.2	65.2	65.9	63.7	
4		3 + 2	58.0	58.6	59.6	61.1	63.2	64.9	62.8	
5		4 + 1	55.1	56.1	56.9	58.6	61.1	62.1	62.0	
6		5 + 0	51.8	53.1	53.9	56.2	58.6	60.7	60.8	50.8
1		0 + 5	64.8	65.4	65.7	66.7	68.1	68.3	65.0	

importance for reproducing the experiments. The boundary (e) between the bridge solution and the calomel electrode was made by simple submersion, which is considered justifiable as the corresponding diffusion potential is small (maximum 2 mV according to the Henderson formula — also when used in Guggenheim's [8] modification pertaining to solutions of equal conductance (*i. e.* rendering the quantity C of equation (10) equal to zero)).

It is pointed out that the true electrode potentials are the same in a column of Table 2. The variations through such a column are entirely due to variations in the diffusion potentials at (d) and (e) (formula (5) and Fig. 1). With the weakest solution (0.001 N HCl) using 3 N KCl as bridge solution, 64.9 mV were measured and with 3 N KNO_3 51.8 mV, *i. e.* a difference of as much as 13 mV. This difference between the extremes decreases as the concentration of the test solution increases and *e. g.* for a 1 N solution we find only a difference of 4 mV.

DISCUSSION OF EXPERIMENTAL FINDINGS

Judging from their transference numbers potassium chloride and potassium nitrate should be equally good as bridge solutions when used in the same concentrations, and the results obtained must be expected to lie on either side of the true value. The latter must be sought somewhere between the two extremes obtained by measurements with pure KCl- and KNO_3 solutions as salt bridges. The bridge solutions given in Table 2 may be evaluated by examining which of the rows of the table is in best agreement with the activity laws. For this object the Debye-Hückel-Brønsted equation has been used:

$$-\log f = 0.5 * \sqrt{c} - \beta c \qquad (6)$$

In the following only concentrations of the test solution equal to 0.1 normal or less have been considered. In this concentration-interval equation (6) may be applied with good approximation.

The emf of the cell is equal to the sum of the potential differences at a, b, c, d, e, and f in formula (5). These potential differences are constants at a, b, and f. At (e) the potential difference varies for each of the 6 different bridge solutions, but it is constant in a row of Table 2. Presuming that for the solutions in question the glass electrode acts like the reversible hydrogen electrode, the potential difference at c is equal to a constant minus $(RT/F \log e) \log a_H$. The potential difference at (d) is denoted $-E_j$.

If the constants are combined in one constant K_1 the emf of the cell may be expressed:

$$E = K_1 - \frac{RT}{F \log e} \log a_H - E_j \qquad (7)$$

By applying equation (6) this may be written:

$$\frac{E}{k} = K_2 + 0.5 \sqrt{c} - \beta c - \frac{E_j}{k} \qquad (8)$$

as $a_H = f c_H$ and $\frac{RT}{F \log e} = k$ and $\frac{K_1}{k} - \log c_H = K_2$

* After Harned and Owen [9] the accurate value of the limiting slope is equal to 0.5036 at 22° C.

E_j may be calculated from the Henderson formula, which, as the present systems contain only uni-univalent electrolytes, may here be applied in the simple form:

$$E_j = \frac{RT}{\mathrm{F} \log e} \cdot \frac{(U—V) - (U_r - V_r)}{(U + V) - (U_r + V_r)} \log \frac{U_r + V_r}{U + V} \tag{9}$$

where $U = \sum_t u_t c_t$ and $V = \sum v_t c_t$

and $U_r = \sum_r u_i c_i$ and $V_r = \sum_r v_i c_i$

u_i and v_j denoting mobilities of cations and anions, respectively, and the indices r and t referring to the bridge solution and the test solution, respectively.

Equation (9) may be written:

$$E_j = k \cdot \Big(\underbrace{\frac{U—V}{(U + V) - (U_r + V_r)}}_{\mathrm{A}} - \underbrace{\frac{U_r - V_r}{(U + V) - (U_r + V_r)}}_{\mathrm{B}}\Big) \log \underbrace{\frac{U_r + V_r}{U + V}}_{\mathrm{C}} \tag{10}$$

or $$E_j = kAC + kBC \tag{11}$$

When the test solution is of low concentration in comparison with the bridge solution we may reason as follows:

The denominator of the fraction A is large compared with the numerator and is relatively independent of the conditions in the test solution. The numerator, on the other hand, depends greatly on the proportions between the ionic mobilities in the test solution and is proportionate to the concentration of the latter solution. The quantity C increases as the test solution becomes more diluted, but this increase is of a lesser order of magnitude than the corresponding decrease in fraction A. Therefore the product kAC will decrease as the test solution becomes more diluted. If dilution becomes infinite the limiting value is equal to zero.

The denominator of the fraction B is approximately constant and the numerator is constant. Therefore, as the test solution becomes more diluted the product kBC increases proportionately with the logarithm of the conductance of the test solution.

In the present experiments the composition of the test solution has been chosen in such a manner that for concentrations below or equal to 0.1 normal

the product kAC does not exceed 0.2 Vm. Thus, in equation (11) variations in the diffusion potential may be considered due to the product kBC alone. Equation (10) may thus be reduced to:

$$E = \frac{-k(U_r - V_r)}{(U + V) - (U_r + V_r)} \log \frac{U_r + V_r}{U + V} \tag{12}$$

or

$$E_j = \frac{-k(U_r - V_r) \log (U_r + V_r)}{(U + V) - (U_r + V_r)} + \frac{k(U_r - V_r) \log (U + V)}{(U + V) - (U_r + V_r)} \tag{13}$$

As the concentration of the test solution in the present investigation did not exceed 0.1 normal the error we introduce by putting $((U + V)-(U_r + V_r))$ equal to $-(U_r + V_r)$ does not exceed 3.5 %; as further the quantity (U_r-V_r) is constant for each of the 6 bridge solutions, equation (13) may with good approximation be reduced to:

$$E_j = K_3 - k\ D \log (U + V) \tag{14}$$

where

$$\mathrm{D} = \frac{U_r - V_r}{U_r + V_r} \tag{15}$$

and $$K_3 = \frac{-k(U_r - V_r) \log (U_r + V_r)}{(U + V) - (U_r + V_r)}$$

If equation (14) is substituted in equation (8) we obtain:

$$\frac{E}{k} = K + 0.5 \sqrt{c} - \beta c + D \log (U + V) \tag{16}$$

the constant terms being combined in the constant K.

The bridge solution which eliminates the diffusion potential best leads to the value zero of the quantity $(D \log (U + V))$, *i. e.* equation (16) becomes:

$$\frac{E}{k} - 0.5\sqrt{c} = -\beta c + K \tag{17}$$

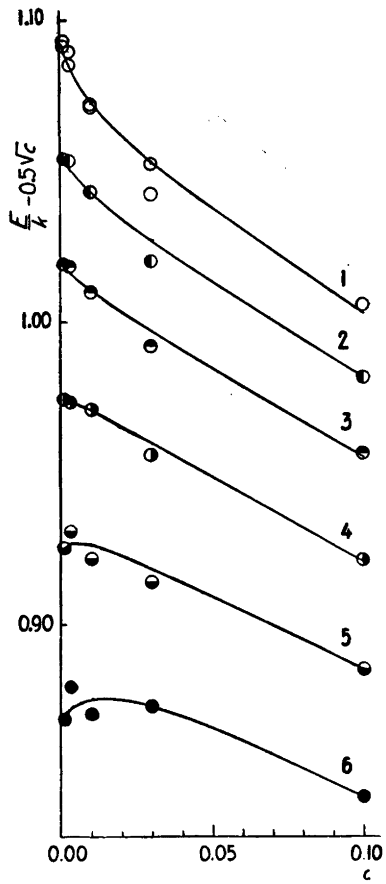

Fig. 2. Curves given by equation (17). Each curve corresponds to a given value of the ratio KCl/KNO_3 in the salt bridge. Presuming that the Debye-Hückel-Brønsted equation is valid, the curve should be a straight line. A straight line is best realized between curves (3) and (4), corresponding to the mol ratio $KCl/KNO_3 = 1$.

In order to examine in which of the 6 systems of Table 2 this elimination is best realized, the quantity $((E/k)—0.5\sqrt{c})$ has been plotted as a function of c in Fig. 2. This should lead to a straight line with slope $-\beta$. The ordinates corresponding to the curves have been entered in columns (a) of Table 3.

Fig. 2 shows that systems (3) and (4) lead to almost straight lines; at the lowest concentrations, however, they deviate slightly in different directions. Thus, it seems reasonable to assume that a straight line would have been obtained between systems (3) and (4), *i. e.* corresponding to approximately equimolar concentrations of potassium chloride and potassium nitrate. Even though the present experiments do not allow of an accurate determination of the ratio between potassium chloride and potassium nitrate, they seem to indicate that mole ratios between 2 : 3 and 3 : 2 are able to eliminate the diffusion potential

Table 3. Column a gives the values of $E/k - 0.5\sqrt{c}$ corresponding to equation (17) and Fig. 2. Column b gives the values of $E/k - 0.5\sqrt{c} + 0.584\ c$ corresponding to equation (18) and Fig. 3.

Concentration		0.001		0.003		0.01		0.03		0.1	
		a	b	a	b	a	b	a	b	a	b
System no.	1	1.093	1.094	1.085	1.086	1.071	1.077	1.042	1.060	1.005	1.063
	2	1.054	1.055	1.054	1.055	1.043	1.049	1.020	1.037	0.981	1.039
	3	1.019	1.020	1.019	1.020	1.010	1.016	0.992	1.010	0.956	1.014
	4	0.975	0.975	0.974	0.975	0.971	0.977	0.956	0.974	0.921	0.979
	5	0.925	0.926	0.931	0.933	0.922	0.928	0.914	0.932	0.885	0.943
	6	0.869	0.870	0.880	0.881	0.871	0.877	0.873	0.890	0.843	0.901
	1	1.091	1.092	1.090	1.091	1.072	1.078	1.052	1.069	1.005	1.063
log $(U + V)$			−0.398		−0.173		0.203		0.618		1.101

to such a degree that — even in extreme dilution — the results obtained are in good agreement with the activity laws.

The curves (3) and (4) have the slopes — 0.632 and — 0.535, respectively; the mean of these slopes, — 0.584 we denote $-\beta$. Substituting in equation (16) this leads to:

$$\frac{E}{k} - 0.5\sqrt{c} + 0.584\ c = K + D\ \log\ (U + V) \tag{18}$$

In Fig. 3 the quantity $((E/k) - 0.5\sqrt{c} + 0.584\ c)$ has been plotted as a function of log $(U + V)$. Thus, D denotes the slope of the obtained lines. The coordinates corresponding to Fig. 3 have been entered in the columns (b) of Table 3. The values of $(U + V)$ have been calculated from data collected by

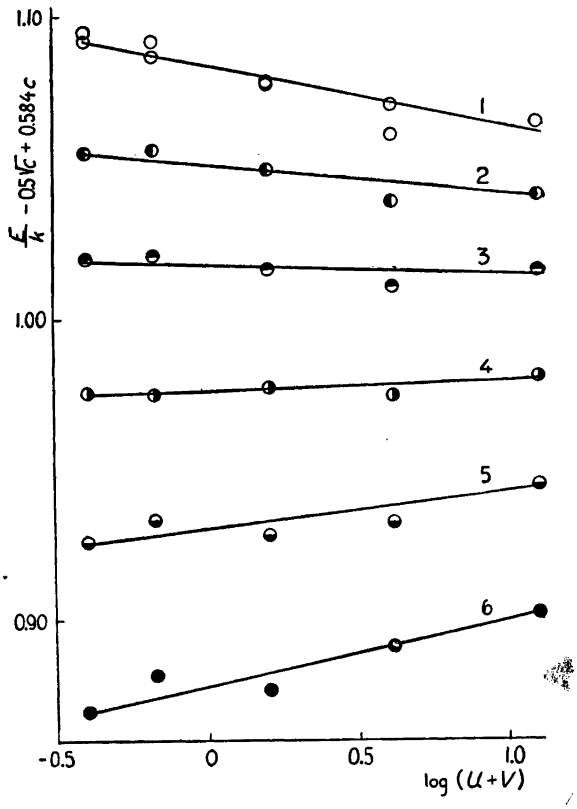

Fig. 3. Transformation of Fig. 2 corresponding to equation (18). The figure justifies the plotting of the curves in relation to the given points. Further, it is possible to calculate the cation transference for each of the bridge solutions used from the slope of the curves. The accuracy of this calculation, however, depends on the accuracy of the value chosen for β.

Harned and Owen [9]. Their values applied to 25° C and have here been corrected to 22° C by subtracting 5 %.

The slopes of the lines plotted for the 6 bridge solutions have been calculated as: $D_1 = -0.0203$, $D_2 = -0.0102$, $D_3 = -0.0036$, $D_4 = 0.0028$, $D_5 = 0.0118$, and $D_6 = 0.0213$.

From these values it is possible to calculate the cation transferences of the bridge solutions. Equation (1) and (15) lead to

$$t = \frac{1 + D}{2}$$

and we thus find the following 6 values for the cation transferences: $t_1 = 0.490$, $t_2 = 0.495$, $t_3 = 0.498$, $t_4 = 0.501$, $t_5 = 0.506$, and $t_6 = 0.511$.

If we compare the value t_1 (pure KCl) and t_6 (pure KNO_3) with the values given in Table 1 we find an agreement which may be considered a good veri-

fication of Henderson's formula. It must be emphasized that the transference values obtained cannot be attributed to any well-defined concentration but must be looked upon as representing the average condition of the boundary.

The slopes of the curves in Fig. 3 and the transference numbers calculated from them are dependent on the value allotted to the quantity β. This implies that the transference numbers determined are not appropriate to a calculation of the optimal ratio KCl/KNO_3. The main use we can make of Fig. 3 is hereafter to find the course of the curves on Fig. 2 in relation to the plotted points, as one of these is a transformation of the other.

The choice of the mole ratio 1 : 1 is solely based on the estimate that the curvatures of the curves (3) and (4) in Fig. 2 take opposite directions and are equal in size. Unfortunately, it is not possible with the technique used here to obtain results so accurate as to allow a computation of the optimal ratio from the curvatures of the curves. It must therefore suffice to know that both curves (3) and (4) give a comparatively good agreement with the activity laws. When we consider how sensitive such very dilute solutions as we are dealing with here are to influences from without it seems doubtful whether the use of another kind of electrode could verify our presumption. It is a fact that in so diluted solutions it is often difficult to use the hydrogen electrode with satisfactory accuracy. Even though the glass electrode is known to give less reproducible results in general than the hydrogen electrode the present author has found that the former gives better results than the latter in solutions that are very dilute with respect to hydrochloric acid.

APPLICATION

By using an equitransferent bridge solution instead of saturated potassium chloride we gain partly the advantage that the formula for calculation is simplified, partly that we are working with smaller diffusion potentials on the whole, and it is not the least consequence that the diffusion potential decreases as the test solution becomes more diluted. As already mentioned the simplification obtained is that in equation (11) the quantity kBC becomes zero. In the above the problem has been elucidated through an example with uni-univalent ions. If the test solution contains polyvalent ions the equation corresponding to equation (10) takes the form:

$$E_j = k\left(\underbrace{\frac{U-V}{(\bar{U}+\bar{V})-(U_r+V_r)}}_{A} - \underbrace{\frac{U_r-V_r}{(\bar{U}+\bar{V})-(U_r+V_r)}}_{B}\right) \log \underbrace{\frac{U_r+V_r}{\bar{U}+\bar{V}}}_{C} \quad (19)$$

where $\bar{U} = \sum_i u_i z_i c_i$ and $\bar{V} = \sum_t v_j z_j c_j$

c denotes the molarity of the test solution and z the valency of the ions, taken positive.

In equation (19) the quantity B will be equal to zero. Thus we have the following general equation for the diffusion potential between an equitransferent uni-univalent bridge solution and a diluted solution:

$$E_t = \frac{RT}{F \log e} \cdot \frac{U-V}{(\bar{U}+\bar{V})-(U_r+V_r)} \log \frac{U_r+V_r}{\bar{U}+\bar{V}} \qquad (20)$$

Equation (20) shows that the diffusion potential decreases as the concentration of the bridge solution increases. In the above experiments the concentration has been 3 normal on account of the solubility of potassium nitrate. For equimolar mixtures of potassium chloride and potassium nitrate it is possible to use somewhat higher concentrations. In a thermostat at 25°C it is possible to use a 4 normal solution, but this has the drawback that the solution crystalises when it is left at room temperature. A 3.6 normal solution (1.8 N KCl + 1.8 N KNO_3) may be recommended because it is stable also at room temperature.

The present work cannot be concluded without an emphatic warning against the use of the common standard values for calomel electrodes in connection with the bridge solution suggested here, as these values are based on the use of saturated potassium chloride and thus include the corresponding diffusion potentials. The error which this implies may be estimated from Fig. 2 in which an unit in the ordinate is equal to one pH-unit. We must possibly except the E_0-value given by Guggenheim and Schnindler [10] for the decinormal calomel electrode as this value has been corrected for the diffusion potential with application of the new value for the transference number of potassium chloride.

Acree and collaborators [6] have demonstrated that it is possible to obtain constant and reproducible calomel electrodes with mixtures of potassium chloride and potassium nitrate. The present author is not so far able to state an accurate E_0-value for such a calomel electrode when using the above suggested bridge solution as reference electrode solution. This, however, does not prevent the use of such an electrode for ordinary work; *e. g.* when measuring hydrogen ion concentrations, a determination is simply made in a solution with known hydrogen ion activity and then the E_0-value is calculated or the pH-value is calculated directly from the difference between the potentials of the standard and the test-solutions (due allowance being made for the diffusion potential).

The order of magnitude of the diffusion potentials found when we are working with equitransferent bridge solutions will be investigated later. Probably they are often so small that it is justifiable to disregard them altogether if the desired accuracy allows it.

On the whole when saturated potassium chloride is used as salt bridge or as reference electrode solution it has been recommended to standardise the determination of the hydrogen ion concentrations with standard buffer solutions instead of using the E_0-value of a calomel electrode [7, 11, 12]. It seems probable that in certain cases the results obtained will equal those obtained with an equitransferent bridge solution or reference electrode solution, namely when the conductance of the standard- and the test-solution are of the same order of magnitude. If this is the case the contribution to the diffusion potential which is due to the differences between the cation and anion mobilities in the bridge solution (the quantity kBC in equation (11)) will be of no consequence as it will appear as a positive correction in one measurement and a negative one in another.

SUMMARY AND CONCLUSIONS

1. If the bridge solution is not equitransferent the diffusion potentials are large in very dilute solutions.

2. A concentrated equimolar solution of potassium chloride and potassium nitrate is considered equitransferent; since when it is used as bridge solution, the measurements are in agreement with the activity laws even when the test solution is extremely dilute. A 3.6 normal solution (1.8 N KCl + 1.8 N KNO_3) is suggested as bridge solution or reference electrode solution.

3. The bridge solution suggested should not be applied in connection with the known standard values for calomel electrodes. A solution of known hydrogen ion activity should be used as standard.

My best thanks are due to Rektor, Professor C. Faurholt for the kind interest he has taken in the present work. Further I wish to thank Professor J. A. Christiansen for discussions of the manuscript.

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Received March 20, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 459—464

A New Technique in Paper Chromatography

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Paper partition chromatography, originally introduced by Consden, Gordon, and Martin [1], has proved an effective method of separating chemical compounds from complex mixtures such as protein hydrolysates and of identifying them on a micro scale. A review of paper chromatography has recently appeared [2]. For several months we have used in this laboratory the original method of Consden, Gordon, and Martin for qualitative analysis of mixtures of amino acids. In practice certain technical improvements — to be reported in this paper — have suggested themselves.

By means of the original technique a complete separation of only a few amino acids from protein hydrolysates can be attained by *one-dimensional* chromatography. For example, when phenol is used as a solvent, only aspartic acid, and when s-collidine is used as a solvent, only valine is often separated as an individual spot whereas all the other amino acids partly overlap one another. Certain alcohols would have a good resolving power but the amino acids move relatively slowly with them. The R_f-values of the fastest moving amino acids in benzyl alcohol (phenylalanine), in *n*-butanol (leucine) and in *tert.* amylalcohol (leucine) are 0.36, 0.43, and 0.30 respectively. So, when the solvent frontier reaches the lower edge of the strip of filter paper, the amino acids have advanced only short distances forming a tightly packed chromatogram (Fig. 1, A) which contains no one-acid spots. For a more effective separation the chromatograms must be developed longer. This can be effected by the following, very simple »continuous developing technique» which gives chromatograms of any desired length.

THE NEW PROCEDURE

A thick pad of cotton wool, cellulose tissue, or other absorbing material is sewed or stapled to the foot of the strip of filter paper. This pad must be able to soak up an abundant amount of developing solvent. The development of

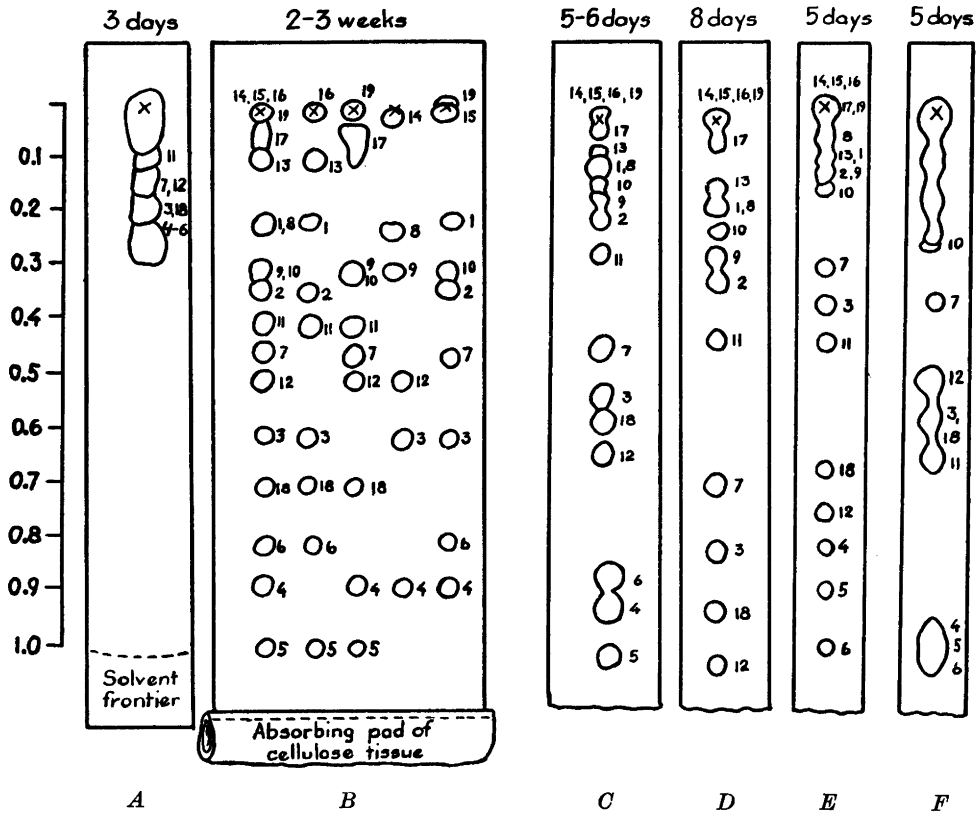

Fig. 1. Diagrams of one-dimensional paper chromatograms of mixtures of amino acids

1. Gly 4. Ileu 7. Tyr 10. Hypro 13. His 16. Asp 19. (CyS-)$_2$
2. Ala 5. Leu 8. Ser 11. Pro 14. Arg 17. Glu
3. Val 6. Phe 9. Thr 12. Try 15. Lys 18. Met

(The symbols proposed by E. Brand [3])

A. A chromatogram of 19 amino acids, developed by the original technique with tert. amylalcohol. The solvent frontier has advanced abt. 50 cm in 3 days. The length of the chromatogram is abt. 13 cm. In experiments A—E the hydrolysate was treated with NH_3-vapour on the paper.

B. A chromatogram corresponding to A but obtained by the 'continuous developing technique' in 3 weeks. The length of the chromatogram is abt. 50 cm. It contains 9 one-acid spots.

C. A chromatogram of 19 amino acids obtained in 5—6 days with n-butanol and by the 'continuous developing'.

D. The same as in C after 8 days' development.

E. A chromatogram of 19 amino acids obtained in 5 days with benzyl alcohol and by the 'continuous developing'.

F. The same as in E, but this time the drop of hydrolysate was not neutralized with NH_3-vapour while applied to the filter paper.

the chromatogram is continued until the fastest moving compound has advanced near the pad. Using big sheets (60 by 60 cm) of Whatman no. 1 filter paper, the approximate developing times for amino acids are: in *tert.* amylalcohol 2—3 weeks, in *n*-butanol as well as benzyl alcohol 5—6 days. The corresponding chromatograms are to be seen in Fig 1.

The spots are identified best by known control mixtures. The rates of advance can be given, *e. g.*, by comparing the final distances of the compounds from the starting line with that of the fastest moving one; these values can be easily read by the use of the scale in Fig. 1, left, but even they — although considerably more consistent than the so-called R_f values — have not been found to be very reliable. The use of one 'complete mixture' containing all compounds that can be in question and of several 'partial mixtures' as controls on the very paper on which the unknown mixture is run eliminates all errors. Such a set of controls is in Fig. 1, B.

RESULTS

Tert. amylalcohol (Fig. 1, B) has the best resolving power. Eight amino acids are completely separated as individual spots (*leucine*, *isoleucine*, *phenylalanine*, *methionine*, *valine*, *tryptophan*, *tyrosine*, and *proline*). *Hydroxyproline* can be identified by its yellowish colour only in the absence of threonine. *Threonine* and *alanine* are often partially separated allowing a sure identification. When there is a marked predominance of either of them, an individual identification is impossible. *Glycine* and *serine* form one spot in common. *Histidine* is not usually completely separated from glutamic acid but if it is present in abundance, it can be identified with certainty, because of its nice grey colour with ninhydrin. We have identified even minute amounts of histidine by using a duplicate chromatogram and the Pauly reaction, a more sensitive colour reaction for histidine than that with ninhydrin [4, cf. 2]; this reaction can be very easily made on filter paper and it is in this case quite specific, tyrosine being in another part of the chromatogram. Even *glutamic acid* can often be identified as in Fig. 1, B, but the other 5 amino acids form one complex spot.

n-Butanol (Fig. 1, C) has a resolving power nearly equal to *tert.* amylalcohol. After developing for eight days the first three amino acids have entered the pad (Fig. 1, D), the other amino acids being somewhat more effectively separated. This is advantageous especially if their quantitative determination [5] is in question.

When the original technique is used, one of the most effective solvent combinations in *two-dimensional* chromatography is perhaps phenol-collidine.

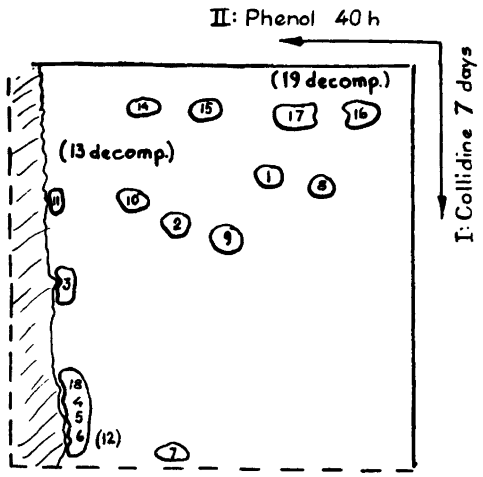

Fig. 2. Diagram of a two-dimensional chromatogram (abt. 50 by 50 cm) of 19 amino acids, obtained with phenol (40 h) and s-collidine (7 days). The absorbing pads torn away. Collidine run first.

By the new technique a somewhat more effective separation can be obtained in the direction of collidine when the development is continued for seven days. The leucines, phenylalanine, and methionine still form a complex spot but most of the other amino acids are completely separated from one another. Histidine and cystine are decomposed by collidine (Fig. 2.) This mode of technique is practical only if a one-dimensional test is made simultaneously with some alcohol and by the continuous development.

The new technique gives good possibilities for alcohols as solvents in two-dimensional chromatography. An example is given in Fig. 3. The location of some compounds not usually present in protein hydrolysates is marked by dotted lines in Fig. 3, A. — The use of alcohols as solvents is to be preferred especially in the quantitative paper chromatography [5] because they cause less decomposition of amino acids than solvents such as collidine, pyridine, *etc.*

GENERAL REMARKS

The great influence the 'reaction' of the system has on the flow of the amino acids has been shown already by Consden, Gordon, and Martin. For the sake of conformity in this respect the drops of hydrolysates and control mixtures have usually been put on the filter paper over an open Petri dish containing ammonia. Without this neutralization the chromatograms may have rather different structures. An example (benzyl alcohol) is to be seen in Fig. 1, E and F. The arrangement of the neutral amino-acids is changed. — In phenol lysine, arginine, and glutamic acid are the most sensitive ones.

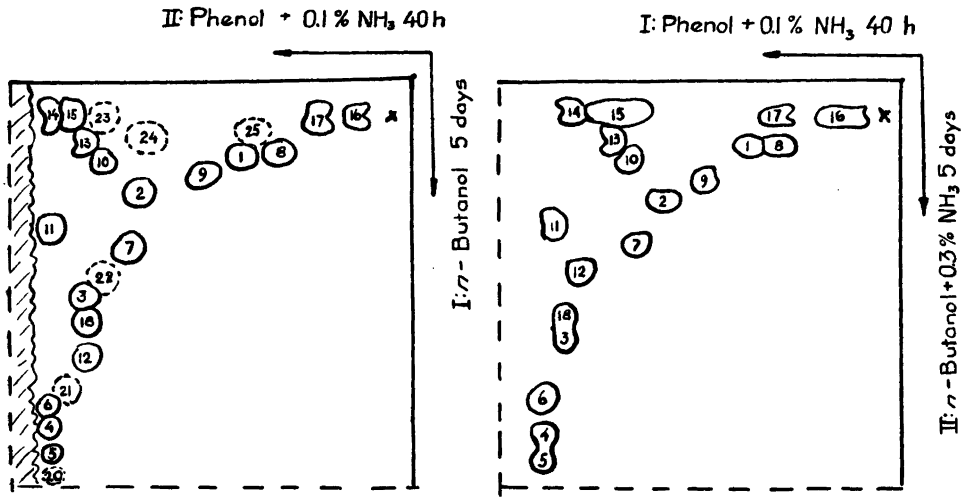

Fig. 3. Diagrams of two-dimensional chromatograms (abt. 40 by 40 cm) of amino acids obtained with phenol + 0,1 % NH_3 (40 h) and n-butanol (5 days). (A) Butanol run first; (B) butanol + 0.3 % NH_3 (5 days); phenol run first.

20. Norleucine *22. α-Amino-isobutyric acid* *24. Glutamine*
21. Norvaline *23. Ornithine* *25. Asparagine*

We have used exclusively Whatman no. 1 filter paper. The two-dimensional experiments have been made in a special constant-temperature room (20° C). Glass-sided boxes (aquaria) of 40 by 70 by 70 cm containing 4 throughs and automatic filling apparatuses have been used. The one-dimensional experiments have been made in stoneware drainpipes [1]; constant temperature is not necessary in this case. Ascending technique [6] has been found practical for preliminary experiments because it does not require much work. For instance, with isobutyric acid a short chromatogram is obtained in an over-night experiment giving a good idea of the complexity of the material to be analyzed.

SUMMARY

1. A 'continuous developing technique' in descending paper chromatography is described, in which a thick pad of cellulose tissue capable of absorbing a large amount of solvent is fastened at the foot of the strip of filter paper by stapling or sewing. The development is continued until the fastest moving compound nearly reaches the pad, or even longer. The identification is made by the use of several differently composed control mixtures.

2. Developing times for amino acids in various solvents and the structures of the corresponding chromatograms are given.

3. *Tert.* amylalcohol has the best resolving power: in 2 — 3 weeks 8 amino acids (leucine, isoleucine, phenylalanine, methionine, valine, tryptophan, tyrosine, proline) are completely separated from protein hydrolysates as individual spots.

4. Being relatively inert solvents the alcohols cause less decomposition than collidine, isobutyric acid, pyridine, *etc.*

We are indebted to Mr. U. K. Virtanen and Mr. T. Moisio for their assistance in this work.

ADDITION TO PROOF.

Using Whatman no. 4 filter paper the developing times are reduced to half of those above, but the spots are sometimes less sharp.

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Received April 6, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 465—473

A simple Equation for the Viscosity and the Rate Constant of High-polymer Substances under Depolymerization

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Several attempts have been made to evaluate simple formulas for the calculation of reaction constants from viscosity measurements on solutions of high-polymer substances subjected to depolymerization. Sillén [1, 2] has reviewed shortly the literature on this subject and has established a very inspiring mathematical treatment of this problem.

The present author, who has been interested in this subject in connection with his studies on viscosimetrical methods for the assay of enzymic activity [3, 4, 5] has made some attempts to attain further simplification for the calculation of rate constants from viscosity measurements, and the results will be given in this article.

THEORETICAL

Staudinger and Heuer [6] have established the following formula for the specific viscosity of diluted solutions of polymeric homologous substances:

$$\eta_{sp} = K_m c_{gm} M \tag{1}$$

where:

η_{sp} = the specific viscosity,
K_m = the viscosity-molecular weight constant,
c_{gm} = the concentration in basic moles per litre, and
M = the molecular weight.

This equation, however, is only valid for polymeric homologous substances whose viscosity is not influenced by the pH of the solution nor by the ionic strength of the various salts present. For other polymeric homologous sub-

stances, Kern [7] has introduced an ionic factor J. Hence a more correct expression for the specific viscosity of the solutions of these substances is:

$$\lim_{c_{gm} \to 0} \eta_{sp} = JK_m c_{gm} M \tag{2}$$

For the further mathematical treatment, the following notation is employed.

α = the degree of depolymerization (the number of broken linkages devided by the number of linkages at complete polymerization),
k = the rate constant for the break down of the linkages,
M_0 = the basic molecular weight,
N = the number of molecules per mole (Loschmidt's number),
N = the total number of basic molecules,
n = the number of basic molecules per molecule,
P_v = the degree of polymerization, determined viscosimetrically = $\frac{\Sigma n^2 z_n}{\Sigma n z_n}$,
R = the original number of basic molecules in the molecules,
t = the time,
V = the volume in liters,
z_n = the number of molecules with n basic molecules.

The rate constant

On observing that $M = nM_0$, and that for each degree of polymerization $c_{gm} = nz_n/NV$, we can write equation (2) in the following form:

$$\eta_{sp} = \frac{JK_m M_0}{NV} \sum_{n=1}^{R} n^2 z_n \tag{3}$$

The sum $\sum_{1}^{R} n^2 z_n$ has been calculated by Sillén [1] under the assumption that the molecules with different chain lengths have all originated by decomposition of one kind of molecule with the original number R of basic molecules. The break down is postulated to take place under such conditions that all linkages are broken with equal ease and that the rate constant is k. If $x = e^{-kt}$,

$$\sum_1^R n^2 z_n = N\left(\frac{1+x}{1-x}\right) - \frac{2\,Nx(1-x^R)}{R(1-x)^2} \tag{4}$$

and

$$\lim_{R \to \infty} \frac{\sum_1^R n^2 z_n}{N} = \frac{1+x}{1-x} \tag{5}$$

The following equation is obtained for the derivative $\frac{\mathrm{d}\frac{1}{\eta_{sp}}}{\mathrm{d}t}$:

$$\frac{\mathrm{d}\frac{1}{\eta_{sp}}}{\mathrm{d}t} = -\,k\,\frac{x\mathrm{d}\frac{1}{\eta_{sp}}}{\mathrm{d}x} = \frac{2\,kNV}{JK_m M_0 N} \cdot \frac{x}{(1+x)^2} \tag{6}$$

$$k = 2\,JK_m c_{gm} M_0 \frac{\mathrm{d}\frac{1}{\eta_{sp}}}{\mathrm{d}t} \cdot \frac{(1+x)^2}{4\,x} \tag{7}$$

The function $\frac{(1+x)^2}{4\,x}$ will in all cases — except where the depolymerization is followed extremely far — assume the value 1, which can be demonstrated as follows.

Equation (5) gives for the degree of polymerization

$$P_v = \frac{1+x}{1-x} \qquad \text{(Sillén 12')}$$

For $P_v = 19$ we get $x = 0.9$, and the function has the value 1.0028. For $P_v = 9$ we get $x = 0.8$, and the function has the value 1.0125. For $P_v = 4$, we get $x = 0.6$, and the function has the value 1.0667. Hence the function $(1+x)^2/4\,x$ may be omitted. If we assume the ionic factor to have the value 1, which also means that this factor is included in K_m, we have

$$k = 2\,K_m c_{gm} M_0 \frac{\mathrm{d}\frac{1}{\eta_{sp}}}{\mathrm{d}t} \tag{8}$$

From the form of this differential equation we gather that we get a straight line when plotting the inverse of the specific viscosity against time. We may also write equation (8) in the following form

$$k = 2\,K_m c_{gm} M_0 \frac{\Delta \frac{1}{\eta_{sp}}}{\Delta t} \tag{9}$$

and counting the time from the moment corresponding to the point where the straight line cuts the time axis, we may write

$$k = 2\,K_m c_{gm} M_0 \frac{\frac{1}{\eta_{sp}}}{t} \tag{10}$$

Equation (8) may also be directly calculated from Silléns equation (12'), for

$$\frac{\eta_{sp}}{K_m c_{gm} M_0} = P_v = \frac{1+x}{1-x} \tag{11}$$

and from this we get a derivative, identical with equation (6).

Sillén has the equation

$$k = \frac{1}{t} \ln \frac{P_v + 1}{P_v - 1} \qquad \text{(Sillén 55)}$$

which was calculated from Silléns equation (12'), and which seems to be a little more laborious, for the function $\frac{P_v + 1}{P_v - 1}$, where $P_v = \eta_{sp}/K_m c_{gm} M_0$ must be calculated for each measurement with great accuracy, and the logarithm must be looked up. The time must be corrected to be counted from the moment corresponding to the point where the function $\ln \frac{P_v + 1}{P_v - 1}$ cuts the time axis (Sillén 55 a). Instead of this time correction it is of course possible to count with the differences in the values of the function and the difference in the corresponding times. — The function $1/\eta_{sp}$, however, can easily be calculated on a slide rule with sufficient accuracy.

The degree of depolymerization

It follows from the definition of the degree of depolymerization and from the law of decomposition that

$$1 - \alpha = x \qquad \text{(Sillén 13)}$$

Combined with (11) this equation gives the following expression for the degree of depolymerization:

$$\frac{\alpha}{1 - \frac{\alpha}{2}} = 2 K_m c_{gm} M_0 \frac{1}{\eta_{sp}} \tag{12}$$

For low degrees of depolymerization it simplifies to

$$\alpha = 2 K_m c_{gm} M_0 \frac{1}{\eta_{sp}} \tag{13}$$

The inverse of the specific viscosity (or preferably the function c_{gm}/η_{sp}) is concequently directly proportional to the degree of depolymerization, and as it is a linear function of the time in depolymerization processes, calculations may be made very rapidly graphically by the break down procedure.

EXPERIMENTAL

Comparison between the formulas for the rate constant

The calculation of rate constants from formula (10) will be compared with the calculation using the more accurate formula (Sillén 55 a) in the following example.

Ekenstam[8, Table 65] investigated the break down of cotton in 65.1 % sulphuric acid at 20° C ($c_{gm} = 0.01267$; $K_m = 13.9 \cdot 10^{-4}$). From his viscosity measurements, Sillén [2] calculated the reaction constant. To Ekenstam's time notations Sillén added a constant, $t_0 = 20$ minutes, obtained by drawing the straight line of the function $\ln \frac{P_v + 1}{P_v - 1}$ plotted against the time until it cut the time axis. Sillén's values and the values obtainable with equation (10) from the corrected zero time, where $1/\eta_{sp}$ is assumed to have the value 0, to each of Ekenstam's measurements are given in Table 1.

To faciliate an accurate comparation of the mathematical differences obtained when the equations (Sillén 55 a) and (10) are employed for the calculation of the rate constant, the last 5 values, where the difference should be greatest, were computed to an accuracy far beyond any chemical significance. The differences, however, may be omitted even for the lowest degrees of polymerization in this experiment.

Table 1. Rate constants for the depolymerization of cotton in sulphuric acid, calculated by Sillén from his equation (55 a) and by the present author from equation (10).

$t + t_0$	η_{sp}	P_v	$k \cdot 10^6$	
			(55 a)	(10)
20	0.5009	175.6	570	570
26.6	0.3915	137.2	549	548
32.95	0.3191	111.9	544	543
39.3	0.2671	93.6	544	544
45.15	0.2356	82.6	536	536
62.85	0.1651	57.9	551	550
79.2	0.1308	45.85	550	551
143.3	0.0677	23.73	588.4	588.2
167.7	0.0557	19.53	611.2	610.8
224.0	0.0408	14.32	624.6	624.3
288.0	0.0315	11.04	630.8	628.9
366.0	0.0241	8.447	649.8	646.8

In Table 1 the rate constant was calculated for every viscosity measurement, and the time was thereby counted from the corrected zero time, which was obtained from a graph. For most practical purposes this way of calculation is unnecessarily time-consuming. It is simpler to plot the inverse of the specific viscosity against the uncorrected time, for which it is valid and draw a straight line through the points. The derivative of this line is easily read off. It should perhaps be mentioned that one can obtain the time, for which a viscosity measurement is valid, by adding half the flow time to the time when the measurement was commenced [9].

Experiments at different concentrations

From equation (8) we gather that the product of the concentration and the derivative $d\frac{1}{\eta_{sp}}/dt$ is constant. Equation (8) includes some postulations and approximations, and its applicability will be illustrated by the following experiment. This experiment is not selected from a larger series.

A solution of cellulose in sulphuric acid was prepared according to Smith's [10] directions (an application of Ekenstam's methods): 1 g of filter paper was treated with 10 ml of 61 % sulphuric acid (equal parts of water and concentrated sulphuric acid). After 5

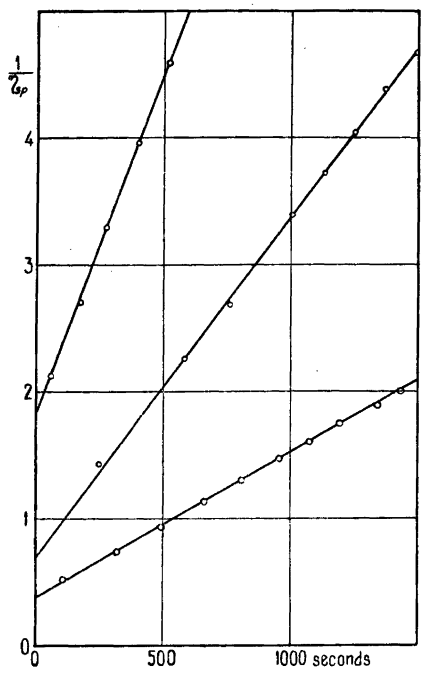

Fig. 1. Depolymerization of cellulose at 30° C in 67 % sulphuric acid.

minutes, the cellulose was dissolved on addition of 20 ml of 70 % sulphuric acid (1 part of concentrated sulphuric acid and 3 parts of 61 % sulphuric acid). From this original cellulose solution, two other solutions were prepared by dilution of 1 part of the original solution with 1 part and 3 parts respectively of sulphuric acid of the same concentration. The solutions were preserved in a refrigerator before the viscosity measurements. In this way the reaction velocities were retarded so much that the solutions could afterwards be easily measured, one after the other.

Table 2. Depolymerization of cellulose at 30° C in 67 % sulphuric acid. Time in seconds.

c_{gm}	$\mathrm{d}\frac{1}{\eta_{sp}}/\mathrm{d}t \cdot 10^3$	$\mathrm{d}\frac{1}{\eta_{sp}}/\mathrm{d}t \cdot c_{gm}$
0.206	1.15	0.237
0.103	2.66	0.274
0.0515	5.40	0.278

The viscosity measurements and the calculations were performed mainly in the same way as described previously by the present author[11]. The results are given in Figure 1 and summarized in Table 2.

In the original solution, the concentration seems to have been too high with regard to the degree of polymerization, for the value of the derivative is here too low. It is frequently observed in depolymerization experiments that Staudinger's formula, upon which the theoretical treatment is founded, is not valid for high concentrations and high degrees of polymerization. With continued depolymerization the agreement usually increases.

SUMMARY

1. For depolymerization processes the following differential equation is established: $k = 2\,K_m c_{gm} M_0 \cdot \mathrm{d}\frac{1}{\eta_{sp}}/\mathrm{d}t$, where k = the rate constant, K_m = the viscosity-molecular weight constant, c_{gm} = the concentration of basic moles per litre, M_0 = the basic molecular weight, n_{sp} = the specific viscosity and t = the time, for which the viscosity measurement is valid. When the inverse of the specific viscosity is plotted on square paper against the time, the points will lie on a straight line, the slope of which gives the derivative.

2. If the time is counted from the moment corresponding to the point, where this straight line cuts the time axis, the equation simplifies to $k = 2\,K_m c_{gm} M_0 \cdot \frac{1}{\eta_{sp}}/t$.

3. The degree of depolymerization (the number of broken linkages divided by the number of linkages at complete polymerization) is approximately $\alpha = 2\,K_m c_{gm} M_0 \cdot \frac{1}{\eta_{sp}}$.

4. The theory is exemplified by some experiments on the depolymerization of cellulose in sulphuric acid. When the cellulose concentration and its degree of polymerization are high, the experimental values differ somewhat from those calculated from the formulas.

For this investigation the author received financial support from *Statens Naturvetenskapliga Forskningsråd*. Prof. Karl Myrbäck kindly granted me the use of his laboratories. The English translation was revised by Mrs William Cameron. For all this help I wish to express my cordial thanks.

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Received April 9, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 474—480

Stereochemistry of 5-Coordinated Compounds

II.* On the Configuration of the Nickel Compound $[NiBr_3(Et_3P)_2]$

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By adding trialkylphosphines to nickel halogenides Jensen[1] in 1936, prepared red compounds of the type $[NiX_2(R_3P_2)]$. These compounds add halogens and NO_2 to form dark-green compounds; the latter are rather unstable, but by the isolation of the compound $NiBr_3,2Et_3P$ they could be shown to contain formally tervalent nickel. The compound $NiBr_3,2Et_3P$ is easily soluble in organic solvents, could be recrystallized from petroleum ether and forms dark violet-black crystals with melting point 83—84°. By determination of the molecular weight in benzene solution the compound was shown to be monomolecular. The formulation of the compound as a binuclear complex with bi- and quadrivalent nickel may therefore be excluded. It is unlikely that the compound is a polybromide or that part of the bromine is bound to triethylphosphine since Et_3PBr_2 does not combine with nickel halogenides; it was, therefore, concluded that the compound in fact contains tervalent nickel.

This conclusion has now been substantiated by magnetic measurements. Values ranging from 1.72 to 1.90 Bohr magnetons were found for the magnetic moment, the lowest values being found just after preparation. This is in good agreement with the value computed for tervalent nickel with covalent bonds (one unpaired electron), *viz.* 1.73 Bohr magnetons, assuming that the magnetism is determined by spin alone, the orbital moment being negligible.

By the magnetic measurements on the solid substance the possibility that the complex might be binuclear (the molecular weight of the dissolved substance being caused by dissociation) is definitely ruled out, as such a compound would be diamagnetic.

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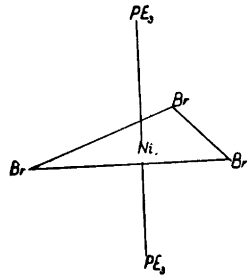
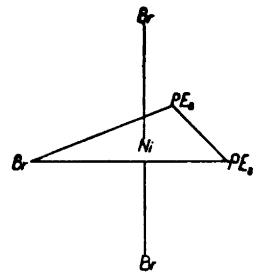
* As no. 1 of this series we wish to consider the paper *Z. anorg. u. allgem. Chem.* **250** (1943) 257.

The compound $NiBr_3,2Et_3P$ accordingly contains tervalent nickel and moreover is one of the rare compounds in which the central atom has coordination number five. For some of the compounds of this type (*e. g.* PF_5) the configuration has been shown to be that of a trigonal bipyramid (*cf.* Jensen [2]). As the compound here discussed is formed by the addition of bromine to the compound $NiBr_2(Et_3P)_2$ which has a trans-planar configuration, it seems possible on the other hand, that it might have the configuration of a tetragonal pyramid (IV).



I.



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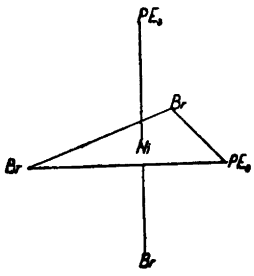

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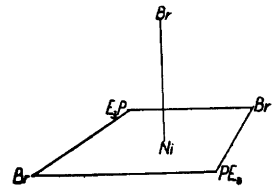
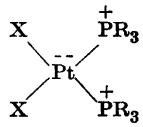


IV.

A choice between these possibilities may be made by means of dipole moment measurements. Because of the instability of the compound $[NiBr_3(Et_3P)_2]$ it is not possible to determine a very exact value for its dipole moment, but we have found a value of about 2.5 D, which at any rate indicates the order of magnitude. If the compound has the configuration of a trigonal bipyramid there are three possibilities for its structure I, II and III. In the first the bond moments compensate each other, and a compound with this structure should therefore have zero dipole moment (like the compounds SbR_3X_2). In the two other cases the two triethylphosphine molecules are in cis positions, and to judge from platinum complexes of the type $[PtX_2(R_3P)_2]$ with two trialkyl-

phosphine molecules in cis position [3] the moment should be in the order of 7—10 D. These large moments are not due to a large moment of the Pt-halogen bonds, but to large moments of the Pt—P bonds, the phosphorus atoms having a large positive charge, when they are coordinated to a metal atom, so that the structure approaches the formula:



The possibility that the configuration of $[NiBr_3(Et_3P)_2]$ might be that of a trigonal bipyramid can therefore be excluded. The configuration of a tetragonal pyramid, on the other hand, is in good agreement with the dipole moment found. With this configuration the ligands in the base are in trans position and the bond moments accordingly compensate each other, so that the dipole moment of this compound should only be that of the Ni-Br bond. One should not expect this to be very large; on the contrary the value 2.5 D seems very plausible. This comparatively low value excludes the possibility that the nickel atom might be situated in the centre of a tetragonal pyramid; because of the large values of the Ni-P bond moment this configuration would give a much higher value for the dipole moment. It is of course not possible to exclude the possibility that the nickel atom is raised somewhat above the base, but the deviation can only be of the order of magnitude of 0.1—0.2 Å (*cf.* Jensen [4]).

It is of great interest that this configuration, which has not hitherto been found for any complex, was predicted by Daudel and Bucher [5]. These authors conclude from quantum mechanical calculations that compounds of the type XY_5 should have the trigonal bipyramidal structure if the bonds involved are formed by means of s-, p- and d-electrons having the same principal quantum number, but the tetragonal pyramidal structure if the dsp-bonds are formed by means of d-electrons having a lower and s- and p-electrons having a higher principal quantum number, as in the case of tervalent nickel, where the bonds are formed from 3d, 4s and 4p orbitals. The bonds may be dsp^3 bonds; the odd electron may, however, also occupy a 4p orbital, so that the bonds are d^2sp^2 bonds.

The problem of the bond arrangement for the coordination number 5 has also been treated by Kimball [6] from a group theory point of view. Kimball comes to the conclusion that the trigonal bipyramid is the stable configuration for dsp^3 bonds, whether the d orbital has the same principal quantum number as the s- and p-orbitals or a lower one. For the configuration d^2sp^2 the bonds,

according to Kimball, are directed from the centre to the corners of a tetragonal pyramid. According to the theory of Daudel and Bucher, on the contrary, the central atom is situated in the centre of the base in the case of the tetragonal pyramidal configuration. Our measurements, therefore, are in better accordance with the predictions of Daudel and Bucher than with those of Kimball.

The theory of Daudel and Bucher generally predicts the tetragonal pyramidal structure for compounds with dsp^3 bonds; this structure should therefore also be possible for compounds of bivalent nickel and copper, although in this case the fifth bond should only be weak. Bjerrum [7] has shown that the diethylendiamine cupric ion in strongly ammoniacal solution takes up an ammonia molecule with the formation of a pentammine complex $[Cuen_2NH_3]^{++}$, and in the triethylendiamine cupric ion $[Cuen_3]^{++}$ one of the ethylendiamine molecules is only bound by one amino group to the cupric ion. The configuration of these complexes is not known, but as the 4-coordinated cupric complexes has a planar configuration [8], it seems very plausible that the fifth bond is perpendicular to the plane formed by the first four ligands.

It may further be predicted from the theory of Daudel and Bucher that iron pentacarbonyl should have the tetragonal pyramidal structure, the bonds being dsp^3-bonds, while Kimball expects the bipyramidal structure. The dipole moment of $Fe(CO)_5$ was found by Graffunder and Heymann [9] and by Bergmann and Engel [10] to be greater than zero, but may be zero, if the atomic polarisation is properly allowed for. Duncan and Murray [11] attempted to obtain Raman spectra of iron pentacarbonyl, but without success. Ewens and Lister [12], however, conclude from electron diffraction measurements that the molecule has the trigonal bipyramidal structure. This evidence can hardly be considered as conclusive, and it would be of great importance to get an exact determination of the structure of iron pentacarbonyl.

EXPERIMENTAL

$[NiBr_2(Et_3P)_2]$. To an ice-cold solution of 4 g of anhydrous nickel bromide in 50 ml of ethanol were added 2.30 g of triethylphosphine. Ruby-red crystals separated at once. The crystals were filtered off and washed with a little ethanol and dried over P_2O_5 (at room temperature and atmospheric pressure). Yield 5.3 g = 85 %. M. p. 103—105°. For purification 2.5 g were dissolved in 35 ml of dry petroleum ether, the solution was filtered and concentrated in vacuo without heating. Large, dark violet-red crystals separated. Yield 1.14 g. M. p. 106—107°.

$[NiBr_3(Et_3P)_2]$. To a solution of 2.5 g of $[NiBr_2(Et_3P)_2]$ in 4.5 ml of benzene (dried over sodium) was added 0.45 g of bromine dissolved in 1.5 ml of benzene. The dark-red solution turned dark-green. The benzene was removed as fast as possible in vacuo without

heating (the receiver was cooled with solid carbon dioxide + ethanol). The residue was dissolved in 25 ml of petroleum ether (dry), the solution was filtered and evaporated in vacuo at 0°. Glittering violet-black crystals separated. The crystals were transferred to a desiccator with P_2O_5 and paraffin, but measurements thereon must be carried out as quickly as possible. In the course of two hours it generally decomposed with evolution of hydrogen bromide and triethylphosphine and formation of nickel bromide. Yield 1.10 g = 63 %. M. p. 83—84°.

Calc.	Br 44.83,	Ni 10.98
Found	» 44.66 (Volhard)	» 10.83 (as dimethylglyoxim nickel)

Magnetic measurements

The magnetic measurements were performed by the Gouy method. After some orienting measurements (which gave values from 1.78 to 1.90 Bohr magnetons) the preparation, of which the analysis is given above, was measured as quickly as possible after isolation (T = 293.3° K):

Field strength in Ørsted	3750	3750	6970	6970	6970
$\chi_g \times 10^6$	2.33	2.35	2.62	2.62	2.63
$\chi_M \times 10^6$	1246	1256	1400	1400	1406
$\mu_{eff} = 2.84\sqrt{T \times \chi_M}$	1.72	1.72	1.82	1.82	1.82

The higher values at the higher field strenght are probably due to the onset of decomposition during the first measurements. The paramagnetism increases considerably with the time.

The first values are in close agreement with the value 1.73 Bohr magnetons, calculated for one unpaired electron. The accordance is of course much better than could be expected, because the values of χ_M are not corrected for the diamagnetic susceptibility (χ_M = about − 300), nor for the Curie temperature. The first correction would change the value of μ_{eff} from 1.72 to 1.91, but a positive Curie temperature might compensate for this. No attempt was made to determine the Curie temperature by measurements at lower temperature; these measurements would namely have to be carried out on different samples, on account of the instability of the compound and the time involved in the measurements, and the difference between the susceptibilities of different samples might completely compromise this determination. The value 1.91 of μ_{eff} may be real and be caused by the start of decomposition or be due to an orbital contribution to the moment. It was not deemed of importance to investigate this question further, because the tervalency of the nickel atom has been proved, whether the value 1.72 or 1.91 be accepted.

Dipole moment

The measurement of the dielectric constants of solutions of $[NiBr_3(Et_3P)_2]$ in benzene was carried out in an apparatus from Kipp and Zonen (*cf.* Jensen and Friediger [13]). The measurements were carried out as fast as possible (ca. 15 min.) after the isolation of

the compound; it slowly decomposed in solution as indicated by an increase in the dielectric constant of the solution in the course of some minutes. This decomposition goes more quickly in dilute solutions and in these a deposite of nickel bromide separates in the course of 1—2 hours. Extrapolation of the dielectric constants found to the concentration 0 is therefore probably without meaning, and the best values are those found in the strongest solutions, which contain about 10 % of the complex. It has previously been found by cryoscopic measurements that the compound is monomolecular in benzene.

Because of the very intensive colour of the solutions, the refractive index could not be determined, and would also be valueless because of anomalous dispersion. The molecular polarizations were therefore calculated from the dielectric constants only, using the formula [14]:

$$P_M = \frac{1000}{c}\left[\frac{\varepsilon_{12}-1}{\varepsilon_{12}+2} - \frac{\varepsilon_1-1}{\varepsilon_1+2}\right]$$

where c is the *molar* concentration of the solute, ε_{12} the dielectric constant of the solution and ε_1 the dielectric constant of the solvent, benzene (2.2725 at 25°). For large dipole moments this formula gives values almost identical with those calculated from the formula $P_M = P_O - P_E - P_A$, and even for moments about 2 D the deviation is only a few percent.

After some orienting measurements, in which values of about 2.5 D were obtained, the following measurement was performed:

c	$\Delta\varepsilon$	ε_{12}^{25}	P_M
0.0297	0.0244	0.2969	134.3
0.0624	0.0443	0.3168	115.4
0.1333	0.0914	0.3639	110.4

From the lowest value of P_M, which is probably the best, the value $\mu = 2.30$ D is calculated. The value of P_M extrapolated to the concentration 0, *viz.* 150, gives $\mu = 2.68$ D. We may therefore safely conclude that the dipole moment of $[NiBr_3(Et_3P)_2]$ is not greater than $\mu = 2.7$ D and not much lower than 2.3 D.

SUMMARY

The magnetic moment and dipole moment of the compound $[NiBr_3(Et_3P)_2]$ have been measured. According to these measurements the compound contains tervalent nickel and has the configuration of a tetragonal pyramid.

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Received April 8, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 481—486

Nickel Compounds of Aminoguanidine, Diaminoguanidine and Triaminoguanidine

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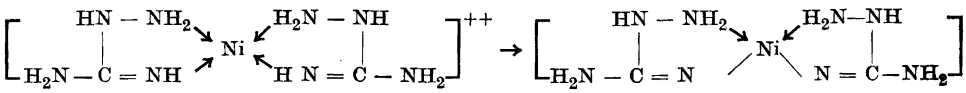
In the course of investigations conducted in 1934—36 on coordination compounds of thiosemicarbazide and semicarbazide[1] it was noted that aminoguanidine also is able to combine with nickel sulphate to give a red complex salt, $[Ni(CH_6N_4)_2]SO_4$, (this was later described by Stanley Smith [2]). By addition of sodium hydroxide to this compound, sulphuric acid was split off, and another red compound, presumably an inner complex salt, was formed. Whereas the corresponding derivatives of thiosemicarbazide and semicarbazide are quite stable, the red aminoguanidine compound rapidly turned black during attempts to filter and decomposed with the evolution of nitrogen and ammonia. It was obvious that this transformation was due to oxidation, and the blackening of the red compound could also be effected by the addition of hydrogen peroxide to a suspension of the compound in water. This phenomenon has now been subjected to a closer examination.

When oxygen is very carefully excluded, it is possible to isolate the above-mentioned red compound. The preparation, filtration and drying must be performed in an atmosphere absolutely free of oxygen. When dry, the compound is somewhat less susceptible to oxidation, but in the course of some hours it generally turns black.

The analyses of the red compound indicate the composition $Ni(CH_5N_4)_2$, and accordingly it is an inner complex compound derived from the cation of the red sulphate by the splitting off of two hydrogen ions:



According to the theory of Pauling [3], compounds of this type must have a planar configuration and must be diamagnetic. Measurements of the magnetism showed the sulphate to be diamagnetic; for the inner complex compound small paramagnetic values were found, but as the compound is very unstable and the black oxidation product is strongly paramagnetic, there is a little doubt that the pure compound really is diamagnetic.

The oxidation of the inner complex compound (suspended in water) in an atmosphere of oxygen was examined in the Van Slyke apparatus for gasometric determination of amino nitrogen. There was only a small change in the volume, because nitrogen was split off during the oxidation. The resulting gas was then analysed for nitrogen by passing it through a tube with hot copper filings and measuring the gas in an azotometer. Judging from these results, the oxidation mainly proceeds in accordance with the following equation:

$$[Ni(CH_5N_4)_2] + 2O_2 + H_2O \rightarrow NiCO_3 + 4NH_3 + CO_2 + 2N_2$$

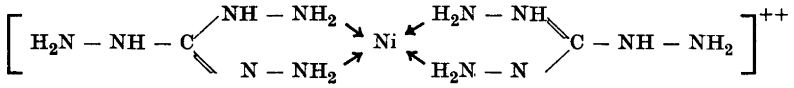
Almost all of the ammonia and carbon dioxide remains in the water. Part of the ammonia is bound to the nickel carbonate. A small trace of the nickel, however, forms nickel peroxide, which liberates iodine from potassium iodide in acidic solution. The reaction product further contains a trace of cyanide. It was shown that oxidation of aminoguanidine with potassium permanganate mainly proceeds in the same way. In that case, also, a small amount of cyanide is formed, especially in alkaline solution. The main process, however, is:

$$CH_6N_4 + O_2 \rightarrow CO_2 + 2NH_3 + N_2$$

We think that the nickel atom functions as a catalyst during the oxidation in the following way: The nickel complex derived from divalent nickel is oxidized to a nickel compound with tervalent or quadrivalent nickel (in the last case a 6-coordinated complex, $[Ni(CH_5N_4)_2(OH)_2]$, might be formed), but it is unstable and in its turn oxidizes aminoguanidine to ammonia and carbon dioxide. This hypothesis receives some support from the fact that the black compound liberates iodine from iodide and accordingly contains some nickel with a higher valency than two. It is remarkable that the compound should be black, as it only contains a trace of ter- or quadrivalent nickel. It has, however, been shown by le Blanc and Sachse [4] that NiO containing a few per cent Ni_2O_3 is quite black. This has been explained by de Boer and Verwey [5] by suggesting the possibility of electron transmission across the whole crystal lattice as soon as there is some deviation from the stoichiometric composition NiO.

With diaminoguanidine nickel sulphate forms complexes quite analogous to the derivatives of aminoguanidine, viz. a red, slightly soluble sulphate, $[Ni(CH_7N_5)_2]SO_4$, and a red inner complex salt, $[Ni(CH_6N_5)_2]$, which is still more easily oxidable than the aminoguanidine compound. In this case, too, the sulphate was found to be diamagnetic, while for the inner complex salt a slight paramagnetism was found, which however, without a doubt is due to beginning decomposition.

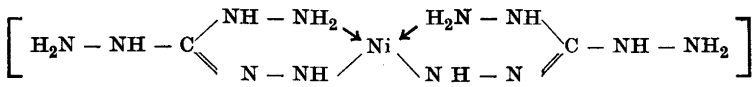
Finally we have investigated the reaction of nickel salts with triaminoguanidine. To judge from the change of colour to red-violet, a complex is formed by the addition of triaminoguanidine to a solution of a nickel salt, but the sulphate is easily soluble. The explanation of this difference is perhaps that in this case a six-membered ring may be formed:



or that triaminoguanidine behaves as a tridentate group, so that a 6-coordinated complex is formed.

By the addition of an excess of sodium hydroxide or potassium hydroxide to the above-mentioned solution, a red inner complex compound, $[Ni(CH_7N_6)_2]$, is formed, as in the case of mono- and diaminoguanidine. The triaminoguanidine compound is somewhat soluble in water. It is still more easily oxidable than the two lower homologues. In a glass tube with a well-fitting stopper it may be kept for some time without decomposition, but as soon as the stopper is removed the oxidation starts with perceptible evolution of heat; when the stopper is replaced, the evolution of heat stops, and the content soon regains room temperature. This experiment may be repeated several times. When the red compound is filtered on a Büchner-funnel in air, the heat evolution is so great that the compound deflagrates. (The corresponding mono- and diaminoguanidine compounds also deflagrate on gentle heating).

By making magnetic measurements just after the preparation, the triaminoguanidine compound was found to be practically diamagnetic. This circumstance, in addition to the colour of the compound, indicates that the nickel atom is 4-covalent, not 6-covalent and that the complex has the square configuration. Presumably a six-membered, not a five-membered, ring is formed, so that the formula of the complex is the following:



Several resonance forms of this formula are of course possible.

EXPERIMENTAL

Aminoguanidine compounds

$[Ni(CH_6N_4)_2]SO_4$. Aminoguanidine sulphate[6] (3.5 g = 0.02 mole) and nickel sulphate (2.9 g = 0.01 mole) were dissolved in 50 ml of water. Upon the addition of sodium hydroxide the green solution at first turned blue, but when the amount equivalent to the aminoguanidine sulphate (10 ml of 2 *N* NaOH) had been added, a red precipitate separated. This was filtered off on a Büchner-funnel, washed with water and ethanol, and dried at 50—60°. The compound is a heavy, brick-red, crystalline powder, which is almost insoluble in water. Yield 3 g ~ 100 %.

Calc.	Ni 19.37	N 37.00	
Found	» 19.15 (as dimethylglyoxime-nickel)	» 36.60	(Micro-Dumas)
Magnetism:	$\chi_g = -0.35 \times 10^{-6}$ (3750 Ørsted and 21.5°)		
	$\chi_g = -0.33 \times 10^{-6}$ (6970 Ørsted and 21.5°)		

$[Ni(CH_5N_4)_2]$. As this compound is extremely sensitive to oxygen, the preparation and filtering were carried out in an atmosphere of nitrogen. The synthesis was performed in an apparatus similar to that employed by Steinkopff[7]. Oxygen must be removed from the nitrogen employed by passing the nitrogen over copper filings. It is important that all solvents be thoroughly boiled to expel all air.

To a solution of 1 g of aminoguanidine sulphate and 0.9 g of $Ni(NO_3)_2$, $6H_2O$ in 25 ml of water and 25 ml of 10 *N* NaOH were added. A red solution was formed, and then almost instantaneously a red precipitate separated, which was filtered as quickly as possible and thoroughly washed with water, alkohol, and ether (dried over sodium).

In one case we succeeded in preparing a sample which gave correct values for nickel and nitrogen:

Calc.	N 54.7	Ni 28.6
Found	» 55.6	» 28.3

In most cases, however, the compound turned more or less black during drying and then gave smaller values for the nitrogen content.

We did not succeed in preparing a diamagnetic sample of this compound. For all preparations a weak paramagnetic susceptibility, varying from 1×10^{-6} to 2×10^{-6} ($\chi_M = 200-400 \times 10^{-6}$) was found. When the preparations turn black, the paramagnetism is increased and approaches the theoretical values for Ni^{++}, calculated from nickel analyses. As the red compound is very unstable, we conclude that the pure compound must be diamagnetic.

Diaminoguanidine compounds

$[Ni(CH_7N_5)_2]SO_4$. A dilute (about 0.1 N) solution of sodium hydroxide was added dropwise to a solution of 1 g of diaminoguanidine-hydrobromide[8], 0.25 g of $Ni(NO_3)_2$, $6H_2O$ and 1 g of Na_2SO_4, $10H_2O$ in 10 ml of water (tepid) until the green colour turned violet. By rubbing with a spatula and heating, a dark-red, crystalline substance was precipitated. This was filtered, washed with water, ethanol, and ether, and dried in vacuo over H_2SO_4.

Calc. Ni 17.63 N 42.09
Found » 17.56 » 42.17
Magnetism: $\chi_g = -0.02 \times 10^{-6}$ (6970 Ørsted and 24.1°)

$[Ni(CH_6N_5)_2]$. The compound was prepared with the same precautions as employed in the preparation of the monoaminoguanidine compound: 1.70 g of diaminoguanidine-hydrobromide and 0.30 g of nickel nitrate were dissolved in 30 ml of water and a concentrated solution of 8 g of sodium hydroxide was added. At first the solution turned dark-red, then a red, crystalline precipitate separated. The compound is very unstable, and although it was analysed just after preparation, we were not able to obtain values in exact agreement with the theoretical ones:

Calc. Ni 25.00 N 59.7
Found » 23.2—23.7 » 58.0—59.1

In air the compound — especially when moist — soon darkens and smells of ammonia.

By measuring magnetically, small paramagnetic values were found ($\chi_g = 2-3 \times 10^{-6}$). When the substance turns black, the paramagnetism increases. At the same time the nitrogen content falls to about 30 %. The nickel content of the black preparations was between 21 and 28 %. From corresponding values of the paramagnetic susceptibility and the nickel content, it was calculated that the values of μ_{eff} increases until about 2.5.

Triaminoguanidine compound

$[Ni(CH_7N_6)_2]$. Upon the addition of triaminoguanidine hydrochloride to a solution of nickel sulphate or nickel nitrate and neutralization, the colour turns red-violet; the complex formed could not be isolated. Upon the addition of sodium hydroxide in excess, a red solution is obtained and from this a pink crystalline precipitate separated: To a solution of 1.40 g of triaminoguanidine hydrochloride and 0.50 g of nickel nitrate in 50 ml of water a solution of 8 g NaOH in 50 ml of water was added. Pink needles separated. The compound is somewhat soluble in water, and the washing water was pink. In 50 % water-alcohol it is almost insoluble. When potassium hydroxide was employed instead of sodium hydroxide, the crystals had a darker red colour; the compound did not contain alkali. During the preparation and isolation of the compound, oxygen was carefully excluded. In air the compound soon gets warm and sometimes deflagrates.

Calc. Ni 22.15 N 63.5
Found » 21.60 » 51.7—52.9

As in the case of the other two inner complex salts, the samples of this compound were found to be weakly paramagnetic.

SUMMARY

Nickel compounds containing mono-, di- and triaminoguanidine have been prepared, *viz.* $[Ni(CH_6N_4)_2]SO_4$, $[Ni(CH_7N_5)_2]SO_4$, $[Ni(CH_5N_4)_2]$, $[Ni(CH_6N_5)_2]$ and $[Ni(CH_7N_6)_2]$. The three last-mentioned compounds were formulated as

inner complex salts. Magnetic measurements indicate that these compounds have a square configuration. They are highly oxidable and are quickly decomposed in air under the formation of nitrogen, ammonia, and carbon dioxide. It is supposed that the oxidation is initiated by the formation of a complex with tervalent or quadrivalent nickel.

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Received April 8, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 487—492

Über einige N-substituierte Dimethyl-diamino-silane und verwandte Verbindungen

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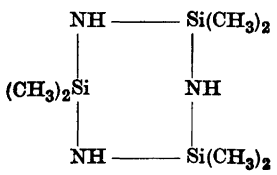
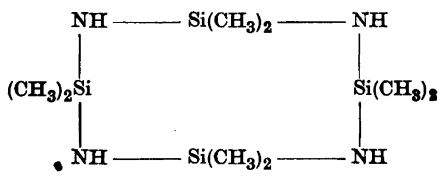
Vor etwa einem Jahre war in der Literatur keine Verbindung beschrieben, in der ein Siliziumatom gleichzeitig zwei Kohlenstoffatome und zwei Aminostickstoffatome band. Wir haben daher einige solche Verbindungen darzustellen versucht, indem wir Dimethyl-dichlor-silan mit Ammoniak und einigen primären Aminen reagieren liessen. Die ersten Resultate dieser Untersuchungen konnte der eine (E. L.) von uns in einem Vortrag vor den chemischen Gesellschaften in Lund und Kopenhagen mitteilen, der im Mai 1948 in Kopenhagen gehalten wurde.

Neulich haben Brewer und Haber [1] u. a. die Reaktionen zwischen einigen Alkyl-dichlor-silanen bzw. Dialkyl-dichlor-silanen und Ammoniak studiert. Dieses veranlasst uns im folgenden einige unserer bisherigen Resultate mitzuteilen. Die Resultate von Brewer und Haber über die Reaktion zwischen Dimethyl-dichlor-silan und Ammoniak stimmen mit den unsrigen überein. Dieser Teil unserer Arbeit wird daher im folgenden nur vorübergehend behandelt.

Beim Einleiten von Ammoniak in eine Lösung von Dimethyl-dichlor-silan in Benzol (Brewer und Haber), Diäthyl-äther (Larsson und Smith) sowie Kohlenstofftetrachlorid (Larsson und Smith) oder beim Hinzutropfen von Dimethyl-dichlor-silan zu kaltem, flüssigem Ammoniak erhält man teils Hexamethylcyclotrisilazin $[(CH_3)_2SiNH]_3$ (I), teils Octamethylcyclotetrasilazin $[(CH_3)_2SiNH]_4$ (II):



I



II

Diese beiden Verbindungen werden durch Wasser hydrolysiert. Brewer und Haber bestimmten den Stickstoffgehalt in der Weise, dass die Verbindungen mit verdünnter Salzsäure einige Stunden digeriert wurden, wonach die verbrauchte Säure durch Titration mit Alkali ermittelt wurde. Wir haben die Verbindungen in wässerigem Äthylalkohol oder Aceton gelöst und dann direkt mit Salzsäure titriert.

Wenn man Dimethyl-dichlor-silan zu Methylamin oder Äthylamin in dem Molverhältnis 1 : 4 in Diäthyläther oder Benzollösung zutropft, erhält man als Hauptprodukte Dimethyl-bis-(N-methylamino)-silan $(CH_3)_2Si(NH \cdot CH_3)_2$ (III) bzw. Dimethyl-bis-(N-äthylamino)-silan $(CH_3)_2Si(NH \cdot C_2H_5)_2$ (IV). Daneben werden kleine Mengen von noch nicht näher Untersuchten Produkten gebildet, die wahrscheinlich die den I und II entsprechenden Verbindungen $[(CH_3)_2 SiN \cdot CH_3]_n$ (V) bzw. $[(CH_3)_2SiN \cdot CH_2 \cdot CH_3]_n$ (VI) und die nichtcyclischen Verbindungen $CH_3 \cdot NH \cdot Si(CH_3)_2 \cdot N(CH_3) \cdot Si(CH_3)_2 \cdot NH \cdot CH_3$ (VII) bzw. $CH_3 \cdot CH_2 \cdot NH \cdot Si(CH_3)_2 \cdot N(CH_2 \cdot CH_3) \cdot Si(CH_3)_2 \cdot NH \cdot CH_2 \cdot CH_3$ (VIII) enthalten.

Dasselbe Resultat erhält man wenigstens bei dem Äthylamin, wenn das Äthylamin zu dem Dimethyl-dichlor-silan im Molverhältnisse 4 : 1 zugetropft wird.

Die Verbindung IV kann ohne Veränderung bei Atmosphärendruck destilliert werden. Die Verbindung III spaltet zum Teil bei einer solchen Destillation Methylamin ab, wobei wahrscheinlich z. B. V und/oder VII gebildet werden dürften.

Wenn eine Lösung von Dimethyl-dichlor-silan in Kohlenstofftetrachlorid zu Anilin in demselben Lösungsmittel (Molverhältnis 1 : 4) zugesetzt wurde, wurde nur die Verbindung $(CH_3)_2Si(NH \cdot C_6H_5)_2$ (IX) erhalten.

In den oben erwähnten Versuchen wurden auf 1 Mol Dimethyl-dichlor-silan 4 Mole Amin (Methylamin, Äthylamin oder Anilin) verwendet. Diese Aminmenge genügt zu der Umsetzung

$$4R \cdot NH_2 + (CH_3)_2SiCl_2 \rightarrow (CH_3)_2Si(NH \cdot R)_2 + 2R \cdot NH_3Cl$$

Bei der Verwendung von einer geringeren Aminmenge sind andere Reaktionen stöchiometrisch denkbar, z. B.

$$2R \cdot NH_2 + (CH_3)_2SiCl_2 \rightarrow (CH_3)_2SiCl(NH \cdot R) + R \cdot NH_3Cl$$
$$3nR \cdot NH_2 + n(CH_3)_2SiCl_2 \rightarrow [(CH_3)_2SiNR]_n + 2nR \cdot NH_3Cl$$

In einem Versuch mit dem Molverhältnis 3 : 1 zwischen Äthylamin und Dimethyl-dichlor-silan wurden keine nennenswerten Mengen von Dimethyl-

bis-(N-äthylamino)-silan gebildet. Es wurden höher siedende Fraktionen erhalten, aus welchen keine reine Verbindung isoliert werden konnte.

In einem Versuch, wo 3 Mol Anilin pro Mol Dimethyl-dichlor-silan verwendet wurden, konnte aus dem Reaktionsgemisch eine geringe Menge der cyclischen Verbindung $[(CH_3)_2SiN \cdot C_6H_5]_3$ (X) isoliert werden. Als Hauptprodukt hatte sich wahrscheinlich die Verbindung IX gebildet.

Die beiden Verbindungen IX und X sind bei Zimmertemperatur fest. IX ist in den gewöhnlichsten Lösungsmitteln leichtlöslich, scheint aber in heissen Lösungen zersetzt zu werden. X ist dagegen sehr schwerlöslich.

Durch Umsatz von Dimethyl-bis-(N-äthylamino)-silan mit Anilin, Benzylamin und Heptylamin wurden Dimethyl-bis-(N-phenylamino)-silan, Dimethyl-bis-(N-benzylamino)-silan und Dimethyl-bis-(N-heptylamino)-silan in guten Ausbeuten dargestellt. Aus Octamethylcyclotetrasilazin und Anilin wurde Dimethyl-bis-(N-phenylamino)-silan dargestellt. Diese Umsetzungen entsprechen denjenigen zwischen Triäthyl-(N-äthylamino)-silan und Aminen, die Larsson [2] untersucht hat.

In der Tabelle 1 sind die Dichten d^{20}, Brechungsexponenten n_D^{20} (D-Linie) und Molekularrefraktionen MR_D^{20} (D-Linie) bei 20,0° für einige der dargestellten Verbindungen zusammengestellt. Bestimmungen an Dimethyl-bis-(N-methylamino)-silan sind nicht mitgenommen, da ihre Ausführung von der Luftfeuchtigkeit gestört wurde.

Tabelle 1. Dichten, Brechungsexponenten und Molekularrefraktionen für einige der dargestellten Verbindungen.

	d^{20}	n_D^{20}	MR_D^{20}
$(CH_3)_2Si(NH \cdot C_2H_5)_2$	0,8067	1,4151	45,4
$(CH_3)_2Si[NH \cdot (CH_2)_6 \cdot CH_3]_2$	0,8297	1,4425	91,5
$(CH_3)_2Si(NH \cdot CH_2 \cdot C_6H_5)_2$	1,0090	1,5409	84,2

BESCHREIBUNG DER VERSUCHE

Dimethyl-bis-(N-methylamino)-silan (III)

27,1 g (0,21 Mol) Dimethyl-dichlor-silan in 30 ml abs. Diäthyläther wurden zu einer Lösung von 31,1 g (1,0 Mol) Methylamin in 200 ml abs. Diäthyläther hinzugetropft, wobei das Reaktionsgemisch auf − 15° gekühlt wurde. Nachdem alles Dimethyl-dichlorsilan zugegeben worden war, wurde das Reaktionsgemisch während 45 Minuten zum Sieden erwärmt. Nach dem Erkalten wurde das ausgefällte Methylaminhydrochlorid abgesaugt und mit Äther gewaschen. Das Filtrat und der Waschäther wurden vereinigt und destilliert. Es wurden 14,4 g Rohprodukt vom Sdp. 65—70° (175 mm) und Äquiv.-Gewicht (Titration mit HCl) 60,9 erhalten. Die Umdestillation ergab Dimethyl-bis-(N-methylamino)-silan vom Sdp. 66° (165 mm).

$(CH_3)_2Si(NHCH_3)_2 = C_4H_{14}N_2Si(118,2)$

Ber.	Si 23,7	Äquiv.-Gew.	59,1
Gef.	» 24,1	» »	59,8

Bei der Destillation des reinen Dimethyl-bis-(N-methylamino)-silans bei 776 mm wurde der Sdp. 105° erhalten, aber die Verbindung wurde zum Teil zersetzt und ein Destillationsrest erhalten, der bei Destillation in Vakuum ein Produkt vom Sdp. 75—76° (10 mm), Äquiv.-Gew. 72,7, % N 19,0 und % Si 25,6 ergab. Diese Zusammensetzung liegt sehr nahe derjenigen des Tri-N-methyl-diamino-tetramethyldisilazins (VII).

$[(CH_3)_2SiNH \cdot CH_3]_2\ N \cdot CH_3 = C_7H_{23}N_3Si(205,4)$

Ber.	N 20,5	Si 27,3	Äquiv.-Gew.	68,5
Gef.	» 19,0	» 25,6	» »	72,7

$[(CH_3)_2SiN \cdot CH_3]_n = (C_3H_9NSi)_n = 87,2 \cdot n$

Ber.	N 16,1	Si 32,2	Äquiv.-Gew.	87,2
Gef.	» 19,0	» 25,6	» »	72,7

Dimethyl-bis-(N-äthylamino)-silan (IV)

a) 58,1 g (0,45 Mol) Dimethyl-dichlor-silan in 50 ml Benzol wurden bei etwa — 5 zu 81,2 g (1,8 Mol) Äthylamin in 150 ml Benzol hinzugetropft. Danach wurde das Reaktionsgemisch während 1 Stunde bei gelindem Sieden gehalten. Es wurde dann wie in der vorangehenden Synthese aufgearbeitet. Das Rohprodukt wog 45,0 g und hatte den Sdp. 70—84° (78 mm) und das Äquiv.-Gew. 75,0 (Titration mit HCl). Die Umdestillation ergab reines Dimethyl-bis-(N-äthylamino)-silan vom Sdp. 139° (775 mm). Die Verbindung kann bei diesem Druck ohne Zersetzung destilliert werden.

$(CH_3)_2Si(NH \cdot C_2H_5)_2 = C_6H_{18}N_2Si(146,3)$

Ber.	Si 19,2	Äquiv.-Gew.	73,1
Gef.	» 19,4	» »	73,8

b) 36,1 g (0,8 Mol) Äthylamin von etwa — 10° wurden zu 25,8 g (0,2 Mol) Dimethyl-dichlor-silan in 100 ml Benzol hinzugetropft. Das Reaktionsgemisch wurde dabei bei etwa 18° gehalten. Dann wurde es während 1 Stunde bei gelindem Sieden gehalten und in gewöhnlicher Weise aufgearbeitet. Das Rohprodukt wog 19,6 g und hatte den Sdp. 74—77° (90 mm) und das Äquiv.-Gew. 76,1.

c) 40,5 g (0,9 Mol) Äthylamin von etwa — 10° wurden zu 38,7 g (0,3 Mol) Dimethyl-dichlor-silan in 100 ml Benzol zugetropft. Das Reaktionsgemisch wurde hierbei bei Zimmertemperatur gehalten. Dann wurde es während 1 Stunde bei gelindem Sieden gehalten und in gewöhnlicher Weise aufgearbeitet. Bei der Destillation wurden zwei Fraktionen aufgenommen. Die erste von diesen wog 13,0 g und hatte den Sdp. 85—95° (10 mm). Sie siedete bei Umdestillation hauptsächlich bei 90—91° (12 mm) und ergab

dann % N 13,3, % Si 24,1, % Cl 6,6 und Äquiv.-Gew. 137 (Titration mit HCl). Die zweite Fraktion wog 1,8 g und enthielt 1,0 % Cl. Aus diesen Fraktionen konnte kein chlorfreies Produkt isoliert werden.

Dimethyl-bis-(N-phenylamino)-silan (IX)

a) 32,3 g (0,25 Mol) Dimethyl-dichlor-silan in 50 ml Kohlenstofftetrachlorid wurden bei Zimmertemperatur zu 93,1 g (1,0 Mol) Anilin in 50 ml Kohlenstofftetrachlorid zugetropft. Das Reaktionsgemisch wurde während 30 minuten zum Sieden erwärmt. Die Aufarbeitung des Gemisches in gewöhnlicher Weise ergab 40,0 g Rohprodukt vom Sdp. 174—176° (4—5 mm), das bei Zimmertemperatur fest war. Das Rohprodukt wurde in kleinen Mengen in Petroläther bei etwa 25° zur Sättigung gelöst. Die Lösung wurde auf etwa — 20° gekühlt, wobei reines Dimethyl-bis-(N-phenylamino)-silan vom Schmp 56° auskristallisierte.

$$(CH_3)_2Si(NH \cdot C_6H_5)_2 = C_{14}H_{18}N_2Si(242{,}4)$$

Ber. N 11,6 Gef. N 11,6

Bei Erwärmung der Verbindung in Petroläther oder bei längerem Aufbewahren in diesem Lösungsmittel wird die Verbindung verändert, so dass sie nicht daraus kristallisiert erhalten werden kann. Dasselbe gilt wenigstens auch für Benzollösungen.

b) 37,2 g (0,4 Mol) Anilin in 25 ml Kohlenstofftetrachlorid wurden bei Zimmertemperatur zu 12,9 g (0,1 Mol) Dimethyl-dichlor-silan in 25 ml Kohlenstofftetrachlorid zugetropft. Das Reaktionsgemisch wurde während 30 Minuten zum Sieden erwärmt. Die Aufarbeitung des Reaktionsgemisches in gewöhnlicher Weise ergab 16,5 g Rohprodukt vom Sdp. 190—193° (11 mm), Schmp. 41° und % Si 11,8.

c) Ein Gemisch von 2,9 g (0,01 Mol) Octamethylcyclotetrasilazin und 15,0 g (0,16 Mol) Anilin wurde auf etwa 180° während 2 Stunden erwärmt, wobei Ammoniak entwickelt wurde. Das Reaktionsgemisch ergab dann bei Destillation 6,1 g Dimethyl-bis-(N-phenylamino)-silan vom Sdp. 174—179° (5—6 mm) und Schmp. 47—51° (ohne Umkristallisation).

d) Ein Gemisch von 5,0 g (0,034 Mol) Dimethyl-bis-(N-äthylamino)-silan und 30,0 g (0,32 Mol) Anilin ergab wie in dem vorangehenden Versuch 7,8 g Dimethyl-bis-(N-phenylamino)-silan vom Sdp. 171—172° (4 mm). Nach vorsichtiger Umkristallisation wurden Schmp. 56°, % N 11,6 (gef.), 11,6 (ber.), % Si 11,8 (gef.), 11,6 (ber.) erhalten.

Tri-N-phenyl-hexamethylcyclotrisilazin (X)

111,7 g (1,2 Mol) Anilin wurden zu 51,6 g (0,4 Mol) Dimethyl-dichlor-silan in 100 ml Kohlenstofftetrachlorid hinzugetropft, wobei das Reaktionsgemisch durch Selbsterwärmen auf 45—60° gehalten wurde. Nachdem alles Anilin zugesetzt worden war, wurde das Reaktionsgemisch während 1 Stunde zum Sieden erwärmt. Die Aufarbeitung ergab 30,9 g Flüssigkeit vom Sdp. 190—193° (11 mm). Dieses Produkt war bei Zimmertemperatur fest. Durch Behandlung mit Petroläther konnten daraus etwa 2 g in Petroläther schwerlösliche Krystalle isoliert werden. Die Hauptmasse war leicht löslich in Petroläther und chlorfrei. Daraus konnte keine einheitliche Substanz isoliert werden. Die erwähnten Krystalle hatte den Schmp. 249—250°. Die Analysen wiesen auf Tri-N-phenyl-hexamethylcyclotrisilazin hin.

$[(CH_3)_2SiN \cdot C_6H_5]_3 = C_{24}H_{33}N_3Si_3$(447,7)

Ber.	H 7,4	N 9,4	Si 18,8	Mol.-Gew. 448
Gef.	» 7,5	» 9,2	» 18,8	» » 430, 480 (Äther)

Dimethyl-bis-(N-benzylamino)-silan

Ein Gemisch von 5,8 g (0,04 Mol) Dimethyl-bis-(N-äthylamino)-silan und 14,0 g (0,13 Mol) Benzylamin wurde während 60 Minuten auf etwa 150° erwärmt und dann destilliert. Es wurden 9,2 g Dimethyl-bis-(N-benzylamino)-silan vom Sdp. 174—178° (5 mm) erhalten.

$(CH_3)_2Si(NH \cdot CH_2 \cdot C_6H_5)_2 = C_{16}H_{22}N_2Si$(270,4)

Ber.	Si 10,4	Äquiv.-Gew. 135,2
Gef.	» 10,1	» » 137,0

Dimethyl-bis-(N-heptylamino)-silan

5,8 g (0,04 Mol) Dimethyl-bis-(N-äthylamino)-silan und 12,3 g (0,11 Mol) *n*-Heptylamin ergaben wie in dem vorangehenden Versuch 7,6 g Dimethyl-bis-(N-heptylamino)-silan vom Sdp. 169—170° (12 mm).

$(CH_3)_2Si(NH \cdot C_7H_{15})_2 = C_{16}H_{38}N_2Si$(286,5)

Ber.	Si 9,8	Äquiv.-Gew. 143,3
Gef.	» 9,7	» » 145,6

ZUSAMMENFASSUNG

Es wurden einige Verbindungen von der allgemeinen Formel $(CH_3)_2Si(NH \cdot R)_2$ mit R = $-CH_3$, $-CH_2 \cdot CH_3$, $-(CH_2)_6CH_3$, $-C_6H_5$, $-CH_2 \cdot C_6H_5$ dargestellt. Ausserdem wurde die cyclische Verbindung $[(CH_3)_2Si \cdot N \cdot C_6H_5]_3$ in geringer Menge erhalten.

Die Untersuchung ist mit Unterstützung von *Uddeholms AB* und *Allmänna Svenska Elektriska AB* ausgeführt worden, wofür wir bestens danken.

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Eingegangen am 11. April 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 493—504

On a Method of Determining the Mechanism of an Enzymatic Reaction the Kinetics of Which is Known

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In 1903 Henri in his thesis for the doctorate [1] concluded from measurements on the enzymatic inversion of saccharose and some related reactions that an enzyme may combine with the »substrate« or with the reaction products. The occurrence of such combinations will have a definite influence on the kinetics of the reaction, which may therefore be used to prove their existence.

Ten years later Michaelis and Menten published a renowned paper [2] in which they drew attention to Henri's work and made an extensive series of experiments on the same reaction, avoiding some sources of error which had escaped Henri's attention. The effect is now known as the Michaelis effect, and the constant relating to the formation of the compound between enzyme and substrate is usually called the Michaelis constant k_m, which symbol dates from Henri's paper.

Although the connection with the work of Henri and Michaelis may not be obvious, the trend of the following is to extend the interpretation of the kinetics of enzymatic reactions on the basis given by these authors. In its main features the method will be the same as that which has been used for many years by many different authors, namely the method of stationarity. It will be given in about the same form as that used in two papers in *Handbuch der Katalyse* [3] with amendments which the application to the special case of an enzymatic reaction has made natural.

In the following we shall treat only one enzymatic reaction, but it is hoped that the treatment may serve as an example of the application of some very simple principles on other reactions and thus be helpful in the elucidation of the mechanisms of other types of enzymatic reaction.

The reaction in question is the well known transamination reaction, *e. g.*

glutamic acid + pyruvic acid $\rightleftharpoons$ keto-glutaric acid + alanine

which proceeds at ordinary temperature only in the presence of a certain enzyme, transaminase. This reaction and a few analogous reactions have recently been experimentally investigated by Sv. Darling, M.Sc., at the Biochemical Institute of the University of Aarhus. The present author has taken no part at all in the experimental investigation, but he has discussed the results and their utilization for unveiling the reaction mechanism with Darling, who has kindly permitted the use of some of the results of the investigation in this paper.

The reaction is obviously of the type:

$$A + B \rightleftharpoons C + G$$

In the following we shall use four facts which appear from Darling's investigation, *viz.*:

1) The equilibrium constant is $(3/2)^2$ at all temperatures from 20° C to about 70° C, *i. e.* ΔH for the reaction is zero or rather experimentally not discernible from zero.

2) Starting with equivalent concentrations a and b of A and B, respectively, the reaction follows approximately the unimolecular law, the constant, of course, being proportional to the total amount of enzyme.

3) Under the same condition the reciprocal velocity constant increases linearly with a, but is not proportional to a, *i. e.* a graph with $1/k$ and a as coordinates will be a straight line which does not pass through the origin.

4) When one experiment is started with different values of a and b ($c_A = a$, $c_B = b$), and another with exchange of the values ($c_A = b$, $c_B = a$), it appears that the course of the reaction in the two cases is the same or very nearly the same, *i. e.* we may say that the course is symmetrical in a and b.

We now attempt to find by trial and error a mechanism which is in harmony with the kinetics.

Beginning with the simplest possibility, we may assume that the reaction is:

$$E + A + B \rightleftharpoons C + G + E$$

where E denotes the enzyme.

From this assumption we conclude that the reaction is bimolecular with respect to A and B, which certainly is not the case. Therefore this possibility is ruled out.

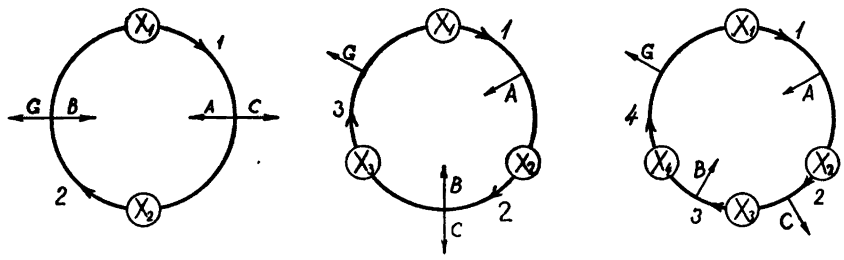


Fig. 1. Geometrical representations of the sequences pp. 495, 499, 500.

Next we assume that the reaction is:

$$\begin{array}{lcll} A + X_1 & \rightleftharpoons & X_2 + C & (\pm 1) \\ B + X_2 & \rightleftharpoons & X_1 + G & (\pm 2) \end{array}$$

Here X_1 and X_2 are symbols for two different forms of the enzyme. If their corresponding concentrations x_1 and x_2 are added, we get, of course, the total enzyme concentration, which will be denoted E.

This (closed) sequence may also be represented geometrically by means of diagram I in Fig. 1. This diagram is intended to mean that by reaction (+ 1) A disappears (from the world outside the circle), while C appears, and similarly for reaction (+ 2). A reaction in the opposite direction is symbolized by the same diagram only with all arrows reverted.

To describe the reaction quantitatively we say that *e. g.* X_1 has a certain probability per unit time $\bar{\omega}_1$ * to react according to reaction (+ 1) and the reaction probability $\bar{\omega}_{-2}$ to react according to (—2) *etc.*, the $\bar{\omega}$'s being either constants or constants multiplied by one or (rarely) two concentrations. In our scheme $\bar{\omega}_1 = k_1 a$ or $\bar{\omega}_1 = k_1(a—x)$ if a is the concentration of A at $t = 0$, and x is the amount which has reacted at time t.

Now it is well known to-day that with time in seconds the constants occurring in these expressions are either very large, *i. e.* about 10^{13} for reactions of the unimolecular type, abt. 10^{11} for reactions of the bimolecular type or the same large numbers multiplied by an exponential of the form $e^{-A/T}$, which for reactions with measurable velocities is a very small fraction of 1, *e. g.* 10^{-11} or less.

From this it follows that if sums like $\bar{\omega}_1 + \bar{\omega}_{-1}$ occur in our expressions, and we know that there is a difference in energy-level for the two systems between

* $\bar{\omega}$ should be read as the greek letter pi. The correct type, (*e. g.* Guggenheim and Fowler, *Statistical thermodynamics*, 1939) was not available.

which the reaction takes place, we may, with an accuracy usually greater by far than the accuracy of our experiments, omit either one or the other member of the sum. On the other hand, when the two systems are on the same level, we may according to present views assume that both are large, *i. e.* that they do not contain the exponential factor.

In the case considered here we have the extra simplification that ΔH of the reaction is zero, which means that the effect of the activation-energies disappears in the equilibrium expression. From these considerations it follows that we may ascribe a meaning to the orientation of the diagram. As it stands it is intended to mean that the reaction probabilities of the »upward» reactions (— 1) and (+ 2) are immensely small as compared to those of the »downward» reactions (+ 1) and (— 2). If we had placed x_1 and x_2 on the same level in the diagram, this would mean that all four probabilities are large, but this again would mean that the reaction would be immeasurably fast unless the enzyme concentration is practically nil. In the following we shall not consider this case.

We shall now proceed to discuss the partition of the enzyme on the two states: X_1 and X_2. This partition will, of course, depend on the momentary values of the concentrations of A, B, C, and G, but besides this it may depend explicitly on time. To find this dependence on time of x_1 and x_2, we treat the problem tentatively as if the reaction-probabilities were constant in time. Of course, this assumption is not strictly true, but they may vary so slowly with time that their dependence on time is of no consequence.

The mathematical treatment of a problem of this type is well known [4].

Denoting differentiation with respect to time with a dot, we obviously get:

$$-\dot{x}_1 = x_1\ (\bar{\omega}_1 + \bar{\omega}_{-2}) - x_2\ (\bar{\omega}_2 + \bar{\omega}_{-1})$$
$$-\dot{x}_2 = -x_1\ (\bar{\omega}_1 + \bar{\omega}_{-2}) + x_2\ (\bar{\omega}_2 + \bar{\omega}_{-1})$$

Putting $-\dot{x}_1 = \lambda\ x_1$, $-\dot{x}_2 = \lambda\ x_2$, we get the characteristic equation:

$$\begin{vmatrix} \bar{\omega}_1 + \bar{\omega}_{-2} - \lambda & -(\bar{\omega}_2 + \bar{\omega}_{-1}) \\ -(\bar{\omega}_1 + \bar{\omega}_{-2}) & \bar{\omega}_2 + \bar{\omega}_{-1} - \lambda \end{vmatrix} = 0$$

the roots of which are $\lambda_0 = 0$, $\lambda_1 = \bar{\omega}_1 + \bar{\omega}_{-2} + \bar{\omega}_2 + \bar{\omega}_{-1}$.

The general solution becomes:

$$x_1 = E\ (\bar{\omega}_2 + \bar{\omega}_{-1})/\lambda_1 + A\ \exp\ (-\lambda_1 t)$$
$$x_2 = E\ (\bar{\omega}_1 + \bar{\omega}_{-2})/\lambda_1 - A\ \exp\ (-\lambda_1 t)$$

where A is a constant which can be determined when, and only when we know the partition of the enzyme on the two states at time zero. In most cases this knowledge is difficult or impossible to obtain, but fortunately it is unnecessary, for when we remember the orientation of the diagram, λ_1 can to all intents and purposes be put equal to $\bar{\omega}_1 + \bar{\omega}_{-2}$ which are both very large. If for instance the concentrations applied in the experiment are of the order of magnitude 10^{-3} molar, $\bar{\omega}_1 + \bar{\omega}_{-2}$ will be something like 10^8 reciprocal seconds, which means that the exponentials above have practically disappeared at the same moment the reaction is started, *i. e.* the partition on the two forms is stationary practically from the start.

This being so, we may safely apply the method of stationarity to calculate the reaction velocity s:

$$s = x_1\bar{\omega}_1 - x_2\bar{\omega}_{-1}$$
$$s = x_2\bar{\omega}_2 - x_1\bar{\omega}_{-2}$$

the solution of which may be written:

$$Lx_1/s = \bar{\omega}_2 + \bar{\omega}_{-1} \qquad L = \bar{\omega}_1\bar{\omega}_2 - \bar{\omega}_{-1}\bar{\omega}_{-2}$$
$$Lx_2/s = \bar{\omega}_1 + \bar{\omega}_{-2}$$

or, as $x_1 + x_2 = E$

$$LE/s = M$$
$$M = \bar{\omega}_2 + \bar{\omega}_{-1} + \bar{\omega}_1 + \bar{\omega}_{-2}$$

If we express the concentration at time t of A by $a - x$, where a is the value at $t = 0$, and similarly for the other participants then obviously $s = \mathrm{d}x/\mathrm{d}t$, and the equation is a differential equation for the determination of x as a function of t, or what is more convenient for the determination of t as a function of x.

The integration is fairly easy, but unnecessary as we see at a glance that the expression is hopelessly unsymmetrical in a and b. For if the orientation of the diagram is as given, $\bar{\omega}_2$ and $\bar{\omega}_{-1}$ disappear from M so that $M = k_1(a - x) + k_2(g + x)$, whereas if it is turned upside down $\bar{\omega}_1$ and $\bar{\omega}_{-2}$ disappear with a similar consequence. As L is always symmetrical in a and b, this proves our case.

The next step is to assume a sequence containing three partial reactions as represented by diagram II or by the sequence:

$$\begin{array}{lcll} A + X_1 & \rightleftharpoons & X_2 & (\pm 1) \\ B + X_2 & \rightleftharpoons & X_3 + C & (\pm 2) \\ X_3 & \rightleftharpoons & X_1 + G & (\pm 3) \end{array}$$

As in the former case we start with an investigation of the dependence on time of the partition of the enzyme on the three states. The characteristic equation becomes of the third degree and has the roots $\lambda_0 = 0$, λ_1 and λ_2. λ_1 and λ_2 may contain an imaginary part, but it can easily be seen that their real part must be positive and large so that again the members containing the exponentials exp $(-\lambda_1 t)$ and exp $(-\lambda_2 t)$ disappear in practically no time.

The conditions of stationarity become:

$$s = x_1\bar{\omega}_1 - x_2\bar{\omega}_{-1}$$
$$s = x_2\bar{\omega}_2 - x_3\bar{\omega}_{-2}$$
$$s = x_3\bar{\omega}_3 - x_1\bar{\omega}_{-3}$$

The solution is:

$$Lx_1/s = \bar{\omega}_2\bar{\omega}_3 + \bar{\omega}_{-1}\bar{\omega}_3 + \bar{\omega}_{-1}\bar{\omega}_{-2}$$
$$Lx_2/s = \bar{\omega}_3\bar{\omega}_1 + \bar{\omega}_{-2}\bar{\omega}_1 + \bar{\omega}_{-2}\bar{\omega}_{-3}$$
$$Lx_3/s = \bar{\omega}_1\bar{\omega}_2 + \bar{\omega}_{-3}\bar{\omega}_2 + \bar{\omega}_{-3}\bar{\omega}_{-1}$$

$$L = \bar{\omega}_1\bar{\omega}_2\bar{\omega}_3 + \bar{\omega}_{-1}\bar{\omega}_{-2}\bar{\omega}_{-3}$$

and consequently:

$$LE/s = M$$

where M is the sum of the members in the 9-membered »partition matrix»:

$$\begin{Bmatrix} x_1 \\ x_2 \\ x_3 \end{Bmatrix} \qquad \left\{ \begin{array}{ccc} \bar{\omega}_2\bar{\omega}_3 & \bar{\omega}_{-1}\bar{\omega}_3 & \bar{\omega}_{-1}\bar{\omega}_{-2} \\ \bar{\omega}_3\bar{\omega}_1 & \bar{\omega}_{-2}\bar{\omega}_1 & \bar{\omega}_{-2}\bar{\omega}_{-3} \\ \bar{\omega}_1\bar{\omega}_2 & \bar{\omega}_{-3}\bar{\omega}_2 & \bar{\omega}_{-3}\bar{\omega}_{-1} \end{array} \right\}$$

the form of which is easy to remember (start with $\bar{\omega}_2$). It is called the partition matrix because the sum of the members in each line is proportional to the quantity of the form of the enzyme which is indicated in the three-membered matrix to the left of M.

As the total amount of enzyme is known (in principle) and constant in time in each experiment, the amounts of the three forms at any time can be easily calculated by means of this matrix when the different constants have been determined.

For the following it will be convenient to write down a matrix of the same form (9 members) containing only the simultaneous concentrations of the four substances A, B, C, and G appearing as factors in the expressions with omission of the constants, the respective concentrations being named a, b, c, and g.

In the case considered this matrix (the c-matrix) becomes:

$$\left\{\begin{array}{ccc} b & 1 & c \\ a & \boxed{ca} & \boxed{cg} \\ \boxed{ab} & \boxed{gb} & g \end{array}\right\}$$

When the diagram is orientated as shown in II, the members outside the frames disappear as compared to those inside.

It appears on consideration of the c-matrix that in this case M may be symmetrical in a and b, namely if $c = g$, and $k_{-2}k_1 = k_{-3}k_2$.

Further inspection of the six different possibilities of orientation:

$$\left|\begin{array}{cc} & x_1 \\ x_3 & x_2 \end{array}\right| \left|\begin{array}{cc} x_2 & x_3 \\ & x_1 \end{array}\right| \left|\begin{array}{cc} & x_2 \\ x_1 & x_3 \end{array}\right| \left|\begin{array}{cc} x_3 & x_1 \\ & x_2 \end{array}\right| \left|\begin{array}{cc} & x_3 \\ x_2 & x_1 \end{array}\right| \left|\begin{array}{cc} x_1 & x_2 \\ & x_3 \end{array}\right|$$

will show that this is also the only orientation of the diagram which can lead to the desired symmetry in a and b.

For comparison with the experiments we shall introduce the $\bar{\omega}$-values which follow from the sequence assumed:

$\bar{\omega}_1 = k_1\ (a—x)$; $\bar{\omega}_{-1} = k_{-1}$; $\bar{\omega}_2 = k_2\ (b—x)$; $\bar{\omega}_{-2} = k_{-2}\ (c + x)$;
$\bar{\omega}_3 = k_3$; $\bar{\omega}_{-3} = k_{-3}\ (g + x)$; and furthermore: $1/s = \mathrm{d}t/\mathrm{d}x$

Now most of Darling's experiments were made with $a = b$; $c = g = 0$. On these assumptions integration of the differertial equation leads to the expression:

$$2qk_3Et = -a\left(\frac{p+q}{1+q}\right)^2 \ln\,(1-\alpha\,(1+q)) + a\left(\frac{p-q}{1-q}\right)^2 \ln\,(1-\alpha\,(1-q)) + 2a\alpha q\frac{(1-p)^2}{1-q^2}$$

where:

$$p = k_{-2}/k_2 = k_{-3}/k_1,\ q^2 = k_{-1}k_{-2}k_{-3}/k_1k_2k_3 \text{ and } \alpha = x/a.$$

On comparison of this formula with the actual experiments, it appears that the first member in the sum on the right hand side is the leading one, *i. e.* the reaction follows with good approximation the unimolecular law (for a reversible reaction). Further it appears from the formula that the reciprocal constant

of the reaction increases with a, which is qualitatively in agreement with the experiments. The formula, however, disagrees with the experiments as it shows that the reciprocal constant should be proportional to a, while as a matter of fact the experiments show that it increases linearly with a, the straight line connecting the empirical points in the $(a, 1/k)$-plot not passing through the origin.

We must, therefore, also discard this mechanism and proceed to investigate the consequences of the sequence:

$$\begin{array}{rcll} A + X_1 & \rightleftharpoons & X_2 & (\pm 1) \\ X_2 & \rightleftharpoons & X_3 + C & (\pm 2) \\ B + X_3 & \rightleftharpoons & X_4 & (\pm 3) \\ X_4 & \rightleftharpoons & X_1 + G & (\pm 4) \end{array}$$

which is represented by diagram III.

The investigation follows the same lines as in the former cases, that is we start with the investigation of the dependence on time of the partition of the enzyme on the different states, and having proved that the partition must become stationary in a time which is negligible compared to the time in which the over-all reaction has proceeded perceptibly, we write down the expression:

$$LE/s = M$$

which is a differential equation connecting the degree of reaction x with time.

In this case it is a little complicated to estimate the values of the roots in the characteristic equation which determine the time for attainment of stationary conditions, simply because the equation is of the third (fourth) degree. If the equation is written:

$$a_0\lambda^4 - a_1\lambda^3 + a_2\lambda^2 - a_3\lambda + a_4 = 0$$

it is at once seen that $a_0 = 1$ and $a_4 = 0$, so that $\lambda_0 = 0$ is as always one of the roots.

The other constants are positive. We find $a_3 = M$, *i. e.* a_3 equals the sum M of the 16 members in the below partition matrix (p. 503).

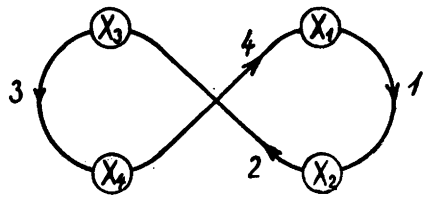
Furthermore a_1 equals the sum of the eight reaction probabilities. a_2 is a sum of 20 products of the form $\bar{\omega}_i\bar{\omega}_j$ $(i \neq \pm j)$:

$$a_2 = \sum \begin{Vmatrix} \bar{\omega}_2\bar{\omega}_3 & \bar{\omega}_{-1}\bar{\omega}_3 & \bar{\omega}_{-1}\bar{\omega}_{-2} \\ \bar{\omega}_3\bar{\omega}_4 & \bar{\omega}_{-2}\bar{\omega}_4 & \bar{\omega}_{-2}\bar{\omega}_{-3} \\ \bar{\omega}_4\bar{\omega}_1 & \bar{\omega}_{-3}\bar{\omega}_1 & \bar{\omega}_{-3}\bar{\omega}_{-4} \\ \bar{\omega}_1\bar{\omega}_2 & \bar{\omega}_{-4}\bar{\omega}_2 & \bar{\omega}_{-4}\bar{\omega}_{-1} \end{Vmatrix} + \sum \begin{Vmatrix} (\bar{\omega}_1 + \bar{\omega}_{-4}) & (\bar{\omega}_3 + \bar{\omega}_{-2}) \\ (\bar{\omega}_2 + \bar{\omega}_{-1}) & (\bar{\omega}_4 + \bar{\omega}_{-3}) \end{Vmatrix}$$

Now if the diagram is as indicated, *i. e.* if we assume that $\bar{\omega}_{-1}$ and $\bar{\omega}_4$, and only they contain the exponential factor, we get for a_1 a sum of 6 quantities $\bar{\omega}$, for a_2 a sum of 11 products $\bar{\omega}\bar{\omega}$, and for a_3 a sum of 6 products $\bar{\omega}\bar{\omega}\bar{\omega}$, none of which contains the exponential factor exp $(-A/T)$.

This shows that the three roots are all large, so that the exponentials exp $(-\lambda t)$ will disappear practically instantaneously.

If, however, the diagram is assumed to be IV in Fig. 2.



IV

Fig. 2. Geometrical representation of the sequence p. 500, second form.

x_2 and x_4 are obviously confined to two »valleys» between two »hills», and it must be expected that the transition from x_2 to x_4 and *vice versa* is »slow». This appears, too, when we try to solve the characteristic equation. In this case $\bar{\omega}_{-1}$, $\bar{\omega}_2$, $\bar{\omega}_{-3}$, and $\bar{\omega}_4$ all contain the exponential term exp $(-A/T)$. This has the following consequences:

Of a_1 a sum of 4 $\bar{\omega}$'s remains: $\bar{\omega}_1 + \bar{\omega}_{-4} + \bar{\omega}_3 + \bar{\omega}_{-2}$,
and of a_2 only the product: $(\bar{\omega}_1 + \bar{\omega}_{-4})\ (\bar{\omega}_3 + \bar{\omega}_{-2})$ remains. None of these members contains the exponential term. In a_3, however, the exponential term is retained, as in the lines corresponding to x_1 and x_3 in the partition matrix it appears in the second power, while in the x_2 and x_4 lines it appears in the first power. This corresponds to the fact that we have assumed x_2 and x_4 to be situated in »valleys», while x_1 and x_3 have been placed on the top of the »hills».

What is left of a_3 is a sum of eight members all containing one exponential term, four in the x_2-line and four in the x_4-line of (M). It is, therefore, obvious that in this case two roots are large, and one is small. As the equation becomes:

$$\lambda^3 - (\bar{\omega}_1 + \bar{\omega}_{-4} + \bar{\omega}_3 + \bar{\omega}_{-2})\lambda^2 + (\bar{\omega}_1 + \bar{\omega}_{-4})\ (\bar{\omega}_3 + \bar{\omega}_{-2})\lambda - M = 0$$

the two large roots must be very nearly:

$$\lambda_2 = \bar{\omega}_1 + \bar{\omega}_{-4} \quad ; \quad \lambda_3 = \bar{\omega}_3 + \bar{\omega}_{-2}$$

while the third is determined (approximately) by neglecting the first two members of the equation and solving for λ.

What we now need is only an estimate of the small root λ_1. For this purpose we consider the situation at the start of an experiment, where the concentrations c and g are zero. Under these conditions the $\bar{\omega}$'s of the »negative» reactions (—2) and (—4) disappear, and we get (compare the expression for M p. 503):

$$\bar{\omega}_1\bar{\omega}_3\lambda - \bar{\omega}_1\bar{\omega}_3\ (\bar{\omega}_2 + \bar{\omega}_4) = 0$$

i. e.:

$$\lambda_1 = \bar{\omega}_2 + \bar{\omega}_4 = k_2 + k_4$$

Under the same conditions we get for s from $LE/s = M$:

$$E\bar{\omega}_1\bar{\omega}_2\bar{\omega}_3\bar{\omega}_4 = s\bar{\omega}_1\bar{\omega}_3(\bar{\omega}_2 + \bar{\omega}_4)$$

i. e.:

$$s = E\,\frac{\bar{\omega}_2\bar{\omega}_4}{\bar{\omega}_2 + \bar{\omega}_4} = E\,\frac{k_2k_4}{k_2 + k_4}$$

an expression for the velocity of the over-all reaction. From this we get the decay-constant, which is to be compared with λ_1 by dividing by a, the initial concentration of A:

$$\frac{s}{a} = \frac{E}{a}\,\frac{k_2k_4}{k_2 + k_4}$$

If $k_2 = k_4 = k$, we find $\lambda_1 = 2k$; $s/a = \frac{1}{2}k\ E/a$. As E/a is in most experiments a very small fraction of 1, it appears that even if the absolute value of λ_1 is small, it will be large as compared to the »decay-constant» of the over-all reaction, and this knowledge is sufficient for our purpose. In the case that k_2 and k_4 are different, for instance $k_2 \gg k_4$, the ratio between λ_1 and s/a becomes still larger, as in that case $\lambda_1 = k_2$ and $s/a = k_4\ E/a$. Thus we have proved that even in this rather disadvantageous case it must be assumed that the partition of the enzyme on the four different states becomes stationary a relatively very short time after the start of the experiment.

The rest is easy. We shall use the equation:

$$EL/s = M$$

where

$$L = \bar{\omega}_1\bar{\omega}_2\bar{\omega}_3\bar{\omega}_4 - \bar{\omega}_{-1}\bar{\omega}_{-2}\bar{\omega}_{-3}\bar{\omega}_{-4}$$

and M is the sum of the 16 members in the partition matrix:

$$\begin{Bmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \end{Bmatrix} \begin{Bmatrix} \bar{\omega}_2\bar{\omega}_3\bar{\omega}_4 & \bar{\omega}_{-1}\bar{\omega}_3\bar{\omega}_4 & \bar{\omega}_{-1}\bar{\omega}_{-2}\bar{\omega}_4 & \bar{\omega}_{-1}\bar{\omega}_{-2}\bar{\omega}_{-3} \\ \bar{\omega}_3\bar{\omega}_4\bar{\omega}_1 & \bar{\omega}_{-2}\bar{\omega}_4\bar{\omega}_1 & \bar{\omega}_{-2}\bar{\omega}_{-3}\bar{\omega}_1 & \bar{\omega}_{-2}\bar{\omega}_{-3}\bar{\omega}_{-4} \\ \bar{\omega}_4\bar{\omega}_1\bar{\omega}_2 & \bar{\omega}_{-3}\bar{\omega}_1\bar{\omega}_2 & \bar{\omega}_{-3}\bar{\omega}_{-4}\bar{\omega}_2 & \bar{\omega}_{-3}\bar{\omega}_{-4}\bar{\omega}_{-1} \\ \bar{\omega}_1\bar{\omega}_2\bar{\omega}_3 & \bar{\omega}_{-4}\bar{\omega}_2\bar{\omega}_3 & \bar{\omega}_{-4}\bar{\omega}_{-1}\bar{\omega}_3 & \bar{\omega}_{-4}\bar{\omega}_{-1}\bar{\omega}_{-2} \end{Bmatrix}$$

The corresponding c-matrix is:

$$\begin{Bmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \end{Bmatrix} \begin{Bmatrix} b & b & c & c \\ ba & ca & ca & cg \\ a & a & g & g \\ ab & gb & gb & gc \end{Bmatrix}$$

The members which according to diagram III do not contain exponentials are inside the frames. It is seen that the expression for s cannot be made exactly symmetrical in a and b, but the constants may have such values that the dissymmetry is small. As a matter of fact Mr. Darling has found by his experiments that under certain starting conditions a dissymmetry exists.

To use the expression it is integrated as before, but as the aim of this paper is only to discuss the method, and as the author has no part in the experiments, we shall not enlarge on details.

It must, however, be added that by repetition of the three first lines in the partitionmatrix below the matrix, and displacement of the frame by two lines downward, it can be seen that another possibility exists for getting an expression of a similar form. Inspection of the diagram and the corresponding sequence shows, however, that there is no real difference between these two possibilities, as the latter can be arrived at from the former simply by changing the meaning of the symbols.

A more essential addition is that diagram IV discussed above might also lead to kinetics which harmonize with the experimental facts. As mentioned above all the members in the x_1 and x_3 lines are in this case to be omitted from the partition matrix (for the application in the expression for s). Comparison with the c-matrix shows that by an appropriate choice of the constants the expression can be made symmetrical in a and b (when $c = g$). It is for the experiments to show whether one or the other mechanism is the right one, the essential thing being that the consequences are sufficiently different to make a decision possible.

So far we have only discussed the analysis of the kinetic results and have completely disregarded the information which may be gained by chemical considerations. The reason for this is that the results become more conclusive when arrived at independently in different ways.

Not to forget completely that we are dealing with a chemical reaction, we may, however, add that from a chemical point of view the mechanism expressed in diagram IV might seem to be more probable than that in diagram III, as (1) and (3) are associations, while (2) and (4) are dissociations.

SUMMARY

The kinetics of an enzymatic reaction:

$$A + B \rightleftharpoons C + G$$

are discussed by means of the so-called partition-matrix, a matrix from which the stationary partition of the enzyme on its different possible forms and the stationary reaction velocity can be derived.

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Received April 12, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 505—519

Electrometric Investigation of Equilibria between Mercury and Halogen Ions

VII.* Complexes between Hg^{2+} and I^-, and some Equilibria involving Solid Mercury(I) Iodide aud Mercury(II) Iodide

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In 1902, Morse [7] measured the solubility of AgI in $Hg(NO_3)_2$ solutions. Assuming that only HgI^+ and no HgI_2 was formed, he calculated with the aid of the solubility product of AgI: $[Hg^{2+}]\,[I^-]\,[HgI^+]^{-1} = \varkappa_1^{-1} = 0.4 \cdot 10^{-13}$. From the solubility of HgI_2 in water and in 1 C $Hg(NO_3)_2$ he calculated $[HgI_2][Hg^{2+}]\,[HgI^+]^{-2} = \varkappa_2\varkappa_1^{-2} = k_{12}^{-1} = 0.016$, and by combining this value, which he considered very inaccurate, with $\varkappa_1$, he obtained $[Hg^{2+}][I^-]^2[HgI_2]^{-1} = \varkappa_2^{-1} = 10^{-25}$.

Sherrill [8], in 1903, measured the emf of cells with a Hg electrode in a solution of HgI_2 and KI, concluded that at the concentrations used (0.04—1.00 C KI, 2.5—305 mC HgI_2) the predominant complex is HgI_4^{2-}, and calculated the equilibrium constant $\varkappa_4 = [HgI_4^{2-}]\,[Hg^{2+}]^{-1}\,[I^-]^{-4} = 1.9 \cdot 10^{30}$. According to Frl Hamburger (quoted in [8]), the equilibrium constant for $Hg_2I_2(s) + 2\ I^- \rightleftharpoons Hg(l) + HgI_4^{2-}$ is $[HgI_4^{2-}]\,[I^-]^{-2} = 2.02$; from this value, in combination with $k_0 = [Hg_2^{2+}]\,[Hg^{2+}]^{-1} = 120$, and a single value of $\varkappa_4$ (not the average), Sherrill calculated the solubility product for Hg_2I_2 as $[Hg_2^{2+}]\,[I^-]^2 = 1.2 \cdot 10^{-28}$. He also measured the partition of HgI_2 between benzene and aqueous KI solutions (0.050—1.0 C) and calculated values for $q_2 = [HgI_4^{2-}][HgI_2]^{-1}[I^-]^{-2}$ ranging from $5.9 \cdot 10^5$ to $31 \cdot 10^5$. Of these values for q_2, he considered the most probable to be $q_2 = 7.3 \cdot 10^5$ which together with $\varkappa_4$ gives $\varkappa_2^{-1} = [Hg^{2+}]\,[I^-]^2\,[HgI_2]^{-1} = 3.8 \cdot 10^{-25}$, thus $\varkappa_2 = 2.6 \cdot 10^{24}$. From catalytical measurements of $[I^-]$ in solutions containing 31.25 mC KI and 0—13.15 mC HgI_2 and in solutions with 18—233 mC KI, saturated with HgI_2, he concluded somewhat surprisingly that at these concentrations the predominant complex is $Hg_2I_7^{3-}$. The freezing

* Part I—VI, see References 1—6.

points of solutions with 0.24—0.74 C KI, saturated with HgI_2, could equally well be interpreted by assuming HgI_4^{2-} and $Hg_2I_7^{3-}$ ions. Abegg and Sherrill [9] have recalculated q_2 by the same vicious circle as for Br^- (*c cf.* [6]).

As far as we know, there are no later measurements of the equilibria involving Hg^{2+}, HgI^+, and HgI_2, except those recently made at this Institute. The work on the higher complexes is also scanty and contradictory. By optical methods, Fromherz and Lih [10] found $q_2 = 1.1 \cdot 10^5$, and Job [11] $q_2^{-1} = 1.2 \cdot 10^{-8}$ (at 16° C) thus $q_2 = 0.8 \cdot 10^8$. These three authors considered only HgI_4^{2-}; as did Malyugina *et al.*[12], who computed $\varkappa_4 = 10^{27}$ from polarographic measurements.

Garrett [13] measured the solubility of HgI_2 in solutions of different $[I^-]$. He could explain his results by assuming both HgI_3^- and HgI_4^{2-} to be present, with the equilibrium constants $[HgI_3^-]\,[I^-]^{-1} = 0.48$ and $[HgI_4^{2-}]\,[I^-]^{-2} = 35$. If the solubility of HgI_2 is assumed to be $1.32 \cdot 10^{-4}$ C (the value accepted by Garrett) we can calculate $q_1 = 3600$, $q_2 = 2.7 \cdot 10^5$.

SURVEY

The equilibria between Hg^{2+} and I^- were studied under the same conditions as were chosen for previous investigations of Hg^{2+}—Cl^- and Hg^{2+}—Br^- equilibria; 25° C, $[H^+] = 10$ mC, and ionic strength close to 500 mC, which was achieved by the addition of $NaClO_4$. The apparatus was the same in principle as in earlier work [1–6]. In the latter part of our work, the thermostat was placed in a thermostat room.

However, the methods used for studying the Hg^{2+}—Cl^- and Hg^{2+}—Br^- equilibria could not be taken over unchanged. Thus the equilibria involving HgI_3^- and HgI_4^{2-} (equilibrium constants $\varkappa_3$ and $\varkappa_4$, definition see Table 4) could not be studied by means of redox emfs in solutions containing Hg_2I_2, HgI_3^-, HgI_4^{2-} and I^-; in this case (as later on for the corresponding Br^- equilibria) emfs were measured with a Hg electrode in (HgI_3^-, HgI_4^{2-}, I^-) solutions.

For Hg^{2+}—Cl^- and Hg^{2+}—Br^- the first two complex products, $\varkappa_1$ and $\varkappa_2$, had been obtained by measuring the maximum redox emf during a titration of a Hg^{2+}—Hg_2^{2+} mixture with X^-, and combining this result with the equilibrium constants $k_{12} = [HgX^+]^2\,[HgX_2]^{-1}\,[Hg^{2+}]^{-1}$ measured by Sillén and Infeldt [2]. For I^- this was not possible, since the sparingly soluble HgI_2 precipitated before the desired maximum E had been reached, and since the value for k_{12} was so uncertain in this case that an independent check was necessary. Finally the desired data could be obtained from the redox emf curve using more complicated formulae than for Cl^- and Br^-.

The solubility product k_s of Hg_2I_2 was calculated from titrations of a Hg_2^{2+} solution with I^-, using a Hg electrode. The calculations were analogous to those for Hg_2Cl_2 and Hg_2Br_2, although the corrections for the presence of Hg^{II} complexes were larger than for the other halogens.

The equilibrium constants obtained are listed in Table 4. The equilibria involving Hg^{2+}, the Hg^{2+}—I^- complexes, Hg_2^{2+}, Hg_2I_2, and I^- are analogous to those studied for Cl^- and Br^-. In addition, a few equilibria involving $HgI_2(s)$ are given.

As a by-product we obtained ΔG (25° C) for the reaction $Hg(l) + HgI_2(s) \rightarrow Hg_2I_2(s)$, a figure that should of course be independent of the special composition of the solutions in our experiments.

In the following, the experiments will be recorded in an order different from that followed in the previous parts of this series. The reason is that we have wanted to present straightforward calculations with as little use as possible of constants derived at a later stage.

TITRATIONS FOR $\varkappa_3$ AND $\varkappa_4$

We tried to study the redox potential of solutions of HgI_3^-, HgI_4^{2-} and I^- in equilibrium with solid Hg_2I_2. However, the emf between a Pt electrode in such solutions, and a calomel electrode, proved to be still less reproducible than the corresponding emf with Hg^{II}, $Hg_2Br_2(s)$, and excess of Br^-.

Thus another method had to be used. By means of a Hg electrode we measured the concentration of free Hg^{2+} in a solution containing HgI_3^-, HgI_4^{2-} and I^- (later on analogous measurements were carried out with Br; they were described in Part VI [6]). In such titrations:

$$[Hg^{II}]_{total} = a = [HgX_2] + [HgX_3^-] + [HgX_4^{2-}] = [Hg^{2+}]X^2\ (\varkappa_2 + \varkappa_3 X + \varkappa_4 X^2) \qquad (1 = \text{VI, } 17)$$

$$X_e = [X^-] + [HgX_3^-] + 2\ [HgX_4^{2-}] = X + [Hg^{2+}]X^3\ (\varkappa_3 + 2\ \varkappa_4 X) \qquad (2 = \text{VI, } 18)$$

It was necessary to use such concentrations (a and X) that Hg_2X_2 did not precipitate. The condition for equilibrium with Hg and Hg_2X_2:

$$a = [HgX_2] + [HgX_3^-] + [HgX_4^{2-}] = k_s k_0^{-1}(\varkappa_2 + \varkappa_3 X + \varkappa_4 X^2) \qquad (3 = \text{VI, } 19)$$

gave when the constants were known for X = I, the following maximum values for a: $|X$ mC, a_{max} mC$|1$ 0.014$|10$ 0.29$|20$ 0.93$|50$ 5.03$|100$ 19.1$|$. Accordingly higher total Hg^{II} concentrations, a, could be used than for Br.

Just as for Br, the concentration of Hg^{2+} was calculated from

$$E^{st} = \tfrac{1}{2}\,(E_{20} + E_{10}) + 29.58 \log [Hg^{2+}] = 496.95 + 29.58 \log [Hg^{2+}] \qquad (4 = III,\ 20)$$

and the function

$$a\ [Hg^{2+}]^{-1}X^{-3} = \varkappa_2 X^{-1} + \varkappa_3 + \varkappa_4 X \approx \varkappa_3 + \varkappa_4 X \qquad (5 = VI,\ 20)$$

was computed and plotted against X. Here, too, $[HgX_2]$ can be neglected. As a first approximation we put $X = X_e - 2\,a$, and from this first diagram calculated approximate values for $\varkappa_3$ and $\varkappa_4$. Then we calculated the correction

$$X_e - X = [HgX_3^-] + 2\,[HgX_4^{2-}] = \frac{a\,(\varkappa_3 + 2\,\varkappa_4 X)}{\varkappa_3 + \varkappa_4 X} = 2\,a - a\,(1 + \varkappa_4\varkappa_3^{-1}X)^{-1} \qquad (6)$$

We used three values for $\varkappa_4\varkappa_3^{-1}$ (0.1; 0.2 and 0.3 mC^{-1}), one within and one on each side of the range that we thought possible. Formula (6) is easily derived from (2) if $[HgI_2]$ is neglected, which can be shown to be permissible.

In Fig. 1 are plotted the points of (5) for two different titrations for each of the a values 0.5, 2, and 5 mC. Those for 2 mC are recorded in Table 1. The corrections have been made assuming $\varkappa_4\varkappa_3^{-1}$ to be 0.2 mC^{-1}. (With the other values the differences were insignificant.) From these measurements we estimated

$$\varkappa_3 = (4.0 \pm 1.5) \cdot 10^{18}\ mC^{-3} = (4.0 \pm 1.5) \cdot 10^{27}\ C^{-3}$$
$$\varkappa_4 = (6.8 \pm 0.3) \cdot 10^{17}\ mC^{-4} = (6.8 \pm 0.3) \cdot 10^{29}\ C^{-4} \qquad (7)$$

It seems that Sherrill [8] found the right order of magnitude for $\varkappa_4$ ($1.9 \cdot 10^{30}$), whereas Malyugina's [12] value (10^{27}) was too low. In the literature we have found no direct determination of $\varkappa_3$.

SOLUBILITY OF HgI_2

The solubility of HgI_2 (s, red) at 25° C in a solution containing 490 mC $NaClO_4$, and 10 mC $HClO_4$ has been determined by George Biedermann, mag. chem., using a colorimetric method [14]. He found 0.074 ± 0.003 mC, which value is accepted in our calculations:

$$s = 0.074 \pm 0.003\ mC \qquad (8)$$

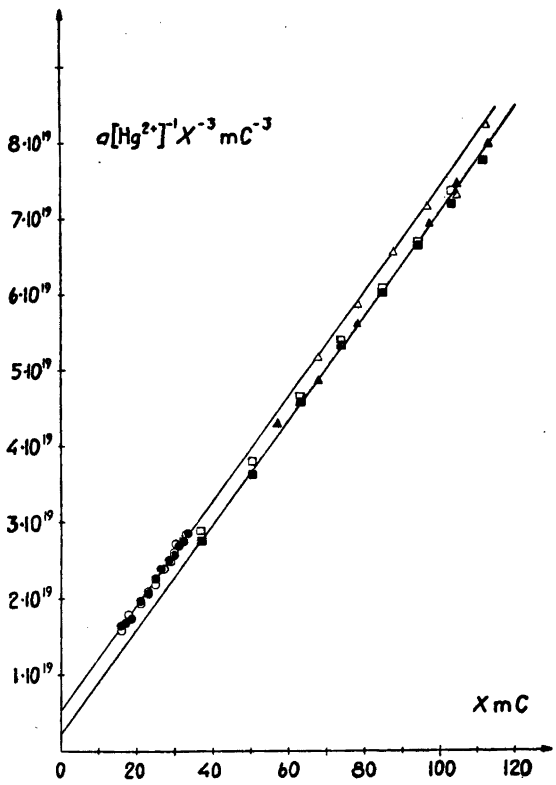

Fig. 1. Titrations for $\varkappa_3$ and $\varkappa_4$ (See Table 1), For ○ and ● $a = 0.497_5$ mC; □ and ■ $a = 1.999$ mC, △ and ▲ $a = 5.049$ mC. Calomel electrode G was used in all titrations except that marked △, where A was used. Upper line: $\varkappa_3 = 5.5 \cdot 10^{18}$ mC^{-3}; $\varkappa_4 = 6.8 \cdot 10^{17}$ mC^{-4}. Lower line: $\varkappa_3 = 2.5 \cdot 10^{18}$ mC^{-3}; $\varkappa_4 = 6.8 \cdot 10^{17}$ mC^{-4}.

TITRATIONS FOR b_1 AND b_2

If Cl^- or Br^- is added to a Hg_2^{2+}—Hg^{2+} solution, the redox potential rises slowly to a maximum value, which is attained at the point where $[HgX^+]$ is maximal, and $[Hg^{2+}] = [HgX_2]$. After this maximum point the redox potential decreases, first slowly and then with increasing slope [4]. From this maximum emf and the value of k_{12} obtained previously [2], we could calculate the values of the first two complex products, $\varkappa_1$ and $\varkappa_2$.

When we tried to make analogous experiments with I^-, however, there was a break in the curve E versus v, the volume of I^- solution added (*cf.* Fig. 2 where the halogen excess X_e is used as coordinate instead of v). The precipitate, which had previously consisted of pure yellow Hg_2I_2, after the break

Table 1. Titrations for $\varkappa_3$ and $\varkappa_4$.

Hg electrode, calomel electrode »G»
$S = 1.99_9$ mC HgI_2, 9.99_8 mC I^- (100 ml)
$T = 1.99_9$ mC HgI_2, 351.4 mC I^- (v ml)
■ *4. 9. 1946* □ *6. 9. 1946*

V ml	X_e mC	X mC	$-E$ mV ■	$-E$ mV □	$-\log [Hg^{2+}]$ ■	$-\log [Hg^{2+}]$ □	$a[Hg^{2+}]^{-1}$ ■	X^{-3} mC^{-3} □ 10^{-19}
5.00	26.25	22.62	(191.9)	(190.5)	(23.289)	(23.242)	(3.364)	(3.015)
10.00	41.03	37.27	208.6	209.25	23.854	23.876	2.760	2.904
15.00	54.53	50.71	224.2	224.65	24.381	24.396	3.689	3.821
20.00	66.89	63.05	235.4	235.6	24.760	24.766	4.593	4.664
25.00	78.27	74.40	243.7	243.95	25.040	25.049	5.330	5.434
30.00	88.78	84.89	250.4	250.4	25.267	25.267	6.044	6.043
35.00	98.50	94.61	255.8	255.95	25.449	25.454	6.644	6.723
40.00	107.5	103.6	260.35	260.6	25.603	25.612	7.219	7.360
45.00	115.9	112.0	264.35		25.738		7.797	

The values for X were calculated assuming $\varkappa_4 \varkappa_3^{-1} = 0.2$ mC^{-1}. For $v = 5$, solid Hg_2I_2 was still present, so the values were not used in our calculations.

also contained red HgI_2. Thus at the concentrations a used by us, the point where $[Hg^{2+}] = [HgI_2]$ could not be attained because of the low solubility of HgI_2. It would have been possible to overcome this by using very low a, but then the attainment of equilibrium could be expected to be slow and the potentials unsteady.

We tried instead to get as much information as possible from the broken curves obtained.

A number of preliminary experiments showed that the attainment of equilibrium was rather slow. Thus, in the titrations which were performed for the calculations and which are represented in Fig. 2, the first point was measured only after 2—5 hours, and the subsequent points each after $\frac{3}{4}$—2 hours' waiting; after these intervals the E seemed to be constant.

For both parts of the curve we have (cf IV, 7), neglecting the concentrations of I^-, HgI_3^-, HgI_4^{2-}, and soluble I^- complexes of Hg_2^{2+},

$$[Hg_2^{2+}]_{total} = [Hg_2^{2+}] + [Hg_2I_2]_{solid} \qquad (9\ a)$$

$$[Hg^{2+}]_{total} = [Hg^{2+}] + [HgI^+] + [HgI_2] + [HgI_2]_{solid} = a \qquad (9\ b)$$

$$[I^-]_{total} = 2\ [Hg_2I_2]_{solid} + [HgI^+] + 2\ [HgI_2] + 2\ [HgI_2]_{solid} \qquad (9\ c)$$

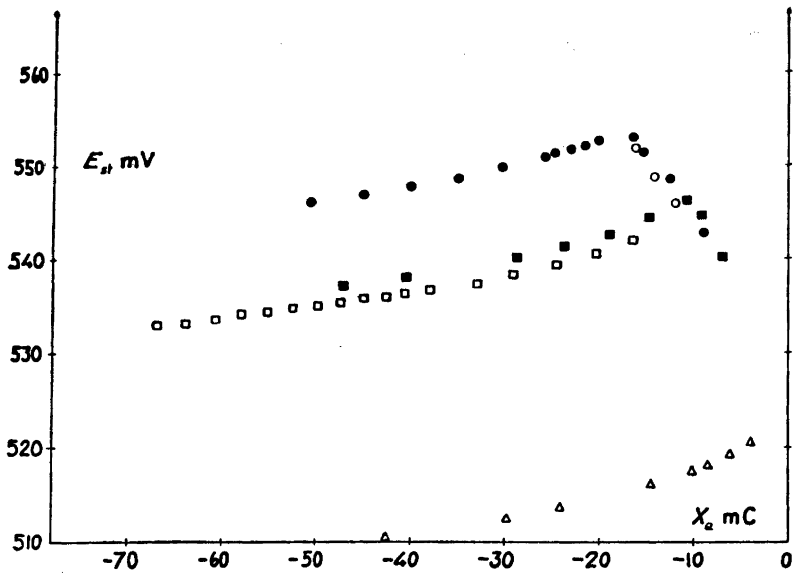

Fig. 2 Titrations for b_1 and b_2, E^{st} (standardized, see [3]) with Pt electrode plotted against X_e.

Symbol	Date	S (100 ml) mC Hg^{2+}	mC Hg_2^{2+}	mC Hg_2I_2	T (v ml) mC Hg^{2+}	mC I^-
△	15. 12. 1947	1.298	20.00	20.01	1.298	100.00
□	1. 12. 1947	3.536	30.00	10.00	3.536	100.00
■	9. 12. 1947	3.536	20.00	20.01	3.536	100.00
○	29. 7. 1947	5.140	7.78	19.00	5.140	47.40
●	17. 12. 1947	5.181	20.21	10.00	5.181	150.00

The excess of halogen added, X_e (which is negative in these experiments), is defined by

$$X_e = [I^-]_{total} - 2\ [Hg_2^{2+}]_{total} - 2\ [Hg^{2+}]_{total} \qquad (10 = IV,\ 1)$$

It can easily be calculated from known quantities: the volumes added of the solutions S and T, and the concentrations in them of the Hg^{2+}, Hg_2^{2+}, and I^- ions. From (9) and (10)

$$-X_e = 2\ [Hg^{2+}] + [HgI^+] + 2\ [Hg_2^{2+}] \qquad (11 = IV,\ 8)$$

From the emf measured we can calculate directly the ratio

$$q = [Hg^{2+}]^2[Hg_2^{2+}]^{-1} \qquad (12)$$

since $E = E_{20} + 29.58 \log q$ (13 = III, 3)

Thus each titration provides us with a series of corresponding values of X_e and q for the a in question.

We shall find it convenient to introduce the two equilibrium constants:

$$Hg_2I_2(s) + 2\ Hg^{2+} \rightleftharpoons Hg_2^{2+} + 2\ HgI^+$$
$$b_1 = [Hg_2^{2+}]\ [HgI^+]^2\ [Hg^{2+}]^{-2} = \varkappa_1^2 k_s \tag{14}$$

$$Hg_2I_2(s) + Hg^{2+} \rightleftharpoons HgI_2(s) + Hg_2^{2+}$$
$$b_2 = [Hg_2^{2+}]\ [Hg^{2+}]^{-1} = k_s k_{2s}^{-1} = \varkappa_2 k_s s^{-1} \tag{15}$$

where k_{2s} is the solubility product of HgI_2:

$$HgI_2(s) \rightleftharpoons Hg^{2+} + 2\ I^-;\ k_{2s} = [Hg^{2+}]\ [I^-]^2 = s\varkappa_2^{-1} \tag{16}$$

In the first part of the curve, before HgI_2 has precipitated, only the equilibrium (14) exists, and not (15). If, for brevity's sake, we denote $[Hg^{2+}]$ by x, we find from (12) and (14)

$$[Hg^{2+}] = x;\ [HgI^+] = \sqrt{b_1 q};\ [Hg_2^{2+}] = x^2 q^{-1} \tag{17}$$

and from (9 b), (11) and (17), introducing the quantity a'

$$a - [HgI_2] = x + \sqrt{b_1 q} = a' \tag{18}$$

$$-X_e = 2\ x + \frac{2\ x^2}{q} + \sqrt{b_1 q} \tag{19}$$

From the definitions of the equilibrium constants it is seen that, in equilibrium with solid Hg_2I_2,

$$[HgI_2] = k_s \varkappa_2 \cdot \frac{[Hg^{2+}]}{[Hg_2^{2+}]} = \frac{b_2 s [Hg^{2+}]}{[Hg_2^{2+}]} = \frac{b_2 s q}{x} =$$

$$= \frac{b_2 s q}{a - \sqrt{b_1 q} - [HgI_2]} \tag{20}$$

Since, in our experiments, $[HgI_2]$ was only a small correction to a, it could be calculated from (20) with all the accuracy needed as soon as approximate values of s, b_1, and b_2 were known.

We eliminate $x = a' - \sqrt{b_1 q}$ from (18) and (19) and find

$$f_1 = b_1 - f_2\sqrt{b_1} \tag{21}$$

$$f_1 = -\tfrac{1}{2}\ X_e - a' - a'^2 q^{-1} \tag{22}$$

$$f_2 = \tfrac{1}{2}\ \sqrt{q} + 2a' q^{-\frac{1}{2}} \tag{23}$$

The calculations were made as follows:

First f_1 and f_2 were calculated by means of (22) and (23) from the known quantities a, X_e, and q neglecting $[HgI_2]$, thus using a instead of a'. In a diagram the points $f_1(f_2)$, and a set of lines $y = k - x\sqrt{k}$ (*cf.* 21) were drawn. The k value corresponding to the points of lowest q (where the correction for HgI_2 is negligible) was an approximate value for b_1. Now with this value for b_1 we calculated an approximate value for b_2 in the way to be described below. With these preliminary values for b_1 and b_2 we computed the correction $[HgI_2]$ in (18) using (20). Corrected values a' were used for calculating f_1 and f_2, and a new diagram was constructed (Fig. 3). A new approximation was not found necessary and we concluded

$$b_1 = 18.5 \pm 2.5 \text{ mC} \tag{24}$$

For the second part of the curve, after solid HgI_2 has appeared, we assume that the equilibria (14) and (15) both exist. From (12), (14) and (15) we find

$$[Hg^{2+}] = b_2 q;\ [HgI^+] = \sqrt{b_1 q};\ [Hg_2^{2+}] = b_2^2 q \tag{25}$$

and thus with (11)

$$-X_e = 2\ q(b_2 + b_2^2) + \sqrt{b_1 q}$$

or

$$-X_e q^{-\frac{1}{2}} = \sqrt{b_1} + 2(b_2 + b_2^2)\sqrt{q} \tag{26}$$

From (26) it follows that q should be the same function of $(-X_e)$, independent of the original Hg^{II} concentration a. This is fulfilled to a certain extent, as is seen from Fig. 2, where the latter parts of the various curves E^{st} (X_e) almost coincide. The deviations we are inclined to ascribe to the slowness with which solid HgI_2 and Hg_2I_2 attain their stable equilibrium states and to

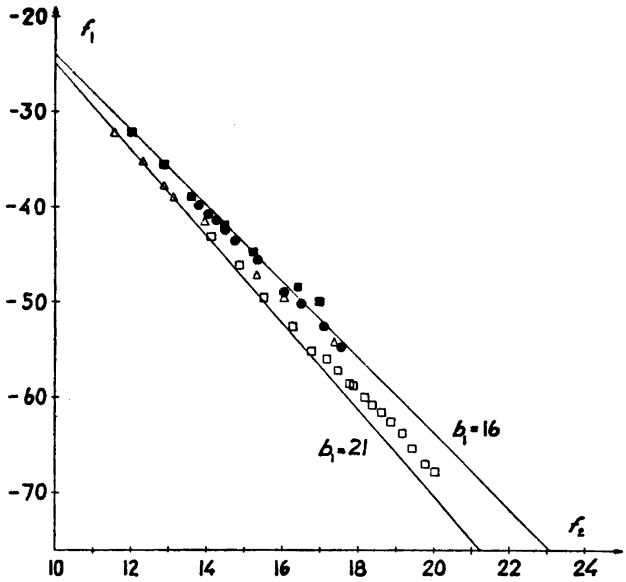

Fig. 3. Diagram for calculating b_1 by means of (21), (22), and (23). The notations for the points from differen ttitrations are the same as in Fig. 2. Upper line: $b_1 = 16$; lower line: $b_1 = 21$. On the addition of more I^- (shift to the right in Fig. 2), f_2 decreases (shift to the upper left in Fig. 3).

the inaccuracy in X_e rather than to the formation of complexes of other types than hitherto assumed, *e. g.* Hg_2I^{3+} or Hg_2I^+.

In Fig. 4 ($-X_e q^{-\frac{1}{2}}$) has been plotted against $\sqrt{q}$. The points which should according to (26) be situated on a straight line are seen to spread considerably. Thus it is quite impossible from this diagram to compute b_1 and b_2 independently. If, however, the value for b_1 from (24) is accepted, we see that the experimental points all lie between the straight lines corresponding to $b_2 = 2.8$ and $b_2 = 3.28$. We thus conclude

$$b_2 = 3.05 \pm 0.25$$

In these calculations no special weight has been put on the position of the peak in q, since the precipitation of HgI_2 might have been slightly delayed.

Experimental note: The solution S was mixed by adding first the calculated volumes of $NaClO_4$, $HClO_4$, and NaI solutions, then Hg^{2+} solution, and finally Hg_2^{2+}. In this way a yellow precipitate of Hg_2I_2 was obtained, and the emfs were steady. If Hg_2^{2+} was

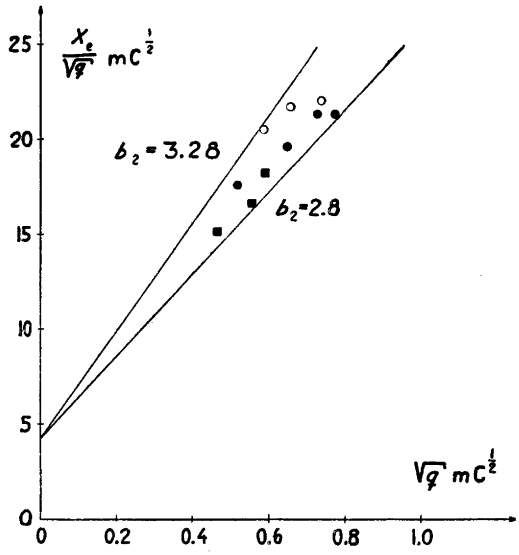

Fig. 4. Diagram for calculating b_2 by means of (26). The notations for the points from different titrations are the same as in Fig. 2. Upper line: $b_1 = 18.5$, $b_2 = 3.28$; lower line: $b_1 = 18.5$, $b_2 = 2.8$.

added before Hg^{2+}, a greenish mixture of Hg_2I_2 and Hg first precipitated, which did not change its colour in a reasonable time, and creeping potentials were obtained.

It was important that all solutions added had already been liberated from air very carefully by nitrogen bubbling before the mixing, since otherwise free iodine appeared.

TITRATIONS FOR k_s

The solubility product k_s of Hg_2I_2 was determined in the same way as for Cl^- and Br^-, by adding I^- to a Hg_2^{2+} solution and measuring E between a Hg electrode in this solution and a calomel electrode with 4 C NaCl. From the first point of the titration, where Hg_2^{2+} is in excess, the quantity E_{10} is calculated by the equation

$$E_{10} = E\text{—}29.58 \log [Hg_2^{2+}] = E - 29.58 \log (-\tfrac{1}{2}X_c) + \delta E \qquad (27 = \text{III}, 12)$$

where δE is the correction for the amounts of Hg^{2+} and HgX^+ present at equilibrium

$$\delta E \approx 12.85\ k_0^{-1} + 6.42\ \varkappa_1 k_0^{-1}\sqrt{k_s}[Hg_2^{2+}]^{-\frac{1}{2}} = 12.85\ k_0^{-1} + 6.42\ k_0^{-1}\sqrt{b_1}\,[Hg_2^{2+}]^{-\frac{1}{2}} \qquad (28 = \text{III}, 13)$$

By inserting the numerical values for k_0 and b_1 with $X^- = I^-$ we find $|[Hg_2^{2+}], \delta E|4$ mC, 0.21 mV$|1$ mC, 0.31 mV$|0.25$ mC, 0.53 mV$|$. Before any I^- has been added,$\delta E = 0.10$ mV as usual. These corrections are practically the same as those calculated from preliminary values in (III, 14).

After the equivalence point there is excess of I^- and

$$X_e = [X^-] + [HgX_3^-] + 2\,[HgX_4^{2-}] = X(1 + \varkappa_3 k_s k_0^{-1} + 2\,\varkappa_4 k_s k_0^{-1} X) \qquad (29 = \text{III, } 21)$$

The quantity E_{1X} is calculated from

$$E_{1X} = E + 59.16 \log X = E + 59.16 \log X_e - \delta'' E$$
$$\delta'' E \approx 25.7\,(\varkappa_3 k_s k_0^{-1} + 2\,\varkappa_4 k_s k_0^{-1} X) \qquad (30 = \text{VI, } 9)$$

If the numerical values for $X^- = I^-$ are inserted, we find

$$\delta'' E = 0.27 + 0.093\,X \text{ mV} \qquad (31)$$

The corrections (28, 31) are thus much larger than for Cl^- and Br^-. However, for the small values of X used by us, the approximations implied in (28) and (31) are still permissible.

Table 2 gives a titration chosen at random, and Table 3 summarizes our measurements of E_{10} and E_{1X} for I^-. As average value we have chosen

$$E_{10} - E_{1X} = -29.58 \log k_s = 546.2 \pm 0.3 \text{ mV} \qquad (32)$$

Table 2. Titration for E_{1X}.

$S = 4.68$ mC Hg_2^{2+}, 0.045 mC Hg^{2+}
$T = 49.65$ mC I^-

v ml	X_e mC	59.16 log X_e mV	E mV	$E_{1X} + \delta'' E$ mV	$\delta'' E$ mV	E_{1X} mV
22.80	1.523	10.80	− 123.4	− 112.60	0.41	− 113.01
23.93	1.961	17.31	− 129.75	− 112.44	0.45	− 112.89
24.02	1.995	17.75	− 129.8	− 112.05	0.46	− 112.51
26.24	2.834	26.76	− 138.7	− 111.94	0.53	− 112.47
26.29	2.852	26.92	− 139.0	− 112.08	0.54	− 112.62
29.03	3.845	34.60	− 146.3	− 111.70	0.63	− 112.33
29.07	3.860	34.70	− 146.5	− 111.80	0.63	− 112.43
31.32	4.645	39.46	− 151.2	− 111.74	0.70	− 112.44
33.77	5.469	43.65	− 155.4	− 111.75	0.78	− 112.53
37.16	6.561	48.33	− 160.1	− 111.77	0.88	− 112.65
42.00	8.030	53.52	− 165.2	− 111.68	1.02	− 112.70

Average: $E_{1X} = -112.6$ mV

Table 3. Measurements of E_{10} and E_{1X}.

Month	E_{10}	E_{1X}	$E_{10}-E_{1X}$
7. 1945	434.8	− 111.5	546.3
3. 1946	433.7	− 112.6	546.3
3. 1946	433.8	− 112.4	546.2
11. 1947	433.8	− 112.3	546.1
11. 1947	433.7	− 112.2	545.9
11. 1947	433.75	− 112.7	546.45

Average: 546.2 ± 0.3

thus $\log k_s = -18.465 \pm 0.010$

$$k_s = (3.43 \pm 0.08) \cdot 10^{-19} \text{ mC}^3 = (3.43 \pm 0.08) \cdot 10^{-28} \text{ C}^3 \quad (33)$$

The activity product of Hg_2I_2 at 25° C has been determined previously by Sherrill [8] ($1.2 \cdot 10^{-28}$) and by Brodsky and Scherschewer [15] ($5.0 \cdot 10^{-29}$). From Bates and Vosburgh's [16] data, Latimer[17] has computed $4.5 \cdot 10^{-29}$. Thus the product of the ionic activity factors in our solutions would be about 0.13 for Hg_2I_2, as compared with about 0.11 for Hg_2Br_2 [6] and 0.09 for Hg_2Cl_2 [5].

DISCUSSION

From the results above we obtain, using (14) and (15),

$$\begin{aligned} \varkappa_1 &= \sqrt{b_1 k_s^{-1}} = (7.3_5 \pm 0.5) \cdot 10^{12} \text{ C}^{-1} \\ \varkappa_2 &= b_2 s k_s^{-1} = (6.6 \pm 0.6) \cdot 10^{23} \text{ C}^{-2} \\ k_{12} &= b_1 b_2^{-1} s^{-1} = 82 \pm 14 \end{aligned} \quad (34)$$

Thus Morse [7] and Sherrill [8] found values of the right order of magnitude (Morse $\varkappa_1 = 2.5 \cdot 10^{13}$, $\varkappa_2 \approx 10^{25}$; Sherrill $\varkappa_2 \approx 2.6 \cdot 10^{24}$). The value 115 ± 25 was found for k_{12} by Sillén and Infeldt [2] under conditions identical with ours We consider our value to be more accurate, though the limits of error overlap

We can moreover calculate:

$$\begin{aligned} q_1 &= \varkappa_3 \varkappa_2^{-1} = \varkappa_3 k_s b_2^{-1} s^{-1} = 6100 \pm 2400 \text{ C}^{-1} \\ q_2 &= \varkappa_4 \varkappa_2^{-1} = \varkappa_4 k_s b_2^{-1} s^{-1} = (1.03 \pm 0.11) \cdot 10^6 \text{ C}^{-2} \end{aligned} \quad (35)$$

Fromherz and Lih's value for q_2, $(1.1 \cdot 10^5)$[10], is of the same order of magnitude as ours but not Job's $(0.8 \cdot 10^8)$[11]. A number of equilibrium constants derived from our measurements are listed in Table 4.

Table 4. Summary of equilibrium constants.

Reaction	Equilibrium constants	log of equil. const. (C scale)
$Hg^{2+} + I^- \rightleftharpoons HgI^+$	$\varkappa_1 = \sqrt{b_1 k_s^{-1}} = (7.3_5 \pm 0.5)\ 10^{12}\ C^{-1}$	12.866 ± 0.028
$Hg^{2+} + 2I^- \rightleftharpoons HgI_2$	$\varkappa_2 = b_2 s k_s^{-1} = (6.6 \pm 0.6)\ 10^{23}\ C^{-2}$	23.818 ± 0.040
$Hg^{2+} + HgI_2 \rightleftharpoons 2HgI^+$	$k_{12} = b_1 b_2^{-1} s^{-1} = 82 \pm 14$	1.914 ± 0.067
$Hg^{2+} + 3I^- \rightleftharpoons HgI_3^-$	$\varkappa_3 = (4.0 \pm 1.5)\ 10^{27}\ C^{-3}$	27.602 ± 0.138
$Hg^{2+} + 4I^- \rightleftharpoons HgI_4^{2-}$	$\varkappa_4 = (6.8 \pm 0.3)\ 10^{29}\ C^{-4}$	29.832 ± 0.019
$HgI_2 + I^- \rightleftharpoons HgI_3^-$	$q_1 = \varkappa_3 k_s b_2^{-1} s^{-1} = 6100 \pm 2400\ C^{-1}$	3.784 ± 0.144
$HgI_2 + 2I^- \rightleftharpoons HgI_4^{2-}$	$q_2 = \varkappa_4 k_s b_2^{-1} s^{-1} = (1.03 \pm 0.11)\ 10^6\ C^{-2}$	6.014 ± 0.044
$2HgI_2 \rightleftharpoons HgI^+ + HgI_3^-$	$\varkappa_3 b_1^{\frac{1}{2}} k_s^{\frac{3}{2}} b_2^{-2} s^{-2} = (6.8 \pm 2.8)\ 10^{-8}$	-7.168 ± 0.152
$HgI_2(s) \rightleftharpoons HgI_2$	$s = (7.4 \pm 0.3)\ 10^{-5}\ C$	-4.131 ± 0.018
$HgI_2(s) + I^- \rightleftharpoons HgI_3^-$	$\varkappa_3 k_s b_2^{-1} = 0.45 \pm 0.18$	-0.347 ± 0.143
$HgI_2(s) + 2I^- \rightleftharpoons HgI_4^{2-}$	$\varkappa_4 k_s b_2^{-1} = 76 \pm 8\ C^{-1}$	1.883 ± 0.040
$Hg_2I_2(s) \rightleftharpoons Hg_2^{2+} + 2I^-$	$k_s = (3.43 \pm 0.08)\ 10^{-28}\ C^3$	-27.465 ± 0.010
$Hg_2I_2(s) + 2Hg^{2+} \rightleftharpoons Hg_2^{2+} + 2HgI^+$	$b_1 = 0.0185 \pm 0.0025\ C$	-1.733 ± 0.055
$Hg_2I_2(s) + Hg^{2+} \rightleftharpoons HgI_2(s) + Hg_2^{2+}$	$b_2 = 3.05 \pm 0.25$	0.484 ± 0.034
$Hg(l) + Hg^{2+} \rightleftharpoons Hg_2^{2+}$	$k_0 = 129.2 \pm 1.0$	2.111 ± 0.003
$Hg_2I_2(s) \rightleftharpoons Hg(l) + HgI_2$	$s b_2 k_0^{-1} = (1.75 \pm 0.16)\ 10^{-6}\ C$	-5.758 ± 0.039
$Hg_2I_2(s) + I^- \rightleftharpoons Hg(l) + HgI_3^-$	$\varkappa_3 k_s k_0^{-1} = 0.010_6 \pm 0.004$	-1.974 ± 0.138
$Hg_2I_2(s) + 2I^- \rightleftharpoons Hg(l) + HgI_4^{2-}$	$\varkappa_4 k_s k_0^{-1} = 1.80 \pm 0.10\ C^{-1}$	0.256 ± 0.022

Our equilibrium constants for $HgI_2(s) + I^- \rightleftharpoons HgI_3^-$, and $HgI_2(s) + 2\ I^- \rightleftharpoons HgI_4^{2-}$; 0.45 ± 0.18, and 76 ± 8, can be compared with Garrett's [13]: 0.48 and 35. Our equilibrium constant for $Hg_2I_2(s) + 2\ I^- \rightleftharpoons Hg(1) + HgI_4^{2-}$, 1.80 ± 0.10 is not far from Hamburger's [8] value 2.02. It should be remembered that our measurements refer to a constant ionic strength larger than that usually used by these previous workers.

In addition, our measurements permit us to calculate a value for ΔG for the reaction between Hg metal, solid HgI_2, and solid Hg_2I_2.

We have studied the two equilibria

$$Hg^{2+} + Hg(l) \rightleftharpoons Hg_2^{2+}\ ;\ [Hg_2^{2+}]\ [Hg^{2+}]^{-1} = k_0 = 129.2 \pm 1.0$$
$$\Delta G_0 = -RT\ln k_0 \qquad \text{(III, 18 = 36)}$$

$$Hg^{2+} + Hg_2I_2(s) \rightleftharpoons Hg_2^{2+} + HgI_2(s)\ ;\ [Hg_2^{2+}]\ [Hg^{2+}]^{-1} = b_2 = 3.05 \pm 0.25$$
$$\Delta G_0 = -RT\ln b_2 \qquad (37)$$

Here, ΔG_0 means ΔG for the reaction in question at 25° C with $[Hg_2^{2+}] = 1$ mC, $[Hg^{2+}] = 1$ mC, and the ionic medium used in our experiments. By combination we find

$Hg(l) + HgI_2(s) \rightarrow Hg_2I_2(s)$ (25° C)

$$\Delta G = -RT \ln k_0 + RT \ln b_2 = -RT \ln 10 (\log k_0 - \log b_2) = -5.708$$

$$(1.627 \pm 0.034) \text{ kj} = -9.29 \pm 0.19 \text{ kj} = -2.22 \pm 0.05 \text{ kcal} \quad (38)$$

This value should be quite independent of the ionic medium and only influenced by temperature and pressure. Obviously HgI_2 is not stable in the presence of metallic Hg.

SUMMARY

By electrometric measurements, a number of equilibrium constants involving Hg^{2+}, HgI^+, HgI_2, HgI_3^-, HgI_4^{2-}, $HgI_2(s)$, Hg_2^{2+}, $Hg_2I_2(s)$, and Hg(l) have been determined and listed in Table 4. They are valid under the special conditions 25° C, $[H^+] = 10$ mC, ionic strength 500 mC.

For $Hg(l) + HgI_2(s) \rightarrow Hg_2I_2(s)$, ΔG (25° C) $= -9.29 \pm 0.19$ kj $= -2.22 \pm 0.05$ kcal

In a concluding paper, the results of this paper and the previous parts I—VI of this series will be discussed and visualized by means of diagrams.

Our thanks are due to Magister Sirkka Hietanen, Mr. Erik Ekedahl, and Mr. George Biedermann for their valuable aid.

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Received April 19, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 520—524

Plastein, a Mixture of Higher-molecular Polypeptides Synthesized by Proteolytic Enzymes

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The investigations carried out in this laboratory on the formation of plastein have led to results which can be interpreted in two different ways depending on how large peptides compose the plasteins. (1) If the mol. wt. of plasteins is low (some hundreds), cyclopeptides must be concerned since the amount of α-amino nitrogen in preparations thoroughly extracted with water has been only 2—3 per cent of total nitrogen. The explanation of the occurrence of this small amount of α-amino nitrogen in plasteins presupposes already by-hypotheses. It may be assumed that either open peptides are absorbed in the precipitate or a part of the cyclopeptides contain a side chain with a free α-amino group. (2) If the molecular weight is several thousands, the plasteins must be open chains. All our experimental results will also suit this opinion. The presence of α-amino nitrogen in the preparations would then be easily explicable, and amino nitrogen would also express the size of peptides.

Alternative (1) seemed at first most probable because the molecular weight of plastein could be considered low both on the basis of our cryoscopic determinations [1, 3] and the ultracentrifuge determinations of Svedberg [4] and Ecker [5] recorded in the literature. Collier [6] found in his ultracentrifuge determinations particles of the size of proteins from plastein preparations in a 6.7 M urea solution. The plasteins were, however, not characterized in regard to their α-amino nitrogen content. As was shown in this laboratory [3] a large amount of peptides which are probably not synthetic products are precipitated also with raw plastein.

After dividing by electrophoresis the peptides formed in pepsin hydrolysis of zein into electrically more homogeneous fractions we were able to precipitate from them, with pepsin, plasteins not accompanied by peptides found in the hydro-

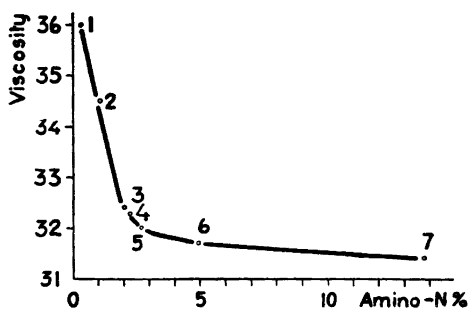

Fig. 1. Viscosity of 1 % solutions in millipoises at 18°C. Solvent 0.1 N NaOH in 60 % ethanol. (1) Zein, (2) Zein hydrolyzed with water at 100°, fraction with 1.05 % amino-N, (3) Zein plastein with 1.97 % amino-N, (4) Zein hydrolyzed with water at 100°, fraction with 2.21 % amino-N, (5) Zein plastein with 2.65 % amino-N, (6) Zein hydrolyzed with water at 100°, fraction with 4.90 % amino-N, (7) Zein hydrolyzed with water at 100°, fraction with 13.73 % amino-N.

lysate. Such plastein precipitates contained, without extraction with water, only 1.5 to 2.0 % of the total nitrogen as α-amino nitrogen. This amino nitrogen cannot be removed with water extractions, hence, it seems evident that it belongs to the plasteins. Occurrence of α-amino nitrogen in cyclopeptides is difficult to understand (*cf.* above) and therefore the low molecular weight and cyclic structure of plasteins began to seem improbable. Comparative viscosity determinations from peptides (average α-amino nitrogen 1.05 to 13.7 per cent of the total nitrogen) formed by prolonged hot water hydrolysis from zein, and from plastein preparations (α-amino nitrogen 1.97 per cent and 2.65 per cent of the total nitrogen) have yielded values which can be explained only by assuming plasteins to have an open peptide structure like the peptides formed in the hydrolysis [8] (Fig. 1).

Moreover, it was ascertained in this laboratory [8] that even rather large-sized peptides formed in the hydrolysis of zein, whose average molecular weight judged from α-amino nitrogen should be several thousands, cause both in formic acid, acetic acid, and phenol depressions of freezing point which indicate that the mol. wt. of peptides would be only a few hundreds. Therefore the values for plasteins obtained earlier by the cryoscopic method in polar solvents have no power of evidence in regard to the molecular weights of plastein. The latest determinations of mol. wt. by the cryoscopic method using a non-polar compound (lactam of 4-amino-cyclo-hexane-carboxylic acid [7]) for the solvent have yielded mol. weight values of about 4000 for plastein preparations with 1.6 % α-amino nitrogen. Judging from the amino nitrogen the average mol. wt. should be about 6000. The accuracy of the cryoscopic method for so large molecules is already poor, but the value obtained indicates, at any rate, the order of magnitude. The values obtained by the diffusion method also show that the average mol. wt. of plasteins is several thousands.

The low mol. weight would thus be supported only by the ultracentrifuge determinations mentioned above [4, 5]. The plasteins used in them have, how-

ever, been of indefinite composition and not more closely characterized. According to our present experience they have evidently contained a large number of smallsized peptides. The determinations are consequently, not convincing although the total lack of larger particles in the plastein preparations used does not agree with the idea that plastein comprises high-molecular peptides.

As was reported already in our previous communication [3] the only quantitative criterion for the formation of plastein in protein hydrolysate — the hydrolysis of zein performed with pepsin at pH 1—2 and the plastein formation with pepsin at pH 4 — is the decrease of α-amino nitrogen. But this can be noted only in such concentrated hydrolysates in which the enzymatic hydrolysis has proceeded so far that amino nitrogen does no longer essentially increase (free amino acids are split from the pepsin hydrolysate to some extent, though slowly even after cessation of the hydrolysis proper). If plastein is precipitated before the hydrolysis has ceased decrease of amino nitrogen cannot often be noted because the larger peptides are simultaneously hydrolyzed. In completely hydrolyzed concentrated pepsin hydrolysates the decrease of amino nitrogen through the effect of pepsin can be shown also at pH 1—2, though no precipitate is then formed, at least not rapidly. At a higher pH, precipitate is formed almost momentaneously. The optimum for precipitation is pH 4. Decrease of amino nitrogen is also greatest in this acidity.

We have also hydrolyzed zein with pepsin at pH 4 and after concentration of the clear solution precipitated plastein with pepsin at the same pH. The yield of raw plastein was then much smaller than when zein was hydrolyzed at pH 1—2. This seems to be largely due to the fact that raw plastein which is obtained from zein hydrolyzed at pH 4 does not contain small-molecular products of hydrolysis, at any rate not appreciably, whereas the zein hydrolysate concentrated at pH 1—2 yields at pH 4 raw plastein the nitrogen of which may be composed up to the half of small-sized peptides removable with water. In the first experiment, when zein was hydrolyzed at pH 4, 92.2 per cent of the total nitrogen in zein was brought into solution in 26 days. α-Amino nitrogen in the hydrolysate was 16.4 % (NH_3-N, amide-N, and N of free amino acids subtracted) corresponding on the average to 5—6 peptides. In the concentrated hydrolysate (38.4 mg N per ml) precipitation of plastein was 8.8 % (N % of the total N of the solution). The decrease of amino N was 9.7 %, thus the precipitate evidently contained synthesized peptides only. The amino N of the precipitate was 2.0 % of the total N. The part of zein which was not brought into solution by peptic hydrolysis (7.8 % N of total N) was dissolved in 60 % alcohol up to 45.9 % N of total N. This can

be taken for zein. The fraction insoluble in alcohol contained 1.6 per cent α-amino N, hence it corresponded in size to »plasteins».

In the second experiment in which the hydrolysis of zein was also performed at pH 4, 19.2 per cent of the nitrogen brought into solution was α-amino nitrogen (NH_3-N, amide-N, and N of free amino acids subtracted) corresponding on the average to 5-peptides. Precipitation of plastein from the concentrated solution (38.6 mg N per ml) was in this case 12.9 % (N % of the total N of the solution). The decrease of amino N was 12.2 %. The plastein precipitate contained 1.6 % of the total nitrogen as amino nitrogen.

After the small molecular size of plasteins was proved invalid all the observations so far can be interpreted in the following way. The formation of plasteins by pepsin is a reaction reversed to hydrolysis. In dilute solutions (in our experiments the amount of zein corresponded to 2—3 mg N per ml) the equilibrium lies very far on the side of hydrolysis. When the hydrolysate is concentrated (in our plastein experiments the concentrated solution usually contained 30—45 mg N per ml) the reaction reversible to hydrolysis comes forth. At pH 4 this reaction proceeds farthest because the reaction product precipitates optimally and is thus removed from the system. The following scheme presents our concept of the action of pepsin.

Zein ⟶ High-molecular peptides (very different mol. size, highest peptide demonstrated by us in zein hydrolysis abt. 40-peptides, in plastein synthesis abt. 50-peptides) ⟶⟵ Low-molecular peptides (average tetra-penta-peptides)

The plasteins which have been precipitated from electrophoretically divided peptide fractions and contain least amino nitrogen (1.5 % of total N) correspond, on the basis of amino nitrogen, to the average mol. weight of abt. 6000, those containing most amino nitrogen (4.4 %) to abt. 2500. All the precipitates are unhomogeneous and contain obviously larger and smaller peptides.

The most abrupt decrease of α-amino nitrogen effected by pepsin in concentrated pepsin hydrolysate at pH 4 (zein hydrolyzed at pH 1) was in our experiments about 19 %. If, after removal of the precipitate, the filtrate is concentrated again, pepsin does no longer cause precipitation of plastein. This suggests that all peptides are not suitable for the synthesis.

SUMMARY

The former idea of the low molecular weight of plasteins (less than 1 000) does not agree with the more recent findings of this laboratory. The parallel viscosimetric determinations with plastein preparations and with polypeptide fractions formed in the hydrolysis of zein as well as the cryoscopic determinations using a non-polar solvent (lactam of 4-amino-cyclo-hexane-carboxylic acid) support the concept that the average molecular weight of plasteins is several thousands. The diffusion experiments also lead to similar results. Accordingly, the plasteins must be regarded as open polypeptides whose α-amino nitrogen results from the end groups. Thus pepsin causes both the hydrolysis and synthesis of polypeptides.

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Received May 20, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 525—538

Paper Chromatographic Analysis of Amino Acids and other Ninhydrin-Reacting Substances in Deproteinized Human Plasma

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In a recent paper the positions on a two-dimensional chromatogram of 14 ninhydrin-reacting substances from protein-free human plasma were determined [1]. 12 of the spots were shown to correspond to the positions of lysine, arginine, aspartic acid, glycine, serine, glutamine, alanine, threonine, proline, valine, tyrosine and probably α-aminobutyric acid. The positions of some other amino acids and ninhydrin-reacting compounds from protein hydrolysates were also demonstrated. Dent [2] has recently published a similar map. A comparison with his map showed that we obtained other positions for some of the amino acids. The probable explanation to the differences seems to be that we use Swedish filter paper sheets and somewhat different organic solvents. In our chromatograms we still missed some amino acids which from microbiological and chemical analysis are known to be present in the protein-free human plasma [3–5]. An attempt was therefore made to identify the positions of some of the missing amino acids.

EXPERIMENTAL

Solvents. With the exception of lutidine the organic solvents were always purified by distillation. The lutidine was a commercial product of Swedish source. Phenol was used as the first solvent in the two-dimensional runs and a mixture of pyridine and technical amyl alcohol in the second direction. Several substitutes for the pyridine-amyl alcohol mixture were tested. Equal parts of tertiary amyl alcohol and lutidine saturated with water gave the same good resolution or perhaps a little better separation of the spots. Mixtures

of tertiary amyl alcohol and pyridine or amyl alcohol and lutidine saturated with water were less successful. Phenol could be replaced by *o*-cresol in the two-dimensional runs but this solvent had no special advantages. Several two-dimensional runs were made with *o*-cresol and ammonia in the first direction and a mixture of benzyl alcohol and butanol in the second direction. These were carried out in the hope of obtaining a satisfactory resolution of the single spot given by leucine, isoleucine and phenylalanine. Acceptable results were not obtained.

In one-dimensional chromatography a satisfactory separation of the leucine and isoleucine spots were obtained either with the lutidine-tertiary amyl alcohol mixture in runs of 50 hours or with the lutidine-amyl alcohol mixture in runs of 60—80 hours. With the last mentioned solvent mixture a spot corresponding to the position and greyish green colour of phenylalanine could be distinguished from the leucine and isoleucine spots when a 0.1 per cent diethylamine solution was present at the bottom of the glass tray. 5, 10 or 50 per cent solutions of diethylamine gave more diffuse spots.

Paper qualities and dimensions. Of the Munktell filter paper sheets no. 0B and 00 the following three sizes 24 × 24 cm, 28 × 37 cm and 48 × 48 cm were compared with regard to resolving capacities. Number 0B is a quick-filtering paper while the fronts of the organic solvents move more slowly in no. 00 (de Verdier and Ågren [1]). In accordance with previous reports (Dent [6], Pratt and Auclair [7]) it was also found that some amino acids showed a tendency to destruction during the development of the chromatograms. From this point of view the quickfiltering paper no. 0B would generally be preferred. It was also found, when the 48 × 48 cm papers of the two qualities were compared, that it was necessary to work with at least 2 ml of protein-free human plasma on the no. 00 papers to obtain the usual 14 spots while 1 ml was a satisfactory amount on the no. 0B papers. Descending chromatography gave a better resolution of the spots than the ascending procedure. With the 24× 24 cm papers the ascending procedure gave better results than the descending method. 0.5 ml of protein-free plasma was a suitable amount to apply on the papers. Larger volumes of filtrates gave diffuse spots. However, in the pyridine-amyl alcohol direction the resolution of the spots were better when the 48 × 48 cm papers were used.

At last the following procedure was adopted which so far has given the best resolution of the spots. No. 0B papers of the dimension 37 × 28 cm are used. With ascending chromatography the phenol front is allowed to proceed along the 37 cm side of the paper as far as to the upper edge. From the dried paper the yellowish brown material deposited by phenol is cut away. The paper with a dimension of 28 × 28 cm is developed in the second direction

using pyridine-amyl alcohol and descending chromatography. The sheets are dried at room temperature overnight in a cabinet combined with the compressed air system. The dried papers are sprayed with a solution of 0.25 per cent ninhydrin in butanol containing 1 per cent of acetic acid. Drying at room temperature, also used by Dent [2], resulted in larger and more coloured spots as compared with some of the procedures previously used [1].

The protein-free filtrates. Detailed comparisons between filtrates obtained by five different deproteinizing methods (*cf.* de Verdier and Ågren [1]) were carried out. The following short comments may be made. Filtrates obtained by dialysis or by precipitation with trichloracetic acid, tungstic acid, ferric hydroxide or ethanol showed the same general pattern of spots on the two-dimensional chromatograms. However, the most clearcut pictures were obtained with the trichloracetic acid, ferric hydroxide and ethanol filtrates and with the dialysates, while the tungstic acid filtrates gave more diffuse spots. Several spots suspected to be caused by peptides were found close to the starting point in the two-dimensional chromatograms of the trichloracetic acid filtrates. Concentrating the investigation at first hand on the free amino acids, ethanol filtrates were used in most of the experiments. It is interesting to note that the ethanol filtrates which always showed lower amino nitrogen values than the other filtrates still gave somewhat larger and more coloured spots. An explanation to this behaviour may possibly be found in the recent find of Martin and Mittelmann [8] that the coloured ninhydrin product consists of several components whose proportions depend upon the conditions under which the colour is developed. The dialysates proved to be of special value for the identification of some amino acids.

The identification of amino acids by the use of colour tests. Pratt and Auclair [7] recently tested the minimum quantities of amino compounds which will give a visible colour with ninhydrin on a two-dimensional chromatogram when viewed by transmitted light. Comparing the listed values with those obtained by chemical and microbiological determinations carried out by Gutman and Alexander [9], Hier and Bergeim [3], Sheffner *et al.*[5] on protein-free plasma some conclusions could be drawn. When 1 ml volumes of protein-free plasma are used it could be assumed that histidine, methionine and tryptophan would not appear on a two-dimensional chromatogram. Weak spots could be expected from arginine and phenylalanine. Accordingly duplicate two-dimensional chromatograms were carried out. — One paper was developed in the usual way with ninhydrin. On the other specific colour reactions for these amino acids were tested.

Histidine. The Pauly diazo reagent was used. The diazotized sulphanilic acid was prepared according to Blatt [10]. Attempts with alcoholic or aceton

solutions of the reagent were unsuccessful. Freshly prepared water solutions (0.1 % of the diazo compound in N $NaCO_3$) had to be used. The amino acid was detectable in amounts of about 10 μg. The bromine reaction carried out according to Woolley and Peterson [11] was less sensitive.

Methionine. The methionine-nitroprusside reaction according to Mc Carthy and Sullivan was carried out in different modification (*cf.* Csonka and Denton [12]) but the sensitivity was to low. The iodoplatinate reaction was used by Consden *et al.* [13] to identify methionine. Recently the reaction was investigated by Winegard and Toennies [14]. According to their figures the reaction is not more susceptible than the ninhydrin reaction. We can corroborate this result.

Tryptophan. The bromine-water reaction [15] was positive only with amounts of 20 μg. The glyoxylic acid reaction according to Shaw and Mc Farlane [16] was tested in the following way. 7 μl of the glyoxylic acid-copper sulphate reagent was applied to the expected position of the amino acid on the paper. The sheet was dried overnight at room temperature and then 7 μ l of 12.5 per cent H_2SO_4 were added. Black spots were obtained after about 4 days. The amino acid was detectable in amounts of about 10 μg. The dimethylaminobenzaldehyde reaction according to Bates [17] was perhaps a little more susceptible and the greenish coloured spots were visible after 48 hours.

Arginine. Sometimes the arginine spot was not clearly separated from the lysine spot. In such cases the presence of arginine had to be verified by use of the Sakaguchi reaction carried out according to Dubnoff [18]. To the expected position of arginine on the cold paper 14 μl of the α-naphtol-urea reagent were added by means of a micro-pipette followed by 7 μl of the NaOBr solution. The amino acid was detectable in amounts of about 5 μg.

Citrulline. 5—10 μg of the amino acid were visible on a two-dimensional chromatogram when the dimethylaminobenzaldehyde reaction (*cf.* Dent [2]) was applied to the paper. The sensitivity of the reaction could be increased by purification of the reagent.

RESULTS

The following routine method is proposed to be used for the detection and identification of a maximum number of amino acids and amino acid derivatives.

1. Munktell papers no. 0B cut to dimensions of 37 × 28 cm are used. Duplicate two-dimensional runs are carried out with phenol and the pyridine amyl alcohol mixture. 1 ml of desalted protein-free plasma (ethanol filtrate) is used. One of the papers is developed with ninhydrin. On the other the presence of

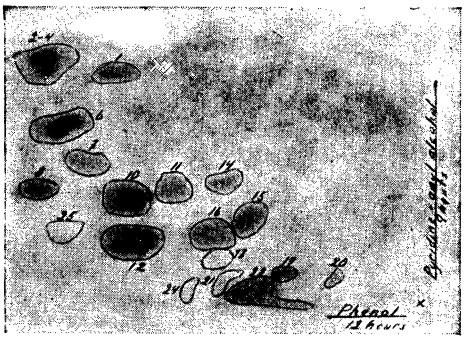

Figure 1. Photograph of a phenol/pyridine-amyl alcohol chromatogram, showing the positions of the amino acids from 1 ml of human plasma filtrate (80 % ethanol precipitation).

1 = Tyrosine	*13 = Citrulline*
2 = Phenylalanine	*14 = Taurine*
3 = Isoleucine	*15 = Serine*
4 = Leucine	*16 = Glycine*
5 = Methionine	*17 = Asparagine*
6 = Valine	*18 = Histidine*
7 = α-Amino-n-butyric acid	*19 = Glutamic acid*
	20 = Aspartic acid
8 = Proline	*21 = Arginine*
9 = Hydroxyproline	*22 = Lysine*
10 = α-Alanine	*23 = Ornithine*
11 = Threonine	*24 = Fast-arginine*
12 = Glutamine	*25 = Peptide*

Figure 2. Photograph of a one-dimensional lutidine-amyl alcohol chromatogram. A = 1 ml of plasma dialysate + 20 μg of tryptophan. B = 1 ml of dialysate + 27 μg of tyrosine. C = 2 × 1 ml of plasma dialysate. D = 1 ml of dialysate + 22 μg of phenylalanine, 6 μg of leucine and 6 μg of isolucine.

26 = Tryptophan	*34 = Ethanolamine-phosphoric acid*
27 = Cystine	*35 = Serine-phosphoric acid*
28 = β-Alanine	
29 = Glucosamine	
30 = Histamine (neutralized)	
31 = Histamine (free base)	
32 = Glutathione	
33 = Peptide	

arginine, histidine and tryptophan may be established by means of the specific colour reactions. A comparison with the paper sprayed with ninhydrin shows the places which should be tested with the different reactions. In this connection we may confirm the statement by Consden *et al.*[19] that when strict duplicates are run simultaneously in the same chamber, differences in R_F values do

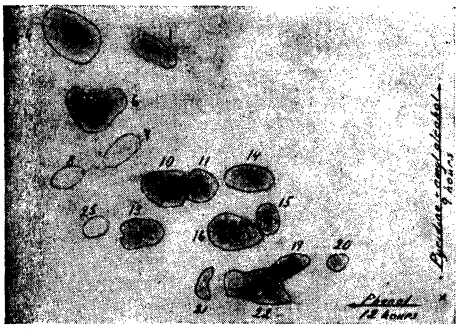

Figure 3. Photograph of a phenol/pyridine-amyl alcohol chromatogram, showing the positions of the amino acids from 1 ml of human plasma dialysate.

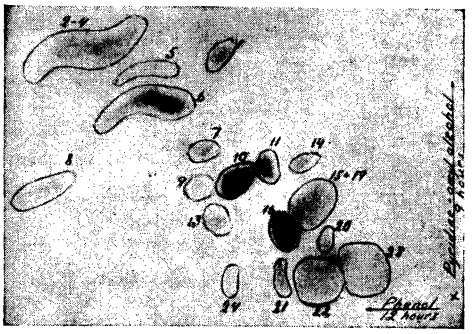

Figure 4. Photograph of a phenol/pyridine-amyl alcohol chromatogram, showing the positions of the amino acids from 1 ml of hydrolyzed plasma filtrate (80 % ethanol precipitation).

not exceed 4 %. It may be mentioned that the arginine and histidine reactions usually are not inhibited by a previous spraying with ninhydrin. In this way nearly all of the plasma amino acids are detected. A typical chromatogram is shown in Fig. 1.

2. A one-dimensional chromatogram is run with the lutidine-amyl alcohol mixture. 0.1 per cent diethylamine is placed on the bottom of the tray. In this way the presence of leucine, isoleucine and phenylalanine may be established. Better results have been obtained with dialysates than with ethanol filtrates of human plasma. Filtrate volumes corresponding to 0.5—1 ml of protein-free plasma have given the best pictures. A typical chromatogram is shown in Fig. 2.

3. For the identification of citrulline and β-alanine a two-dimensional chromatogram of a dialysate must be run in the same way as in (1). During the dialysis glutamine is hydrolyzed to glutamic acid. Citrulline and β-alanine which occupy the same position as glutamine on the chromatogram may be detected. Spontaneous hydrolysis of the labile amide nitrogen of glutamine has previously been observed (Archibald [20]). That a proteolysis usually takes place during our dialysing conditions (8 hours at + 2°) was realized when it was found that the non-dialysing proteins of plasma were practically soluble in 80 per cent ethanol at the end of the dialysis. However, the proteolysis is not reflected by a more pronounced increase of the α-amino nitrogen. The hydrolysis of glutamine may also be of enzymatical nature. A volume of the desalted

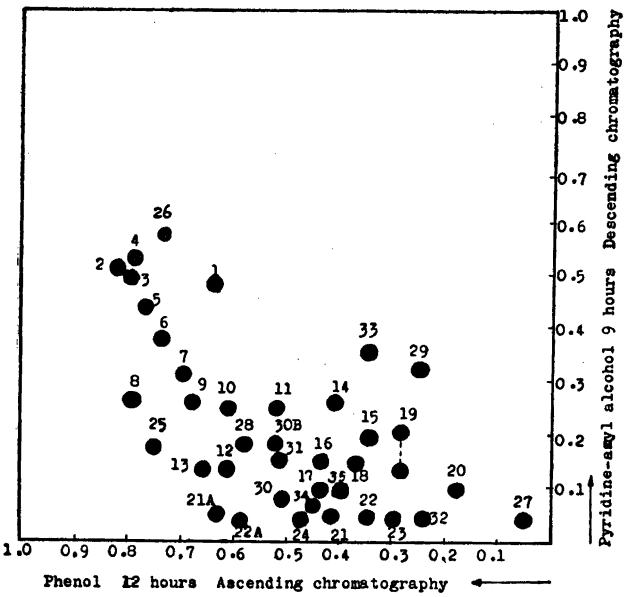

Figure. 5. Phenol/pyridine-amyl alcohol diagram, showing the average positions of the spots given by 23 amino acids and amino acid derivatives together with 2 peptides which have been found in 1 ml of deproteinized plasma. The positions of the spots given by 10 other amino acids and amino acid derivatives which may occur in blood and tissue extracts are also given.

dialysate corresponding to 1 ml of protein-free plasma is used for these chromatograms. The typical pattern is illustrated by Fig. 3.

4. Spots due to peptides disappear from the paper chromatogram after hydrolysis of the material. The hydrolysis is carried out with 2 *N* HCl for 5 hours at 120° or with 6 *N* HCl for 24 hours at atmospheric pressure. The acid is removed by evaporation to dryness followed by desalting. A two-dimensional chromatogram is run as in (1) with hydrolyzed material corresponding to 1—2 ml of protein-free plasma. Chromatograms of our five types of deproteinized plasma have unanimously shown a spot suspected to be caused by a peptide, no. 25 in Figure 1, and more irregularly a second spot no. 33 in Fig. 5. After hydrolysis the two spots disappear and spots corresponding to methionine and hydroxyproline appear on the papers. The typical picture is demonstrated by Fig. 4.

The identity of the different spots was verified by addition of known amino acids to the protein-free, desalted human plasma. Most of these experiments were carried out with ethanol filtrates. Fig. 5 shows the positions of the amino acids which are normally found in such filtrates (*cf.* Figs. 1 and 2). In addition the positions of some other amino acids and amino acid compounds which may occur in tissue extracts are given. The map is supposed to serve as a guide in further experiments.

DISCUSSION

With regard to the ninhydrin colours of the amino acids and their positions on the two-dimensional chromatograms some further remarks may be made. The usual colour of the spots is purple. In the following colour descriptions are given only when a deviation from this rule occurs.

β-Alanine. A spot corresponding to this amino acid has so far not been observed with the amounts of deproteinized plasma used in our two-dimensional runs. If present in sufficient amounts it should have been visible as a spot on the chromatograms of the plasma dialysates and the hydrolysates of deproteinized plasma. When 5 μg of β-alanine was added to a plasma dialysate a small blue coloured spot appeared on the chromatograms close to the citrulline spot but clearly separated from this spot. On the other hand β-alanine can not be observed on the chromatograms of the other types of deproteinized plasma where the glutamine spot overlaps both the β-alanine and the citrulline spots.

α-Amino-n-butyric acid. The position of this amino acid was verifed by addition of 5 μg of synthetic material to the protein-free human plasma. According to Pratt and Auclair [7] the compound should be detectable in amounts of 0.2 μg. The figure given by Dent [2], 4 μg, is more in agreement with our experience. There is no microbiological or chemical method available for the determination of the small amount of free amino acid present in human plasma. However, since the spot is barely visible when 1 ml of protein-free human plasma is used only a few μg of the amino acid should be present in this volume. α-amino-*n*-butyric acid has not been found in acid hydrolysates of proteins. According to Fromageot and Clausen [21] it should be formed *in vivo* by decomposition of methionine.

Arginine. The two basic amino acids arginine and lysine travel faster when ammonia is present during the phenol runs. The position of their spots are then changed and they will occupy a place on the chromatograms below the glutamine spot while the histidine spot is overlapped by the glutamine spot (*cf.* spots no. 21 A and 22 A in Fig. 5). The arginine and lysine spots are not better separated by this procedure and the Pauly diazo reaction presents some difficulties with the large amounts of glutamine present in blood plasma. On similar chromatograms of dialysates histidine will occupy nearly the same position as citrulline. Accordingly we prefer not to use ammonia in the phenol runs. Arginine is detectable in amounts of 10 μg. On a two-dimensional chromatogram of an arginine solution two spots appear, one in the expected position but a weaker spot is also found close to the citrulline spot. However, the spot does not coincide with the citrulline spot when both amino acids are

run together. Previous investigators [7] have already stated that arginine is decomposed by the solvents used in chromatography. The ninhydrin-positive decomposition product found by us is so far of unknown nature.

Asparagine gives an orange-brown coloured spot and is easily detectable on the two-dimensional chromatograms when amounts of about 5 μg are added to 1 ml of an ethanol filtrate of human plasma. The spot corresponding to this small amount of amide moves close to the glycine spot (*cf.* Fig. 5) but is not overlapped by this large spot caused by the high glycine content of human plasma (*cf.* Dent [2], Gutman and Alexander [9]). A small spot giving the same colour and occupying the same position as the asparagine spot is also observed on the chromatograms of the filtrates from 1 ml of ethanol precipitated human plasma without any addition of the amide. This spot is not observed after hydrolysis of the ethanol filtrates. Accordingly it seems highly probable that free asparagine is present in small amounts in normal human plasma. The asparagine spot may sometimes be overlapped by a blue purple spot formed by the decomposition of arginine.

Aspartic acid gives a small blue coloured spot (*cf.* no. 20, Figs. 1, 3, and 4). According to previous investigators [4] the amounts of aspartic acid in human plasma should be neglible. Our experience is that a small aspartic acid spot is a rather constant phenomenon on the two-dimensional chromatograms of human plasma.

Citrulline. The citrulline spot is not detectable on the chromatograms of protein-free filtrates obtained by the precipitation methods. These filtrates contain large amounts of glutamine which occupy about the same place as citrulline on the chromatograms. However, on two-dimensional chromatograms from 1 ml of human plasma dialysates a spot is observed which by addition experiments and colour reagent (Dent [2]) has been verified to be citrulline. When two-dimensional chromatograms of this amino acid were carried out two spots were observed, one in the expected position, the other occupying the same place as the norvaline spot. Nitrogen determinations of the citrulline preparation (Hoffman-La Roche) gave the theoretical yield. A partial decomposition of this amino acid during the development of the chromatogram must occur. The amino acid is detectable in amounts of 5 μg.

Cystine and cysteine. Spots denoting the presence of these two compounds are usually not seen on two-dimensional chromatograms when 1 ml samples of ethanol filtrates are analyzed. According to Alexander [4] cystine plus methionine should comprise about 5 per cent of the free α-amino nitrogen in human plasma. The methionine content of 1 ml of protein-free plasma is estimated to about 4 μg (Sheffner *et al.*[5]). Accordingly an amount of about 15 μg of cystine plus cysteine could be present in the same volume. A comparison between

one-dimensional chromatograms of 10—15 μg samples of cystine or cysteine with either phenol or the pyridine-amyl alcohol mixture as organic solvents showed for both compounds very faint, reddish purple coloured streaks in the phenol runs and weak but more sharply defined purple coloured spots in the pyridine-amyl alcohol runs. A decomposition of the amino acids obviously took place in the phenol solvent. After treating the amino acid solutions with hydrogen peroxide larger spots of cysteic acid were obtained but still a decomposition of cysteic acid took place in the phenol runs. With regard to cysteine it may be further pointed out that this amino acid easily forms a thiazolidine compound during the conditions prevailing in the protein-free plasma (Ågren [22]). The conclusion was drawn that the amounts of cysteine or cystine present in 1 ml of protein-free plasma, wether treated with hydrogen peroxide or not, would not give clearly visible spots on the two-dimensional chromatograms.

Ethanolamine is detectable in amounts of 10 μg and gives a bluish purple spot. It has never been observed in our experiments with protein human plasma. When added to the plasma filtrates it occupies the position shown in Fig. 5.

Ethanolamine-phosphoric acid. A sample synthesised in this laboratory was used. The compound gives a purple coloured spot and is detectable in amounts of about 20 μg. It has never been observed on our paper chromatograms of plasma filtrate. The position of the spot is given in Fig. 5.

Glucoseamine is detectable in amounts of about 10 μg when added to deproteinized human plasma. It gives a purple-brown colour. It has not been observed on the chromatograms with the amounts of protein-free plasma used in the present investigation. A spot close to the position of glucoseamine (no. 33, Fig. 5) was at first thought to be this compound. However, the spot did not move when ammonia was present during the phenol run (*cf.* Dent [2]). Moreover, after hydrolysis the spot disappeared. The most probable explanation is that the spot is related to a peptide present in the filtrates from ethanol precipitated human plasma.

Glutamic acid is mainly present as the amide in the protein-free human plasma. The large amounts of the amide, 60—120 μg, found in 1 ml of the plasma (Hamilton [20]) give an easily recognized spot on the papers. On the chromatograms of dialysates or hydrolysates the glutamine spot has disappeared and a spot corresponding to the position of added glutamic acid appears. In his recent paper Dent [2] has described several irregularities with regard to the position of the glutamic acid spot. We can corroborate his findings. The amino acid is detectable in amounts of about 2 μg.

Glutathione of blood is present in the red blood corpuscles [24, 25] and accordingly can not be extected to appear on the chromatograms of deproteinized plasma. When added to such samples it will occupy the position shown in Fig. 5.

Glycine gives a reddish purple colour. It is one of the main amino acids in plasma.

Histamine gives a bluish purple spot which after some time changes to dull greenish. It is detectable in amounts of 5—10 μg. So far it has not been observed on the chromatograms of protein-free plasma. As demonstrated in Fig. 5 the free base and the hydrochloride do not occupy the same position on the paper when they are added to an ethanol filtrate of plasma.

Histidine. According to Hier and Bergeim [3] 1 ml of deproteinized human plasma contains 14 μg of histidine. Previous investigators [2, 7] have stated that about 20 μg of this amino acid is the smallest amount which will give a spot on a two-dimensional chromatogram. It is therefore not surprising to find that the histidine spot usually is missing on our papers. When a further 10 μg of histidine is added to 1 ml of ethanol precipitated plasma a greyish blue spot will appear as shown in Fig. 5. Hence the presence of histidine in 1 ml samples of deproteinized plasma is better detected by means of the Pauly diazo reaction as described previously in this communication.

Hydroxyproline gives a brownish yellow spot and is detectable in amounts of 5 μg. The spot touches that of alanine but the two spots are easily distinguished by differences in colours. Hydroxyproline can not be detected on a two-dimensional chromatogram of 1 ml of protein-free human plasma without further proceedings. Following hydrolysis it appears at the same time as two ninhydrine positive substances disappears (spots no. 25 and no. 33 *cf.* Fig. 5). The most probable explanation is that hydroxyproline occurs in peptide bound form in deproteinized plasma.

Lysine gives a greyish purple spot. It is larger than any of the two closely situated arginine and ornithine spots and often shows streaking to the right. It sometimes partially overlaps the ornithine spot. The two spots are best separated on the chromatograms of the hydrolyzed and desalted ethanol filtrates. (Fig. 4.)

Methionine. According to Sheffner *et al.*[5] 1 ml of deproteinized human plasma contains about 4 μg of the amino acid. This amount obviously is not large enough to give a spot in our two-dimensional chromatograms. Dent [2] also states that the amino acid is detectable in amounts of 10 μg. When 4 μg were added to 1 ml of protein-free plasma a methionine spot appeared on the papers. A methionine spot was also observed when 1 ml of deproteinized and

hydrolyzed plasma was run (*cf.* Fig. 4). It seems possible that a certain amount of the amino acid is bound in peptide form.

Ornithine. In agreement with previous investigators we have estimated the detectable amount of this amino acid to be about 5—10 μg. The ornithine spot is most clearly separated from the lysine spot on chromatograms of deproteinized plasma which has been hydrolyzed (*cf.* Fig. 4). The increase of the ornithine spot on these papers makes it probable that in addition to the preformed free amino acid some amount is formed during the hydrolysis either from arginine or possibly from the peptides present in the plasma filtrate.

Phenylalanine. According to Hier and Bergeim [3] the phenylalanine concentration amounts to about 14 μg per ml of deproteinized normal human plasma. This quantity of amino acid is barely sufficient to give a small greenish blue spot clearly separated from the leucine and isoleucine spots on the one-dimensional chromatograms.

Proline is detectable in amounts of about 5 μg. It gives a yellow spot. According to Hier and Bergeim (quoted by Alexander [4]) the concentration of proline in plasma should be neglible. Our impression is that the size of the proline spot on the chromatograms indicates a concentration higher than 5 μg per ml of protein-free plasma.

Serine-phosphoric acid. A sample synthezised in this laboratory was used. The compound gives a purple colour and is detectable in amounts of 5—10 μg. The position of the spot is given in Fig. 5. It has not yet been found in deproteinized plasma.

Tryptophan. The concentration of free tryptophan is estimated to 11 μg per ml of human plasma. This amount of amino acid will not give a ninhydrin spot on our two-dimensional chromatograms. The colorimetric tryptophan reaction of Bates [17] has been used by us and seems to be somewhat more sensitive than the ninhydrin reaction. However, the results with 1 ml of deproteinized plasma have been irregular. In experiments with 2 ml samples of deproteinized human plasma the ninhydrin spot appears in the expected position.

Unidentified substances. Protein-free human plasma contains at least two peptides (*cf.* spots no. 25 and 33, Fig. 5). The amino acid composition of these substances has so far not been investigated in any detail. However, when the deproteinized samples are hydrolyzed and subsequently analyzed with two-dimensional chromatography hydroxyproline and methionine spots will appear on the papers. The spots given by lysine and especially by ornithine are enlarged (*cf.* Figs. 1 and 4). It is probable that these four amino acids are integral parts of the peptides. In his recent paper Dent [2] describes a spot given by a substance tentativelly named fast-arginine. The suggestion is

made that the compound should be the guanidine analogue of lysine. A spot in a similar position appears on our chromatograms. Since the Sakaguchi reaction when applied to this spot is negative the proposed structure seems less probable.

SUMMARY

1. By combining one- and two-dimensional paper chromatographic procedures in the analysis of the amino acid content in 1 ml samples of deproteinized human plasma the presence of the following 21 compounds have been established: α-alanine, α-amino-*n*-butyric acid, arginine, asparagine, aspartic acid, citrulline, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

2. Apart from the free amino acids the following 4 ninhydrin positive substances have been found: two peptides probably containing hydroxyproline, lysine, methionine, ornithine and possibly other amino acids, thirdly taurine and finally the unidentified spot described as »fast-arginine» by Dent. This substance seems not to be a guanidine compound.

3. A map is shown which gives the positions of these 25 substances and 10 other biologically occurring and ninhydrin-positive substances.

The investigation was supported by grants for medical research from the Swedish Medical Research Council and the Ferrosan Corporation. The technical assistance of Dr. G. Ekström and Ing. S. Eklund is greatfully acknowledged.

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Received May 11, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 539–553

Electrometric Investigation of Equilibria between Mercury and Halogen Ions. VIII. Survey and Conclusions

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In the seven preceding publications [1–7] the work of a team investigating the equilibria between Hg^{2+} and the halogen ions Cl^-, Br^-, and I^- has been described. Now that the work is concluded for the time being, it seems appropriate to collect the main results and to try to visualize them in the form of diagrams.

SURVEY OF PARTS I–VII

A review of previous work on mercury-halogen complexes showed that a new investigation was desirable for many reasons, *e. g.* the equilibria involving Hg^{2+}—HgX^+—HgX_2 have as far as we know not been investigated since 1908. On the equilibria involving HgX_2—HgX_3^-—HgX_4^{2-} somewhat more work has been done. However, the investigators of the latter equilibria can be divided into two groups. Some have assumed that only HgX_3^- appears in appreciable amounts, and neglected HgX_4^{2-}. The others have, on the contrary, neglected HgX_3^- and assumed that only HgX_4^{2-} is formed.

Moreover the influence of acidity and ionic strength on these equilibria, which can be expected to be rather great, has as a rule been neglected.

It was decided to study the Hg^{2+}-halogen equilibria at 25° C by electrometric methods, using solutions of constant acidity (10 mC) and with ionic strength as constant as possible (500 mC), using $NaClO_4$ as salt medium. In this way the activity factors were kept approximately constant; otherwise the calculations would have been hopelessly complicated.

At high concentrations $[Br^-]$ and $[I^-]$ it was possible to measure the concentration of free Hg^{2+}, using a Hg electrode (Parts VI and VII). In no other

instance, however, was it possible to measure the concentrations $[Hg^{2+}]$ or $[X^-]$ directly, using electrodes of the first or second kind. Instead indirect methods must be used. A large part of our investigations was founded on measurements of redox emfs using solutions with both Hg_2^{2+} and Hg^{2+}, generally in equilibrium with solid Hg_2X_2. For interpreting these emfs it was necessary also to know the solubility products of the mercury(I)halides, Hg_2X_2, under the special conditions of our work.

In Part I earlier work is reviewed, the general plan of the work is given, and the apparatus and analytical methods are described. Part II describes measurements of the equilibria $Hg^{2+} + HgX_2 \rightleftharpoons 2\,HgX^+$ (the value given for I^- is only preliminary). Part III deals with measurements of the solubility products for the Hg_2X_2, and of the equilibrium $Hg^{2+} + Hg(l) \rightleftharpoons Hg_2^{2+}$. Part IV is theoretical and gives the formulae for the variation of the redox emf, when halogen ions X^- are added to a $Hg_2^{2+} - Hg^{2+}$ mixture. The equilibria are rather complicated, since solid Hg_2X_2 appears in addition to the various $Hg^{2+} - X^-$ complexes. Finally, parts V—VII deal with the measurements for the individual halogens, Cl^- (part V), Br^- (part VI), and I^- (part VII). For no two halide systems has it been possible to use exactly the same experimental and computational methods, because of the great differences between the equilibrium constants for Cl^-, Br^-, and I^-.

EQUILIBRIUM CONSTANTS

Our measurements could be explained assuming that the following molecular species, and only these, appear in the solutions:

$$X^-,\ Hg_2^{2+},\ Hg^{2+},\ HgX^+,\ HgX_2,\ HgX_3^-,\ HgX_4^{2-} \qquad (1)$$

It was not possible to neglect the existence of any one of the $Hg^{2+} - X^-$ complexes. On the other hand there was no need to assume that, for instance, univalent mercury also forms single ions Hg^+ or soluble complexes Hg_2X^+, or that there are higher complexes of bivalent mercury such as HgX_5^{3-} and HgX_6^{4-} or polynuclear complexes such as $Hg_2X_5^-$, $Hg_3X_8^{2-}$, and the like. This is not to deny the presence of a few of these molecular species in small amounts, or even in considerable amounts under special conditions; thus it is possible that polynuclear Hg^{II} complexes may appear at higher total Hg^{II} concentrations than those used by us, which were generally $\leqq 10$ mC.

The most concise way of giving our results is a table of the complex products $\varkappa_1$—$\varkappa_4$, defined by

$$Hg^{2+} + X^- \rightleftharpoons HgX^+ \qquad \varkappa_1 = [HgX^+]\,[Hg^{2+}]^{-1}\,[X^-]^{-1}$$
$$Hg^{2+} + 2X^- \rightleftharpoons HgX_2 \qquad \varkappa_2 = [HgX_2]\,[Hg^{2+}]^{-1}\,[X^-]^{-2}$$
$$Hg^{2+} + 3X^- \rightleftharpoons HgX_3^- \qquad \varkappa_3 = [HgX_3^-]\,[Hg^{2+}]^{-1}\,[X^-]^{-3}$$
$$Hg^{2+} + 4X^- \rightleftharpoons HgX_4^{2-} \qquad \varkappa_4 = [HgX_4^{2-}]\,[Hg^{2+}]^{-1}\,[X^-]^{-4} \quad (2{=}I,1)$$

and of the solubility products

$$Hg_2X_2(s) \rightleftharpoons Hg_2^{2+} + 2X^- \qquad k_S = [Hg_2^{2+}]\,[X^-]^2 \qquad (3 = III,6)$$

The logarithms of these quantities for Cl^-, Br^-, and I^- (on the C scale) are listed below:

	X = Cl	Br	I	
log $\varkappa_1$	6.74 ± .02	9.05 ± .03	12.87 ± .03	
log $\varkappa_2$	13.22 ± .02	17.33 ± .04	23.82 ± .04	
log $\varkappa_3$	14.07 ± .15	19.74 ± .11	27.60 ± .14	
log $\varkappa_4$	15.07 ± .06	21.00 ± .03	29.83 ± .02	
log k_S	−16.88 ± .01	−21.29 ± .04	−27.47 ± .01	(4)

Together with the equilibrium constant k_0:

$$Hg^{2+} + Hg(l) \rightleftharpoons Hg_2^{2+} \qquad k_0 = [Hg_2^{2+}]\,[Hg^{2+}]^{-1} = 129.2 \pm 1.0$$
$$\log k_0 = 2.11_1 \pm .00_3 \qquad (5 = III,18)$$

and the solubility of HgI_2 according to Biedermann and Sillén [8]:

$$s = (7.4 \pm .3) \cdot 10^{-5}\ C;\ \log s = -4.13 \pm 0.02 \qquad (6)$$

these constants permit the calculation for each halogen of all equilibria involving the above mentioned molecular species (1) in solution, Hg metal, and solid Hg_2X_2 (for I also solid HgI_2).

DISTRIBUTION OVER DIFFERENT COMPLEXES

Since it may be difficult to grasp immediately the significance of these figures, we have tried to illustrate them by means of diagrams.

Figs. 1 a—c have as abscissa the logarithm of the concentration of *free* halogen ions, Cl^-, Br^-, or I^-. The ordinate goes from 0 to 100 and shows the percentage of the total amount of Hg^{II} present in the form of different com-

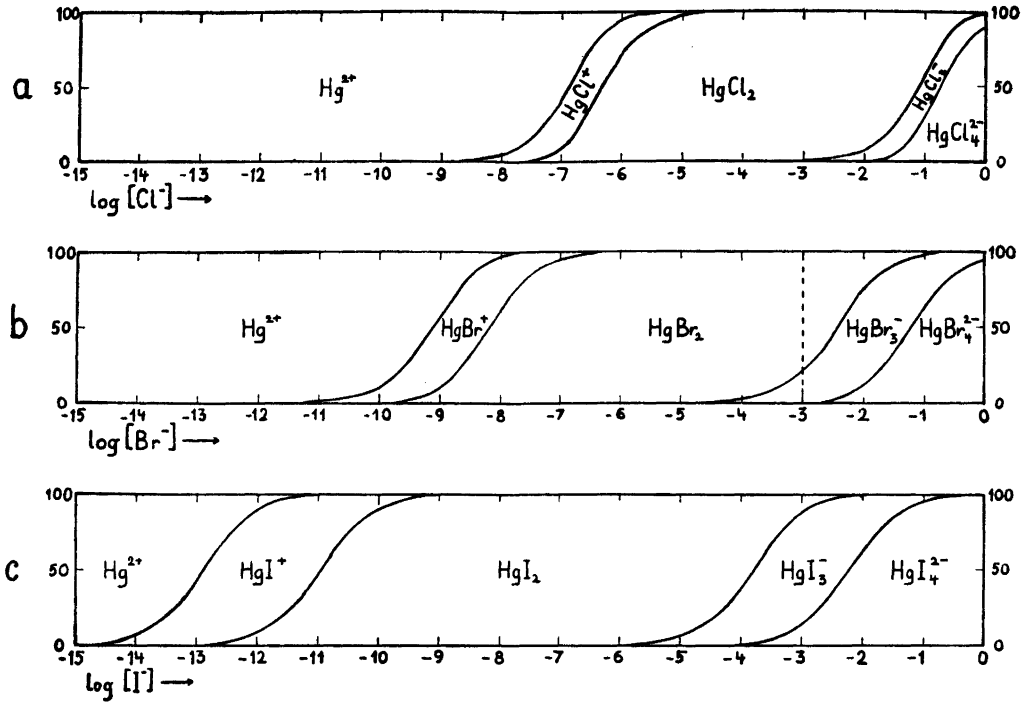

Fig. 1. Distribution of Hg^{II} over different complexes with a) Cl^- b) Br^- c) I^- for varying $[X^-]$. The abscissa is log $[X^-]$. On the ordinate axis the distance 0—100 represents the total amount of Hg^{II} present. If for a given value of $[X^-]$, a vertical line is drawn at the corresponding log $[X^-]$, the segment of this line falling in a certain area, e. g. HgX_3^-, represents the fraction of the total amount of Hg^{II} present as that complex.

plexes at the $[X^-]$ given. For example, we see that in a bromide solution containing 1 mC free Br^- (log $[Br^-] = -3$, dotted line in Fig. 1b) about 20 % of all Hg^{II} is present as $HgBr_3^-$, and about 80 % as $HgBr_2$; other complexes are negligible. With 10 mC free Br^- (log $[Br^-] = -2$), about 12 % of all Hg^{II} is present as $HgBr_4^{2-}$, about 63 % as $HgBr_3^-$, and about 25 % as $HgBr_2$. At higher $[Br^-]$, $HgBr_4^{2-}$ becomes the predominant complex. Between $[Br^-] = 10^{-4}$ and 10^{-7}, there is a broad range with $HgBr_2$ dominating. At lower $[Br^-]$, the amounts of $HgBr^+$ and Hg^{2+} become considerable. At $[Br^-] = 10^{-9}$, we have, for instance, 9 % $HgBr_2$, 48 % $HgBr^+$, and 43 % Hg^{2+}. Below $[Br^-] = 10^{-11}$ C practically only Hg^{2+} is present.

These percentages must be independent of the total concentration of Hg^{II}, as can be seen from the formulae used for the derivation of the curves.

According to (2), denoting (X^-) by X

$$[HgX^+] = \varkappa_1\,[Hg^{2+}]X; \qquad [HgX_2] = \varkappa_2\,[Hg^{2+}]X^2$$
$$[HgX_3^-] = \varkappa_3\,[Hg^{2+}]X^3; \qquad [HgX_4^{2-}] = \varkappa_4\,[Hg^{2+}]X^4$$

$$\text{Thus } [Hg^{II}]_{total} = [Hg^{2+}] + [HgX^+] + [HgX_2] + [HgX_3^-] + [HgX_4^{2-}] =$$
$$= [Hg^{2+}]\,(1 + \varkappa_1 X + \varkappa_2 X^2 + \varkappa_3 X^3 + \varkappa_4 X^4) =$$
$$= [Hg^{2+}]\,(1 + \textstyle\sum \varkappa_n X^n) \qquad (7)$$
$$[Hg^{2+}]\;[Hg^{II}]^{-1}_{total} = (1 + \textstyle\sum \varkappa_n X^n)^{-1} \qquad (8_0)$$
$$[HgX^+]\,[Hg^{II}]^{-1}_{total} = \varkappa_1 X\;(1 + \textstyle\sum \varkappa_n X^n)^{-1} \qquad (8_1)$$
$$[HgX_2]\;[Hg^{II}]^{-1}_{total} = \varkappa_2 X^2\;(1 + \textstyle\sum \varkappa_n X^n)^{-1} \qquad (8_2)$$
$$[HgX_3^-]\,[Hg^{II}]^{-1}_{total} = \varkappa_3 X^3\;(1 + \textstyle\sum \varkappa_n X^n)^{-1} \qquad (8_3)$$
$$[HgX_4^{2-}][Hg^{II}]^{-1}_{total} = \varkappa_4 X^4\;(1 + \textstyle\sum \varkappa_n X^n)^{-1} \qquad (8_4)$$

All the ratios in (8_{0-4}) are seen to be functions of the single variable X, and independent of $[Hg^{II}]_{total}$.

Fig. 1. shows certain general trends. In each of the complex systems there is a large range of log X where almost only HgX_2 is present. This shows that the first and second halogen ions are added much more easily than the third and fourth — probably by a different type of bond. The same prevalence of the second complex was noticed for Hg^{2+}—NH_3 by J. Bjerrum [9]. For the Cd^{2+} complexes with halogen ions and other ligands, the second complex has no dominant position (Leden [10]). On the contrary, the existence range of CdI_2 is unusually narrow.

It is also evident from Fig. 1 that the stability of all complexes increases as we go from Cl^- to I^-.

Of all the domains in Fig. 1, those for HgX_3^- are the most uncertain since the values for log $\varkappa_3$ are less accurate than any of the other log $\varkappa_n$. However, the increase in the breadth of the HgX_3^- area from Cl^- to I^- seems to be as real as the broadening of the HgX^+ area from Cl^- to I^-.

TYPES OF BONDS

There is abundant evidence from structure investigations of vapours [11, 12] and crystals [13–16] that the molecules HgX_2 are linear or almost linear X—Hg—X; according to Pauling [17] the bonds are of *sp* type. It is sometimes assumed that the higher complexes are tetrahedral with sp^3 bonds [27]. Tetrahedral bonds around a Hg are certainly present in red HgI_2, which is built up [18–20] of sheets with coordination Hg—4I,I—2 Hg. The crystal structure of Ag_2HgI_4 (Cu_2HgI_4) can be interpreted as built up of Ag^+ (Cu^+) and tetrahedral HgI_4^{2-}

ions, although there may be some electron sharing between Ag (Cu) and I atoms, too [21] *. On the other hand, the crystal structures of NH_4HgCl_3 and $K_2HgCl_4H_2O$ [23] seem to be built up of $HgCl_2$ molecules, Cl^- ions, (H_2O molecules), and NH_4^+ (K^+) ions. In these structures every Hg is surrounded by six chlorine atoms: apart from the two firmly bound Cl, there are four Cl^- at larger distances around the »equator» of the $HgCl_2$ molecule. The evidence from Raman spectra does not seem to be conclusive as to whether tetrahedral HgX_4^{2-} ions exist or not [24–26]. As pointed out by Wells [27] the early structure determination [28] for $CsHgCl_3$ and $CsHgBr_3$ ought to be checked.

Thus it does not seem quite decided whether the HgX_4^{2-} ions in solution can be tetrahedral or always consist of linear HgX_2 molecules with loosely attached X^- ions. That higher complexes such as HgX_5^{3-} have not been observed in our experiments, whereas around the equator of HgX_2 there should be room for three or four X^-, may argue in favour of the tetrahedral bonds.

It would be desirable to study the crystal structures of a number of complex halogeno-mercurates to see whether tetrahedral $HgCl_4^{2-}$ and $HgBr_4^{2-}$ ions can be found or not.

MERCURY FLUORIDES

All available evidence indicates that if Hg^{2+} and F^- form complexes at all, they are much weaker than the complexes of Hg^{2+} with Cl^-, Br^-, and I^-. No solid fluo-mercurates seem to be known. Whereas all the other mercury halides have crystal structures with linear HgX_2 molecules ($HgCl_2$ [13], $HgBr_2$ [14, 15], yellow HgI_2 [16]), or with tetrahedral bonds Hg—4 I (red HgI_2 [18–20]), HgF_2 has the CaF_2 structure with coordination Hg—8 F, which is typical of ionic compounds [29]. From the compound $HgF_2(H_2O)_2$ the water cannot be removed without decomposition and formation of HF [See *e.g.* 30], which also indicates a bond type different from that in the other mercury(II)halides. In aqueous solutions, HgF_2 is strongly hydrolysed, the only solid hydrolysis product observed being HgO [31]. This is easily understood if Hg^{2+} and F^- are present as free ions, since Hg^{2+} is an acid which is even slightly stronger than HF [32].

LOGARITHMIC DIAGRAMS. REDOX EMFS

Figs. 2—4 give another mode of representation, which may be advantageous for some purposes. They show how the equilibrium concentrations of the various complexes (given on a logarithmic scale) vary with X, the concentration of *free* X^-, in solutions where the total concentration of bivalent mercury, $[Hg^{II}]_{total}$, is kept constant at 10 mC. With increasing X the complexes

* See note, p. 552.

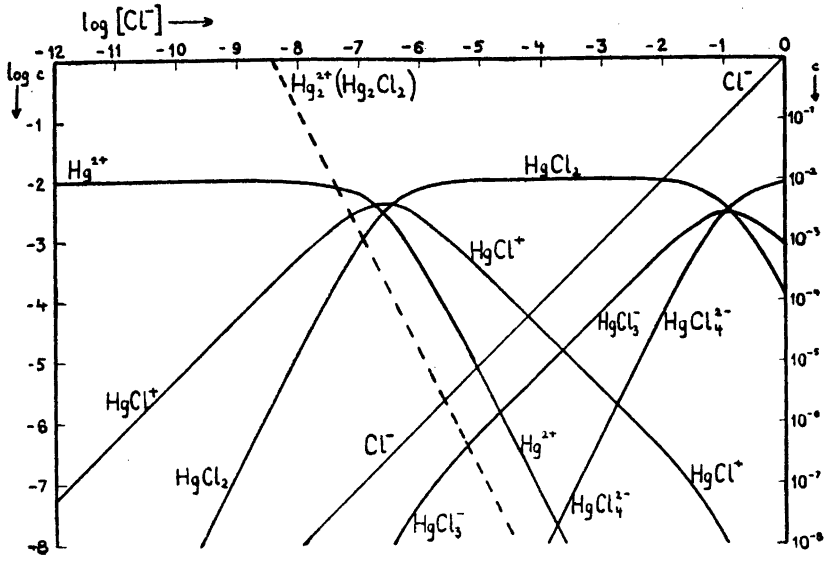

Fig. 2. Logarithmic diagram for $[Hg^{II}]_{total} = 10$ mC and varying $[Cl^-]$ showing the concentrations of the different $Hg^{2+} - Cl^-$ complexes. Broken line = concentration of Hg_2^{2+} in equilibrium with solid Hg_2Cl_2.

HgX^+, HgX_2, and HgX_3^- are seen to increase, attain their maximum concentration and then vanish till finally HgX_4^{2-} predominates. If another $[Hg^{II}]_{total}$ is chosen, the whole set of curves will move upwards or downwards without changing their relative positions.

There are in these figures also broken lines »Hg_2^{2+} (Hg_2X_2)», representing the concentration of Hg_2^{2+} in equilibrium with solid Hg_2X_2. Thus for varying X, sets of concentrations of Hg_2^{2+}, Hg^{2+}, and HgX^+, corresponding to the equilibrium

$$Hg_2X_2(s) + 2\ Hg^{2+} \rightleftharpoons Hg_2^{2+} + 2\ HgX^+$$

can be read off from the diagrams.

If a solution containing Hg_2^{2+} and Hg^{2+} is titrated with X^-, and the redox emf measured between a Pt electrode in the solution and a standard electrode, E will be given by

$$E = E_{20} + 29.58\ \log\ q \qquad (9 = I,5)$$

where E_{20} is a constant, and the quantity q is defined by

$$\log\ q = 2\ \log\ [Hg^{2+}] - \log\ [Hg_2^{2+}] \qquad (10)$$

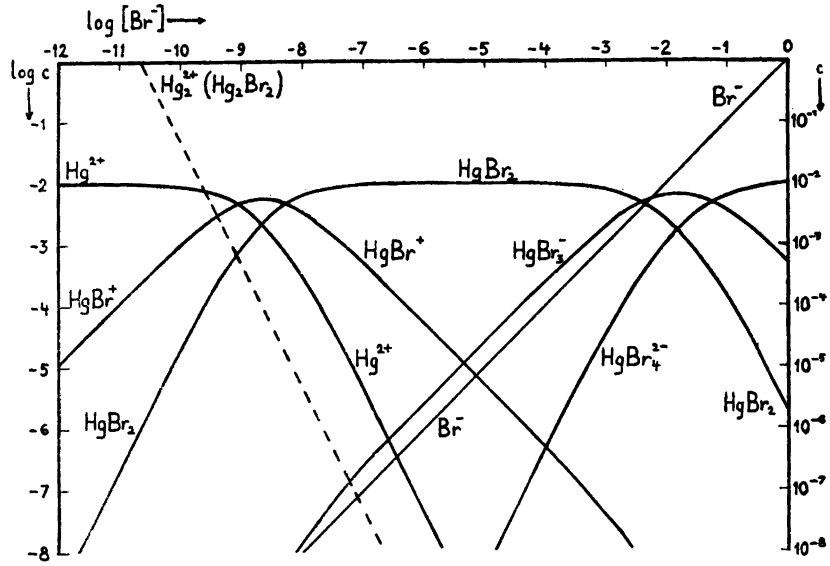

Fig. 3. Logarithmic diagram for $[Hg^{II}]_{total} = 10$ mC and varying $]Br^-]$ showing the concentrations of the different $Hg^{2+} - Br^-$ complexes. Broken line = concentration of Hg_2^{2+} in equilibrium with solid Hg_2Br_2.

It can be proved from the definitions of k_s and $\varkappa_1$ that in the presence of solid Hg_2X_2

$$\log q = 2 \log [HgX^+] - \log k_s - 2 \log \varkappa_1 \qquad (11)$$

Thus log q will follow the course of log $[HgX^+]$ in Figs. 2—4, first rise with increasing X, attain a maximum, and then decrease again (Parts IV—VI).

In the range of log X where HgX_2 predominates, the solution has a very low buffer value for X^- ions, as measured by the small increase in the quantity X_e (excess of halogen):

$$X_e = [X^-] + 2\,[HgX_4^{2-}] + [HgX_3^-] - [HgX^+] - 2\,[Hg^{2+}] - 2\,[Hg_2^{2+}] \qquad (12,\ cf.\ IV,\ 1,\ 8,\ 20)$$

needed for effecting a large increase in log X. Thus during a titration, the solution will hurry across this range for a very small addition of X^-, and a sharp fall will occur in the curve E (or log q) *versus* volume of X^- solution added (Part IV, Fig. 1).

At high X, the course of such a redox titration will be broken because Hg metal is precipitated by dismutation of Hg_2^{2+}; the precipitation of Hg metal

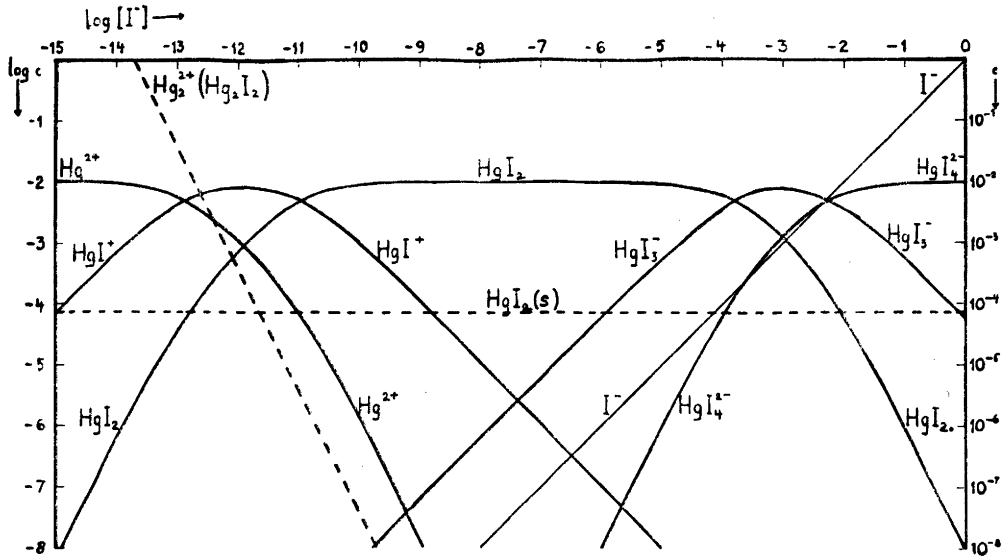

Fig. 4. Logarithmic diagram for $[Hg^{II}]_{total} = 10$ mC and varying $[I^-]$ showing the concentrations of the various $Hg^{2+}-I^-$ complexes. Broken line = concentration of Hg_2^{2+} in equilibrium with solid Hg_2I_2. Horisontal dotted line = solubility of HgI_2.

occurs at higher X for high $[Hg^{II}]_{tota}$. The resulting equilibria are dealt with in the next section.

For I, the whole system of Hg^{II} curves is rendered meaningless in the range $[I^-] = 10^{-12.8}$—10^{-2} C by the precipitation of solid HgI_2, since the concentration of HgI_2 in the solution would exceed the solubility $s = 10^{-4.13}$ C (line 'HgI_2(s)'). This line does not move if $[Hg^{II}]_{total}$ is changed; at low $[Hg^{II}]_{total}$, the whole system of complex curves can thus be realized.

EQUILIBRIA WITH Hg_2X_2 AND Hg

If a solution is in equilibrium with Hg metal and with solid Hg_2X_2, the concentrations of the different complexes of Hg^{II} are given by the formulae

$$\begin{aligned}
[Hg^{2+}] &= k_S k_0^{-1} X^{-2} \\
[HgX^+] &= \varkappa_1 k_S k_0^{-1} X^{-1} \\
[HgX_2] &= \varkappa_2 k_S k_0^{-1} \\
[HgX_3^-] &= \varkappa_3 k_S k_0^{-1} X \\
[HgX_4^{2-}] &= \varkappa_4 k_S k_0^{-1} X^2
\end{aligned} \qquad (13)$$

as can easily be shown from the definitions of the various equilibrium constants.

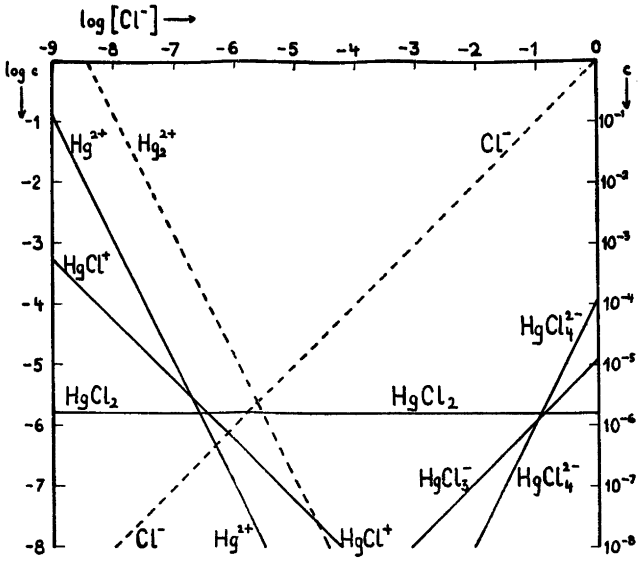

Fig. 5. Concentrations of different ionic (molecular) species in equilibrium with Hg Hg metal and solid Hg_2Cl_2 for varying [Cl⁻].

In Figs. 5—7 these equilibrium concentrations (as well as $[Hg_2^{2+}] = k_S X^{-2}$) have been plotted on a logarithmic scale as functions of X for Cl^-, Br^-, and I^-.

For very low X, Hg^{II} is present chiefly as Hg^{2+}. This part of the diagram is valid for solutions of high $[Hg_2^{2+}]$. With increasing X, the total concentration of Hg^{II} decreases till it reaches a minimum value, namely the concentration of HgX_2 corresponding to the equilibrium

$$Hg_2X_2(s) \rightleftharpoons Hg(l) + HgX_2$$

This concentration happens to be about the same for all three halogens.

$$\begin{aligned} [HgCl_2] &= 1.7_0 \cdot 10^{-6}\ C & \log [HgCl_2] &= -5.77 \pm .03 \\ [HgBr_2] &= 0.8_5 \cdot 10^{-6}\ C & \log [HgBr_2] &= -6.07 \pm .06 \\ [HgI_2] &= 1.7_4 \cdot 10^{-6}\ C & \log [HgI_2] &= -5.76 \pm .04 \end{aligned} \quad (14)$$

The values in (14) have been made consistent with the two digit approximation in (4), and sometimes differ by one unit in the last figure from those given in Parts V—VII.

At still higher X, HgX_3^- and HgX_4^{2-} are formed in appreciable amounts by the reactions

$$Hg_2X_2(s) + X^- \rightleftharpoons Hg(l) + HgX_3^-; \qquad Hg_2X_2(s) + 2X^- \rightleftharpoons Hg(l) + HgX_4^{2-} \quad (15)$$

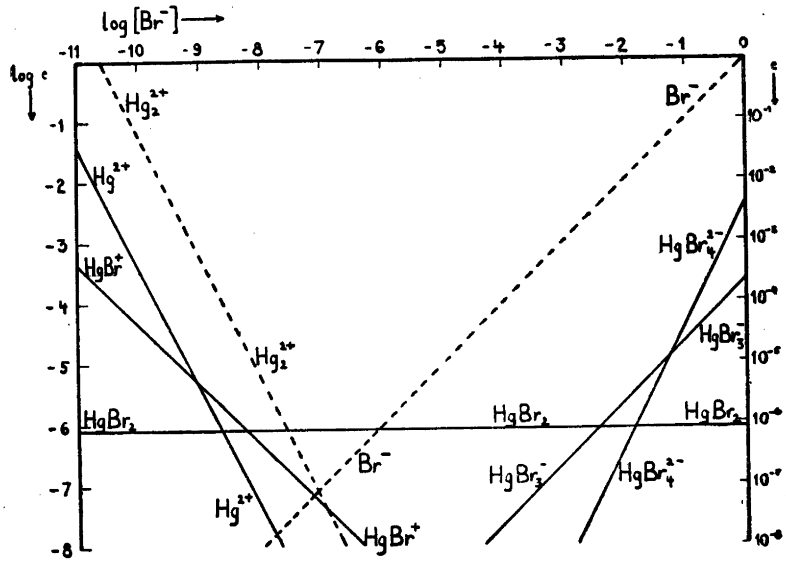

Fig. 6. Concentrations of different ionic (molecular) species in equilibrium with Hg metal and solid Hg_2Br_2 for varying [Br^-].

The equilibrium amounts are highest for I. For instance with $[I^-] = 10^{-2}$ (10 mC), $[HgI_3^-] = 0.10$ mC and $[HgI_4^{2-}] = 0.18$ mC.

A given solution, containing Hg^{II} and X^-, can behave in three different ways with regard to the equilibria with Hg and Hg_2X_2, *e. g.* (15). a) It can be in equilibrium with Hg and Hg_2X_2 simultaneously. In this case its composition is determined by X and the diagrams, Figs. 5—7. b) It can attack Hg metal with the formation of Hg_2X_2, if $[Hg^{II}]_{total}$ is higher than that corresponding to the X of the solution and the equilibrium diagrams. In this case the solution does not attack Hg_2X_2. c) If $[Hg^{II}]_{total}$ is lower than that corresponding to equilibrium with Hg + Hg_2X_2, the solution will attack Hg_2X_2 with the formation of Hg metal and of more Hg^{II}. Such a solution will, on the other hand, not attack Hg metal.

From Figs. 5—7 it can be seen which of these cases applies to a given solution. For instance, a solution with $[I^-] = 10^{-1}$ would be in equilibrium with Hg and Hg_2I_2 with $[Hg^{II}]_{total} = 10^{-1.75} + 10^{-3.0} \approx 0.019$ C (Fig. 7). For lower values of $[Hg^{II}]_{total}$, the reaction $Hg_2I_2 + 2\ I^- \rightleftharpoons HgI_4^{2-} + Hg(l)$ will proceed to the right (Hg_2I_2 attacked but not Hg), for higher $[Hg^{II}]_{total}$ it goes to the left (Hg attacked but not Hg_2I_2).

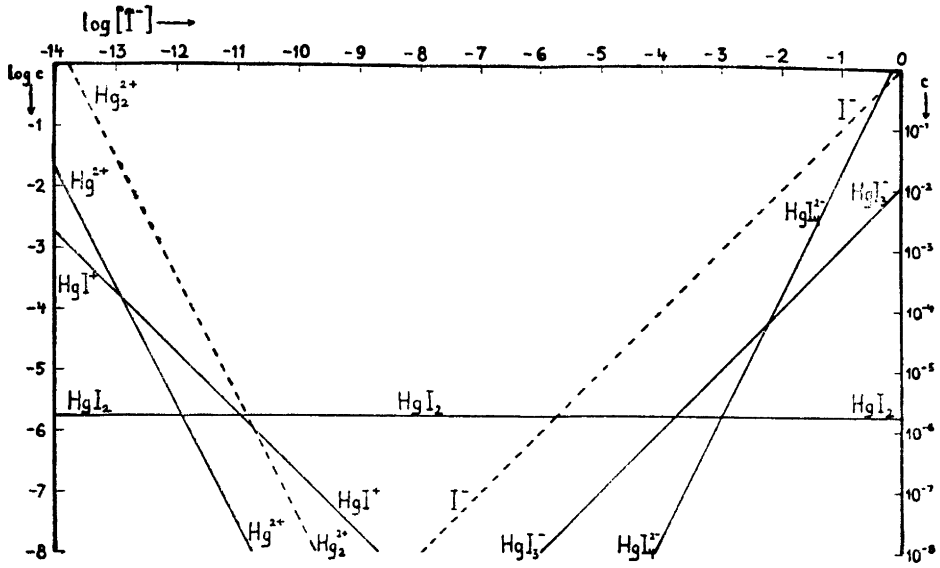

Fig. 7. Concentration of different ionic (molecular) species in equilibrium with Hg metal and solid Hg_2I_2 for varying $[I^-]$.

EQUILIBRIA WITH SOLID HgI_2

In Fig. 8 are shown in a logarithmic scale the concentrations of the complexes in equilibrium with solid (red) HgI_2:

$$\begin{aligned} [Hg^{2+}] &= s\varkappa_2^{-1}X^{-2} \\ [HgI^+] &= \varkappa_1 s\varkappa_2 X^{-1} \\ [HgI_2] &= s \\ [HgI_3^-] &= \varkappa_3 s\varkappa_2^{-1}X \\ [HgI_4^{2-}] &= \varkappa_4 s\varkappa_2^{-1}X^2 \end{aligned} \qquad (16)$$

These formulae can also easily be deduced from the definitions of the various equilibrium constants. Actually the lines in Fig. 8 are identical with those in Fig. 7 only that they are all (except, of course, the I^- curve) displaced upwards by $1.63 = \log sk_0k_S^{-1}\varkappa_2^{-1}$, since the solubility s of red HgI_2 is $10^{1.63} = 43$ times larger than the concentration $\varkappa_2 k_S k_0^{-1}$ of HgI_2 in equilibrium with Hg_2I_2 and Hg.

Of course the equilibrium described in any one of the diagrams in this paper becomes fictitious when the concentration of one of the ions becomes so large that the ionic strength, 0.5 C, is exceeded.

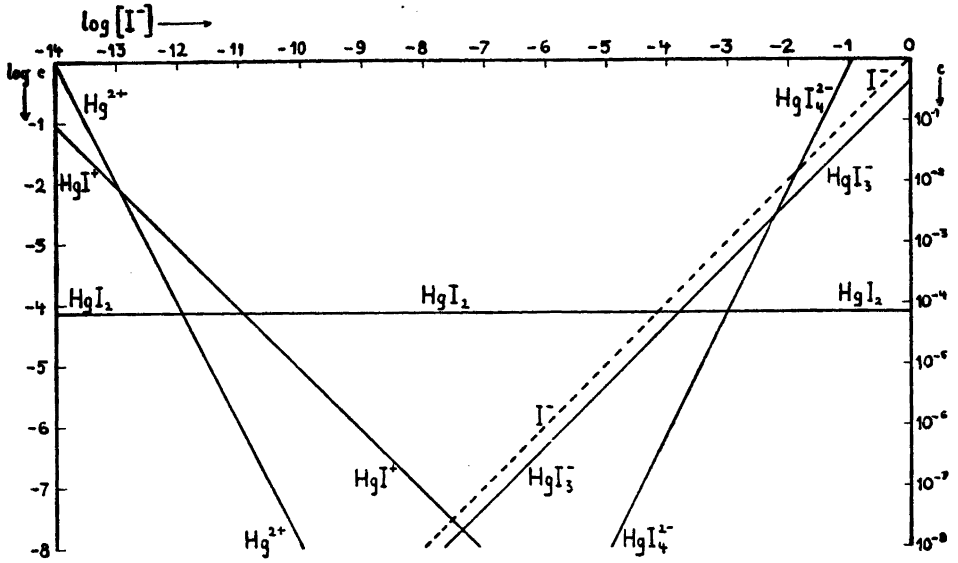

Fig. 8. Concentrations of Hg^{2+} and its complexes with I^- in equilibrium with solid HgI_2 for varying $[I^-]$.

ON THE THERMODYNAMIC CONSTANTS

All the equilibrium constants given are valid under the special conditions of our experiments: 25° C, $[H^+] = 10$ mC, and ionic strength = 500 mC (by addition of $NaClO_4$). Now that it seems certain that we need only count with the ionic species mentioned in (1), one might repeat the measurements for lower ionic strengths in order to find the thermodynamic constants. However, an extrapolation to ionic strength zero must be rendered difficult by the fact that Hg^{2+} is an acid of considerable strength so that the concentration of H^+ must not be too low if its hydrolysis is to be held back. Then the ionic strength will at any rate be at least about 10 mC, so that the assignment of activity factors to the various ionic species cannot be very accurate.

At the ionic strength chosen for our experiments, no general formulae for the activity factors hold good, since the individual properties of the ions cannot be neglected. For our ionic medium, the products of the ionic activity factors for Hg_2Cl_2, Hg_2Br_2, and Hg_2I_2 seem to be about 0.09—0.13 [7,p.517], and the activity factor for uncharged HgI_2 about 1.4 [8]. If it is assumed that Hg_2^{2+} and Hg^{2+} have about the same activity factors, it can be concluded that the values for log $\varkappa_2$ and log $\varkappa_3$ for infinite dilution are about one unit higher than those found for our ionic medium; log $\varkappa_1$ and log $\varkappa_2$ should also be higher for infinite dilution, though the difference should be less.

The acidity of Hg_2^{2+} and Hg^{2+}, the standard potentials of mercury, and the value for k_0 at infinite dilution will be discussed in papers by Hietanen and Sillén [32] and Forsling and Sillén [33].

SUMMARY

The complexes of Hg^{2+} with Cl^-, Br^-, and I^- have been studied at 25° C by electrometric methods using solutions with $[H^+] = 10$ mC and ionic strength 500 mC (by addition of $NaClO_4$).

Complexes of the types HgX^+, HgX_2, HgX_3^-, and HgX_4^{2-} were proved to exist for all three halogens, whereas there was no need for assuming the existence of other complexes.

The logarithms of the complex products $\varkappa_1 \ldots\ldots \varkappa_4$ (defined by 2) were found to be:

	log $\varkappa_1$	log $\varkappa_2$	log $\varkappa_3$	log $\varkappa_4$
for $Hg^{2+} - Cl^-$	6.74 ± .02	13.22 ± .02	14.07 ± .15	15.07 ± .06
for $Hg^{2+} - Br^-$	9.05 ± .03	17.33 ± .04	19.74 ± .11	21.00 ± .03
for $Hg^{2+} - I^-$	12.87 ± .03	23.82 ± .04	27.60 ± .14	29.83 ± .02

The logarithms of the solubility products k_S were, under the conditions chosen,

for Hg_2Cl_2 —16.88 ± .01, for Hg_2Br_2 —21.29 ± .04, and
for Hg_2I_2 —27.47 ± .01.

Diagrams are given showing the distribution of Hg^{II} over the different complexes for varying concentrations of free X^-, and illustrating the equilibria with Hg metal and solid Hg_2X_2, for I also with solid HgI_2.

Finally, I wish once more to express my gratitude for the favour of cooperating with Mr Per Olof Bethge, Mr Gunnar Infeldt (fil.kand.), Mrs Inga Jonevall-Westöö, Mr Arne Jonsson, Mr Bengt Lindgren (fil.lic.), and Miss Ingegerd Qvarfort.

Mrs Inger Brattsten (fil.lic., leg.apot.), Mr Erik Ekedahl, Miss Sirkka Hietanen (fil.mag.), Mr Herbert Larsson, Mr Bengt Liljeqvist (fil.kand.) and Mr Sven Sahlqvist have given valuable aid in many respects, as has been gratefully acknowledged in previous papers. I should also like to thank Miss Karin Ehrnström and Miss Aina Norström for their helpful assistance in the preparation of the present paper.

* *Note added in proof:* According to a private communication, Professor P. M. Harris and Mr. A. F. Foster (Ohio State University, Columbus; Ohio) have found extra lines in the powder photographs of low-temperature Ag_2HgI_4, indicating that the real unit cell contains eight formula units. The crystal structure previously given [21] is thus subject to revision.

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Received May 27, 1949.

ERRATA IN PREVIOUS PARTS

Part II:	p. 64, line 4 from below	for '5.8 ± 0.5' read '5.8 ± 0.3'
Part III:	p. 467, head of Table 3	for '$\frac{1}{2} X_e$' read '$-\frac{1}{2} X_e$'
	p. 470, formula (21)	for '$X = (X^-) + etc.$' read '$X_e = (X^-) + etc.$'
Part V:	p. 487, line 5 from below	for (0.227 ± 0.26) read (0.227 ± 0.026)

ACTA CHEMICA SCANDINAVICA 3 (1949) 554–559

Sur les différentes Moyennes des Grandeurs moléculaires mesurables sur un Echantillon polydispersé

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On sait que dans l'état actuel de nos techniques de préparation et de purification, les produits macromoléculaires que nous pouvons obtenir sont presque toujours constitués de mélanges contenant des molécules de masses différentes. Il est vrai qu'au prix de précautions convenables, nous savons préparer à partir de produits naturels certaines protéines qui se révèlent homogènes quand on les soumet aux critères expérimentaux les plus sensibles. Mais, même si toutes les molécules de tels échantillons ont bien la même masse, elles peuvent prendre en général toute une série de formes.

Les grandeurs qui caractérisent les macromolécules ne peuvent donc en général pas être exprimées par un seul chiffre. Il faudrait pouvoir donner la courbe de distribution de la grandeur considérée entre les molécules. Trop souvent, il faut se contenter d'une certaine valeur moyenne.

§ 1

Certaines valeurs moyennes sont fournies directement par l'expérience. On peut remarquer que si ces moyennes sont, formellement, de types assez variés, elles peuvent pourtant s'obtenir toutes d'après le même schéma.

Sur le mélange à étudier, on mesure une grandeur A que l'on sait être la somme des grandeurs correspondantes A_i attachées à chacune des espèces de molécules (i) présentes dans le mélange.

$$A = \Sigma A_i \tag{1}$$

Cette grandeur A_i est fonction d'une grandeur moléculaire particulière λ_i, d'une série d'autres grandeurs moléculaires $/\xi_i/$ et d'un certain nombre de paramètres extérieurs $/x/$

$$A_i = A\ (\lambda_i,\ /\xi_i/,\ /x/)$$

Par ailleurs, il faut que la fonction A puisse se mettre sous la forme

$$A\ (\lambda_i,\ /\xi_i/,\ /x/) = \varphi\ (\lambda_i) \cdot \psi\ (\lambda_i,\ /\xi_i/,\ /x/) \tag{2}$$

Finalement, il faut que la fonction ψ, tout comme la fonction A soit une fonction additive, c'est à dire que

$$\psi = \Sigma\ \psi_i \tag{3}$$

soit bien l'expression d'une réalité physique. Si toutes les trois conditions (1), (2) et (3) sont remplies, on peut calculer la valeur moyenne de $\bar{\lambda}$ que fournit la méthode de mesure envisagée.

$$\Sigma\ \varphi_i \cdot \psi_i = \bar{\varphi}\ \Sigma\ \psi_i$$

$$\bar{\lambda} = \bar{\varphi}^{-1}\left(\frac{\Sigma\ A\ (\lambda_i,/\xi_i/,/x/)}{\Sigma\ \psi\ (\lambda_i,/\xi_i/,/x/)}\right) \tag{4}$$

φ^{-1} représentant la fonction inverse de la fonction φ.

Voici quelques exemples tirés de la littérature. Nous avons conservé les notations originales et placé un tableau des symboles utilisés à la fin du présent article.

Tableau 1.

$\cdot A \cdot$	$\cdot\lambda \cdot$	$\cdot\varphi \cdot$	$\cdot\psi \cdot$	Référence
π	M	M^{-1}	RTc	
$\eta - \eta_0$	M	M^{α}	$K_m c \eta_0$	
$\frac{\mathrm{d}c}{\mathrm{d}x}$	M	M	$2Axc$	Lansing & Kraemer [1].
$\frac{\mathrm{d}Z}{\mathrm{d}x} - \frac{Z}{x}$	M	M	$4A^2x^2Mc$	Ibid.
m_{n+2}	D	D	m_n	Gralén [2].
$n_e - n_0$	a	a^3	$\frac{32\pi^2(g_1 - g_2)G\eta c}{45nkT(2\ \ln\left(\frac{2a}{b}\right) - 1)}$	Wales [3]. Sadron [4].

§ 2

Dans quelques cas, on dispose d'une série de valeurs moyennes pour les différentes fractions en lesquelles on a pu diviser un échantillon à étudier.

On peut alors calculer la moyenne de ces moyennes d'après le même schéma, à cette différence près toutefois que l'additivité des fonctions A et a ne doit plus nécessairement répondre à une réalité physique. Voici quelques exemples.

A	$\cdot\lambda\cdot$	$\cdot\varphi\cdot$	$\cdot\psi\cdot$	Référence
$M_{zz} \cdot Z \cdot dx$	M	M	$Z \cdot dx$	Lansing & Kraemer [1].
$M_{wz} \cdot c \cdot x \cdot dx$	M	M	$c \cdot x \cdot dx$	Ibid.

Jullander [5] donne une méthode permettant d'obtenir graphiquement la courbe

$$\frac{dc}{ds} = f\ (s)$$

que l'ont peut intégrer graphiquement. A partir de la courbe intégrale, on peut calculer une série de valeurs moyennes d'une façon purement formelle. Par analogie avec les moyennes M_n, M_w et M_z introduites par Lansing et Kraemer [1] et entrées depuis dans la pratique on peut écrire

$$s_n = \frac{\sum \frac{c_i s_i}{M_i}}{\sum \frac{c_i}{M_i}}$$

$$s_w = \frac{\sum c_i s_i}{\sum c_i}$$

$$s_z = \frac{\sum c_i s_i M_i}{\sum c_i M_i}$$

par analogie avec, par exemple

$$M_z = \frac{\sum M_i c_i M_i}{\sum c_i M_i}$$

ce choix étant justifié par le fait que $\frac{c}{M}$ représente le nombre de molécules présentes. Mais on peut aussi bien écrire, en analogie formelle

$$s'_n = \frac{\sum c_i}{\sum \frac{c_i}{s_i}}$$

$$s_w = \frac{\sum c_i s_i}{\sum c_i}$$

$$s'_z = \frac{\sum c_i s_i^2}{\sum c_i s_i} .$$

comme aussi nous pouvons écrire avec Gralén [6]

$$D_n = \frac{\sum c_i}{\sum \frac{c_i}{D_i}}$$

On se rend compte ainsi que la désignation de ce genre de moyennes par un seul indice est insuffisante *. Du reste le nombre de ces moyennes va en augmentant à mesure que se développe la chimie macromoléculaire (voir Wales [3, 9]). C'est pourquoi nous avons essayé de trouver une nomenclature systématique pour ces grandeurs.

§ 3

Observons que les fonctions A et ψ qui apparaissent dans la formule (4) prennent une forme plus simple par suite de l'élimination des paramètres x. Soit ψ * la fonction obtenue à partir de ψ quand on a simplifié A et ψ par les paramètres $/x/$. Représentons la valeur moyenne $\bar{\lambda}$ par le symbole

$$(\varphi)\ \lambda\ (\psi^*)$$

Dans cette symbolique, les exemples présentés au tableau 1 s'écrivent:

$(M^{-1})M(c)$	=	M_n	de Lansing & Kraemer [1].
$(M^{a})M(c)$	=	M_v	
$(M)M(c)$	=	M_w	
$(M)M(cM)$	=	M_z	
$(D)D(cD^{n/2})$	=	$D_{n+2,\,n}$	de Gralén [2].
$(D^{-1})D(c)$	=	D_n	de Gralén [6].
$(a^3)a(c)$	=	a	de Wales [3].

et les différents s moyens calculés de facon formelle au § 2

* Jullander [5] a introduit des poids moléculaires à deux indices qu'il obtient en introduisant dans la première formule de Svedberg [7] les valeurs moyennes dont il a été question jusqu'ici. Ce type de moyenne a été étudié en détails par Singer [8] mais il sort du cadre de cet article.

$$
\begin{aligned}
(s^{-1})s(csM^{-1}) &= s_n \\
(s)s(c) &= s_w \\
(s)s(cM) &= s_z \\
(s^{-1})s(c) &= s'_n \\
(s)s(sc) &= s'_z
\end{aligned}
$$

RESUME

La formation de valeurs moyennes quelconques est discutée, et un certain nombre de différents types de ces valeurs est présenté, spécialement pour prouver qu'il est possible de former deux différentes catégories de moyennes du type M_n, M_w et M_z. Enfin une terminologie nouvelle est proposée.

Nous tenons à remercier ici MM. Gralén et Jullander pour l'aide qu'ils nous ont apportée et les critiques bienveillantes qu'ils nous ont faites au cours du développement de cette étude.

LISTE DES SYMBOLES UTILISÉS

π	Pression osmotique
M	Poids moléculaire
R	Constante des Gaz parfaits
T	Température absolue
c	Concentration
η	Viscosité de la solution
η_0	Viscosité du solvant pur
a	Exposant empirique compris entre 0,5 et 1
K_m	Constante empirique de Staudinger
x	Distance séparant le point considéré de l'axe de rotation de l'ultracentrifugeuse (Svedberg & Pedersen [7])
A	$\frac{(1 + V\varrho)\omega^2}{2RT}$ (ibid.)
Z	Décalage de la division de l'échelle de mesure située au niveau x (ibid.)
m_n	n ième moment de la courbe donnant le gradient de concentration en fonction du niveau
D	Constante de diffusion
$n_e - n_0$	Valeur de la biréfringence
a	Grand axe de l'ellipsoïde de révolution ayant les mêmes propriétés hydrodynamiques que la molécule considérée
b	Petit axe de cet ellipsoïde
G	Gradient de vitesse
k	Constante de Boltzmann
n	Indice de réfraction moyen du milieu
$g_1 - g_2$	Fonction de la forme de la molécule et des indices de réfraction du soluté et du solvant supposée constante pour toutes les sortes de molécules constituant le mélange
s	Constante de sédimentation

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Reçu le 3 mai 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 560–568

The Accuracy of Inter-atomic Distances Obtained in Electron Diffraction Investigations of Molecular Structures

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A large and growing mass of data from several different experimental fields has been obtained about the dimensional details of molecular structures. To co-ordinate and to make the best possible use of this information, it is necessary to free the results of any systematic errors, to estimate the random errors, and to subject any metrical interpretations or comparisons to standard statistical tests of significance. The present paper describes how to treat the results of electron diffraction investigations; its approach to the problem of accuracy has had two sources. A similar theory of errors has been discussed in X-Ray Crystallography by Cox and Cruickshank [1], and Cruickshank [2]; these papers contain a full discussion of the details of the problem, and give the references to those authors who have contributed to its solution in X-Ray Crystallography. On the other hand Finbak, Hassel and co-workers in a number of papers [3] have applied Fourier Analysis in the sector method of electron diffraction, and Viervoll [4] has shown the use of the method of differencing experimental and theoretical $\sigma_m(r)$ curves in eliminating diffraction effects, searching for light atoms and guarding against anomalies.

In the electron diffraction of gaseous molecules interatomic distances may be obtained from the radial distribution functions $\sigma_m(r)$, $\frac{\sigma_m(r)}{r}$ or $D_m(r)$ which are related by $\sigma_m(r) = 4\pi(r)^2 D_m(r)$. The functions $\sigma(r)$ and $\frac{\sigma(r)}{r}$ defined by equation (17) of Viervoll's paper [4] are also used. For definiteness our discussion refers to $\sigma_m(r)$; similar remarks apply, however, to the other radial distribution functions. $\sigma_m(r)$ is obtained from the function $\frac{I_m(s)}{K}$ which is

found experimentally, $I_m(s)$ being an intensity varying with $s = \frac{4\pi}{\lambda} \sin \Theta$. ($\lambda$ = wave length of electron beam; 2Θ = scattering angle.)

$$\sigma_m(r) = \frac{2r}{\pi} \int_0^\infty \frac{I_m(s)}{K} s^5 \sin sr \, ds \tag{1}$$

POSITIONS OF THE MAXIMA

In numerical calculations as an alternative to using $\sigma_m(r)$ the positions of the maxima may be found from the zeros of the function which is the slope of $\sigma_m(r)$ (as after Booth [5] in X-Ray Crystallography). As slope functions are also needed in estimating the accuracy of the positions of the maxima, it may sometimes be convenient to calculate them directly *e. g.*

$$\frac{d}{dr}\left(\frac{\sigma_m(r)}{r}\right) = \frac{d}{dr} \int_0^\infty \frac{2}{\pi} \frac{I_m(s)}{K} s^5 \sin sr \, ds = \frac{2}{\pi} \int_0^\infty \frac{I_m(s)}{K} s^6 \cos sr \, ds \tag{2}$$

The curvatures of the peaks at the maxima, which are also needed in accuracy estimations, may be found from the slopes of the slope functions.

CORRECTION FOR FINITE INTEGRATION

The finite range of integration (s_1 to s_2 instead of 0 to ∞) gives rise to systematic errors known as diffraction effects, after the name proposed by Bragg and West [6] for the similar effects occuring in the Fourier maps of X-Ray Crystallography. The diffraction effects take the form of ripples which create false peaks and shift the positions of the real maxima. By the use of 'normal curves' which represent the distribution function for a single inter-atomic distance with diffraction effects, Viervoll [4] has developed a method for detecting the spurious maxima and for correcting the positions of the displaced maxima.

When provisional interatomic distances have been obtained directly from the experimental radial distribution curve, the correction for finite intergration may be made in the following manner. By taking a complete set of 'normal curves' we may obtain a theoretical $\sigma_m(r)$ for a molecular model based on these provisional distances; this theoretical $\sigma_m(r)$ will be found to have its maxima slightly displaced from the assumed positions due to diffraction effects. Since the maxima of the experimental $\sigma_m(r)$ are obtained from a curve with diffraction effects, their correct positions, without diffraction, are obtained by reversing the displacements found in the theoretical $\sigma_m(r)$. Provided these

shifts are small it is unneccesary to repeat the process with the theoretical $\sigma_m(r)$ based on the (once) corrected positions.

As appears from the discussion on p. 129 of Viervoll's paper [4], even with an infinite range of integration the inter-atomic distances are not exactly given by the maxima of $\sigma_m(r)$. The preceeding method automatically allows for this also. When the maxima corresponding to two different inter-atomic distances are not resolved, the distances must be estimated by »fitting» the normal curves to give the best agreement. A discussion of the conditions of validity of the similar procedure in X-Ray Crystallography, first proposed by Booth [7, 8] is given in § 10 of Cruickshank's paper [2].

RANDOM ERRORS

The distances now obtained are the final estimates, and are in error due to:

1) Inaccuracies in correction for finite integration.

2) Errors in $I_m(s)$.

3) Approximation errors in calculation.

There may also be an additional error due to an error in the linear scale of the intensity diagram. (In X-Ray Crystallography this corresponds to errors in the cell dimensions.) This error is usually negligible in comparison with those mentioned above, but if it is not, an estimate must be added statistically to the other errors.

On the assumption that the effect of the errors 1)—3) is that of many small random errors (as must for instance, occur in the determination of $I_m(s)$ over a large range of s), the errors in the inter-atomic distances have a normal (Gaussian) probability distribution. We shall now show how to estimate the standard deviation of these errors.

The problem cannot be treated in the same way as the corresponding one in X-Ray Crystallography, discussed in § 11 of Cruickshank's paper [2]. In that case estimates were sought of the errors in the electron density of a finite unit cell due to small errors in a set of discrete intensities; we are now seeking to estimate the errors at different points of a radial distribution function of infinite extent due to small errors in a continuous intensity function. Two points may be noticed at once. The errors in $\sigma_m(r)$ depend on r and they must tend to zero as r tends to infinity. The usual range of observation of s is sufficiently large for the root mean square difference of the theoretical and experimental intensities to be a reliable estimate of their r. m. s. error, and hence in a sufficiently large range of r the r. m. s. difference of the experimental and theoretical slopes of $\sigma_m(r)$ is a reliable estimate of the r. m. s. error of the slope in that range of r.

This suggests the following procedure. Let $\gamma(r)$ be the difference between the theoretical and experimental slopes of $\sigma_m(r)$. $\gamma^2(r)$ will be found to oscillate irregularly as in Fig. 1. Draw a smooth curve $p^2(r)$ (indicated by the dotted line) such that in any sufficiently large range of r its mean value is equal to the mean value of $\gamma^2(r)$ in that range. Then the estimate of the standard deviation of the slope of $\sigma_m(r)$ is $p(r)$.

One obvious difficulty is to decide on the length of the ranges of r to take in finding $p(r)$. They must be long enough to give reliable estimates of the r. m. s. error in that range, yet short enough to show variation with r. We suggest (arbitrarily) that ranges of length $\frac{4\pi}{s_2}$ should be used, so that $p^2(r)$ should be close to the mean value of $\gamma^2(r)$ in the range length $\frac{4\pi}{s_2}$ centred at this r, and that no value of $\gamma^2(r)$ should exceed three times the corresponding value of $p^2(r)$ (a position of very large $\gamma^2(r)$ either indicates a position of large error, or, more likely, an anomaly *e. g.* the effects of hydrogens atoms not considered in the theoretical $\sigma_m(r)$.) If there is any doubt as to the value of $p^2(r)$ it is wise to err on the safe side and to use an upper estimate.

By this consideration of the difference between the slopes of the theoretical and experimental $\sigma_m(r)$ we have estimated directly the effects of the experimental errors in $I_m(s)$, the approximation errors in calculation and any inaccuracies in the correction for finite integration.

The estimated standard deviation of an inter-atomic distance is $\frac{p(r)}{A}$ where A is the curvature of the peak of the $\sigma_m(r)$ diagram which gives the distance. When by the symmetry of the molecule several inter-atomic distances are related to one parameter, the standard deviation d of the weighted (*i. e.* inversely as the squares of the deviations) mean estimate of the parameter is given by

$$\frac{1}{d^2} = \frac{1}{d_1^2} + \ldots . + \frac{1}{d_n^2}$$

where $d_1, \ldots d_n$ are the standard deviations of the various determinations of the parameter.

AN EXAMPLE OF THE ESTIMATION OF STANDARD DEVIATIONS

The procedure will be illustrated by the structure determination of CBr_4, Fig. 1. The intensity $I_m(s)$ was in this case measured from $s_1 = 5$ to $s_2 = 16$; the corresponding experimental $\sigma_m(r)$ curve being shown in the upper part of

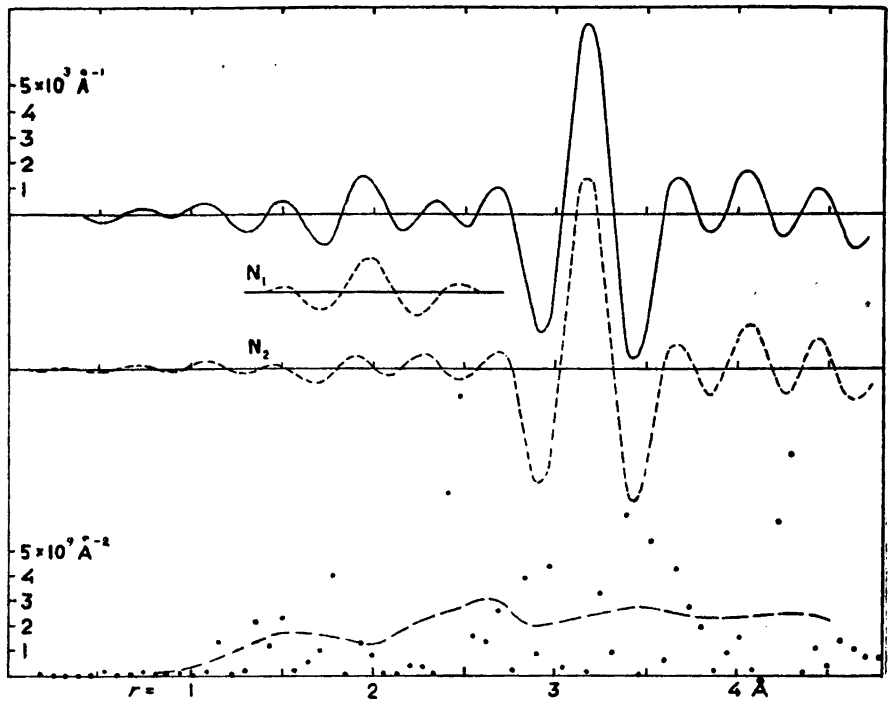

Fig. 1. Determination of standard deviation from the experimental $\sigma_m(r)$ curve of CBr_4. ——— *exp.* $\sigma_m(r)$, – – – »*normal curves*», • $\gamma^2(r)$, — — $p^2(r)$.

Fig. 1. The dominating peak at $r = 3.174$ Å gives the Br--Br distance. The 'normal curve' of this distance is shown by the dotted curve, N_2. By subtracting the function N_2 from the experimental $\sigma_m(r)$ function we obtain a difference function in which the diffraction ripples of the Br—Br distance have been removed. This difference function has its most pronounced maximum at $r = 1.960$ Å which gives the C—Br distance. The corresponding 'normal curve' is shown by the dotted curve N_1.

By subtracting the functions N_1 and N_2 from the experimental $\sigma_m(r)$ function, we obtain a difference curve the slope of which is $\gamma(r)$. The irregular oscillation of $\gamma^2(r)$ is plotted as dots in the lower part of Fig. 1. The curve $p^2(r)$ has been drawn as the mean value of $\gamma^2(r)$ in a range length approximate to $\frac{2\pi}{16}$.

From these curves we obtain the following estimation of the standard deviations of the two distances:

C—Br: $p^2(r) = 1.4 \times 10^9$ Å^{-2} $\quad d_1 = \dfrac{3.741}{204} = 0.0183$ Å

$A = 2.04 \times 10^4$ Å^{-2}

Br—Br: $p^2(r) = 2.3 \times 10^9$ Å^{-2} $\quad d_2' = \dfrac{4.796}{734} = 0.0065$ Å

$A = 7.34 \times 10^4$ Å^{-2}

If we now assume the molecule to have tetrahedral symmetry, we obtain from the Br—Br distance the following value of the C—Br distance:

$$3.174 \times \sqrt{\tfrac{3}{8}} = 1.944 \text{ Å} \qquad d_2 = 0.0065 \times \sqrt{\tfrac{3}{8}} = 0.0040 \text{ Å}$$

The ratio $\left(\dfrac{d_1}{d_2}\right)^2 = 21$; by weighting the results by this factor we get the final estimate:

C—Br: 1.945 Å; $\quad d = 0.0039$ Å

TEMPERATURE FACTORS AND THE METHOD OF LEAST SQUARES

It is often necessary to multiply the theoretical intensity by a temperature factor to get agreement with the experimental data, and thus to make the heights and forms of the experimental and theoretical $\sigma_m(r)$ peaks the same. When this is done the corrections for diffraction should be estimated from normal curves which allow for the temperature factor; the same normal curves should be used to find $\gamma(r)$.

Similarly if an artificial temperature factor is applied to the experimental $I_m(s)$ to reduce the diffraction effects, it should also be applied to the 'normal curves'. It is sometimes found that the use of an artificial temperature factor not only reduces the diffraction effects but also the estimated random errors in the inter-atomic distance. To understand this we consider the method of least squares.

Let $I_{exp.}$ and I_{th} denote the experimental and theoretical intensities, and let $w(s)$ be the weight given to the intensity at any s. According to the method of least squares the best estimates of the inter-atomic parameters R_{ij} are those for which the function L is a minimum

$$L = \int_{s_1}^{s_2} \omega \, (I_{exp.} - I_{th})^2 \, ds \tag{3}$$

I_{th} being calculated for the parameters R_{ij}. When L is a minimum $\left[\frac{\delta L}{\delta R_{ij}}\right] = 0$. Hence

$$\int_{s_1}^{s_2} \omega\ (I_{exp.} - I_{th}) \frac{\mathrm{d}I_{th}}{\mathrm{d}R_{ij}} = 0 \tag{4}$$

Now

$$\frac{\mathrm{d}I_{th}}{\mathrm{d}R_{ij}} = \frac{mK\ (Z_i - F_i)(Z_j - F_j)}{s^4} \frac{\mathrm{d}}{\mathrm{d}R_{ij}} \frac{\sin sR_{ij}}{sR_{ij}} \tag{5}$$

m being a multiplicity; hence, dividing by mK^2

$$\int_{s_1}^{s_2} \frac{\omega\ (Z_i - F_i)(Z_j - F_j)}{Ks^4} \frac{\mathrm{d}}{\mathrm{d}R_{ij}} \left[\frac{\sin sR_{ij}}{sR_{ij}}\right] I_{exp.}\ \mathrm{d}s =$$

$$= \int_{s_1}^{s_2} \frac{\omega\ (Z_i - F_i)(Z_j - F_j)}{Ks^4} \frac{\mathrm{d}}{\mathrm{d}R_{ij}} \left[\frac{\sin sR_{ij}}{sR_{ij}}\right] I_{th}\ \mathrm{d}s \tag{6}$$

i. e. the slopes at R_{ij} of

$$W_{exp.}(r) = \frac{2}{\pi} \int_{s_1}^{s_2} \frac{\omega\ (Z_i - F_i)(Z_j - F_j)}{Ks^4} \frac{\sin sr}{sr} I_{exp.}\ \mathrm{d}s \tag{7}$$

and

$$W_{th}(r) = \frac{2}{\pi} \int_{s_1}^{s_2} \frac{\omega\ (Z_i - F_i)(Z_j - F_j)}{Ks^4} \frac{\sin sr}{sr} I_{th}\ \mathrm{d}s \tag{8}$$

are equal. We may regard $W(r)$ as the $D_m(r)$ obtained by using an artificial temperature factor $\alpha = \frac{\omega}{s^{10}}\ [(Z_i—F_i)\ (Z_j—F_j)]$. The function $\omega(s)$ must be determined from a study of the differences in $I_{exp.}$ and I_{th} and cannot be predicted entirely on theoretical grounds. By the very purpose of the least squares method α is the temperature factor which gives the least error in the determination of R_{ij}; it does this because it weights the intensity according to its reliability for different s. The use of other artificial temperature factors is quite valid, but they will lead to less accurate results, their accuracy depending on how closely they resemble α. In each case the errors will be those estimated by the methods described above. If the F's of the different atoms are not

proportional several different $W(r)$'s will be needed to get the best estimate of all the parameters, though probably little accuracy will be lost by using the same α throughout.

We may notice that when nearly correct parameters have been obtained from $\sigma_m(r)$ it is possible to express approximately $I_{th}(s)$ as a linear function of the parameters. By doing this the usual 'normal equations' of the least squares method can be found and solved. When the maxima are well resolved the least squares method leads to the same results as those given by $W_{exp.}(r)$, corrected for diffraction effects. But when some of the maxima are not resolved it may be preferable to use least squares. (A similar connection in X-Ray Crystallography between the Fourier series and the least squares method has been pointed out by Cochran [9].)

SIGNIFICANCE TESTS

For valid comparisons to be made of experimental determinations of inter-atomic distances in molecular structures, or between experimental and theoretical results it is necessary to use statistical assessments of significance based on the estimated errors. Details of the application of significance tests to the comparison of bond-lengths will be found in Cruickshank's paper [2]; a general outline of the problem is given in § 3, and an example in § 15, where the complications which occur when the errors in two bond-lengths are dependent, are also discussed.

SUMMARY

Methods of correcting the systematic errors of finite integration in the radial distribution functions of electron diffraction are discussed. A method is given for estimating the standard deviations in inter-atomic distances, due to random errors caused by experimental errors, approximation errors in calculation, and any inaccuracies in the correction for finite integration. As an example this method is applied to the CBr_4 structure. The effect of artificial temperature factors on accuracy is discussed by considering the method of least squares.

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Received April 11, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 569–583

Some Investigations in the Crystal Chemistry of Silicates

I. Diffusion of Pb and Ra in Feldspars

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The diffusibilities of ions in ionic crystals and atoms in metals and alloys have been studied during several decades by a great number of scientists, and valuable results on this research have been published. Barrer [1] gives a thorough list of references to the scientists who have worked in this extensive field of science.

The diffusibilities in crystal silicates, however, have chiefly been investigated in a qualitative way. The quantitive investigations have been concentrated on diffusion in zeolites. In *International critical tables* only one single quotation on the diffusion coefficient of ions in crystalline silicates is to be found. This concerns the diffusibility of Na^+ in natrolite at 20 degrees centigrade. The value given is 13.10^{-6} cm^2 per day = 11.10^{-11} cm^2 per sec. There is no statement as to the energy of activation.

For a long time scientists have been aware of the fact that diffusion occurs more rapidly in zeolites like natrolite than in the usual crystalline silicates. This depends on the loose crystal structure of the zeolites and the high water content.

Bowen [3] has measured the diffusion of diopside glass in different plagioclase glasses. He found that at about 1500 °C the diffusion coefficients vary from 0.015 to 0.3 cm^2 per day. His experiments must be considered as very elegant when the laboratory technique of that time is taken into account. As late as 1941 Eitel [4] quoted his results as the only quantitative values of the diffusion coefficient in silicate glasses.

In a recent paper [5] I have dealt with the diffusion of lead in some radioactive minerals. I found the following values:

$D_{Pb} = 4.10^{-19}$ cm^2 per sec in amorphous niobates-tantalates of the metamict samarskite-yttrotantalites type.

$D_{Pb} = 1.10^{-18}$ cm^2 per sec in amorphous ortho-silicates of the metamict uranothorities type.

$D_{Pb} = 2—4.10^{-22}$ cm^2 per sec in crystal uranium 4 oxide of the uraninite type from Ausel and Morogoro.

These values refer to the average diffusion of Pb_{206} since the formation of the minerals up to the present time.

Several serious reasons indicate that the temperature of the minerals never was much above 100° C in this very extensive space of time.

Bowen [6] discussed the possible rates of diffusion in silicate crystals. Basing his calculations on his previous investigations regarding the diffusibility in plagioclase glasses, he found it reasonable that the diffusion coefficient in crystal silicates would be lower than $10^{-9}—10^{-10}$ cm^2 per sec at temperatures of 1400—1500 °C. Such maximal values seem reasonable even though the diffusion coefficient of course must vary greatly for different ions in the same crystal. Bowen [3] states that his experiments on the diffusion in silicate glasses did not support the assumption of selective diffusion. His methods, however, could hardly be considered as very useful in the study of selective diffusion if the difference in diffusibility should not happen to be very high. From other investigations, however, it is known that the diffusion coefficient of different ions in the same crystalline medium may vary by several orders of magnitude at the same temperature.

By means of radioactive tracers it is possible to study diffusion processes by very much higher accuracy than it has been possible by the earlier methods. Hevesey and Seith [7] may be mentioned among those who carried out fundamental research work in this branch. It does not seem, however, as if the radioactive tracer technique has been much empoyed in the case of crystalline silicates, especially not in the quantitative determination of the diffusion constants.

The usual method in the study of diffusion processes in solid state by means of radioactive tracers is to use alpha active tracers and to measure the alpha activity or the recoil particles from the tracer. When the tracer is placed on the surface of crystals, the alpha activity or the recoil activity decreases as the radioactive component diffuses into the crystal. Such a method involves several advantages in the mathematical treatment, as the range of alpha-particles in all solids is very small, and the range of recoil particles still smaller.

When beta and gamma-active radioactive tracers are used, we can not employ the same calculation methods. This is caused by the fact that the range of gamma rays and of beta rays of usual energy in crystals are much above the thickness of the crystal layers through which the radioactive ions will diffuse in a sufficiently short time. According to the method mentioned

below, the diffusion coefficient in solids may be determined by means of beta and gamma active tracers. The experiments are based on the following assumptions:

a) The diffusion coefficient of the radioactive tracerions is much higher in glasses than in crystal silicates at the same temperature. Several experiments have shown that this assumption is correct;

b) The depth of the silicate crystals through which the radioactive ions are diffusing, is small compared to the range of the beta and gamma rays in the mineral. It is easy to conduct the experiments in such a way that this assumption proves to be correct. The range of the beta rays of average energy is several millimeters in the usual silicates, and after three months the average penetration depth of the diffusing ions is only a few hundred microns, provided the diffusion coefficient is as high as 10^{-12} cm^2 per sec.

c) The thermodynamic potential of equal concentrations of the radioactive component is the same independent of whether they are dissolved in the glass or in the crystalline substance. We do not know if this assumption is correct, but we will later return to this question.

Anyhow, I assumed in the beginning that the difference in free energy would be without importance in the experiments referred to. I found it reasonable to assume this chiefly because of the modern theories of structure of the glass. Comp. Eitel [4].

According to these theories the structure of the glass does not differ very much from the structure of the crystalline silicates. In my experiments on the diffusion of Pb^{++} and Ra^{++} in potash feldspars (microcline) and in sodium-feldspars (albite) I thought it reasonable that the ions alien to the lattices would find themselves in nearly the same condition independent of phase. Closer investigations, however, showed that this 3rd assumption was not quite correct, and I had to introduce a factor of correction.

To investigate this, small drops of glass were melted on the surface of the feldspar crystals in the same way as was done in the diffusion experiments described in this paper. The glass drops contained small amounts of the radio-active tracers evenly distributed. The feldspar crystals with the glass drops were heated for sufficiently long time for the radioactive tracers to penetrate aprox. 2/10—3/10 millimeters into the crystals. The glass drops were then carefully removed and the radioactivity of the crystals measured. Later on 1/100 of a millimeter of the crystal surface was removed by grinding, and the radioactivity was measured a second time. The thickness of the removed layer was controlled by micrometer. The difference in activity was compared with the radioactivity of the glass slide of the same thickness and the same cross-section. When such a thin layer as 1/100 mm was ground off, we may assume that the concentration of the radioactive tracer in this layer is equal to the boundary concentration. The ratio between the boundary concentration and the concentration in the glass slide could then be intro-

duced as a factor of correction in the equation 11, page 574 in the following mathematical treatment of the problem.

I will here take the opportunity to express my sincere thanks to Jan V. Garwick, cand.real. for his valuable help in the mathematical treatment of the problems.

We are basing our calculations on the three assumptions mentioned above, employing the following experimental technique: On the surface of the solid where the diffusion coefficient is to be measured, another material with a relatively high content of the radioactive component used as a tracer is placed assuring good contact. The solid, with the glass containing the tracer is heated to the experimental temperature for a sufficiently long time. After this the glass with the tracer is removed and the radioactivity of the crystalline body is measured.

If the radioactive components have not penetrated, very far, we may neglect the absorption in the material, and we have:

$$A = \frac{N}{\lambda} \tag{1}$$

Here N is the number of radioactive atoms which have penetrated into the solid. λ is the disintegration constant and A is the number of disintegrations per time unit. The radioactive atoms introduced in the solid are distributed in such a way that the equation:

$$\frac{\delta N}{\delta T} = D \bigtriangledown^2 N = D \frac{\delta^2 N}{\delta x^2} \tag{2}$$

is fulfilled. D is the diffusion coefficient. As a boundary condition we may find in several cases nearly

$$N = N_0 \text{ for } x = 0 \tag{3}$$

Here N_0 is the concentration in the glass. It is supposed that N_0 varies little with the time. This means that a very low fraction of the radioactive atoms has diffused out of the glass, and the diffusion coefficient in the glass is so high that the concentration variation is equalized by diffusion. When (2) and (3) are laplacetransformed and

$$\mathrm{L}\{N\ (x,\ t)\} = n\ (x,\ s) \tag{4}$$

we have

$$D \frac{d^2 n}{dx^2} = sn \tag{5}$$

$$n = \frac{N_0}{s} \text{ for } x = 0 \tag{6}$$

as

$$N\ (x,\ 0) = 0$$

The solution of (5) passing towards 0 together with $\frac{1}{x}$ is

$$n = A \cdot e^{-\sqrt{\frac{s}{d}}x}$$

Here A is determined by (6) and we have:

$$n = \frac{N_0}{s} e^{-\sqrt{\frac{s}{d}}x} \tag{7}$$

We further have:

$$N\ (t) = q \int_0^\infty N\ (N,\ t)\ dx \tag{8}$$

Here q is the cross section of the surface through which the atoms are passing. This cross section is assumed to be great compared to the average penetration depth. (8) is laplacetransformed and we have

$$\mathrm{L}\{N\ (t)\} = U\ (s) \tag{9}$$

Thus we have

$$U\ (s) = q \int_0^\infty n\ (x_1 s)\ dx = q \frac{N_0}{s^{\frac{3}{2}}} \sqrt{D}$$

where

$$N\ (t) = \mathrm{L}^{-1}\ \{U\ (s)\} = 2\ q\ N_0 \sqrt{\frac{Dt}{\pi}}$$

By direct measurement we have found A in (1). Hence we know

$$\frac{2_q N_0}{\lambda} \cdot \sqrt{\frac{Dt}{\pi}}$$

In order to determine N_0 we prepare a thin slide of the glass with a cross-section equal to q. If the thickness is d, this will contain qdN_0 radioactive atoms. We hence have $B = \frac{qdN_0}{\lambda}$ disintergrations per sec. The measurement of A and B will have the same errors for geometrical reasons, and in the ratio A/B; these errors will then be eliminated. We therefore have

$$\frac{A}{B} = p = \frac{2}{d}\sqrt{\frac{Dt}{\pi}} \tag{10}$$

or

$$D = \frac{\pi}{4}p^2 \cdot \frac{d^2}{t} \tag{11}$$

It is supposed that the boundary concentration is equal to the concentration in the glass. If this is not the case, the factor of correction depending on the distribution coefficient must be introduced. This is most easily done by dividing the diffusion coefficient found, by the square of the distribution coefficient.

LABORATORY PROCEDURE

The first task was to prepare the glass containing radioactive tracers. The following two glass samples were prepared:

1. About 10 g of ordinary window glass was crushed and passed through a sieve (no. 60 U. S. Standard). The glass powder was carefully mixed with about two grams of lead oxide, containing about 4/1000 mC RaD. The mixture was melted in an iron crucible. After cooling, the glass charge was crushed again and remelted. This procedure was repeated 3 times in order to obtain a homogenous composition.

2. A second sample containing radium was prepared in much the same way. About 10 g of glasspowder was mixed with 0.5 g of carbonate of soda and 0.5 g of borax and 0.020 mg of $RaBr_2$. This was dissolved in one milliliter of water. The different components were carefully mixed and after drying the mixture was melted in an iron crucible. In the same way as the first charge, the glass charge was remelted three additional times.

From the two glass charges small samples, weighing about 0.1 gram were taken out. Each sample was melted on a thin platinum sheet at about 1500° C until the sample was quite liquid. By means of a platinum rod the melted sample was spread evenly out on the sheet-metal in a thin layer. After cooling, the thin glass slide could be removed, and the thickness could be controlled by a micrometer.

Sample I an average thickness of 0.036 cm
» II » » » » 0.024 »

This thickness is much below the average range of β and γ rays in the glasses, and no measurable weekening in the radiation because of absorption should occur. The two small glass slides were placed on non-radioactive vermiculite plates after weighing. They

were kept as radioactive standards. The radioactivity was measured 7 days after the preparation, and this radioactivity was noted. All the other samples delt with in this paper were likewise measured 7 days after the last heat treatment. When any radioactivity is quoted, this hence refer to the activity after 7 days. In this way the major part of the errors depending on selective diffusion of different radioactive isotopes is eliminated. Likewise the possible error depending on the loss of Rn during the heat treatment is eliminated. All samples to be measured were placed exactly 0.5 cm below the center of the micawindow of the G. M. counter equipped with an end window. The activity was registered by means of an amplification circuit with scaling from 1 to 64. The mica window had a diameter of 1 inch and a weight of 0.03 gcm^{-2}. The glass slide containing RaD had a weight of 116.4 mg and a specific gravity 2.73. After 7 days the activity was 560.3 counts . min^{-1}. The glass slide containing radium had a weight of 96.2 mg and a specific gravity of 2.65. After 7 days the activity was 2272 counts min^{-1}.

The crystals used in the following diffusion experiments were carefully picked form our mineral collection. The pieces could not be seen to contain any inclusions except pertites. As a sample of microcline, one piece of about 500 gr. from Einerkilen in Evje was chosen; and as a sample of albite, a piece of about 300 g from Bjortjenn in Aust-Agder was used. The pieces were parted into smaller bits varying from about 1 cm^3 to about 15 cm^3. From each mineral some pieces were ground and polished on different faces. The following faces were chosen: (001); (010) and $\perp$ [100]. The pieces were placed in a little furnace with the polished face upwards, and heated to a temperature of about 950° C. Small pieces of the radioactive glasses were placed on the faces. As soon as the glass had reached a sufficiently high temperature to weaken, the mineral pieces were removed from the furnace and cooled to room temperature. Usually less than one minute of heating was sufficient to weaken the glass. This time is too short to cause any measurable diffusion of the radioactive substances into the crystals.

The crystals thus provided with a glass drop on the polished face were placed in furnace to be tempered at constant temperatures until sufficient quantities of the radioactive tracer had diffused into the crystals. The appropriate time had to be calculated from preliminary experiments.

After tempering, the mineral pieces were removed from the furnace and cooled to room temperature. The glass drops could then be removed from the mineral surface by means of a fine steel chisel. By means of a magnification glass the mineral faces could be exmined. All traces of the glass drops had to be removed. Very often it happened that parts of the minerals had loosened when the glass drops were removed. In these cases the experiment was not continued, and in the tables only those results are given were visual examination showed no failures in the experiment. In the case of sodium feldspar it was especially difficult to remove the glass drop from the basis face without destroying the sample.

Seven days after the removal of the glass drops the radioactivity of the crystal face was measured. In the case of the potash feldspar the radioactivity of the feldspar was subtracted from the total activity. In the case of the albite the activity of the feldspar was so low that no subtraction was done. The diffusion coefficient was calculated according to formula 11, page 574. The activities of the standard glass slides mentioned were used as a comparative standard, and the standard glass slides were recalculated into slides of the same cross-section as the contact cross-section between glass and crystals.

THE DISTRIBUTION COEFFICIENT OF RADIUM AND LEAD BETWEEN GLASS AND CRYSTALLINE FELDSPAR

The distribution coefficient resulting from diffusion was measured in the manner mentioned on page 572. The following results were obtained:

The	potash	feldspar	distribution	coefficient	for	Ra = 1.045
»	»	»	»	»	»	RaD = 1.87
»	sodium	»	»	»	»	Ra = 1.3
»	»	»	»	»	»	RaD = 1.15

After some preliminary experiments the following experiment was performed.

Table 1. Diffusion of lead in microclinepertite $\perp \{001\}$.

Expt. no.	Time	T	Tracer	Count min^{-1}	Cross-section of contact area in cm^2	Count cm^{-2} $min.^{-1}$	D	
11	90d	603°	RaD	36.0	2.4	15.0	$3.79 \cdot 10^{-14}$	cm^2/sec
12	90»	603°	RaD	13.6	0.8	17.0	$4.85 \cdot 10^{-14}$	cm^2/sec
15	6»	823°	RaD	8.3	0.25	33.3	$2.81 \cdot 10^{-12}$	cm^2/sec
16	6»	823°	RaD	13.2	0.25	52.8	$8.15 \cdot 10^{-12}$	cm^2/sec
17	44h	1023°	RaD	37.9	0.4	94.8	$7.28 \cdot 10^{-11}$	cm^2/sec
18	44»	1023°	RaD	41.5	0.4	103.5	$9.20 \cdot 10^{-11}$	cm^2/sec
19	44»	1023°	RaD	35.8	0.37	94.5	$7.22 \cdot 10^{-11}$	cm^2/sec
20	20»	1038°	RaD	31.5	0.8	39.4	$2.77 \cdot 10^{-11}$	cm^2/sec
21	20»	1038°	RaD	51.6	0.7	73.9	$0.74 \cdot 10^{-11}$	cm^2/sec

Standard sample 116.4 mg RaD-glass, specific gravity 2.73. Activity 560.3 count. min^{-1}, distribution coefficient 1.87.

Because of the relatively low value of the diffusion coefficient, the difference between parallel experiments may be considerable. Nevertheless, these differences are not so serious that the observations could not be used in calculating the activation energy and the diffusion constant D_0. Fig. 1 shows a diagram where $\frac{1}{T} \cdot 10^4$ is marked along the x-axis and $\log_{10} D$ along the y-axis. The observations can be seen in relation to the theoretical values

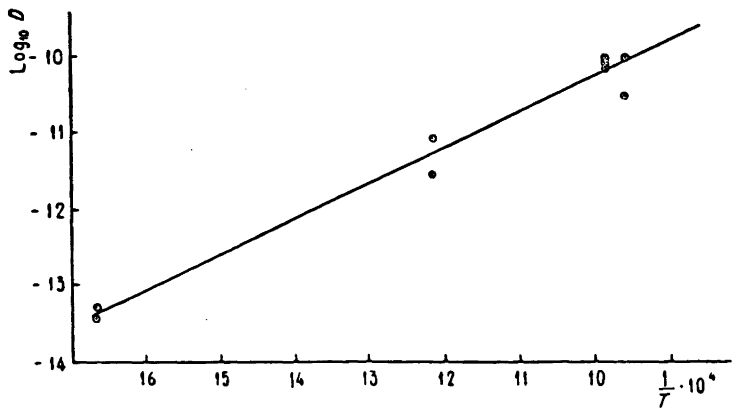

Fig. 1. Diffusion of Pb^{++} in microperthite $\perp \{001\}$.
$D = 2.5 \cdot 10^{-6} e^{-\frac{21300}{RT}}$

when $D_0 = 2.5 \cdot 10^{-6}$ cm² sec and $\omega = 21\,300$ cal. per gramion. These values are introduced in the classical formula $D = D_0 \cdot e^{-\frac{\omega}{RT}}$.

In the later experiments the diffusion coefficient is higher, and the parallel experiments show a better agreement. Therefore I find it sufficient to perform 4 good experiments, at two different temperatures.

Table 2. Diffusion of lead in albite $\perp \{001\}$.

Expt. no.	Time	T	Tracer	Count min^{-1}	Cross-section of contact area in cm²	Count cm^{-2} min^{-1}	D	
31	160h	873°	RaD	5.4	0.23	23.3	$4.68 \cdot 10^{-11}$	cm²/sec
32	160»	873°	RaD	4.2	0.21	19.9	$3.52 \cdot 10^{-11}$	cm²/sec
35	20»	1038°	RaD	30.3*	0.25	121	$3.22 \cdot 10^{-9}$	cm²/sec
36	20»	1038°	RaD	53.3	0.40	133	$3.88 \cdot 10^{-9}$	cm²/sec

Standard sample 116.4 mg RaD-glass, specific gravity 2.73. Activity 560.3 count/min. Distribution coefficient 1.15.

Fig. 2 shows a diagram where $\frac{1}{T} \cdot 10^{-4}$ is marked along the x-axis and $\log_{10} D$ along the y-axis. The observations show good agreement with the theoretical curve by introducing $D_0 = 2.5$ cm² per sec and $\omega = 43\,200$ cal per gramion.

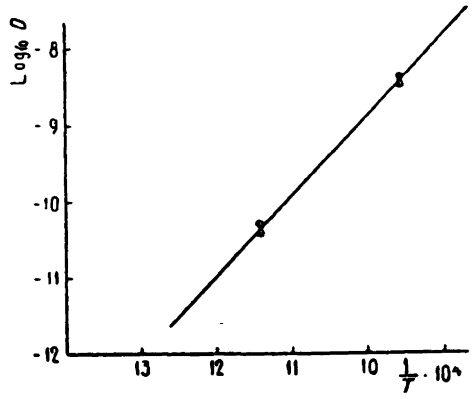

Fig. 2. Diffusion of Pb^{++} in albite $\perp \{001\}$.
$D = 2.5 \cdot e^{-\frac{43200}{RT}}$

SURFACE DIFFUSION

In order to study the possible influence of surface diffusion in poly-crystalline agregates, some experiments were tried. In these experiments a very finegrained leptite rich in feldspar was used. It proved however impossible to remove the glass drop quantitatively from this leptite without loosening parts of the leptite, and no reliable result could be obtained. For the time I therefore had to drop these experiments.

DIFFUSION ANISOTROPY

To investigate how the diffusion processes occur in different crystallographic directions in the two feldspar minerals, I measured the diffusibility of Ra^{++}. This tracer seems to be better suited for the experiment than was RaD. Lead feldspar seems to be isomorphically soluble in potash feldspar, but not in albite. Radium however, seems to be less soluble in potash feldspar, and the two feldspar minerals should therefore exhibit a more equal medium for diffusion. Because of the great radioactivity of the radium glass it was easier to get more accurate measurements in the case of radium than it was in the case of RaD.

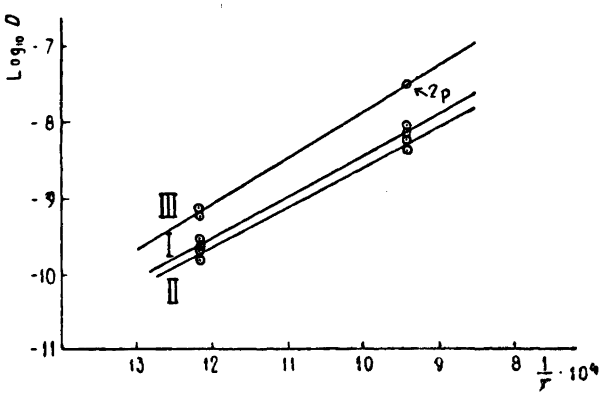

Fig. 3. Diffusion of Ra^{++} in microperthite.

$$I \perp \{001\}. \quad D = 5.1 \cdot 10^{-4} e^{-\frac{24300}{RT}}$$

$$II \perp \{010\}. \quad D = 2.4 \cdot 10^{-4} e^{-\frac{24000}{RT}}$$

$$III \neq [100]. \quad D = 2 \cdot 10^{-2} e^{-\frac{29200}{RT}}$$

Table 3. Diffusion of Ra^{++} in micropertite.

Expt. no.	Time	T	Cryst. dir.	Tracer	Count min^{-1}	Cross-section contact area in cm^2	Count cm^{-2} min^{-1}	D
41	6d	823°	$\perp \{001\}$	Ra	205.3	0.42	854	$2.53 \cdot 10^{-10}$
42	6»	823°	$\perp \{001\}$	Ra	87.3	0.12	725	$1.82 \cdot 10^{-10}$
43	6»	823°	$\perp \{010\}$	Ra	87.5	0.14	620	$1.33 \cdot 10^{-10}$
44	6»	823°	$\perp \{010\}$	Ra	235.6	0.39	820	$2.32 \cdot 10^{-10}$
45	6»	823°	$\neq [100]$	Ra	150.20	0.11	1360	$6.35 \cdot 10^{-10}$
46	6»	823°	$\neq [100]$	Ra	281.30	0.20	1420	$6.94 \cdot 10^{-10}$
47	20h	1063°	$\perp \{001\}$	Ra	177.00	0.10	1770	$8.13 \cdot 10^{-9}$
48	20»	1063°	$\perp \{001\}$	Ra	508.00	0.30	1690	$7.14 \cdot 10^{-9}$
49	20»	1063°	$\perp \{010\}$	Ra	460.00	0.35	1315	$4.32 \cdot 10^{-9}$
50	20»	1063°	$\perp \{010\}$	Ra	500.00	0.35	1430	$5.12 \cdot 10^{-9}$
51	20»	1063°	$\neq [100]$	Ra	524.00	0.15	3490	$3.04 \cdot 10^{-8}$
52	20»	1063°	$\neq [100]$	Ra	1217.00	0.35	3510	$3.08 \cdot 10^{-8}$

Standard sample 96.2 mg Ra-glass, specific gravity 2.65. Activity 560.3 count.min^{-1}. Distribution coefficient 1.045.

Fig. 3 shows the values found, represented graphically in the same way as the previous diagrams. The observations are seen in relation to the theoretical curves based on the following values:

$$\perp \{001\}\ D_0 = 5.1.10^{-4} \quad \omega = 24\,300 \text{ cal/ion}$$
$$\perp \{010\}\ D_0 = 2.4.10^{-4} \quad \omega = 24\,000 \text{ cal/ion}$$
$$\neq [100]\ D_0 = 2.0.10^{-2} \quad \omega = 28\,200 \text{ cal/ion}$$

Table 4. Diffusion of Ra^{++} in albite.

Expt. no.	Time	T	Cryst. direc.	Tracer	Count min^{-1}	Cross-section contact area in cm^2	Count cm^{-2} min^{-1}	D
55	160h	848°	$\perp \{001\}$	Ra	27.4	0.07	390	$4.23 \cdot 10^{-11}$
56	160»	848°	$\perp \{001\}$	Ra	87.6	0.22	395	$4.34 \cdot 10^{-11}$
57	160»	848°	$\perp \{010\}$	Ra	34.3	0.09	375	$3.84 \cdot 10^{-11}$
58	160»	848°	$\perp \{010\}$	Ra	34.7	0.115	310	$2.68 \cdot 10^{-11}$
59	160»	848°	$\neq [100]$	Ra	98.5	0.13	760	$1.77 \cdot 10^{-10}$
60	160»	848°	$\neq [100]$	Ra	110.0	0.10	1100	$3.66 \cdot 10^{-10}$
61	20»	1073°	$\perp \{001\}$	Ra	384.0	0.32	939	$2.60 \cdot 10^{-9}$
62	20»	1073°	$\perp \{001\}$	Ra	170.7	0.085	645	$1.22 \cdot 10^{-9}$
63	20»	1073°	$\perp \{010\}$	Ra	54.9	0.085	812	$1.95 \cdot 10^{-9}$
64	20»	1073°	$\perp \{010\}$	Ra	170.7	0.210	645	$1.22 \cdot 10^{-9}$
65	20»	1073°	$\neq [100]$	Ra	752.0	0.28	2675	$2.04 \cdot 10^{-8}$
66	20»	1073°	$\neq [100]$	Ra	404.2	0.212	1920	$1.08 \cdot 10^{-8}$

Standard sample 96.2 mg Ra-glass, specific gravity 2.65. Activity 560.3 count.min.$^{-1}$. Distribution coefficient 1.03.

Fig. 4 shows the value found, represented graphically in the same way as the previous diagrams. The observations are seen in relation to the theoretical curves based on the following values:

$$\perp \{001\}\ D_0 = 4.8.10^{-3} \quad \omega = 31\,000 \text{ cal/ion}$$
$$\perp \{010\}\ D_0 = 1.6.10^{-3} \quad \omega = 29\,800 \text{ cal/ion}$$
$$\neq [100]\ D_0 = 5.0.10^{-2} \quad \omega = 32\,200 \text{ cal/ion}$$

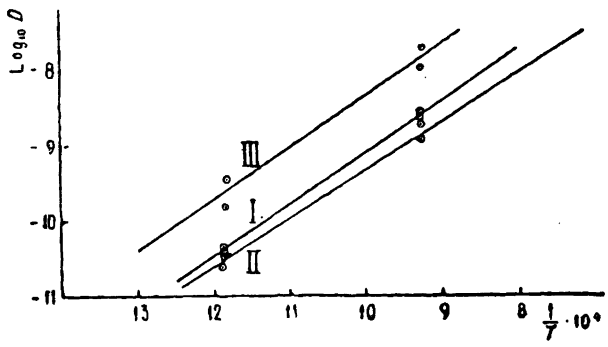

Fig. 4. Diffusion of Ra^{++} in albite.

$$I \perp \{001\}. \quad D = 4.8 \cdot 10^{-3} e^{-\frac{31000}{RT}}$$

$$II \perp \{010\}. \quad D = 1.6 \cdot 10^{-3} e^{-\frac{29800}{RT}}$$

$$III \neq [100]. \quad D = 5 \cdot 10^{-2} e^{-\frac{32200}{RT}}$$

DISCUSSION

The experiments mentioned above clearly indicate that radium is diffusing more rapidly $\neq [okl]$ than in the two other directions at the temperatures where experiments were performed. This is the case both in microcline and in albite. The activation energy, however, is greater in this direction and at a lower temperature, it seems as if the diffusion would have the same rate along the 3 crystallographic directions. In the case of albite this should be the case at the temperature of about 400° K, and in the case of microcline pertite about 475 °K. The emphirical formula $D = D_0 \, l^{-\frac{\omega}{RT}}$ gives no real information as to the mechanism of the diffusion processes. It is, however, reasonable to assume that the energy of activation must be higher by diffusion $\neq [100]$ than by diffusion $\perp \{001\}$ and $\perp \{010\}$. At the same time the possibility for a movement in the direction $\neq [100]$ must be considerably greater than in the two other directions.

Glasstone, Laidler, and Eyring [8] give an equation for diffusion in solid substances under certain conditions. According to this assumption the diffusion constant D_0 may be identified with λ_γ^2. Here λ means the free path for diffusion and γ is the vibration frequency of the considered ion in the direction of diffusion. The fact that the diffusion has the greatest velocity in

the direction where the activation energy has a maximum, seems curious. Similar conditions have, however, been found earlier by Seith [9] in his studies of selfdiffusion in bismuth. The theoretical interpretation of the phenomenon is not quite clear as yet, and in this paper I will not try to deal with this point. There is, however, another point which seems striking; namely, the fact that radium diffuses more slowly in all directions in albite than in microcline pertite at the temperature intervals investigated. At the same time the activation energy in albite is higher than in microcline pertite. In the case of lead, however, the diffusion coefficient is much smaller in microcline than in albite. The activation energy for lead in albite is, however, much higher than in microcline. It is possible that this is due to the fact that lead easily enters isomorphically into potash feldspar lattice, but not into the albite lattice.

It seems reasonable that the activation energies in microcline pertite are lower than in albite. We must from other crystal investigations expect a higher degree of irreversible disorder in microcline pertite than in albite, and even microscopical investigations show that the albite lamellae in the pertites are clear and homogenous. The microcline parts, however, often seem unclear and filled with microcrystals.

A further question is how the radioactive components are passing from the glassdrop into the crystals. We may assume an exchange of cations in the glass phase and in the crystal phase. Potasium ions may diffuse into the glass and lead ions may diffuse from the glass into the crystal. Barth [10] thinks that oxygen provides a fairly stationary medium in the silicates because of the fact that the volume of oxygen is larger than the volume of the cations. In this stationary oxygen medium he thinks that the cations diffuse. Bengtson [11] and Jagitsch [12] have, however, shown by experiments that oxygen diffuses in much the same way as the cations. It seems more likely to me that oxygen as well as metal diffuse either as metal oxide or in an ionised state from the glass phase into the crystal phase, and in the crystal phase the tracer ions and the oxygen ions diffuse independently.

SUMMARY

A method of studying diffusion processes in crystalline silicates is outlined. The diffusion coefficients of Pb and Ra in potash and sodafeldspars are measured at different temperatures. It is shown that the diffusibility depends on the crystalographic directions. The diffusion anisotropy seems to increase with increasing temperature.

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Received May 4, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 584–588

The Influence of Metallic Ions on the Viscosity of Hyaluronic Acid Solutions

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Blix and Snellman [1] mention the possibility that heavy metals cause a depolymerisation of aqueous hyaluronic acid solutions. The purpose of the present work was to prepare hyaluronic acid, which was stable with regard to viscosity in aqueous solution and to examine the influence of some metallic ions on the viscosity.

PREPARATION OF HYALURONIC ACID

Two sources have been used for the preparation: human umbilical cords and synovial fluid from cattle.

The umbilical cords which had been collected under alcohol were carefully minced, the pulp was extracted with 90 per cent actic acid according to Meyer and Palmer [2]. The bulk of the acetic acid was removed by washing with water, and the rest was neutralised to pH 7 by adding saturated potassium hydroxide. After extraction with water the hyaluronic acid was precipitated by 1 ½ volume alcohol. The precipitate was dissolved in water, precipitated by alcoholic potassium acetate (McClean [3]), washed with alcohol and ether, and dried in vacuo over phosphorus pentoxide. 3 to 20 umbilical cords were worked up at the same time. 3 umbilical cords treated in this way yielded about one half gram.

800 ml synovial fluid was filtered through gauze, and 1 per cent acetic acid was added. The precipitate was extracted by glacial acetic acid. The product was worked up as outlined above.

The preparations showed the same rather high viscosity in aqueous solutions for several days. A solution which was 118 mg % (mg per 100 grams) showed a relative viscosity of 4.93, and the same value was found after a

fortnight a room temperature. The reason may be that the iron has been effectively removed by the acetic acid. As will be shown later in the present paper iron has a definite viscosity decreasing effect on hyaluronic acid solutions even if present in very small amounts only.

It was possible to precipitate the potassium hyaluronate by 1 ½ volume alcohol directly from the aqueous extract. The treatment with acetic acid and the strong alcohol has probably denatured the protein in the mucin complex as the precipitate apparently does not contain protein. *Cf.* the relative low nitrogen per cents in Table 1.

It proved impossible to increase the yield by adding serum to the aqueous extract of umbilical cords acidified by acetic acid, as no mucin clot was formed; probably hyaluronic acid is linked only to certain proteins.

It was observed that when hyaluronic acid is precipitated by alcoholic potassium acetate, one part of the product formed is present as a stringy clot, the other as a flocculent precipitate. If the solutions are very dilute the last modification is by far the most prominent.

A very important stage in the preparation is the preliminary mincing of the starting material, which ought to be very careful in order to obtain maximal yield. One method of obtaining this is repeated chopping, another freezing by liquid air, but here we meet with the difficulties of crushing the glasslike material.

Table 1. Nitrogen content of some potassium hyaluronate preparations.

Source of hyaluronate	Nitrogen per cent
Umbilical cords	3.60
Synovial fluid	2.65
Umbilical cords frozen in liquid air	2.71

The preparations did not contain glycogen.

INFLUENCE OF METALLIC IONS ON THE VISCOSITY OF HYALURONIC ACID SOLUTIONS

For this study Ostwald viscosimeters with a capacity of 3 ml have been used, the flowtime for water varies from 12.0 to 65.4 seconds. The composition of the fluids used has been as follows: To 4 ml potassium hyaluronate the concentration of which was about 230 mg % is added 2 ml of buffer, respectively water, plus 2 ml of the metal salt, the influence of which was to be examined. In case buffer solutions have been employed their pH have been about 7 and

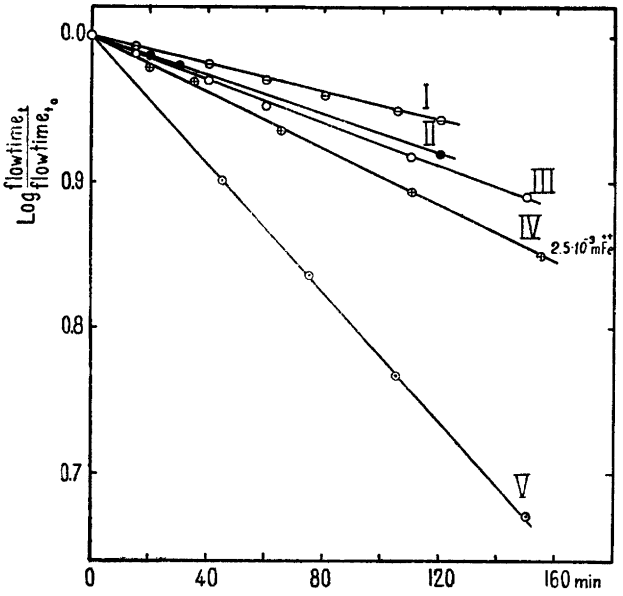

Fig. 1. The logarithm of the flowtime as a function of the time elapsed from the addition of iron; as ordinates are used the difference between the logarithm initial flowtime and the logarithm of the flowtime measured.

I. $2.5 \cdot 10^{-5}$ *M* Fe^{++}
II. $2.5 \cdot 10^{-4}$ *M* Fe^{++}
III. $5.0 \cdot 10^{-4}$ *M* Fe^{++}
IV. $2.5 \cdot 10^{-3}$ *M* Fe^{++}
V. $2.5 \cdot 10^{-2}$ *M* Fe^{++}

their molarity 0.1. If nothing else is mentioned the temperature has been 34° C in all experiments.

The following metal salts showed no measurable effect during 24 hours in 0.025 molar solution: Zinc sulfate, mercury chloride, cobalt nitrate, silver nitrate, lead nitrate, magnesium sulfate, beryllium chloride, nickel nitrate *, and chromic chloride. Experiments were also made at a molarity of 0.0025 with regard to the three last named salts. The measurements were made both in water and buffer solution.

The only cations which displayed any influence on the viscosity were iron and copper.

* At the low pH values which are necessary to keep certain salts in solution a low initial viscosity is observed.

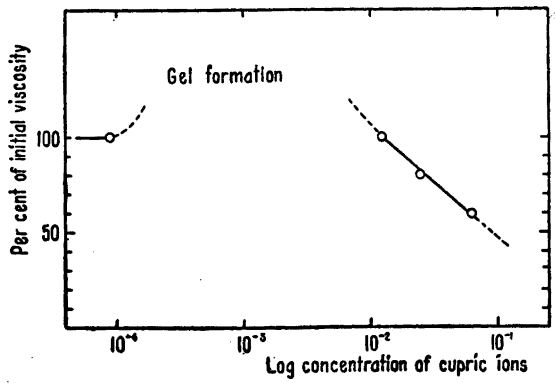

Fig. 2. The relation between viscosity and concentration of copper.

THE INFLUENCE OF IRON

Iron was found to cause a continuous decrease of the viscosity even at a concentration of about 2.5×10^{-5} molar, thus confirming the assumption of Lundquist [4]; the measurements were carried out in maleic acid buffer.

The iron salt used was ferrous sulfate. The solutions were prepared just before use in order to avoid too extensive transformation of ferrous into ferric salt, which is precipitated when present in larger amounts, but the effect of ferric ion is equal to that of ferrous ion as has been observed in case of very dilute solutions. To avoid formation of ferric precipitates in the solution 10 mg hydrazin sulfate may be added to 3 ml hyaluronate solution without influencing the results. Addition of 20 mg hydrazine sulfate to a similar hyaluronate solution causes a decrease of only about 2 per cent in the viscosity in 4 hours.

In Fig. 1 the logarithm of the flowtime is plotted against the duration of the experiment. For further details concerning this technique *cf.* Lundquist [4]. The inclination of the lines is a measure of the influence of the iron salt.

The finding of McClean and Hale [5] that ascorbic acid in very low concentration causes a depolymerisation of hyaluronic acid solutions has been confirmed. Ferricyanide does not change the viscosity of hyaluronate solutions.

THE INFLUENCE OF COPPER

The influence of cupric ions (cupric sulphate was used) on the viscosity of hyaluronate solutions depends on the concentration. Concentrations above 10^{-2} molar cause a decrease, smaller concentrations a gel formation which is detectable even at a concentration of about 10^{-4} molar.

Fig. 2 shows graphically the influence of cupric ions on hyaluronate solutions. The duration of the experiments was 3 hours. If the hyaluronate is dissolved in maleic or phtalic acid buffer the gel formation is observed at a considerably higher copper concentration. Other substances, *e. g.* several dicarboxylic acids have been applied but without effect. The reason for that may be that a complex formation takes place between maleic, respectively phthalic acid and copper thus causing a decrease in the concentration of cupric ions. When a 0.5 molar maleate solution is mixed with cupric sulfate solution a dark blue complex crystallizes on cooling. Photometric measurements indicate that at most one third of the copper is present in ionic form under the experimental conditions employed. It may be mentioned that the gel formation was more prominent after 4 than after 24 hours. Vigorous shaking apparently destroys the gel. Attempts were made to regenerate the hyaluronate from the gel by addition of alcoholic potassium acetate, the product evidently contained copper and displayed a gel-like appearance when treated with water.

It is of some interest to notice that the hyaluronic acid is found in the vitreous humor in a gelatinised form much resembling that produced by the influence of copper ions on potassium hyaluronate solutions.

SUMMARY

Potassium hyaluronate has been prepared which appears to be stable in aqueous solutions.

The influence of some cations on the viscosity has been examined.

Ferrous and ferric ions have been found to cause a decrease in the viscosity of aqueous hyaluronate solutions.

Small cupric ion concentrations cause a gel formation, concentrations above 10^{-2} molar a decrease in the viscosity.

The author is indebted to Dr. Lundquist, chief of the biochemical department, for his great interest in the work, and for his valuable aid without which this investigation had not been possible.

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Received May 9, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 589–594

Notes on the Fractionation and Colorimetric Assay of Commercial Heparin

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The barium salt of heparin was fractionated by means of precipitation with acetone into two different sodium salts as reported by Kuizenga and Spaulding [1]. By means of electrophoresis the present authors separated commercial heparin into two distinctly different fractions, both of which exerted anticoagulant and metachromatic activities [2]. Continued fractionation studies have been performed with a view to gain more insight into the anticoagulant and metachromatic capacities of different fractions obtained, and this forms the subject of the present paper. When comparing the anticoagulant and metachromatic activities of heparin preparations or fractions of purified heparin it should be recalled that these activities are segragated and cannot be ascribed to the same group of the heparin molecule; the anticoagulant effect may be due to a central nitrogen bond [3], the electric charge of the molecule [4], or some other characteristic. The metachromatic reaction is on the other hand referable to the ester sulphate groups of heparin [5]. The segragation mentioned above was studied in previous recrystallization experiments [2]. Additional data on the inhomogeneity of purified heparin with reference to the varying degree of esterification may be obtained from recent report by Jorpes and Gardell [6].

EXPERIMENTAL

Serial precipitation experiments were performed on commercial heparin (Vitrum, Sweden) using the organic precipitants acetone, alcohol, and dioxane. Increasing amounts of the precipitants were added to a row of glass tubes, each containing 1 ml of a 1 per cent heparin solution in 0.9 per cent sodium chloride. The concentration increment of the precipitants between two sub-

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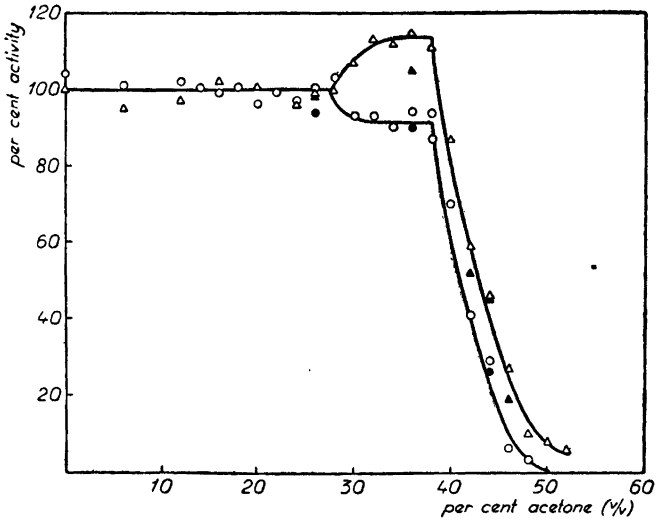

Figure 1. Precipitation diagram of heparin Vitrum (lot F 1946) using acetone as precipitant.

○ *anticoagulatiang activity of the supernatant.*

△ *metachromatic activity of the supernatant.*

solid points: anticoagulant- and metachromatic activity of the samples used for electrophoresis experiments (see Table 1).

sequent tubes was 2 per cent. The precipitants were carefully pipetted into the tubes, which were then gently shaken and immediately corked to avoid evaporation. To prevent contamination with the cork a small piece of soft tin foil was wrapped around the latter, and this thightening proved to be effective. The tubes were allowed to stand in a water thermostat at 20° C until the precipitate had collected. From every tube samples of 0.100 ml. of the supernatant liquid were transferred to a row of 10 ml. volumetric flasks; the organic precipitate was removed by suction in vacuum, and the flasks were then filled with 0.9 per cent sodium chloride solution. This series of solutions was used for the determination of anticoagulant activity by means of the thrombin method of Jaques and Charles [7], and for the assay of metachromatic reaction by the method of MacIntosh [8] using toluidine blue (Grübler, equivalent to Asure A). All experiments were performed at pH 7.

The anticoagulant activity found in the supernatants was expressed in per cent of the calculated activity which would have been found provided no precipitation had occurred, and these values were plotted as ordinates. The corresponding concentrations of the precipitants used in the tubes were

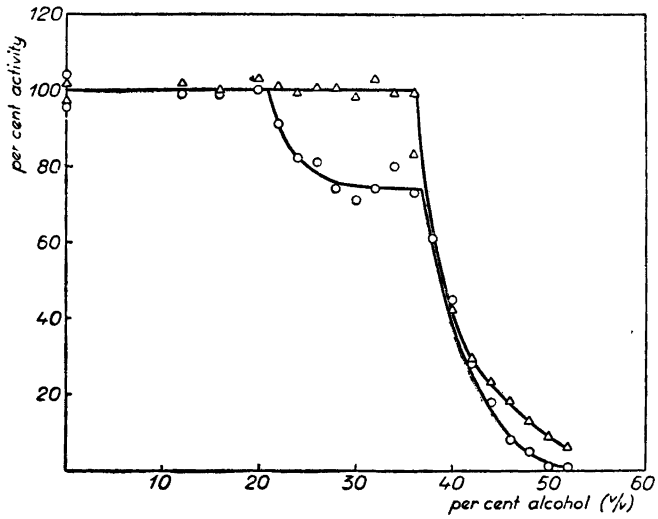

Figure 2. Precipitation diagram of heparin Vitrum (lot F 1946) using alcohol as precipitant.

○ *anticoagulant activity of the supernatant.*

△ *metachromatic activity of the supernatant.*

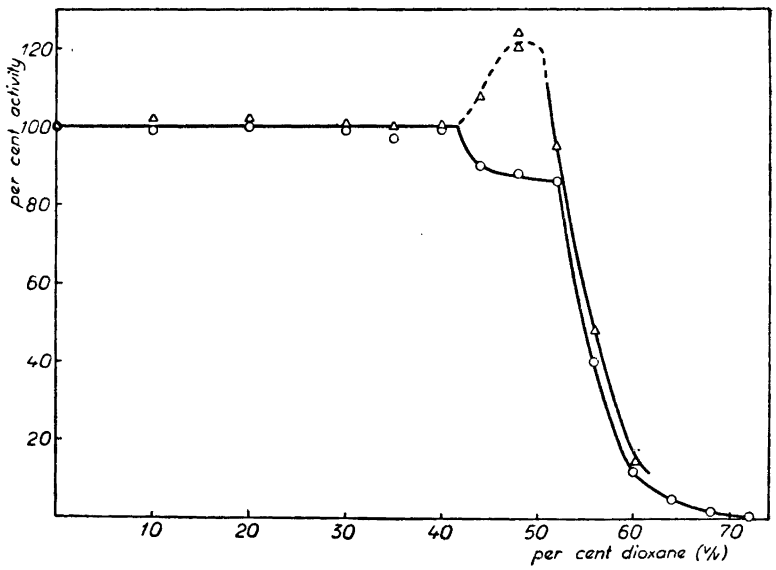

Figure 3. Precipitation diagram of heparin Vitrum (lot VII : 44) using dioxane as precipitant.

○ *anticoagulant activity of the supernatant.*

△ *metachromatic activity of the supernatant.*

plotted along the abscissa, and in this way precipitation diagrams were obtained for acetone, alcohol, and dioxane as precipitants (Figs. 1—3).

All diagrams were similar in type and evidence the inhomogeneity of commercial heparin. Partial precipitation of the active material was found to appear after the addition of 20—40 per cent of alcohol and acetone, and after 40—50 per cent of dioxane, and this was followed by a decrease in anticoagulant activity of about 10—20 per cent. Following the addition of larger volumes of the precipitants, the actual precipitation of active material rapidly took place. The values obtained by means of the metachromatic assay for heparin showed a consistent divergence from those just mentioned, but only during the first phase of partial precipitation (Figs. 1—3).

In order to elucidate whether the first drop in anticoagulant activity could be ascribed to the precipitation of a singel one of the heparin components found during electrophoresis (2, α and β), further precipitation experiments were performed on larger samples of heparin *(Vitrum)* together with additional electrophoresis investigations. As previously described, serial precipitation was performed with acetone but at larger concentration invervals. From each tube 20 ml. samples were taken of the supernatant liquid, freed from acetone by suction in vacuum, and then dialyzed against a phosphate buffer at pH 6.8, and finally subjected to electrophoresis. Smaller samples were used for control determinations of the anticoagulant and metachromatic activities of the supernatants. The latter values were plotted in Fig. 1 (solid points) and corroborate the previous ones.

The results obtained from electrophoresis experiments (Table 1) indicate that the reciprocal concentration of the two main components α and β (2) contained in the supernates is not significantly changed in the course of the precipitation, nor are the concentration values of the components differing from those of the original material.

Table 1. Relative concentrations of heparin components contained in the supernates during precipitation with acetone.

Amount of acetone in per cent		0	38	42	44	46
Relative concentration of heparin components .... in per cent	α	66	72	60	72	73
	β	34	28	40	28	27

These experiments were repeated using alcohol as precipitant. Following the precipitation at an alcohol concentration of 36 per cent, the supernatant

liquid was found to contain 66 per cent of α and 34 per cent of β, and the precipitate obtained held 70 per cent of α and 30 per cent of β. The electrophoresis experiments thus indicate that the first drop in anticoagulant activity reported in the precipitation curves (Figs. 1—3), could not be explained by the precipitation of a single one of the heparin components α or β.

DISCUSSION

Present attempts to separate commercial heparin by means of gentle precipitation into two components corresponding to those previously found [2] in electrophoresis experiments have not been successful. On the contrary, both components were found to be precipitated concurrently, and further in a characteristic two-step fashion. No reasonable explanation of this fact can so far be advanced. Another precipitant acting more smoothly might achieve such a segragation of the actual components *Cf.* [6].

However, the results are of definite interest in other respects. Following the gradual addition of acetone (Fig. 1) and dioxane (Fig. 3) an increase in metachromatic capacity was found in the supernates parallel to the first drop in anticoagulant activity. Later on, during rapid precipitation of the remaining solute both activities are decreasing at the same rates. Available data do not justify an interpretation of this interesting segragation between the anticoagulant and metachromatic capacities of the material contained in the supernates. Several possibilities have to be considered. Commerical heparin is known to be inhomogeneous and impurities may occur, which might be able to obscure the metachromatic reaction. With reference to the last mentioned possibility the following experiments were made.

In two points of the dioxane precipitation diagram (Fig. 3) corresponding to 48 and 52 per cent dioxane content, the amount of the precipitate obtained was roughly estimated by drying an aliquote amount of the supernatant, and found to agree with the observed drop in anticoagulant activity calculated as heparin. This means that a possible admixture could only constitute a smallish fraction of the original substance, which in that case would explain why such an admixture was not observed in previous electrophoresis experiments [2].

In addition, when heparin dialysed for a week was tested according to the method of MacIntosh [8] the anomalous increase in metachromatic activity had disappeared. This might indicate that some impurity was removed by dialysis.

For lack of sufficient information of the mechanism and kinetics of the metachromatic staining reaction further discussion will be postponed. Difficulties evidently exist for the assay of heparin by means of the metachromatic reaction [5] as well as by using more indirect methods of determination [9].

SUMMARY

Serial precipitation experiments on commercial heparin (Vitrum) are reported using the organic precipitants acetone, alcohol, and dioxane. A similar segragation of heparin into two components as demonstrated in previous electrophoresis experiments was not obtained. Both components were found to be precipitated concurrently in a two-step fashion. During the first phase of precipitation a remarkable discrepancy was observed between the anticoagulant and metachromatic activity of the remaining solute material. This may be due to a variety of factors, and some attention was paid to the possible precipitation of some impurity, which might hamper the metachromatic reaction. This unexplained phenomenon constitutes a source of error in colorimetric assays of heparin.

We wish to express our thanks to the Consul General, Axel Ax:son Johnson, Stockholm, for financial support.

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Received May 17, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 595–602

The Binary System Chromium-Boron

I. Phase Analysis and Structure of the ζ- and Θ-phases

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This investigation is part of a study on binary alloys between transition elements and boron, carried out at this institute. A phase analysis of the system chromium-boron and structure determinations of two of the intermediary phases have been made. An attempt to determine the structures of the remaining phases is presently carried out, and the result will appear in a later communication.

Few investigations on the chromium-boron system have been reported. According to older works [1–5] borides of composition Cr_3B_2 and CrB exist. At the moment of publication of this work, a paper »Properties of Chromium Boride and Sintered Chromium Boride» by S. J. Sindeband [6] appeared. Some X-ray data on the boride CrB are mentioned, which are in accordance with those obtained for the ζ-phase. The conclusion, that only one phase exists in the range 12—20 weight % boron (39.5—54.5 atomic %) seems to be wrong, perhaps resulting from the unsatisfactory method for preparing the alloys.

GENERAL METHODS

Starting materials were chromium, deposited electrolytically, and with a purity of 99.4 % and boron with a purity of 98—99 % prepared by reduction of boron tribromide with hydrogen [7]. The alloys were prepared in two different ways. The first method was to heat and melt mixtures of chromium and boron in a high frequency vacuum induction furnace at about 1600° C. The second method was to sinter weighed mixtures of chromium and boron in evacuated silica tubes, usually for 48—72 hours at 1150° C. Single crystals were obtained, however, from mixtures sintered for about 20 days at 1150° C.

Selected alloys were dissolved in perchloric acid or fused with sodium peroxide or nitrate-carbonate mixture. In the solutions chromium and boron were determined by the usual methods.

The phase analysis was carried out by X-ray powder methods as was the structure determination of the Θ-phase. The structure of the ζ-phase was determined, using single crystal methods. The determination was based on Weissenberg photographs taken with Mo-K radiation. The estimation of the intensities was carried out visually, starting from 4 : 2 : 1 as relative values of the intensities of Mo-$K\alpha_1$, Mo-$K\alpha_2$ and Mo-$K\beta$ radiation. Correlation between the intensity scales of different Weissenberg photographs was obtained according to the Weissenberg oscillation method, described by Magnéli [8]. Relative values of F^2_{hkl} were obtained by multiplying the intensity values with the factor $\cos^2\mu \times \sin \Upsilon \times (1 + \cos^2 2\Theta)$ utilizing the curves given by Chia-si Lu [9]. The temperature factor was neglected.

The calculations of Patterson cuts an electron density function projections were carried out by means of the electric machine for the summation of Fourier series, constructed by Hägg and Laurent [10].

GENERAL SURVEY OF THE SYSTEM

Five intermediary phases, δ, ε, ζ, η and Θ were found, all of which had metallic properties. The hardness was remarkable and all the phases seemed to be rather resistant against chemical attack. Of the more common acids, only perchloric acid was able to dissolve them.

The solubility of boron in the chromium lattice was very low, as no displacements of the interferences of the chromium lattice were observed as the boron content increased.

The δ-phase was found to have a probable content of 33 atomic % boron. It crystallized in very thin hexagonal plates.

The ε-phase had a content of 40 atomic % boron.

The ζ-phase was found with a content of 50 atomic % boron. The homogeneity range was narrow and the crystal structure of this phase will be discussed below. It crystallized in needles or rods with a square transverse section.

The η-phase had a content of about 55 atomic % boron, and the crystals were similar to those of the ζ-phase.

The Θ-phase had the composition CrB_2 and crystallized in hexagonal plates. The structure of this phase will be discussed below.

THE ζ-PHASE

This phase appeared pure in powder photographs of alloys with boron content of 50 atomic %. The homogeneity range was narrow, as no displacements of the interferences of the ζ-phase were observed in alloys of different boron content in the range between the ε- and η-phases. A well defined single crystal was investigated. Laue photographs showed the Laue symmetry to be D_{2h}-*mmm*. Rotation and Weissenberg photographs were taken around [0 0 1], the needle axis, using Cu-K and Mo-K radiation. Accurate cell dimensions were obtained from powder photographs, giving the axes of the orthorhombic cell:

$$a = 2.969 \text{ Å}, \quad b = 7.858 \text{ Å}, \quad c = 2.932 \text{ Å}.$$

The volume of the unit cell is $V = 68.40$ Å^3. The density found was 6.05, corresponding to a cell content of 4 CrB (calculated density 6.11).

Reflections $h\ k\ l$ were observed only for $h + k = 2n$, $0\ k\ l$ for $k = 2n$, $h\ 0\ l$ only for $h = 2n$ and $l = 2n$ and $h\ k\ 0$ for $h + k = 2n$. Probable space groups are thus D_{2h}^{17}-*Cmcm*, C_{2v}^{12}-*Cmc* and C_{2v}^{16}-*Ama*.

Chromium positions. From space considerations, the only possible positions for the four metal atoms are: *

in C_{2v}^{12}-*Cmc* the fourfold position 4 : (*a*)
in C_{2v}^{16}-*Ama* the fourfold position 4 : (*b*)
in D_{2h}^{17}-*Cmcm* the fourfold position 4 : (*c*).

The investigation was started by examining wether the structure was consistent with 4 : (*c*) in D_{2h}^{17}, having the highest symmetry. (The axes, given above, haven been chosen so, that the International tables may be used directly for D_{2h}^{17}-*Cmcm*.)

$$4 : (c)\ 0\ y\ 1/4;\ 0\ \bar{y}\ 3/4;\ 1/2\ 1/2 + y\ 1/4;\ 1/2\ 1/2 - y\ 3/4.$$

In order to determine the parameter y, the Patterson-Harker method was used. The space group contains a glide plane perpendicular to the b-axis and thus the section P(0 v 1/2) in the Patterson space was investigated. The only strong maximum appeared at $v = 0.292$. This corresponds to a value of the parameter y in 4 : (*c*) of $v/2 = 0.146$. With this parameter value, the metal

* Notations according to *International tables for the determination of crystal structures*, Berlin 1935.

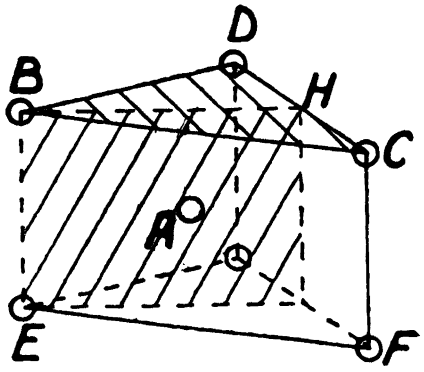

Fig. 1. Trigonal prism of metal atoms in the ζ-phase.

atoms form a lattice, built up of trigonal prisms (Fig. 1) in a manner indicated by Fig. 2. Every metal atom is surrounded by six nearest neighbours, lying at the corners of the trigonal prism, four at distances A—F = 2.65 Å and two at distances A—E = 2.72 Å.

Boron positions. The boron atoms must be situated in holes of the metal lattice. The only holes large enough are situated at the centre of a trigonal prism of metal atoms. These holes are connected to channels, running parallel to the *c*-axis, and their centres occupy the position 4 : (*c*) in D_{2h}^{17} with a value of the parameter $y = 1/2 - c^2/16\ b^2\ y_{Me} = 0.440$. A boron atom placed in such a hole

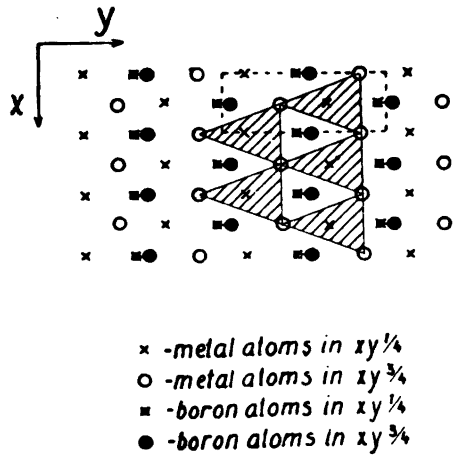

Fig. 2 a. The lattice of the ζ-phase, projected on (001).

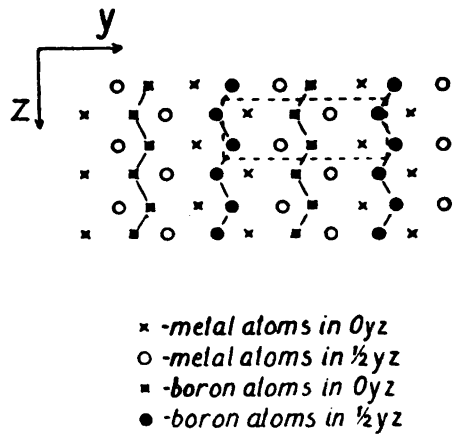

Fig. 2 b. The lattice of the ζ-phase, projected on (100).

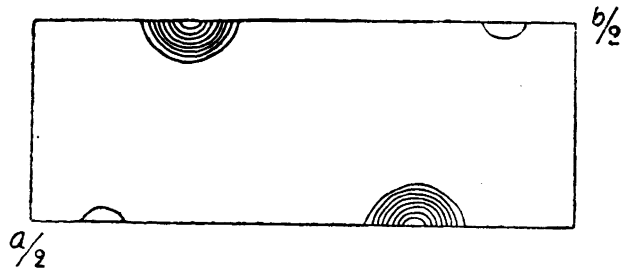

Fig. 3. Projection of the electron density of the ζ-phase on (001).

would be surrounded by six chromium atoms at a distance of 2.19 Å. The boron atoms would be connected to zig-zag chains, running parallel to the c-axis and with a distance boron-boron of 0.86 Å. This value is in close agreement with those previously observed. A comparison between observed and calculated $|F|$ values assuming the positions above for the chromium and boron atoms, is given in Table 1. The agreement is satisfactory. In Fig. 2 the structure is projected on (0 0 1) and (1 0 0). A further support for the positions of the boron atoms is given by the projection of the electronic density on (0 0 1) (Fig. 3). As the position of the heavy chromium was determined from the Patterson cut, the signs of the observed $|F|$ values could be calculated for all reflections. The value of the parameter for the boron atoms, obtained from this projection, was 0.437. The error of this parameter value, however is rather large because of the limited number of reflections and the comparatively small boron maximum. For this reason, the value 0.440 is considered to be more correct. It is of interest to note that the boron chains are parallel to the needle axis of the single crystals.

The ζ-phase, CrB, thus crystallizes in space group D_{2h}^{17}-$Cmcm$ with the metal as well as the boron atoms in:

$$4:(c)\ 0\ y\ 1/4;\ 0\ \bar{y}\ 3/4;\ 1/2\ 1/2+y\ 1/4;\ 1/2\ 1/2-y\ 3/4.$$

The values of the parameters are $y_{\mathrm{Me}} = 0.146$ and $y_{\mathrm{B}} = 0.440$.

This structure may be compared with the δ-phases in the molybdenum and tungsten-boron systems (ideal composition MeB)[11]. The metal atoms of the latter phases have the same coordination as the ζ-phase in the chromium-boron system. The prisms of metal atoms, however, are connected in a different way, giving channels in two directions at right angles. The characteristic zig-zag chains of boron atoms also appear, now running in two directions.

Table 1. ζ-phase. Weissenberg photographs with Mo-K radiation. Comparison between observed and calculated $|F|$ values.

h k l	$\|F\|$ obs.	$\|F\|$ calc.	h k l	$\|F\|$ obs.	$\|F\|$ calc.	h k l	$\|F\|$ obs.	$\|F\|$ calc.
2 0 0	60	62	0 2 1	48	61	0 6 2	24	26
4 0 0	41	38	0 4 1	28	36	0 8 2	—	13
6 0 0	31	27	0 6 1	34	40	0 10 2	32	35
0 4 0	52	55	0 8 1	35	34	0 12 2	—	2
0 6 0	29	31	0 10 1	—	11	0 14 2	29	24
0 8 0	21	17	0 12 1	—	2	1 1 2	28	23
0 10 0	39	39	0 14 1	—	2	1 3 2	46	46
0 12 0	—	2	1 1 1	53	54	1 5 2	—	7
0 14 0	31	32	1 3 1	28	29	1 7 2	48	42
1 1 0	36	43	1 5 1	40	41	1 9 2	—	10
1 3 0	62	64	1 7 1	—	9	1 11 2	16	20
1 5 0	—	8	1 9 1	27	33	1 13 2	17	20
1 7 0	45	50	1 11 1	20	23	2 0 2	56	50
1 9 0	—	9	1 13 1	—	20	2 2 2	—	6
1 11 0	27	22	2 2 1	56	42	2 4 2	43	35
1 13 0	21	21	2 4 1	31	29	2 6 2	18	22
2 2 0	—	7	2 6 1	35	34	2 8 2	—	12
2 4 0	42	41	2 8 1	23	30	2 10 2	36	32
2 6 0	31	27	2 10 1	—	10	2 12 2	—	2
2 8 0	—	13	2 12 1	—	2	2 14 2	16	24
2 10 0	38	35	2 14 1	—	10	3 1 2	19	26
2 12 0	—	2	3 1 1	40	34	3 3 2	42	34
2 14 0	22	25	3 3 1	21	20	3 5 2	—	6
3 1 0	24	20	3 5 1	31	30	3 7 2	34	34
3 3 0	49	40	3 7 1	—	7	3 9 2	—	6
3 5 0	—	6	3 9 1	26	28	3 11 2	—	16
3 7 0	41	37	3 11 1	—	19	4 0 2	48	34
3 9 0	—	7	4 2 1	29	30	4 2 2	—	4
3 11 0	—	18	4 4 1	20	20	4 4 2	23	25
4 2 0	—	4	4 6 1	23	24	4 6 2	16	16
4 4 0	30	27	4 8 1	28	22	4 8 2	—	8
4 6 0	20	18	4 10 1	—	8	4 10 2	22	26
4 8 0	—	9	5 1 1	21	22	5 1 2	—	11
4 10 0	31	28	5 3 1	—	14	5 3 2	25	22
5 1 0	—	12	5 5 1	20	20	5 5 2	—	4
5 3 0	31	27	5 7 1	—	5	5 7 2	18	26
5 5 0	—	4	6 2 1	—	20	6 0 2	22	26
5 7 0	31	27	6 4 1	—	15	6 2 2	—	2
6 2 0	—	2	0 2 2	—	8	6 4 2	16	18
6 4 0	22	19	0 4 2	38	41	0 2 3	40	36

Table 1 (cont.).

h k l	|F| obs.	|F| calc.	h k l	|F| obs.	|F| calc.	h k l	|F| obs.	|F| calc.
0 4 3	18	24	1 13 3	—	18	3 9 3	21	25
0 6 3	30	29	2 2 3	30	31	3 11 3	—	17
0 8 3	18	26	2 4 3	22	22	4 2 3	21	23
0 10 3	—	9	2 6 3	26	26	4 4 3	—	8
0 12 3	—	2	2 8 3	20	24	4 6 3	—	21
0 14 3	—	10	2 10 3	—	8	4 8 3	—	19
1 1 3	34	34	2 12 3	—	2	4 10 3	—	8
1 3 3	18	20	2 14 3	—	9	5 1 3	—	20
1 5 3	30	30	3 1 3	25	26	5 3 3	—	12
1 7 3	—	8	3 3 3	18	16	5 5 3	—	18
1 9 3	20	27	3 5 3	23	24	5 7 3	—	5
1 11 3	—	19	3 7 3	—	6			

THE Θ-PHASE

This phase was obtained at a boron content of 66.7 atomic %. As for the ζ-phase the homogeneity range of this phase was narrow. Powder photographs gave a hexagonal cell with unit dimensions:

$$a = 2.969 \text{ Å}, \quad c = 3.066 \text{ Å}, \quad c/a = 1.03.$$

The agreement between observed and calculated $p\,|F|^2$ values is satisfactory, assuming the metal atoms form a simple hexagonal lattice. If they are placed in 0 0 0, the only place for two boron atoms per cell will be in 1/3 2/3 1/2; 2/3 1/3 1/2. These positions are compatible with space group D^1_{6h}—$C\ 6/mmm$ and the boride thus is isomorphous to AlB_2[12] and ZrB_2[13] (C 32 type). The boron atoms form a plane hexagonal net, similar to that of the carbon atoms in graphite. The distance boron-boron in the same net will be $a\sqrt{3}/3 = 1.72$ Å, giving a radius of the boron atom of 0.86 Å (assuming the atoms to be spherical and in contact).

SUMMARY

The chromium-boron system has been investigated by X-ray methods. Five intermediary phases with contents of boron of respectively about 33 (δ), 40 (ε), 50 (ζ), 55 (η) and 66.7 (Θ) atomic % have been found to exist

Complete structure determinations of the orthorhombic ζ-phase and the hexagonal Θ-phase have been carried out. In the ζ-phase, the boron atoms form parallel chains running through the metal lattice and the structure is related to the δ-phases of the molybdenum- and tungsten-boron systems. The Θ-phase is of C 32 type and the boron atoms form hexagonal nets.

The author wishes to thank Professor G. Hägg for his continued interest in this investigation and Mr. Lars-Henrik Andersson and Mr. Arne Strömberg for their valuable assistance with preparations, analyses and calculations. The *Statens Tekniska Forskningsråd* has supported the research financially, which support is gratefully acknowledged.

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Received May 5, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 603–615

The Borides of Tantalum

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This investigation is part of a study on binary alloys between transition elements and boron. The system tantalum-boron is of special interest as its ratio r_B/r_{Me} is very close to the critical value 0.59, given by Hägg [1, 2].

Only one boride of composition TaB_2 has been mentioned before [3, 4].

GENERAL METHODS

Starting materials were tantalum (Fansteel Met. Corp., North Chicago) and boron, prepared by the reduction of boron tribromide with hydrogen and with a purity of 98—99 % [5]. The alloys were prepared in two ways. Part of the alloys were prepared by sintering mixtures of tantalum and boron in a high frequency vacuum induction furnace at 1800—1900° C for about half an hour. The other method was to heat mixtures in evacuated silica tubes for 100—150 hours at about 1150° C. Selected alloys were analyzed, according to the method, given by Andrieux [3]. It was not possible to melt the alloys. Some experiments on the solubility of boron in tantalum at higher temperatures were made, using quenched specimens. The silica tubes were quenched in water and crushed at the moment of immersion.

The system was investigated by X-ray powder methods. Observed and calculated $p|F|^2$ values were obtained by the method described in a previous publication [6]. The indexing of the two orthorhombic intermediary phases (γ, δ) exclusively from powder data was possible because of the fact that one of them (γ) was found to be isomorphous with a phase in the chromium-boron system, previously indexed by single crystal methods, and the other (δ) had two axes of about the same length as corresponding axes of the γ-phase. The hexagonal ε-phase was indexed by fil. kand. Rolf Hesse, using his method for indexing of powder photographs [7].

All intensity calculations are based solely on the metal lattice, the scattering power of the boron atoms, compared with that of the tantalum atoms, being too low, to be effective. The position of the boron atoms was determined from space considerations.

GENERAL SURVEY OF THE SYSTEM

The system contains four intermediary phases, β, γ, δ and ε, all of which have metallic properties.

The solubility of boron in the body-centred tantalum lattice (α) is low at room temperature, but at higher temperatures the range of solubility is extended.

The β-phase seems to be stable only at lower temperatures. The composition of the phase is impossible to determine because of the slow reaction rate. It always appears together with α-phase.

The boron content of the γ-phase was 50 atomic % and of the δ-phase 57 atomic %. The ε-phase, finally, exists in a homogeneity range of about 64—72 atomic % with the ideal composition TaB_2.

THE α-PHASE

It was found, that the body-centred tantalum lattice could take up small amounts of boron. The amount increased with temperature. An alloy with a boron content of 10 atomic % was quenched at different temperatures and the value of the parameter, which for pure tantalum is 3.303 Å* was increased to 3.309 Å for specimens quenched at 950° C, 3.313 Å at 1170° and 3.321 at 1270°. An attempt to determine the α-phase boundary gave results, which were not reproducible. This probably results from the slow reaction rate and the small variation of the parameter.

THE β-PHASE

When the boron content of the alloys increased, lines of a new phase, the β-phase, appeared. It was not possible to obtain this phase in a pure state. For boron content up to about 14 atomic % the interferences of the β-phase always occurred together with those of the α-phase, even if the alloys were prepared at 1800—1900° C. For higher boron content, lines of the γ-phase appeared and the powder photographs now showed interferences of the three

* True Ångström units = 10^{-8} cm.

Table 1. Ta-B, β-phase, Cr-K radiation.

h k l	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	I obs.	$p\|F\|^2$ obs.*	$p\|F\|^2$ calc. $x=1/6$	h k l	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	I obs.	$p\|F\|^2$ obs.*	$p\|F\|^2$ calc. $x=1/6$
1 1 0	0786	0785	m	2	7	3 1 2	6141	6141	m	45	62
2 0 0	1573	1570	st	21	24	4 0 0	6281	6280	w−	14	15
0 0 2	2215	2216	st	37	45	2 1 3	6948	6948	m	98	131
2 1 1	2515	2516	st +	160	195	3 3 0	7063	7065	m	49	57
1 1 2	3001	3001	w	8	10	4 1 1	7225	7226	m	98	128
2 2 0	3135	3140	w−	6	5	4 2 0	7851	7850	w−	9	7
2 0 2	3786	3786	m	41	38	4 0 2	8492	8496	w +	30	26
3 1 0	3920	3925	m	44	37	0 0 4	8865	8864	w +	26	25
2 2 2	5355	5356	w−	7	8	3 3 2	9275	9281	st	79	99
3 2 1	—	5656	—	0	0	1 1 4	9661	9649	w	9	6

phases $\alpha + \beta + \gamma$. As the boron content increased, the system $\alpha + \beta$ gradually disappeared. The confusing results from the phase analysis may be explained, if the β-phase is supposed to exist only below a certain temperature and if the reaction rate below this temperature is too slow to permit complete equilibrium to be established.

The interpretation of the interferences of the β-phase showed the phase to have tetragonal symmetry, the axes of the elementary cell being:

$$a = 5.778 \text{ Å}, \; c = 4.864 \text{ Å}, \; c/a = 0.842, \; V = 162.4 \text{ Å}^3$$

The value of the length of the axes indicated an isomorphy between the tantalum lattice of this phase and the metal lattices of the borides of the $CuAl_2$-type (*C* 16), Fe_2B, Co_2B, Ni_2B, Mo_2B and W_2B[6, 8–10]. This was verified by further structure analysis.

Reflections *h k l* were observed only for $h + k + l = 2n$, *h k* 0 for $h + k = 2n$, *h h l* for $l = 2n$ and 0 *k l* for $k = 2n$ and $l = 2n$, showing that space group D_{4h}^{18}—*I*4/*mcm* is possible. The structure analysis showed that the tantalum lattice of the phase corresponds to the position of 8 : (*h*) in D_{4h}^{18}. The agreement between observed and calculated $p|F|^2$ values was good with a value of the parameter $x = 1/6$ (Table 1). The metal lattice of the phase thus is isomorphous with the metal lattices of the Me_2B borides mentioned. Each tantalum atom is surrounded by one metal neighbour at 2.72 Å, two at 2.79 Å, four at 3.05 Å and four at 3.10 Å. There are four holes per cell, with

* Referred to α_1 for all lines.

the position 4 : (*a*), which have room for boron atoms. They have place for spherical atoms with a radius less than 1.11 Å and the radius of the boron atom is about 0.86 Å. The isomorphy between the metal lattice of this phase and the metal lattices of the Me_2B-phases suggests that the real composition of the phase should be Ta_2B. The fact that it was impossible to obtain the β-phase pure at this composition may depend on the difficulty to get equilibrium at temperatures, where the β-phase is stable.

THE γ-PHASE

This phase was found homogenous in preparations with a boron content of 50 atomic %. The homogeneity range was narrow, and an inspection of the powder photographs indicated an isomorphy between this phase and the ζ-phase in the chromium-boron system, the structure of which has been determined previously [11]. This was confirmed by further structure analysis. The powder photographs could be indexed from an orthorhombic cell with axes:

$$a = 3.276,\ b = 8.669,\ c = 3.157,\ V = 89.66\ \text{Å}^3.$$

A cell content of 4 TaB per cell would give a calculated density of 14.29, in close agreement with the density found, 14.0.

Reflections $h\ k\ l$ were observed only for $h + k = 2n$, $0\ k\ l$ for $k = 2n$, $h\ 0\ l$ only for $h = 2n$ and $l = 2n$ and $h\ k\ 0$ for $h + k = 2n$. Probable space groups are thus D_{2h}^{17}-*Cmcm*, C_{2v}^{12}-*Cmc*, and C_{2v}^{16}-*Ama*. (The axes have been chosen so that the International tables may be used directly for D_{2h}^{17}.) The further structure analysis showed the phase to be isomorphous with the ζ-phase in the chromium-boron system. The tantalum atoms are thus in 4 : (*c*) of D_{2h}^{17}-*Cmcm* and in Table 2 a comparison between observed and calculated $p|F|^2$ values is given, assuming the parameter $y = 0.146$ (the same value as for the ζ-phase in the chromium-boron system). The agreement is acceptable, except for the reflection 2 0 0, which is too weak compared with the calculated value. The disagreement may be due to some orientation effect. The lattice is fully described elsewhere [11]. Each tantalum atom is surrounded by four metal neighbours at 2.90 Å, two at 2.98 Å, two at 3.16 Å and two at 3.28 Å. The boron atoms occupy the same position 4 : (*c*) with a value of the parameter $y = 0.440$. The distance tantalum-boron is 2.40 Å and boron-boron 1.91 Å. The boron atoms form zig-zag shaped chains, all running parallel to the *c*-axis.

Table 2. Ta-B, γ-phase, Cu-K radiation.

h k l	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	I obs.	$p\|F\|^2$ obs.*	$p\|F\|^2$ calc. $z = 0.146$	h k l	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	I obs.	$p\|F\|^2$ obs.*	$p\|F\|^2$ calc. $z = 0.146$
0 2 1	0908	0910	st	10	21	0 6 2	5224	5215	w—	3	5
1 1 1	1225	1227	st	12	26	2 6 1		5642			10
0 4 0	1259	1261	st	12	8	0 8 1	5642	5640	m	36	8
1 3 0		1262			18	3 1 1		5648			13
0 4 1	1855	1856	w +	8	5	0 2 3	5667	5666	m	18	10
1 3 1		1857			5	3 3 0		5682			9
2 0 0	2210	2209	w—	1	9	2 4 2	5859	5848	w	14	15
0 0 2	2378	2378	w +	8	8	1 1 3	5989	5983	w	14	13
1 5 0	—	2523	—	0	0	3 3 1	6295	6278	w—	4	3
2 2 0	—	2524	—	0	1	0 4 3	6619	6612	w	8	2
0 2 2	—	2693	—	0	1	1 3 3		6613			3
0 6 0	2830	2837	w —	1	4	1 7 2	6802	6793	m	26	19
1 1 2	3011	3010	w	8	11	1 9 0	—	6937	—	0	1
2 2 1	3119	3119	st	63	27	3 5 0	—	6943	—	0	0
1 5 1		3118			29	0 8 2		7422			2
0 6 1	3443	3432	w—	3	6	2 6 2	7429	7424	w +	17	9
2 4 0	3480	3470	w	7	10	3 1 2		7430			7
1 3 2	3644	3640	m	33	23	1 9 1	7535	7531	m	31	15
0 4 2		3639			10	3 5 1		7538			18
2 4 1	4067	4065	w—	3	6	1 5 3		7874			17
1 7 0	4420	4415	w	11	12	2 2 3	7880	7875	st	49	16
2 0 2	4603	4587	w	8	11	010 0		7881			4
2 2 2	—	4902	—	0	2	3 3 2	8066	8060	w	15	15
1 5 2	—	4901	—	0	0	0 6 3	8200	8188	w—	5	4
1 7 1	—	5010	—	0	0	010 1	8497	8496	w—	2	1
0 8 0		5044			1	2 4 3		8821			4
2 6 0	5050	5046	w +	13	6	3 7 0	8835	8835	w	15	8
3 1 0		5052			4	4 0 0		8836			4

The γ-phase, TaB, thus crystallizes in D_{2h}^{17}-*Cmcm*, isomorphous with CrB with the tantalum as well as the boron atoms in:

$$4 : (c)\ 0\ y\ 1/4;\ 0\ \bar{y}\ 3/4;\ 1/2\ 1/2 + y\ 1/4;\ 1/2\ 1/2 - y\ 3/4.$$

The value of the parameters $y_{Ta} = 0.146$ and $y_B = 0.440$.

* Referred to α_1 for all lines.

THE δ-PHASE

When the boron content of the alloys increased above 50 atomic %, lines of a new phase appeared. This phase had the ideal composition Ta_3B_4, as will be shown below. The powder photographs showed similarities with those of the γ-phase, but new lines appeared. The interferences always appeared at fixed angles in alloys with composition between the γ- and ε-phases, showing that the homogeneity range was narrow. The powder photographs could be indexed, assuming the symmetry to be orthorhombic, the axes of the elementary cell being:

$$a = 3.29 \text{ Å}, \; b = 14.0 \text{ Å}, \; c = 3.13 \text{ Å}, \; V = 144.0 \text{ Å}^3 \; *$$

The δ-phase thus has the a- and c-axes of the unit cell of about the same size as corresponding axes of the γ-phase, but the b-axis is longer, the ratio between the b-axes being 1.6. The number of metal atoms in the elementary cell therefore seems to be six.

The density calculated for 2 Ta_3B_4(13.60) is in good agreement with the density found (13.50). Reflections $h\ k\ l$ only appeared for $h + k + l = 2n$, $0\ k\ l$ for $k + l = 2n$, $h\ 0\ l$ for $h + l = 2n$ and $h\ k\ 0$ for $h + k = 2n$. Probable space groups are thus D_{2h}^{25}-*Immm*, D_2^8—*I*222, D_2^9—$I2_12_12_1$ and C_{2v}^{20}-*Imm*.

Tantalum positions. The investigation was started by examining, whether the structure was consistent with space group D_{2h}^{25}, the space group with the highest symmetry. From space considerations, the six metal atoms must be situated in one twofold and one fourfold position and only the two fourfold positions 4 : (*g*) and 4 : (*h*) are possible. 4 : (*g*) may be combined with one of the positions 2 : (*a*), 2 : (*b*), 2 : (*c*) or 2 : (*d*). A comparison between observed and calculated $p|F|^2$ values for some reflections $h\ 0\ l$ makes it possible to exclude 2 : (*a*) and 2 : (*d*) (Table 3). 2 : (*b*) may be excluded if the intensities of some $0\ k\ l$ and $h\ k\ l$ reflections are compared (Table 3). To make the influence of a variation of the parameter y in 4 : (*g*) as small as possible, small k-values have been chosen. From space considerations, the value of the parameter y must be situated between 0.170 and 0.186. The value 0.180 has been chosen for the calculations.

The remaining possibility for placing six tantalum atoms in D_{2h}^{25} thus is 4 : (*g*) + 2 : (*c*) (or, which will be the same, 4 : (*h*) + 2 : (*a*))

4 : (*g*) 0 y 0; 0 $\bar{y}$ 0; 1/2 1/2 + y 1/2; 1/2 1/2 − y 1/2.
2 : (c) 1/2 1/2 0; 0 0 1/2.

* The axes are given with less accuracy than for the other phases because of the rather diffuse reflections and the great number of coincidences (see Table 4).

Table 3. Calculated $p\,|F|^2$ values for different positions of the metal atoms in the δ-phase.

$h\ k\ l$	$p\,\|F\|^2$				
	obs.	calc. for 4 : (*g*) +			
		+ 2 : (*a*)	+ 2 : (*b*)	+ 2 : (*c*)	+ 2 : (*d*)
1 0 1	70	478	53	53	478
2 0 0	210	193	193	193	193
3 0 1	0	238	26	26	238
1 0 3	0	228	25	25	228
0 1 1	0	211	211	2	2
2 1 1	0	260	260	2	2
0 1 3	0	89	89	1	1

The agreement between observed and calculated $p|F|^2$ values is satisfactory for a value of the parameter $y = 0.180$ (Table 4). The interferences were rather broad and α_1 and α_2 were not separated even at high glancing angles. The limits of error for y seems to be $0.175 < y < 0.186$ (Table 5). The distances between the different atoms thus may be slightly altered, but the fundamental building of the structure is not influenced.

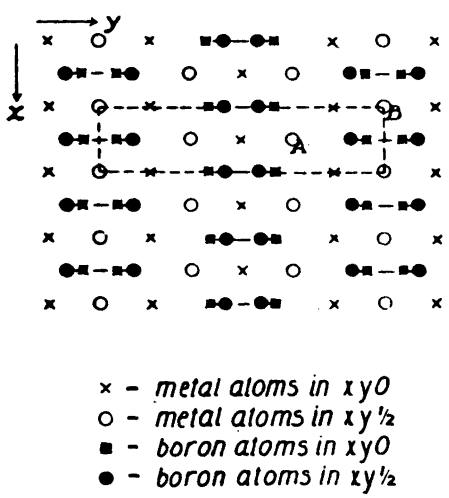

Fig. 1a. Structure of the δ-phase, projected on (001). The elementary cell and the double-chains of boron atoms are indicated.

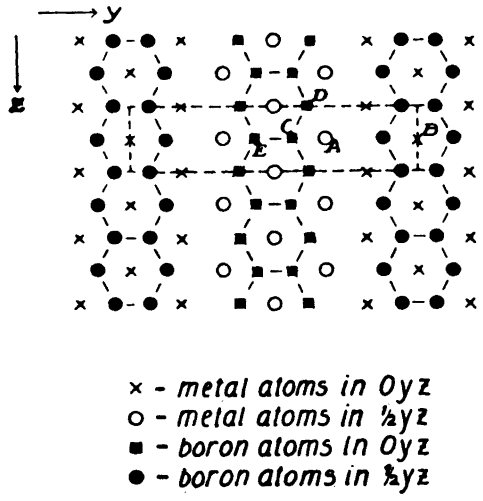

Fig. 1b. Structure of the δ-phase, projected on (100).

Table 4. Ta-B, δ-phase, Cu-K radiation.

h k l	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	I obs.	$p\|F\|^2$ obs.	$p\|F\|^2$ calc. $z = 0.180$	h k l	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	I obs.	$p\|F\|^2$ obs.	$p\|F\|^2$ calc. $z = 0.180$
0 2 0	0123	0121	w—	0.11 *	0.3	1 11 0	4216	4213	w	26	28
0 4 0	0489	0485	w—	1*	2	0 11 1	4288	4270	w—	9	3
1 1 0	0576	0579	st	19*	22	2 7 1		4288			8
0 1 1	—	0636	—	0	0	0 8 2	—	4361	—	0	2
1 3 0	0823	0822	w	4*	5	0 12 0	—	4361	—	0	6
0 3 1	0871	0879	st	61*	50	1 7 2	—	4456	—	0	4
0 6 0	1090	1090	w	20*	20	2 0 2	4626	4621	m	34	26
1 0 1	1149	1155	w—	7*	5	2 2 2	—	4742	—	0	1
1 2 1	1271	1276	st	48	54	2 4 2	—	5106	—	0	3
1 5 0	1305	1306	st	26	36	3 3 0		5218			2
0 5 1	1369	1363	—	0	2	2 10 0	5245	5226	w	26	8
1 4 1	1633	1640	w+	7	5	2 9 1		3257			32
0 8 0	—	1938	—	0	2	1 9 2	—	5425	—	0	1
1 7 0	—	2033	—	0	3	0 10 2	5450	5451	w—	6	8
0 7 1	2085	2090	w+	5	6	0 1 3	—	5482	—	0	0
2 0 0	2198	2198	st	21	19	1 12 1	—	5516	—	0	0
1 6 1	2253	2245	w—	1	5	3 0 1	—	5551	—	0	3
2 2 0	—	2319	—	0	0	1 13 0	5669	5667	w	30	0
0 0 2	2426	2423	m	14	19	3 2 1		5672			27
0 2 2	—	2544	—	0	0	3 5 0		5702			18
2 4 0	—	2683	—	0	2	2 6 2	5714	5711	st	120	39
2 1 1	—	2834	—	0	0	0 3 3		5725			23
0 4 2	—	2908	—	0	2	0 13 1		5724			12
1 1 2		3002			25	0 14 0	—	5937	—	0	1
1 9 0	3011	3008	m	25	1	1 0 3	—	6001	—	0	3
0 10 0		3028			5	3 4 1	6027	6036	w—	8	9
0 9 1		3059			22	1 2 3	6123	6122	w +	35	26
2 3 1	3081	3077	st +	158	64	0 5 3	—	6209	—	0	1
1 8 1		3093			61	3 7 0	—	6429	—	0	2
1 3 2	—	3245	—	0	6	2 11 1	6470	6468	w—	11	5
2 6 0	3290	3288	w	15	26	1 4 3		6486			8
0 6 2	3516	3513	w	18	26	2 8 2		6559			4
2 5 1	—	3561	—	0	3	2 12 0	6620	6559	w	19	10
1 5 2	3733	3729	m	45	46	1 11 2	(dif-	6636			5
2 8 0	—	4136	—	0	2	3 6 1	fuse)	6641			3
1 10 1	—	4183	—	0	1	0 12 2	6801	6784	w—	8	9

* The interferences, marked *) have been obtained in a camera with a bent monocromator of Guinier type.

Table 4 (cont.).

h k l	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	I obs.	$p\|F\|^2$ obs.	$p\|F\|^2$ calc. $z = 0.180$	h k l	$\sin^2\Theta$ obs.	$\sin^2\Theta$ calc.	I obs.	$p\|F\|^2$ obs.	$p\|F\|^2$ calc. $z = 0.180$
0 7 3	—	6936	—	0	3	0 9 3		7905			13
1 6 3	7095	7091	w +	39	3	2 3 3	7923	7923	st	111	37
1 14 1		7092			39	2 13 1		7922			20
1 15 0	—	7363	—	0	1	1 8 3		7939			36
3 1 2		7398			15	1 13 2	—	8090	—	0	0
3 9 0	7413	7398	w	19	0	3 5 2	8124	8125	w +	36	30
0 15 1		7420			5	2 14 0		8135			2
3 8 1	7484	7489	w +	39	37	0 14 2	—	8360	—	0	2
3 3 2		7641	w	19	4	2 5 3	—	8407	—	0	2
2 10 2	7650	7649			13	3 10 1	—	8579	—	0	0
2 11 3	—	7680	—	0	0	3 11 0	8616	8609	w +	22	19
0 16 0	7770	7753	w—	9	7	4 0 0	8803	8790	w	7	9
						3 7 2	—	8852	—	0	3

The tantalum lattice (Fig. 1) contains two kinds of metal atoms. One kind, (A), has its six nearest neighbours at the corners of a surrounding trigonal prism, two at distances 2.97 and four at distances 3.00 Å. These atoms, lying in 4 : (*g*), thus have the same coordination as the tantalum atoms of the γ-phase. The other kind, (B), in the twofold position 2 : (*c*) is surrounded by four neighbours at 2.97 Å, two at 3.13 Å and two at 3.29 Å.

Boron positions. The boron atoms must be situated in holes of the tantalum lattice. The only holes, large enough, are in the centre of a trigonal prism of metal atoms. These holes are connected to channels, all being parallel to the

Table 5. Limits for the parameter of the δ-phase.

h k l	$p\|F\|^2$ obs.	$p\|F\|^2$ calc. $z =$ 0.175	0.180	0.186
0 12 2	77	161	94	34
0 16 0	92	29	70	97
3 11 0	220	160	186	183

c-axis. There are 8 such holes per cell, and in D_{2h}^{25} they possess the two fourfold positions 4 : (g) and 4 : (h) with parameters $y = (1-y_{Me})\,{}^1/_2 - c^2/8\,b^2\,y_{Me} = 0.375$ in 4 : (g) and $y = (1-y_{Me})\,{}^1/_2 + c^2/8\,b^2\,y_{Me} = 0.444$ in 4 : (h).

Boron atoms, placed in these holes, would be connected to double-chains (Fig. 1). Each doublechain may be regarded either as consisting of two usual single chains (compare the γ-phase) or of hexagonal boron rings (compare the ε-phase). The distance boron-boron in the same halfchain (C-D) will be 1.85 Å and the distance between two adjacent boron atoms in different half-chains (C-E) 1.57 Å (assuming the atoms to be spherical and in contact). The distance boron-boron in chains or nets obtained from other determinations has been about 1.72—1.74 Å. The holes in the tantalum lattice, however, are great enough to make a symmetrical arrangement with distances 1.72 Å between adjacent boron atoms in the same doublechain possible.

The boride thus will have the formula Ta_3B_4. It crystallizes in space group D_{2h}^{25}-$Immm$ with two formula units per cell and the tantalum atoms in 2 : (c) and 4 : (g).

2 : (c) 1/2 1/2 0; 0 0 1/2.
4 : (g) 0 y 0; 0 $\bar{y}$ 0; 1/2 1/2 + y 1/2; 1/2 1/2−y 1/2.

The value of the parameter $y = 0.180$.

The eight boron atoms are placed in the two fourfold positions 4 : (g) and 4 : (h).

4 : (h) 0 y 1/2; 0 $\bar{y}$ 1/2; 1/2 1/2 + y 0; 1/2 1/2−y 0.

with the parameters $y = 0.375$ for 4 : (g) and $y = 0.444$ for 4 : (h). The lattice will be further discussed below in connection with the ε-phase.

THE ε-PHASE

In preparations with a boron content of more than 58 atomic % lines of a new phase, the ε-phase, appeared. This phase showed an extended homogeneity range. The powder photographs could be indexed, assuming a hexagonal cell with axes (at the ideal composition TaB_2):

$$a = 3.078 \text{ Å}, \; c = 3.265 \text{ Å}, \; c/a = 1.06.$$

The homogeneity range was determined by studying the variation of the axes with the boron content. The lower limit was at about 64 atomic % ($a = 3.099$ Å, $c = 3.224$ Å), the upper at about 72 atomic % ($a = 3.057$ Å, $c = 3.291$ Å). The agreement between observed and calculated $p|F|^2$ values was satisfactory, assuming the metal atoms to form a simple hexagonal lattice.

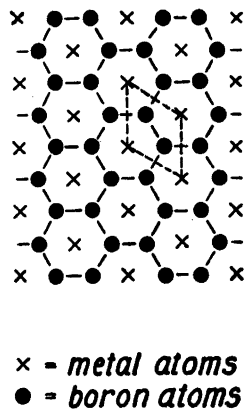

Fig. 2. Structure of the ε-phase, projected on (001). The elementary cell and net of boron atoms are indicated.

If they are placed in 0 0 0, the only place for boron atoms will be in 1/3 2/3 1/2 and 2/3 1/3 1/2, giving an ideal formula of TaB_2 for this phase. The positions are compatible with space group D_{6h}^1—*C* 6/*mmm* and the boride thus is isomorphous to AlB_2, ZrB_2 and CrB_2 (*C* 32 type)[11—13].

The boron atoms form a plane hexagonal net (Fig. 2) with a distance boron-boron of 1.79 Å. It is of interest to compare the structure of this phase with that of the δ-phase. The similarities between the lattices is great, the δ-phase may be regarded as consisting of parallel sheets of simple hexagonal metal lattices with boron nets in the holes (Fig. 1). The difference in potential energy between the lattices of the δ-phase and the ε-phase must be small, and when boron is added to the δ-phase, linkages between the net fragments are formed. The ε-phase will be stable even if the boron net is not complete as indicated by the extended homogeneity range below 66.7 atomic % boron. The homogeneity range, however, is extended above 66.7 atomic %, where the nets are complete, up to about 72 atomic % boron. There are two possibilities for an ε-phase with more than 66.7 atomic % boron. The first is, that in the lattice of the ε-phase some tantalum atoms are replaced by boron atoms. This possibility may be excluded, as it would result in a decrease of the length of the axes, whereas in reality the *a*-axis is decreased but the *c*-axis increased with increasing boron content. The remaining possibility is therefore that boron atoms are added to the simple hexagonal tantalum lattice with complete boron nets. These additional boron atoms may be taken up between the different nets, probably in the positions 1/3 2/3 0, 2/3 1/3 0. They may also be taken up in the centres of the already existing rings of the nets e. g. in the position 0 0 1/2, giving more or less complete sheets of boron atoms as in the ε-phases of the molybdenum- and tungsten-boron systems[6]. The former

possibility would cause an increase of both the a- and the c-axes. The latter would give an increased c- and a decreased a-axis, the decrease depending on the reduction of the distances boron-boron caused by the forces between the boron atoms of the hexagonal rings and the additional atoms in the ring centres. The last possibility thus seems to be more probable.

GENERAL DISCUSSION

The tantalum-boron system belongs to the group of interstitial compounds. It has a ratio $r_B/r_{Me} = 0.59$, equal to the critical value given by Hägg [1, 2]. Compared with the other binary systems between transition metals and boron, which have been investigated [6, 8–11, 13], it has an intermediate position between simple and complicated systems. The solubility of boron in the tantalum lattice is small at room temperature, but the limit of solid solubility is extended at higher temperature. The intermediary phases partly belong to the more complicated kind of phases (β, γ), in part to the simple type (ε) and in part have an intermediate position between simple and complicated lattice types (δ). Further the tendency of the boron atoms to form first chains and then rings with increased boron content of the phases is remarkable. The same tendency has been observed for other systems, for instance the molybdenum- and tungsten-boron systems. In the tantalum-boron system, the β-phase has isolated boron atoms, the γ-phase has zig-zag shaped chains, the δ-phase doublechains, which may be regarded as fragments of boron nets and the ε-phase plane boron nets, which seem to have a tendency to take up more boron to form complete two dimensional sheets.

SUMMARY

The system tantalum-boron has been studied by X-ray methods. The solubility of boron in the tantalum lattice is low at room temperature, but the solubility range is extended at higher temperatures.

Four intermediary phases exist. Complete structure determinations of all the phases have been carried out.

The β-phase is instable at higher temperatures. The metal lattice of the phase is isomorphous with the metal lattices of the borides of the $CuAl_2$ type (C 16). The composition has not been possible to determine, but the isomorphy suggests the composition Ta_2B.

The γ-phase with composition TaB is isomorphous with CrB and the boron atoms form parallel chains through the lattice.

The δ-phase has the composition Ta_3B_4 and the boron atoms form fragments of nets.

The ε-phase with the ideal formula TaB_2 has an extended homogeneity range and is of the AlB_2 type (C 32).

The system is discussed according to the relation r_B/r_{Me}, and to the tendency of the boron atoms to form chains and nets, a tendency, also found in other systems.

The author wishes to thank Professor G. Hägg for his continued interest in this investigation and Mr. Rolf Hesse for the indexing of the ε-phase. He is further indebted to Mr. Georg Andersson and Mr Lars-Henrik Andersson for valuable assistance with preparations and calculations, and to the *Kungl. Svenska Vetenskapsakademien* for a grant from the Edlund fund. The *Statens Tekniska Forskningsråd* has supported the research financially, which support is gratefully acknowledged.

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Received May 25, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 616–619

Investigations on Plasmin

II. On the Determination of the Activity of Instable Enzyme Solutions

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In an investigation recently published by one of us[1] certain difficulties arose in the determination of the activity of an enzyme, plasmin, originating from the fact that the time necessary for the assay was so long and the stability of the enzyme so low that the activity considerably declined during the assay.

In order to render the calculations of enzymic activity more accurate under such conditions, we have established the following mathematical treatment for this problem.

THEORETICAL

In an enzymatic hydrolysis, the number of linkages broken per unit time is directly proportional to the amount of enzyme if the amount of substrate present is sufficiently large and if the affinity of the enzyme to the substrate is considerable. This means that if the enzyme is stable, the number of linkages broken per unit time is always the same. If the activity of the enzyme declines, we can still use the ordinary formulas, if the time is changed into such a function of the time that the number of linkages broken is proportional to this function.

This function of the time will depend on the law of decomposition of the enzyme. This law may be complicated. In the beginning, however, the decomposition may usually be considered as a reaction of the first order. Under these assumptions the problem will be treated with the following notations:

A_t = the enxyme activity at the time t,
c = the proportional factor for the enzymic break down,
C = an integration constant,

k = the rate constant for the inactivation of the enzyme,
n = the number of linkages,
n_0 = the number of linkages originally present,
t = the time, and
$T_{\frac{1}{2}}$ = the half-life period of the enzyme.

The activity of the enzyme under these conditions is

$$A_t = A_0 e^{-kt} \tag{1}$$

Further

$$dn = -cA_0 e^{-kt} \cdot dt \tag{2}$$

$$n = -cA_0 \frac{e^{-kt}}{-k} + C \tag{3}$$

If $t = 0$ we have $n = n_0$. Hence $C = n_0 - \frac{cA_0}{k}$ and

$$n = n_0 - cA_0 \frac{1 - e^{-kt}}{k} \tag{4}$$

If this expression is compared with the corresponding expression for stable enzymes,

$$n = n_0 - cA_0 t \tag{5}$$

we gather that the time should be changed into the function

$$\frac{1 - e^{-kt}}{k}$$

The problem is now to find the rate constant for the inactivation of the enzyme. The present authors have employed the method of successive approximations.

From equation (1) we get

$$\log A_t = \log A_0 - kt \log e \tag{6}$$

and

$$k = -\frac{\Delta \log A_t}{\Delta t \cdot \log e} \tag{7}$$

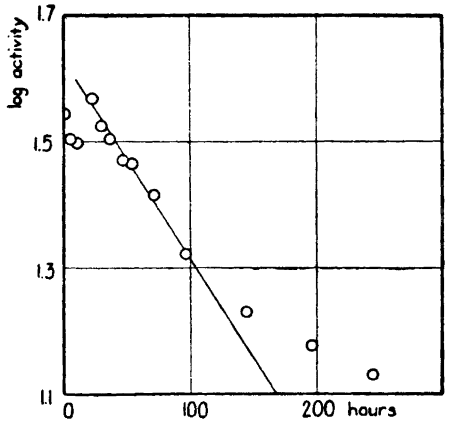

Fig. 1. Graph for the calculation of the rate constant of the inactivation of the enzyme.

and the half-life period

$$T_{\frac{1}{2}} = -\frac{\log 2 \cdot \Delta t}{\Delta \log A_t} \tag{8}$$

EXPERIMENTAL

We have applied the expressions evaluated in the theoretical part to the measurements of plasmin activity reported in the article mentioned above [1]. From the data given there on plasmin activity (*cf.* Fig. 2) we have prepared the present Fig. 1, giving the logarithm of the enzyme activity as a function of the time.

From the graph in Fig. 1 we have calculated $k = 0.0075$ and $T_{\frac{1}{2}} = 93$ (time in hours).

The three last points of the activity determinations lie considerably above the straight line. This may indicate that the stability increases with continued inactivation. If a straight line is fitted to these points and the corresponding calculations of k and $T_{\frac{1}{2}}$ are made, the following values are obtained: $k = 0.0023$ and $T_{\frac{1}{2}} = 300$ (time in hours).

The rate constant for the enzyme inactivation determined in this way cannot be assumed to be accurately valid for the inactivation in the reaction mixture with gelatin. For want of better values at the present early stage of plasmin investigation, this value may however be assumed to be at least of the right magnitude. Hence we have calculated the function $(1-e^{-kt})/k$ with this value. An example of the result is given in Fig. 2.

From the figure we gather that the time may advantageously be corrected according to the given function. It is possible to fit 2 straight lines to the

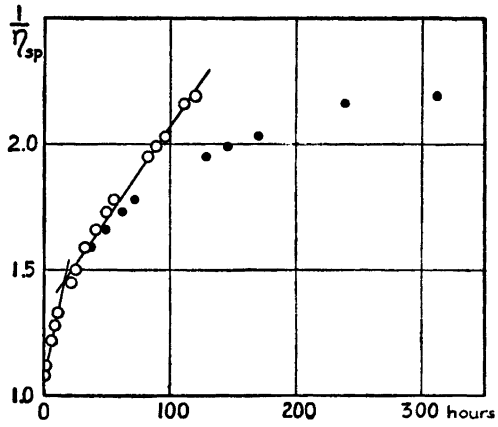

Fig. 2. Viscosimetric assay of plasmin with gelatin. Correction of the time for the spontaneous inactivation of the enzyme. Dots give the uncorrected time. Circels give the corrected time $= (1-e^{-kt})/k$.

measurements as described by one of us [2,3] and thus calculate the enzyme activity for two different steps of the hydrolysis.

For the assay, 1 ml of plasmin solution was mixed with 3 ml of 4 % gelatin solution. Hence [4], remembering that the time was counted in hours, and taking 0.0235 for the derivative of the first line (Fig. 2), the activity in μA units is

$$\tfrac{4}{1} \cdot \tfrac{3}{4} \cdot \tfrac{3}{4} \cdot 0.04 \cdot 0.04 \cdot 0.0235 \cdot \frac{1}{3600} \cdot 10^6 = 0.0235$$

SUMMARY

The spontanous inactivation of an enzyme during the assay can be corrected for by substituting the following function of the time t and the rate constant k for the enzymic inactivation: $(1-e^{-kt})/k$ for the reaction time. The application of the formula is exemplified by the viscosimetric assay of plasmin.

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Received May 21, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 620–624

Investigations on Plasmin

III. On the Formation of Plasmin from Plasminogen

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In a previous paper in this journal [1] one of us reported the strange behaviour of plasmin solutions in several experiments. At first, the proteolytic activity decreased normally, but after a certain time the solutions revealed an activity greater than that of the fresh solution. The activity thereupon again decreased normally.

THEORETICAL

The increase in the activity of a plasmin solution gives raise to two different hypotheses. In both cases we must presume the presence of plasminogen in the plasmin solution, but the mechanism of the plasmin formation will be quite different if we postulate the absence or the presence of an antiplasminogen, which must first be destroyed before the plasminogen can be transformed into plasmin. We will first consider the case when we have plasmin and plasminogen but no antiplasminogen.

The mechanism of the decomposition of the plasminogen may first be assumed to be autocathalytic as in the trypsin formation from trypsinogen. This case has been treated by Kunitz and Northrop [2, 3]. The activity first increases more and more rapidly and after a while decreases slowly. There is no activity minimum so the behaviour of the plasmin solutions cannot be explained in this way.

A reaction may also be postulated to proceed in such a way that the enzyme and the enzymogen are both assumed to decompose according to a reaction of the first order. We apply the following notations:

A = the enzyme activity,
E_0 = the enzymogen amount at zero time (one unit of enzymogen gives one unit of enzyme),
k = the rate constant for the inactivation of the enzyme,
c = the rate constant for the transformation of the enzymogen to enzyme, and
t = the time.

For the enzyme activity we get the following differential equation

$$\frac{dA}{dt} = cE_0e^{-ct} - kA \tag{1}$$

which has the following solution if $c \neq k$

$$A = \frac{cCe^{-kt} - cE_0e^{-ct}}{c - k} \tag{2}$$

where C is an integration constant. If the activity at zero time is A_0, we get

$$A = A_0e^{-kt} + E_0 \frac{e^{-kt} - e^{-ct}}{1 - \frac{k}{c}} \tag{3}$$

If the time is counted so that the activity at zero time is zero, we get the following expression:

$$A = E_0 \frac{e^{-kt} - e^{-ct}}{1 - \frac{k}{c}} \tag{4}$$

If $c = k$, we get

$$A = E_0kte^{-kt} \tag{5}$$

and if $c = \infty$, we get

$$A = E_0e^{-kt} \tag{6}$$

The curves of the equations (4—6) are given in Fig. 1. It can easily be shown that the curves have a maximum at the point where they are cut by the line $A = E_0e^{-kt}$, *i. e.* $k/c = 0$, and no other maximum or minimum points.

From this treatment we gather that the mechanism assumed in this calculation is not valid for an enzyme showing one minimum and one maximum in its activity.

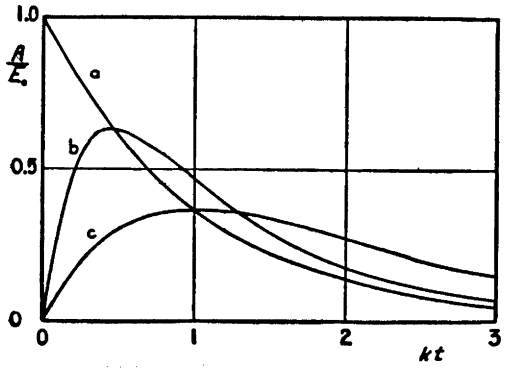

Fig. 1. The activity of an enzyme solution, where one unit of enzymogen is transformed to enzyme in a reaction of the 1st order with the rate constant c, and the enzyme is inactivated in a reaction of the 1st order with the rate constant k.
a. $c = \infty$. *b.* $k/c = \frac{1}{4}$. *c.* $c = k$.

If, however, we postulate the presence of an antiplasminogen, we may assume that this antiplasminogen can be broken down by the plasmin. If the antiplasminogen is present in excess compared with the plasminogen, a great part of the antiplasminogen must first be broken down before the plasminogen can be transformed into plasmin. During this first step, the plasmin inactivation is likely to proceed normally. When the last residues of the antiplasminogen are distroyed, the plasminogen is rapidly transformed into plasmin. When this plasmin formation is completed, the inactivation of the plasmin again proceeds normally. In the activity determinations, however, the antiplasminogen is diluted with the substrate, and so the probability of a given linkage in the antiplasminogen being split is greatly decreased. Thus the activity at the moment when the plasmin solution was mixed with the substrate solution is measured.

EXPERIMENTAL

The experimental values from measurements reported in a previous article by one of us [1] have been recalculated in the way recently described by us [4]. We have thus found the activity values listed in Table 1. The activity values are given for the first and the second step of the break down of gelatin [5].

The initial viscosity of the gelatin solution changed during the experiment, and so we must assume that the later determinations in this series cannot without hesitation be compared with the earlier ones. This is also reflected by the fact that the proportion between the activity values obtained from the first and from the second step of break down of the gelatin solution is not constant. The interesting part of the activity determinations were, however, all performed before the gelatin solution had changed very much. Hence

Table 1. The activity of a plasmin solution at different times, indicating the presence of an antiplasminogen.

Time hours	Gelatin $\frac{1}{\eta_{sp}}$	Activity (μA)	
		1st step	2nd step
0	1.08	0.022_5	0.0074
6	1.11	0.021	0.0070
11	1.06	0.019	0.0066
23	1.15	0.024_5	0.009
30	1.20	0.023	0.009
37	1.33	0.022	0.009
47	1.29	0.020	0.0077
54	1.26	0.018_5	0.0082
71	1.46	0.015	0.0078
96	1.48	0.012_5	0.0065
144	1.67	0.009_5	0.0067
196	1.57	0.009	0.0051
244	1.61	0.007	0.0041

The function $1/\eta_{sp}$ of the initial viscosity of the gelatin solution in each activity assay is given in the second column.

this series may be used to support the hypothesis of the occurence of an antiplasminogen *.

SUMMARY

1. A mathematical treatment is given for the activity of an enzyme solution, where an enzymogen is transformed to an enzyme in a reaction of the first order and the enzyme is also inactivated in a reaction of the first order.

2. Several hypothetical mechanisms for the transformation of plasminogen to plasmin are discussed. Earlier observations showing that the activity of plasmin solutions after a preliminary period of decrease, suddenly increases and thereupon again decreases cannot be explained either as an autocathalytic reaction or as a spontanous decomposition of the plasminogen in a reaction of the first order.

3. The behaviour of the plasmin solutions may, however, be explained by the presence of an excess of antiplasminogen that prevents the transformation of plasminogen to plasmin, and which must first be broken down by the plasmin, before the transformation can take place.

* Called by Loomis *et al.*[6] antiprofibrinolysin.

The activity calculations were mainly performed by Dr G. Kriszat and Miss Irma Sjögårdh, and the English text was revised by Mrs William Cameron. We wish to express our cordial thanks for this help.

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Received May 21, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 625–629

On the Viscosimetric Assay of Enzymic Activity and Rate Constants

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The viscosity of solutions of polymeric homologous substances has been the object of much interest during the last few decades, and many formulas have been suggested. A review of this subject has recently been made by Ewart [1] to which the reader is referred.

As early as 1887 Arrhenius [2] put forward a formula giving the relationship between the relative viscosity η_r of a solution and the concentration c of the solute. We may write it in the following form

$$\eta_r = A^c \tag{1}$$

where A is a constant.

Berl and Bütler [3] found that the logarithmic form

$$\log \eta_r = Kc \tag{2}$$

where K is a constant, was applicable to solutions of starch nitrates.

Staudinger and Heuer [4] found that there exists a simple relationship between viscosity, solute concentration and molecular weight, valid for dilute solutions:

$$\eta_{sp} = K_m c_{gm} M \tag{3}$$

where η_{sp} = the specific viscosity,
c_{gm} = the concentration in basic moles per litre,
K_m = the viscosity-molecular weight constant, and
M = the molecular weight.

Staudinger [5] also called attention to the fact that the constant in equation (2) is proportional to the molecular weight, and Hess and Sakurada [6] showed that Staudinger's formula (equation 3) can be regarded as the first approximation of the logarithmic formula, if natural logarithms are used, *i. e.*

$$\lim_{c \to 0} \frac{\eta_{sp}}{c} = \lim_{c \to 0} \frac{\ln \eta_r}{c} \tag{4}$$

The formula

$$\ln \eta_r = K_m c_{gm} M \tag{5}$$

will be referred to here as the modified Arrhenius-Staudinger formula *.

When the viscosity is used for the determination of molecular weights and for the characterization of depolymerization processes, it must be observed that the limes value (4) is the value required. By increasing concentration, the values of $(\ln \eta_r)/c$ generally differ less from the limes value than do the values of η_{sp}/c, and values of $(\ln \eta_r)/c$ can frequently be used instead of the corresponding limes values. If necessary, a calculation of the limes value may be performed by Mead and Fuoss's method [9], who give the formula

$$\frac{\ln \eta_r}{c} = [\eta] - \beta [\eta]^2 c \tag{6}$$

where $[\eta]$ = the intrinsic viscosity [10], and
β = a constant, which is characteristic for the solute.

The present author [11] has previously deduced ** a formula for the viscosimetric assay of enzymic activity:

$$A = c_s^2 \cdot \frac{\mathrm{d}\frac{1}{\eta_{sp}}}{\mathrm{d}t} \tag{7}$$

where A = the activity of the enzyme in the reaction mixture,
c_s = the concentration of the substrate in grams per gram of solution in the reaction mixture, and
t = the time in seconds.

* There are two logarithmic expressions for viscosity which are sometimes confused, especially as both may be written log η/c. One is $(\log \eta_r)/c$, discussed above, and the other is log (η_{sp}/c), proposed by Bungenberg de Jong, Kruyt and Lens [7] and extensively used by Staudinger *E.g.* [8].

** The equation (5) in that paper inludes an approximation: the number of basic molecules N is substituted for the number of linkages actually present between the basic molecules.

The deduction was based on Staudinger's formula (equation 3). If, however, we apply the modified Arrhenius-Staudinger formula (equation 5), we get

$$A = c_s^2 \cdot \frac{\mathrm{d}\frac{1}{\ln \eta_r}}{\mathrm{d}t} \tag{8}$$

The present author [12] has recently deduced some equations for the rate constant k of the depolymerization of high-polymer substances and for the degree of depolymerization α. These equations are also based on Staudinger's formula, and the same arguments can be applied as to the enzyme assay. In cases where the modified Arrhenius-Staudinger formula is valid, we thus get the following equations, corresponding to the equations (8), (10), and (13) in that article respectively:

$$k = 2K_m c_{gm} M_0 \frac{\mathrm{d}\frac{1}{\ln \eta_r}}{\mathrm{d}t} \tag{9}$$

$$k = 2K_m c_{gm} M_0 \frac{\frac{1}{\ln \eta_r}}{t} \tag{10}$$

$$\alpha = 2K_m c_{gm} M_0 \frac{1}{\ln \eta_r} \tag{11}$$

The functions c/η_{sp} and $c/\ln\eta_r$ are thus of special interest for substances for which Staudinger's formula and the modified Arrhenius-Staudinger formula are valid respectively.

If the expression $1/\eta_{sp}$ is used in those cases where the expression $1/\ln\eta_r$ would be correct, we get appreciable deviations at high viscosities, especially in calculations of molecular weights, and polymerization and depolymerization degrees. In calculations of enzymic activity and rate constants, however, where the derivative is considered, the error is less disturbing, as the depolymerization is carried out for a period when $1/\eta_{sp}$ is practically a linear function of the time. The curve $1/\eta_{sp}$ has one asymptote, since

$$\lim_{\ln \eta_r \to 0} \left(\frac{1}{\ln \eta_r} - \frac{1}{\eta_{sp}} \right) = \tfrac{1}{2} \tag{12}$$

and both the curve and the asymptote are given in Fig. 1.

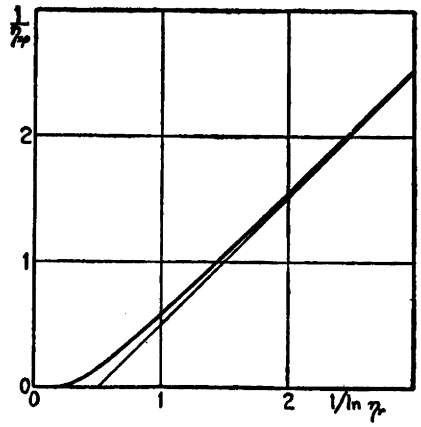

Fig. 1. $1/\eta_{sp}$ *as a function of* $1/\ln \eta_r$.

SUMMARY

1. The modified Arrhenius-Staudinger formula $\ln \eta_r = K_m c_{gm} M$, where η_r = the relative viscosity, K_m = the viscosity-molecular weight constant, c_{gm} = the concentration in basic moles per litre, and M = the molecular weight, is used for the deduction of formulas for the assay of enzymic activity and the determination of rate constants.

2. For the enzymic activity A, the following formula is given: $A = c_s^2 \cdot \mathrm{d}(1/\ln \eta_r)/\mathrm{d}t$, where c_s = the substrate concentration in grams per gram of solution, and t = the time in seconds.

3. For the rate constant k, the following formula is given: $k = 2K_m c_{gm} M_0 \cdot \mathrm{d}(1/\ln \eta_r)/\mathrm{d}t$, where M_0 = the basic molecular weight.

4. For the degree of depolymerization α, the following approximative formula is given: $\alpha = 2K_m c_{gm} \mathrm{M}_0/\ln \eta_r$.

This investigation was financially supported by *Statens Naturvetenskapliga Forskningsråd*. The English translation was revised by Mrs William Cameron. For this help I wish to express my cordial thanks.

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Received May 25, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 630–638

The Use of Surface Active Agents to Prevent 'Precipitate Crawling' and to Speed Filtration in Gravimetric Determinations

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The purpose of this investigation was to determine the relationship, if any, between a reduction in surface tension and the phenomenon known as 'precipitate crawling'. In order to accomplish this, various surface active agents were introduced into the procedure for the determination of nickel as nickel-dimethylglyoxime. This precipitate was chosen because it was known to exhibit the phenomenon of 'crawling' to a marked extent. This tendency to 'crawl' increases the chance for inaccurate results in quantitative determinations by making it difficult and time-consuming to effect the transfer of precipitate from beaker to Gooch.

The problem was twofold: to determine first if 'crawling' could be decreased by a reduction in surface tension; and second, if the precipitation remains quantitative when surface active agents are used.

In order to detect any decrease in 'crawling' it was necessary to use almost completely visual means since no suitable quantitative measurement of this phenomenon is possible. However, the use of these agents would be warranted only if the decrease in 'crawling' was great enough to be plainly visible to the eye.

The effect these agents might have on the accuracy of the determination could be detected by analyzing steel samples of known nickel content and comparing the results with the known value and with 'control' determinations, *i. e.*, determinations using no surface active agents. If the accuracy of the determination is affected by these agents, their use would not be warranted regardless of how effective they might be in preventing 'crawling'.

CLASSIFICATION OF DETERGENTS

A glance at the following classification of surface active agents by McCutcheon [1] will give some idea of the types of agents that may be chosen.

I Alcohol sulfates
II Alkyl-aryl sulfonates
III Alkyl sulfonates
IV Sulfated or sulfonated amides
V » » » esters
VI » » » amines
VII Miscellaneous types
VIII Cationic agents

From these groups it was possible to obtain cationic, anionic or neutral agents that were stable over a wide range of pH and toward most oxidizing and reducing agents.

SUREACE TENSION REDUCTION

The first question that arose was how much agent to use. In order to answer this a surface tension analysis was run testing various agents on a 'blank' solution, that is, a solution identical in all respects to an actual determination except that no steel sample was used. The results of this analysis gave the most effective concentration of surface active agent with respect to an approximately maximum decrease in surface tension.

Table 1. Liquid agents.

Amount per 50 ml 'blank' sol.	Igepal	Santo-merse	Triton 770	Sterox SK	Triton X-155	Triton 720	Triton N-100
	γ	γ	γ	γ	γ	γ	γ
0 drops **	55.56	55.56	55.56	55.56	55.56	55.56	55.56
1 drop	39.64	31.68	54.28	42.6	44.8	32.68	34.38
2 drops	32.30 *	30.55 *	40.35	31.2 *	42.6	31.26 *	33.11
3 drops	32.30	31.36	35.52	34.1	35.8	31.26	31.83 *
4 drops	33.11	—	31.12 *	—	32.1 *	—	32.68
5 drops	—	—	32.11	—	32.7	—	—

* Most effective concentration.

** Concentration of commercial product.

Table 2. Solid agents.

Amount per 50 ml 'blank' solution	Vel	Nacconol NRSF
	γ	γ
.00 g	55.56	55.56
.05 g	35.09	38.08
.10 g	30.69	31.40
.15 g	30.26 *	29.27 *
.20 g	30.69	30.98

* Most effective concentration.

Tables 1 and 2 show the results of the analysis for the nine agents used in the quantitative determinations. Surface tension (γ) is expressed in dynes per centimeter.

QUANTITATIVE RESULTS

The effectiveness of the different agents is illustrated by Figs. 1 and 2.

Two quantitative tests were run with each surface active agent using the standard procedure as outlined by Kolthoff and Sandell [2]. Samples A

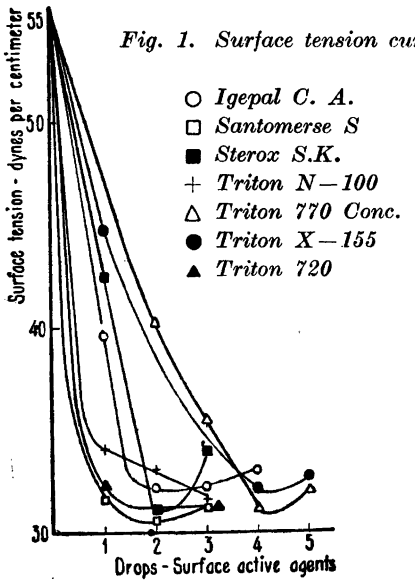

Fig. 1. Surface tension curves.

○ *Igepal C. A.*
□ *Santomerse S*
■ *Sterox S.K.*
\+ *Triton N—100*
△ *Triton 770 Conc.*
● *Triton X—155*
▲ *Triton 720*

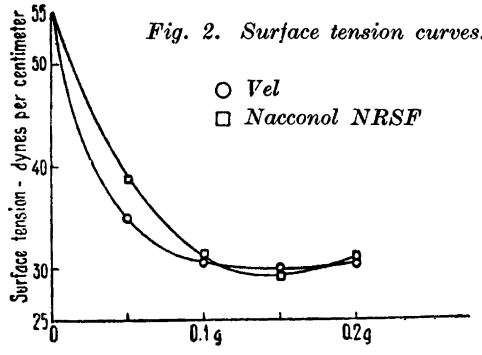

Fig. 2. Surface tension curves.

○ *Vel*
□ *Nacconol NRSF*

had the agent added to the cold acid solution before the addition of dimethylglyoxime, while Samples B had it added to the hot basic solution after precipitation. The only other change from the standard procedure was that the solutions were filtered by decantation and washed twice with a water solution of the same surface active agent used in the determination. The transfer of precipitate was accomplished by using this surface active wash liquid and was so effective that no rubber policeman was required. While in the Gooch, the precipitate was washed twice with hot pure water.

The effectiveness of this method in removing all foreign matter and all excess surface active agent was tested by shaking a water solution of a dried and washed precipitate and a dried but unwashed one in individual test tubes.

Table 3. Quantitative results.

Agent used	Sample	Wt. of sample	Wt. of dimethylglyoxime ppt.	Percent (computed)	Percent (actual)	Error
Control	1	1.6785 g	0.0317 g	0.5973	0.61 *	− 0.013
»	2	1.3649	0.0426	0.6342	0.61	+ 0.024
»	3	1.0847	0.0355	0.6647	0.63 **	+ 0.035
»	4	1.0611	0.0340	0.6507	0.63	+ 0.021
Igepal C. A.	A	1.0766	0.0326	0.6154	0.61	+ 0.005
	B	1.0071	0.0317	0.6395	0.61	+ 0.029
Vel	A	1.1787	0.0364	0.6361	0.61	+ 0.026
	B	0.8992	0.0280	0.6328	0.61	+ 0.023
Triton N-100	A	1.0256	0.0345	0.6835	0.61	+ 0.074
	B	1.0275	0.0338	0.6684	0.61	+ 0.058
Santomerse S	A	1.0460	0.0054	0.1049	0.61	− 0.505
	B	1.0974	0.0175	0.3240	0.61	− 0.286
Triton 770	A	1.0387	0.0323	0.6319	0.61	+ 0.022
	B	Discarded — Filtration unsatisfactory				
Sterox SK	A	1.1106	0.0310	0.5672	0.61	− 0.043
	B	1.0270	0.0315	0.6232	0.61	+ 0.013
Triton X-155	A	Discarded — Filtration unsatisfactory				
	B	1.0389	0.0334	0.6533	0.63	+ 0.023
Triton 720	A	1.1080	0.0371	0.6804	0.63	+ 0.050
	B	1.0168	0.0339	0.6774	0.63	+ 0.047
Nacconol NRSF	A	1.0003	0.0306	0.6216	0.63	− 0.008
	B	1.0419	0.0325	0.6338	0.63	+ 0.004

* Analyzed sample obtained from Smith and Underwood, sample no. 12.

** Analyzed sample obtained from Smith and Underwood, sample no. 14.

The washed and dried precipitate showed no foaming tendency, whereas the other foamed considerably.

The results of these determinations were then compared with four »control» determinations as to 'crawling', ease of precipitate transfer, speed of filtration, and accuracy. The quantitative results were also compared with the percent nickel known to be present in the sample.

Table 3 shows the quantitative results of these determinations.

EFFECT ON 'CRAWLING'

The conclusion from this investigation was that one of the main causes of 'crawling' as exhibited by nickel-dimethylglyoxime is surface tension, since its reduction by use of surface active agents removes all observable tendencies of the precipitate to 'crawl'.

Figures 3 to 6 show the difference in 'crawling' tendencies of the precipitate in a 'control' and a 'surface active' solution. It will be noticed that the 'control' precipitate 'crawls' considerably both in the beaker and in the Gooch, whereas the 'surface active' precipitate shows no tendency to 'crawl'.

This conclusion would naturally lead one to question whether the use of surface active agents is applicable to other determinations in which the precipitate exhibits this phenomenon. Mr. G. Chen, of Drew University, used Igepal C. A. in connection with the determination of nickel in nickel ore and found that all 'crawling' tendencies were removed, filtration and washing time was reduced by one-third, and the accuracy was within $\pm$ 0.03 %. These results tended to confirm the results of the author even in a substance of considerably higher nickel content.

Qualitative tests were run testing the effectiveness of various agents on the 'crawling' tendencies of the ammonium phosphomolybdate precipitate. It was found that some agents, such as Igepal C. A., either had no effect on 'crawling' or even increased it; some, such as Santomerse S and Nacconol NRSF, removed 'crawling' tendencies; some, such as Sterox S. K., caused the formation of a gum-like precipitate; and some, such as Vel, reacted themselves with the molybdate reagent to form insoluble precipitates.

It would appear that certain surface active agents could be found that would remove 'crawling' and facilitate the transfer or removal of almost any precipitate that exhibits this phenomenon.

QUANTITATIVENESS

The use of surface active agents, except for Santomerse S, apparently does not interfere with the quantitativeness of the determination of nickel.

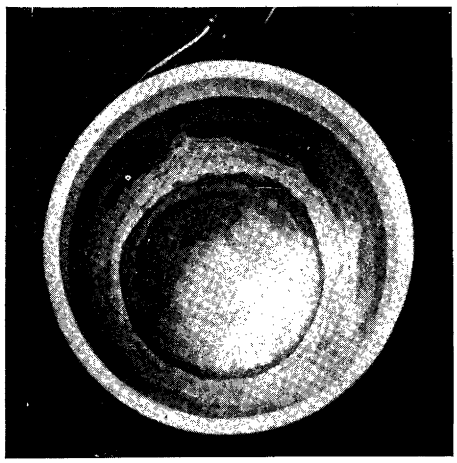

Fig. 3. Gooch crucible after filtration — no surface-active agent used.

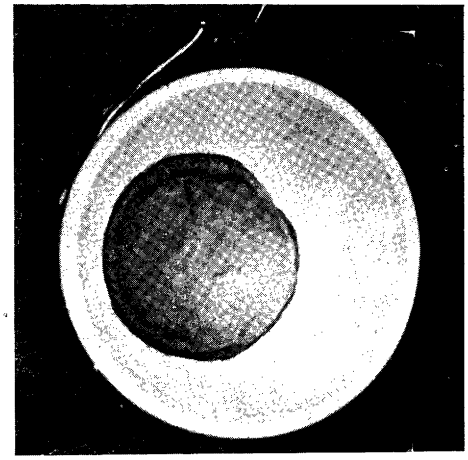

Fig. 4. Gooch crucible after filtration — surface-active agent added.

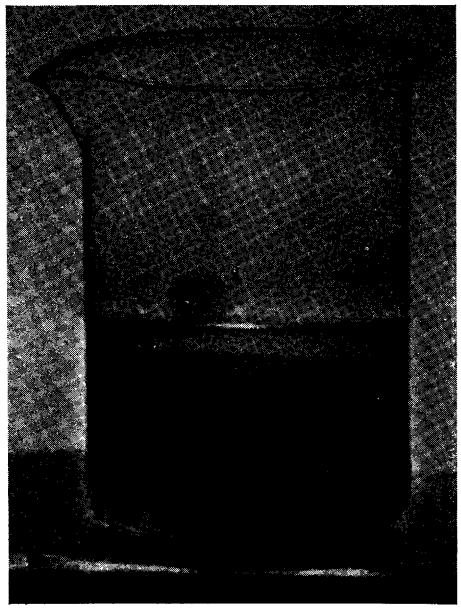

Fig. 5. Precipitate 'crawling' in 'control' solution.

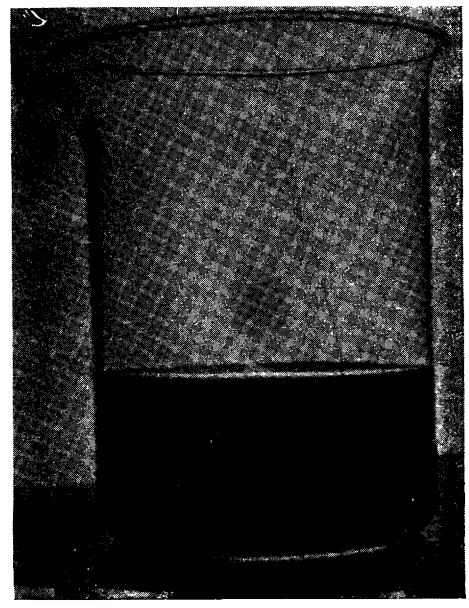

Fig. 6. Absence of 'crawling' in surface-active solution.

In a determination of this sort an error of ± 0.1 % is generally considered to be the maximum which may be conceeded to inherent errors in the determination and personal errors. It will be noticed that, except for the very low results of Santomerse S, the range of error is from 0 to ± 0.07 %, which is well within the allowable limit. This range also compares favorably with the 0 to ± 0.04 % error obtained from the 'control' solutions.

The effect of Santomerse S on the precipitate is not known with certainty. However, a microscopic analysis of the residue after drying showed that very few of the characteristic crystals of nickel-dimethylglyoxime were present. The main bulk of the precipitate was a dark brown amorphous solid. This observation would lead one to believe that Santomerse S either forms a complex ion with nickel and thereby prevents complete precepitation, peptizes the precipitate, or forms a precipitate itself with nickel.

FILTRATION SPEED

An attempt was made to prepare all Gooches with mats of approximately similar thickness and density in order to permit a comparison of the filtration times of the various solutions. Obviously this was practically impossible to accomplish and variations of 10 to 15 minutes in filtration time might very easily be due to variations in the respective mats. This might tend to explain the difference of 19 to 37 minutes filtration time for the »control» solutions; but it would not, in itself, be sufficient to explain the variation of 6 minutes to over 2 hours for the 'Surface active' solutions. Apparently the agents affect the precipitate in some way that causes a great difference in its ability to be filtered. The exact mechanism by which this is effected could not be determined. It is believed, however, that the agglomeration tendencies of the precipitate are varied by the use of surface active agents and this, in turn, affects the speed of filtration.

Whether the variation between the more efficient surface active solutions and the 'control' solutions is due to some change in the properties of the precipitate or only to the increased ease of precipitate transfer is not known. The fact remains, however, that certain surface active agents not only remove 'crawling' tendencies but also increase, to some extent, the speed of filtration.

MICROSCOPIC ANALYSIS

The precipitates from Igepal CA, Sterox SK, Triton 770, and 'control' solutions were examined by means of a microscope equipped with a micrometer eyepiece. The characteristic red monoclinic crystals of nickel-dimethyl-

glyoxime of approximately 25 μ length and 1 μ diameter were apparent in all four cases.

The agglomeration tendencies, however, did seem to vary since in the 'control' and Triton 770 solutions the precipitates seemed to consist of individual crystals, whereas the others were aggregates.

The precipitate from the Santomerse S solution was found to consist mainly of a dark brown amorphous solid with very little nickel-dimethylglyoxime present.

Table 4. Summary.

Surface active agent	Classification (type)	Sample	Deviation from accepted percent nickel	Observed 'crawling' tendencies	Filtration and washing time	Ease of transfer of ppt.
None (Control)	—	1	− 0.013	Bad	31 min	Very poor
» »	—	2	+ 0.024	»	37 »	» »
» »	—	3	+ 0.035	»	20 »	Poor
» »	—	4	+ 0.021	»	19 »	»
Santomerse S	II	A	− 0.505	None	20 »	Excellent
» »	»	B	− 0.286	»	7 »	»
Nacconol NRSF	»	A	− 0.008	»	1 h	»
» »	»	B	+ 0.004	»	6 min	»
Vel	V	A	+ 0.026	»	14 »	»
»	»	B	+ 0.023	»	2 h	Very good
Igepal C. A.	VII	A	+ 0.005	»	12 min	Excellent
» »	»	B	+ 0.029	»	16 »	»
Triton X-155	»	A	—	»	Filtration stopped	—
» »	»	B	+ 0.023	»	22 min	Excellent
Triton N-100	»	A	+ 0.074	»	9 »	»
» »	»	B	+ 0.058	»	11 »	»
Triton 770	»	A	+ 0.022	»	1 + h	»
» 770	»	B	—	»	Filtration Stopped	—
Sterox SK	»	A	− 0.043	»	10 min	Excellent
» »	»	B	+ 0.013	»	9 »	»
Triton 720	»	A	+ 0.050	»	26 »	»
» 720	»	B	+ 0.047	»	17 »	»

SUMMARY

Table 4 summarizes the results of the investigation and shows that certain surface active agents are capable of preventing 'crawling', speeding filtration, and maintaining accuracy in the determination of nickel as nickel-dimethylglyoxime. The agents are classified according to the types given by McCutcheon, listed earlier in this article. Some manufacturers are reluctant to furnish detailed information as to the types to which their products belong.

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Received May 18, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 639—643

Reactivation of Adsorptive Alumina Spent in Organic Chromatography

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During the last ten years chromatography has attained a remarkable position as a tool in organic work. This development is particularly influenced by the investigation of Brockman and Schodder[1] from 1941 concerning the preparation of adsorptive alumina of different activity degrees and the method for defining this degree accurately, which makes it possible to choose a suitable adsorbent according to the adsorbability of the substances to be separated.

Although alumina standardized according to Brockman at present is produced on a large scale by different producers, its relatively high price considerably limits its use in investigations on wider scale. Several investigations have been carried out to restore the activity of spent alumina. The results differ largely from each other, often not being very promising. Strain [2] in his collective book concerning chromatography mentions only that adsorptive clays in the industry are reactivated by burning in special kilns which prevent the sintering of the clay itself. On the other hand Hesse [3] in a similar book advises to avoid the regeneration for the sake of the many difficulties arising. However, the reactivation can be done, according to him, by eluting with a suitable powerfull solvent, and burning thereafter, to which operation a treatment with steam and activated carbon can be adjoined.

Christensen [4] reactivates alumina used in the chromatographic analysis of Tinctura nucis vomicae by heating it for 2 hrs. over an open flame, and Gerstenberg [5] mentions the original activity to be completely restored after the material has been washed with organic solvents, alkali carbonate solutions at an elevated temperature and hydrochloric acid successively, finally having been heated with activated carbon. Sergeant [6] uses a mere heating, maintaining the temperature below 700° C. Alumina which has been used in the purification of transformer oil can, according to Harwood and Davies [7], be reused

after being heated up to 650—700° C in a bed through which a flow of air is blown. Latham [8] mentions that waste alumina from transformers did not attain its original effectiveness in a treatment consisting of washing with paraffins, and burning or drying in air at 500—600° C. The method of Murata and Kakudo [9], which is discontinous and seems to be somewhat elaborate, comprises treatments with potassium bichromate in a sulphuric acid solution, aquous alkali and heat. The product is said to be of a good quality. Summarizing numerous investigations Eckart [10] states that the restoration can be effected in general to 80 per cent at most, and usually to 50—60 per cent only.

From the investigations mentioned above the conclusion can be drawn that the methods available for obtaining good results are either slow or elaborate. To remove strongly adsorbed substances an equally energetic treatment is needed, but the danger of damaging the micro structure of the material simultaneously arises especially if a high temperature acts long upon it. Weitbrecht and Fricke [11] have measured by means of ultramicroscopy and X-rays the dimensions of the pores in active γ-alumina and have obtained values between 25 and 150 Ångström units, the mean value being about 50 Å. For the molecular area they obtained the value of 45,000 square meters per mole, when a heating for 2 h at 500° C was used in the preparation of the sample. If the temperature was raised to 800° C, otherwise maintaining the conditions unchanged, the molecular area was reduced by about a third. A lengthening of the heating time was also found to have an effect in the same direction.

Usually in the preparation of active alumina the starting material is artificial aluminium hydroxide which on dehydration changes to γ-AlOOH. Hüttig and Grubitsch [12] refer to an unpublished paper of W. Schröder in which it is stated, that γ-AlOOH at 120° C, losing its constitution water, is converted into γ-alumina. In this condition the material, however, is found to be amorphous and very unstable, and a slow recrystallisation is observed. The rate of the recrystallisation increases but little when the temperature is raised up to 790° C from which point the speed-temperature curve rapidly rises. Thus the decrease in the molecular area mentioned above and obviously a decrease in the adsorption-activity can be explained.

Another reason for the deteriorating effect of long heating at high temperatures is the allotropic change of γ-alumina into the α-form or corundum. Weitbrecht and Fricke (*l. c.*) have found this to happen rather sharply at 1150° C by studying the X-ray diffraction patterns. On the other hand, Hückel [13] reparts a temperature as low as 750° C at which the α-modifikation begins to form at a measurable speed. The difference in the results might be regarded to be due to differing amount of impurities from one preparation to another particularly when it is known that alkali is left in adsorptive alumi-

nas intentionally. Thus it is evident that conditions under which the irreversibile change to the ineffective corundum is possible, are to be avoided as well as the conditions aiding the recrystallisation of the active γ-form. Hence, a short time of action and low temperatures are favourable.

EXPERIMENTAL

Material and pre-treatment

The alumina used in the regeneration experiments was a 'Savory & Moore' product 'standardized according to Brockman'. It had been exhausted in different chromatographic separations of exclusively organic substances of varying and so far unravelled constitution. Substances left in the adsorbent could not be eluted with an methanol-benzene mixture.

The excess solvent was removed by burning off the alumina in an open kettle and thereafter heating the material with a bunsen burner in batches of about one kilogram until the small fountains caused by the solvents and moisture boiling off disappeared. During this manipulation there was a change in the colour to an evenly light tan. In ultraviolet light the material had a bright white fluorescence whereas the original one had a sandy coulour.

The material thus treated was ready for further reactivation experiments.

Treatment with ozone

As it could be supposed that the browning during the pre-heating was due to cracking reactions causing unsaturated linkages, an idea was at hand to break up and remove the substances by means of ozonization. 50 grams of the material were poured on a G 3 glass sinter filtering funnel forming a 15 mm thick layer. Through the sinter a flow of oxygen containing 5—6 vol. % ozone was lead upwards at a speed of 12 litres an hour. A reaction immediately began, changing the colour in the lowest layer into pure white and the reaction vessel warmed considerably. The escaping gas did not liberate iodine from a potassium iodide solution, thus showing the ozone absorption to be complete. The all-over reaction time rose to 50 min partially because the reaction layer, owing to a varying flowing resistance, advanced unequally. The treated material did not give a positive ozone reaction with potassium iodide-starch solution after heating up to 300° C for one minute.

The product was pure white but still gave a white fluoressence in ultraviolet light and did not belong to any activity class, tested according to Brockman [1]. It is obvious that a number of saturated linkages still have remained in the heating operation which linkages do not react with ozone, and on the other hand some decomposition products of the ozonides may not be easily removed by heat. The work in this direction was checked because of its elaborateness.

Application of direct heating

The brown material was heated with continous mixing in an open iron bowl over a strong gas flame in batches of about 500 g. In half an hour the alumina was changed to a grayish white matter. A sample taken did not belong to any standard class. Only after

a heating of several hours and simultaneous mixing, products were obtained which belonged to different classes. The method proved to be uncertain, elaborate and slow.

Burning in oxygen

With regard to the viewpoints mentioned in the theoretical part an apparatus was constructed in which the organic substances could be burned off without using too high a temperature. The operation was performed, using the countercurrent principle, in a quartz tube surrounded by an electrical resistance and placed at an angle of 35° in respect to the horizon. The running time of the alumina through the tube was about 10 seconds. The length of the tube was 100 cm and the bore 16 mm. The heating mantle was placed on the upper portion and had a length of 75 cm. The lower end was connected with a rubber stopper to a 1 liter distillation flask, the oxygen inlet being through its side tube. The material was lead to the quartz tube from a dropping funnel, and the rate of flow of the alumina was controlled with the stopcock. Previously the material had been screened to remove extraneous particles. The burnt alumina was collected in the distilling flask. Temperature measurements were performed using a Pt—PtRh thermocouple inserted into the quartz tube.

The heating resistance was wound of Kanthal D 0.7 mm resistance wire in a spiral, the total resistance being 40 ohms. The whole construction was inserted into a Pyrex tube of an inner diameter of 40 mm and wound on the outside with asbestos tape. Using a 220 V current a temperature of 850° C could be achieved.

A number of regeneration experiments were run, changing the flow rates of alumina and oxygen at several temperatures. It was found that below 700° C the working rate had to be uneconomically slow, and on the other hand at about 800° C a slight sintering seemed to occur causing an uneven flow of the material. In the table test values from the

Table 1.

No.	Colour in ultraviolet light	Colour in day-light	cm^3 O_2/g alu-mina	g Alu-mina/min	Tempe-rature ° C	Activity class acc. to Brockman	Elutable per cent
Original	S	W	—	—	—	I	0.001
1	L	G—W	307	1.3	750	I	0.005
2	S—L	G—W	144	1.4	720	I	0.001
3	S—L	G—W	105	3.8	760	I	0.002
4	S	G—W	63	5.4	770	I	0.002
5	S	G—W	55	7.6	750	I	0.004
6	S	G—W	51	3.7	760	I	0.004
7	S—W	G—W	67	3.2	710	I	0.002
8	W	B	20	12.5	750	II	0.002
9	W	B	16	12.2	770	II	0.001

Significances of the letters: S = sandy, L = lilac, W = white, G = grayish, B = brownish (tan).

interval 700—800° are collected. Values in the column »elutable per cent» are obtained as follows: about 12 grams of the regenerated alumina were washed on a sintered glass filter forming a column 19 × 50 mm. At first 30 cm^3 of ether, containing 3 % alcohol were used, and thereafter 20 cm^3 petroleum ether 40—60° C with the same amount of alcohol. The collected filtrates were evaporated to dryness, and the residue weighed.

The results show, that using 50 cm^3 or more oxygen for each gram of alumina, regeneration products belonging to the first standard class are obtained. A large excess of oxygen or too slow a rate of flow of the alumina, both cause an abnormally dark fluoressence colour which can render it difficult to perform chromatographic analyses in ultraviolet light. Changes in temperature between 700 and 800° C seem not to have any remarkable effect. The amount of eluable remains in every case insignificantly low. The grayish tinge of the reactivated adsorbent might be due to elementary carbon left in the pores and hardly causes any troubles.

By this method about 15 kilograms have been treated at our laboratory, a part of it three times, and the product has proved to be equal to the original. When handling oxygen sensitive substances it is preferable to remove the oxygen left by leading nitrogen through the material.

SUMMARY

1. The reactivation of alumina, used in the chromatographic separation of organic substances, has been investigated by treating it with ozone by burning in an open kettle, and by burning in oxygen.

2. The burning in countercurrent oxygen gave products of the first activity class »according to Brockman». The two other methods proved unsatisfactory.

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Received May 9, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 644–652

Short Communications

The Synthesis of Tetraethylthiouram Disulphide (Antabus) labelled with Radioactive Sulphur

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Tetraethylthiouram disulphide is at present in frequent use in Scandinavia as an adjuvans in the treatment of alcoholism. A year only has passed since the discovery [1] of its peculiar physiological action on the metabolism of ethyl alcohol, and relatively little is yet known about its turnover and way of action.

In order to investigate its fate in the organism, the compound labelled with radioactive sulphur was synthezised in the following way:

S^{35} was available as sulphuric acid in 0.25 *N* hydrochloric acid from Oak Ridge National Laboratory, Oak Ridge, Tennessee.

The labelled sulphate together with carrier sulphate was precipitated as calcium sulphate and reduced at 850° C with carbon monoxide [2] to calcium sulphide.

The calcium sulphide was transformed to potassium sulphide by liberating the hydrogen sulphide with hydrochloric acid and receiving it in potassium hydroxide in a diffusion chamber.

The labelled sulphur was brought into carbon disulphide by an exchange reaction [3] between labelled potassium sulphide in water and unlabelled carbon disulphide:

$$S^{*--} + CS_2 \rightleftharpoons CS_2S^{*--} \rightleftharpoons S^{--} + CSS^*$$

When equilibirum is established, the labelled sulphur will be distributed between the two phases according to their sulphur content. In this way, a yield of 50 % per run of radioactive carbon disulphide was easily obtained.

The labelled carbon disulphide was reacted with diethylamine and potassium hydroxide to give potassium diethyldithiocarbamate. The oxidation to tetraethylthiouram disulphide was achieved by means of sodium tetrathionate[4].

The compound was recrystallized from absolute ethyl alcohol to give faintly yellow crystalline needles, m. p. 70.4° C.

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Received June 29, 1949.

On the Origin of the Basic Amino Acids

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The classification of arginine, histidine and lysine as the 'basic' fraction of amino acids does, naturally, not imply any closer metabolic relationship between these substances. In fact, very little is known as to their origin. Som evidence point to ornithine, and consequently arginine, as being derived from glutamic acid via proline [1,2]. As for histidine and lysine very few hints are given as to the formation of the carbon sceleton of these substances. Recently, however, work with *Neurospora* mutants show that α-amino adipic acid might be one of the members of the precursor chain for the formation of lysine [3,4].

In this situation, some experiments on amino acid metabolism of yeast may give some points of interest. Feeding a strain of *Torulopsis utilis* with ammonia and acetic acid, the latter being the only carbon source, the acetate, labelled with C^{13} in the methyl group and with C^{14} in the carboxyl, enters the metabolism of the yeast, partly appearing in the protein fraction [5]. The yeast, after 3 hours treatment with the labelled acetate, was killed, fats, lipids, carbohydrates and proteins separated, the latter hydrolyzed and subjected to electrodialysis. Using the method of Kossel [6,7] arginine, histidine and lysine were separated, then recrystallized as flavianate and picrates and finally isolated as mono-hydrochlorides. By combustion of minor samples of the (chromatographically pure) substances, and by ninhydrine treatment [8] and hydrolysis with 20 %, baryta CO_2- free, a series of CO_2-samples, trapped as $BaCO_3$, were obtained, representing total carbon, carboxyl carbon and, in the case of arginine, guanido-carbon. Isotope analysis of these samples show the following picture:

Table 1.

Carbon atoms from:		C^{13} Atom per cent excess	C^{14} Counts/min per 15 mg of $BaCO_3$
Acetic acid (substrate)	C^{CH_3}	2.42	—
	$C^{Carboxyl}$	—	9850
Arginine	C^{total}	0.082	218
	$C^{carboxyl}$	0.065	356
	$C^{guanido-}$	0.027	353
calc:	$C^{2,3,4,5}$	0.100	150
Histidine	C^{total}	0.090	81
	$C^{carboxyl}$	0.172	3
calc:	$C^{2,3,\ ring}$	0.074	97
Lysine	C^{total}	0.155	477
	$C^{carboxyl}$	0.024	1070
calc:	$C^{2,3,4,5,6}$	0.181	360

In the case of arginine the high C^{14}- and the low C^{13} content of the carboxyl and the guanido-group is what could be expected, the relatively high turnover-rate of the carboxyl group being already earlier observed [9]. Calculation of the average isotope values of the remaining atoms C^2, C^3, C^4, C^5, gives $C^{13} = 0.150$ and $C^{14} = 150$. Assuming two atoms of high C^{14}- and low C^{13}-content (round 300 and 0.06) the two remaining carbon atoms should be markedly of the C^{13}-type (round 0.20). Another possibility is one atom containing the main part of C^{14} (round 600) and the remaining three, emanating from the methyl group of the acetate, having a C^{13}-average of about 0.14. Incidentally, analysis of the glutamic acid from the same experiment gives the C^{14}-value of 1500 and 840 for the γ- and the α-carboxyls respectively, the three middle atoms being C^{14}-free and with a C^{13}-content of 0.22. This means that, actually, *if* the ornithine

part of the arginine originates from glutamic acid, the observed values for $C^{2,3,4,5}$ are compatible with the corresponding pattern of glutamic acid $= HOOC - \underset{0,22}{CH_2} - \underset{0,22}{CH_2} - \underset{0,09}{CH(NH_2)} - \underset{0,17}{COOH}\ \frac{1500}{840}\ \frac{(C^{14})}{(C^{13})}$, (the intensity of the arginine labelling being round half of that of glutamic acid).

The histidine values, on the other hand, are surprising in view of the absence of C^{14} in the carboxyl, the high C^{13}-content of which decidedly points towards its origin from the methyl group of the acetate of the substrate, probably through decarboxylation and dehydrogenation of some intermediate α-ketoacid structure. Incidentally, during the metabolic breakdown of α-ketoglutaric acid to succinic acid, *one* carboxyl of the latter actually originates from the keto group of the keto acid, and should thus, in view of the type of labelling of the acetate be of C^{13}-type[10]. Now, it must be taken into account that the other succinic acid carboxyl will be of C^{14}-type and thus the labelling of *both* carboxyls will be mixed. Considering the pure C^{13}-labelling of the histidine carboxyl, this points to its origin from a α-ketoacid structure, the metabolic breakdown of which should form a *non-symmetrical carboxylic acid derivative*. Decarboxylation and dehydrogenation of the keto-analogue of glutamine, furnishing the half-amide of succinic- or fumaric acid might fit in with this purely speculative view.

Of the non-carboxylic moiety of histidine part of it must have derived from the carboxyl of acetic acid. Assuming the whole C^{14}-content of histidine being located to a single carbon atom of the structure, the C^{14}-value would be about 400; if located to *two* atoms, consequently about 200, the latter being reasonable in view of the low turnover rate of histidine in general.

Remain lysine with an unexpected high C^{14}-content of its carboxyl group, and so high a C^{14}-content of the non-carboxylic part, that at least two atoms of decidedly C^{14}-type must be located there. In that case each of them might have a C^{14}-value round 900, which is about the same magnitude as the estimated content of the carboxyl = 1070. In view of the labelling of the latter and the high C^{13}-C^{14}-overall value for the rest of the molecule (0.18/360) it seems reasonable to assume that the whole carbon sceleton of lysine has been formed from acetic acid by a direct route, correlated with the head-to-tail condensation of acetyl residues to fatty acids[11]. This view is supported by the fact that the C^{14}/C^{13}-quotient of fatty acids isolated from the yeast is 0.80 (the isotope quotient of the acetic acid taken as unity), which is about the same as that for lysine = 0.76. As to the incorporation of the nitrogen atoms during the formation of lysine, the mechanism might involve double bond amination.

The primary data given above show that, at least in yeast, the formation of the basic amino acids must pass over quite different metabolic pathways. In order to make a total check-up of the isotope content of all separate atoms of the amino acids in question, the above simple degradation procedures have to be extended to a total, stepwise break-down of the compounds. Methods with this end in view are by now worked out, and it is hoped to publish the final results gained in the near future.

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The Identity of Karrer and Gränacher's '2-Phenyl-5(4*H*)-imidazolone' and Hippuronitrile

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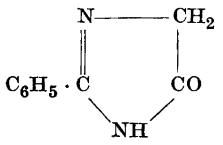
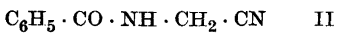
In the course of an investigation of various types of intramolecular anhydride formation in polypeptides Karrer and Gränacher [1] studied the reaction between hippuramide and phosphorus pentachloride in dry ether. From the reaction mixture they isolated in unstated yield a well crystallizing colorless compound melting at 141−3° with the analytical composition $C_9H_8ON_2$. This finding in conjunction with the fact that the compound was hydrolyzed by acid to hippuric acid and small amounts of benzoic acid and that it possessed only weakly basic properties prompted Karrer and Gränacher (*l. c.*) to formulate it structurally as 2-phenyl-5(4*H*)-imidazolone (I).



I



II

It was pointed out by the authors that (I) should be considered as a very simple representative of the poorly explored 5-imidazolones. In the course of a study of these compounds in this laboratory it became imperative to repeat the preparation of the above mentioned phenylimidazolone for comparison. By following the procedure of Karrer and Gränacher (*l. c.*) there was isolated in 40 % yield a substance melting at 142°, giving the same analytical figures and showing further characteristics in accordance with the published data. However, a study of the ultraviolet spectrum revealed that the compound could not be represented by formula (I) because it showed in methanolic solution a typical benzamido-absorption with a peak at 228 mμ (ε = 11700) and not the split peak which will be shown in a forthcoming paper to be characteristic for this type of compounds.

A closer investigation of the presumed 2-phenyl-5(4*H*)-imidazolone rapidly disclosed that this compound is in fact simply hippuronitrile (II), a substance reported in the literature as early as 1902. The identity of the two compounds was secured by preparation of an authentic sample of hippuronitrile by benzoylation of aminoacetonitrile following the procedure of Klages and Haack [2]. Analysis, spectroscopic data, melting point and mixed melting point of the two samples showed no discrepancies. Thus there can be no doubt that the compound claimed by Karrer and Gränacher as being the 2-phenyl-5(4*H*)-imidazolone is in fact hippuronitrile. As to the authentic phenylimidazolone it is quite certain from analogy considerations that its stability and chemical properties will differ quite widely from those here reported.

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Received July 6, 1949.

Concerning the Supposed Absorption of Ultraviolet Energy by the Peptide Linkage

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Gelatin, which contains only traces of tyrosine and no tryptophane exhibits a small but definite absorption band near 2 800 Å, which is shifted towards the red in alkaline solution. This band which has also been found in egg albumin, has been ascribed to peptide bonds by Anslow and Nassar [1]. The evidence is considered insufficient by Crammer and Neuberger, however [2]. In view of the importance of this subject to the problem of the photochemistry of proteins [3], clupein, a polypeptide of molecular weight ca. 4 000 [4], which lacks aromatic amino acid residues, has been examined.

In Fig 1, the lower curve is that of an aqueous solution containing 1.25 % clupein at pH 7 for a 1 cm path length, as obtained with a Beckman spectrophotometer. The wavelength is plotted in Ångström units and the ordinate is optical density as usually defined. The upper curve is for a solution of the same concentration at ca. pH 12 as read against an aqueous solution of 0.06 *N* sodium hydroxide. The middle curve is for a solution of the same concentration of clupein and at a pH ca. 10.5.

These data show that there is no specific absorption of peptide bonds at 2 800 Å and confirm the fact that no aromatic amino acids are present in clupein. At the higher pH only about half the guanidine groups of the arginine residues are ionized which may account for the difference in absorption as compared with the solution at pH 7.

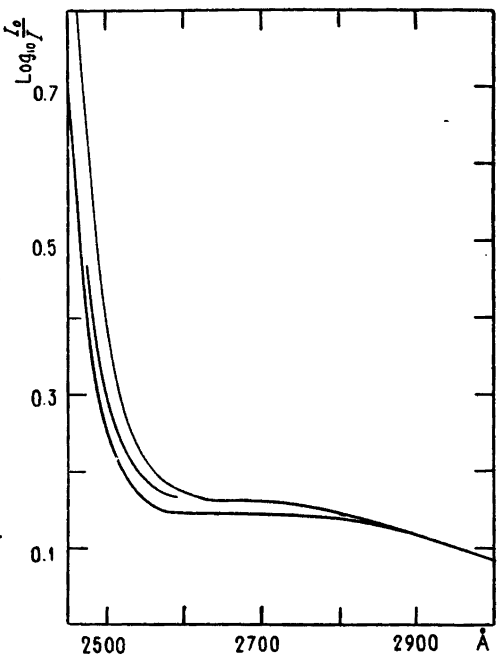

Fig. 1. Ultraviolet absorption spectrum of clupein chloride.

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Received July 18, 1949.

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Aqueous Colloidal Solutions of Cellulose Micelles

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In 1920 [1,2] it was postulated from X-ray investigations that native cellulose fibres contain crystalline areas or micelles, and more recently, attention has been concentrated on the dimensions, shape and position of these micelles in the cell wall. From X-ray diffraction measurements [3,4] a width of approximately 60 Å and a minimum length of 600 Å have been calculated for such micelles. They have now been obtained in aqueous colloidal solution, and have been observed as isolated morphological units by means of the electron microscope. A brief report on this work was given by Svedberg [5] in a recent lecture and a more comprehensive paper will shortly be published by Rånby and Ribi [6].

After considerable degradation of the wood or cotton cellulose fibres, the sols are prepared by peptization. This degradation may be performed by boiling with 2.5 *N* sulphuric acid [7] for 1—8 hours and then washing out the acid in a preparative centrifuge with distilled water. The first 2—3 portions of wash liquid (pH<2) are almost clear, then peptization begins at pH~3 with maximum opacity (maximum cellulose concentration in the sol) occuring at pH~4. Concentrations of 0.5 % have been obtained with a peptization of 25—40 % of the hydrolysed cellulose sample. A similar peptization can also be obtained from the alkaline side, if the cellulose is immersed in 0.1 *N* sodium hydroxide after hydrolysis, in which case maximum opacity occurs between pH 8.5 and pH 7.

After sterilization by heating to 100° C. the cellulose sols are stable for several weeks if their pH values lie between 3.5 and 9.5. Outside this pH range the sol coagulates. The pH is controlled by means of 0.01 *N* hydrochloric acid and 0.01 *N* sodium hydroxide. The sols are also coagulated by very small amounts of neutral electrolytes, *e. g.*, $4 \cdot 10^{-4}$ *N* sodium chloride caused coagulation in a few seconds and $1 \cdot 10^{-4}$ *N* in about two hours. Thus, the cellulose sol is definitely *hydrophobic*. The stability of such a sol, prepared as above, is probably due to the protective action of a substance of low molecular weight, possibly oxydized oligosaccharides, which are dissolved during the peptization process. When dialysed against distilled water, the sols coagulate in about half an hour. Only dilution 5—10 times causes a slow coagulation. Other protective substances are being sought among the polyuronides.

The peptisized cellulose has a rather high content of carboxyl groups (3—5 milliequivalents/100 g cellulose) which give it a negative charge in aqueous solution.

This colloid particles have been extensively studied morphologically in the electron microscope and structurally by X-Ray and electron diffraction, all in cooperation with Ribi [6]. From this work the following results can be quoted. After suitable hydrolysis an accumulation of rodlike particles having the same dimensions as the micelles, earlier calculated from X-ray diffraction diagrams [3,4] of cellulose fibres, has been found. The particles are free or in resolvable aggregates. They are very well crystallized — as shown by their X-ray and electron diffraction patterns — and they have exactly the same structure and lattice dimensions as the fibrecellulose [6]. Undoubtedly, the rodlike particles are identical with the earlier postulated micelles of the cellulose fibre. The micelles are well-defined, fundamental morphological units of the cell walls.

The colloidal cellulose has a high electrophoretic mobility ($\sim 10^{-5}$ cm/sec. volt), a high sedimentation constant (500—700

S), a low intrinsic viscosity ($\lim_{c \to 0} \eta_{sp}/c \sim 0.3$, c in g/100 ml) and a high turbidity and light scattering. It seems to be a useful model substance for such physico-chemical measurements. The sorption of water by isolated micelles is lower than that of native cellulose [8]. Chemical reactions have also been carried out with the colloidal cellulose.

It is of interest to note that Herzog [9] in 1925 and Meyer and Mark [10] in 1928 made the assumption that cellulose and cellulose derivatives generally were dissolved as micelles. This assumption could not be proved by later experiments, and only now, more than twenty years later, has it been shown that such micellar cellulose solutions can be produced.

The author wishes to express his thanks to Professor The Svedberg for stimulating discussions and kind interest in this work, and to Dr. Edgar Ribi for the co-operation in the morphological and structural interpretation. The author is also indebted to the Swedish cellulose firms *Billeruds AB, Mo och Domsjö AB, Stora Kopparbergs Bergslags AB, Svenska Cellulosa AB* and *Uddeholms AB* for generous financial support.

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Received July 19, 1949.

A New Type of Copper Acetylene Compounds

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When acetylene is introduced into a sufficiently concentrated aqueous solution of cuprous chloride and potassium or ammonium chloride, yellow or orange crystalline precipitates appear. These compounds have hitherto been regarded as addition complexes, *i. e.* as containing acetylene with retained hydrogen together with cuprous and potassium (or ammonium) chloride. For the potassium complex Chavastelon [1] gives the formula $C_2H_2(CuCl)_8(KCl)_2$. Tzyrikh and Ginzberg [2] state the formula $C_2H_2(CuCl)_6(NH_4Cl)_3$ for the ammonium complex.

Investigations concerning the conditions for the formation and dissolution of these compounds were performed at this laboratory in 1945, and the conclusion drawn was that the complexes must have some composition other than that suggested by the quoted formulas. A complete analysis however encounters many difficulties. *E. g.* the mother liquor which contains a considerable portion of the inorganic compounds cannot be completely removed without altering the precipitate. It is necessary to perform the analysis in such a way (determination of all components in one and the same sample, parallel analysis of the mother liquor) that the contributions from the mother liquor can be calculated. The result will include a cumulative error, for potassium estimated to be $\pm$ 5 %, and for chlorine $\pm$ 2 %. The data for copper and carbon (determined as acetylene after conversion with cyanide [3]) are but slightly influenced by the correction. The table shows the results of some analyses.

S), a low intrinsic viscosity ($\lim_{c \to 0} \eta_{sp}/c \sim 0.3$, c in g/100 ml) and a high turbidity and light scattering. It seems to be a useful model substance for such physico-chemical measurements. The sorption of water by isolated micelles is lower than that of native cellulose [8]. Chemical reactions have also been carried out with the colloidal cellulose.

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Table 1. Results of analyses.

	Moles/8.00 moles of Cu			Cu+K —Cl
	C	Cl	K	
I	2.08	8.00	2.00	2.00
II	2.16	8.01	2.22	2.21
III	2.20	8.11	1.93 (?)	1.82
IV	2.14	8.20	2.33	2.13
V	2.11	7.96	2.17	2.10

Approximate composition of solutions (molalities):

1.5 m CuCl, 4 m KCl, 0.06 m C_2H_2
Preparations: I 1.45 m CuCl, 3.80 m KCl, 0.07 m C_2H_2; II Do: but 0.10 m C_2H_2; III Do. but 0.06 m C_2H_2; IV 1.40 m CuCl, 4.00 m KCl, 02. m KAc, 0.05 m C_2H_2; V 1.60 m CuCl, 3.90 m KCl, 0.10 m LiCl, 0.04 m C_2H_2.

More accurate results can be achieved by use of a marking method involving addition of a small known amount of a tracer. Determination of tracer in the sample gives the quantity of mother liquor present. Work on this is continuing.

The last column of the table shows a kation surplus well corresponding to the content of acetylenic carbon. Thus the hydrogen atoms of the acetylene must be substituted by metal. That the compounds are *carbidic* is proven also by the fact that an amount of acid, approximately equivalent to the acetylene introduced, is liberated on their formation. The analyses also show that the complexes contain an *equal number* of copper and chlorine atoms. To suggest an unambigious formula is however difficult. Best corresponding with the results is the formula $C_2(CuCl)_8K_2$. There is however a systematic deviation: the acetylene content is somewhat too high (4—10 %). The potassium values may also be too high, but the greater possible error in this analysis makes a decision difficult. The results and the appearance indicate that the main body of the precipitate has the formula stated, but that it also contains varying amounts of another compound, possibly also of the type $C_2(CuCl)_nK_2$, but with $n < 8$.

If the carbidic nature of the compound is to be stressed the formula could be written $C_2Cu_2(CuCl)_{n-2}(KCl)_2$. So far however it seems preferable to regard the molecules of cuprous chloride as equivalent.

A liquid phase in equilibrium with a solid compound of this type is yellow. The dissolved colouring copper acetylene compound has a very high molar extinction. In a solution composed of 2.3-molal cuprous chloride, 5.75-molal ammonium chloride and 0.10-molal ammonia, where we have reason to assume that the acetylene absorbed (if the concentration is low) is quantitatively combining as the dissolved yellow complex, the acetylene concn. necessary for a visible yellow coloration amounts only to a 10^{-5} solution. For spectrophotometric measurements solutions have been used giving no precipitates even at an acetylene pressure of 1 atm. (*e. g.* 0.6-molal CuCl, 3.8-molal KCl, 0.2-molal HCl). The concn. of of the dissolved yellow compound is low, but optically measurable. The main part of the acetylene entering the solutoin is present in the form of a dissolved colourless addition complex containing 1 mole of copper per mole of acetylene. (Its existence has been demonstrated by Manchot [4].)

Computations from the measurements have been carried out under the assumption that there exists only one compound contributing to the extinction. The assumption is unproven but supported by the fact that the colour curves are identical in all measurements. The *results* which are sketched under a)—c) indicate a close relationship between the dissolved yellow compound and the solid yellow precipitate, and that the concentration of the former is determined by an equilibrium which can be expressed as

$$C_2H_2 + n\mathrm{CuCl} = C_2(\mathrm{CuCl})_n^{-2} + 2\,\mathrm{H}^+$$
where $n \geqq 6$

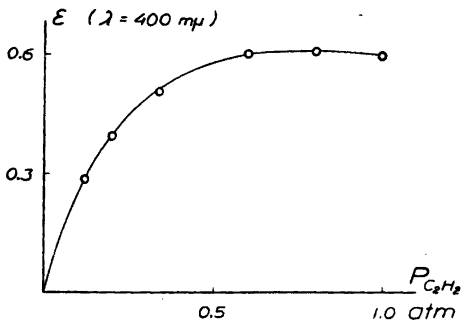

Fig. 1. Concentration of dissolved yellow complex at different acetylene pressures.

a) Variation of hydrochloric acid concn. at a constant concn. of cuprous chloride and chloride ion and constant acetylene pressure (the CuCl-activity of the solution and the concentration of the simple addition complex remaining unchanged) gives values of extinction proportional to $(H^+)^{-2}$.

b) Variation of the cuprous chloride concn. at constant potassium chloride and hydrochloric acid concns. (the relative depression of the CuCl-activity through formation of the simple addition complex remaining unchanged) gives extinction values proportional to the cuprous chloride concn. raised to its 6th—8th power.

c) According to the formula there should be a linear relation between acetylene pressure and extinction, but variations of the acetylene pressure over the same solution give the results illustrated by the figure.

The concentration of the yellow complex thus initially increases rapidly with the acetylene pressure, but then approaches a flattened maximum. The shape of the curve can be completely explained by considering that an increasing acetylene pressure indirectly causes a decrease in the CuCl-activity by augmenting the formation of the simple addition complex. Although the reduction of the CuCl-activity is moderate — 22 % at the highest acetylene pressure in the experiment illustrated by the curve — it considerably influences the concn. of the yellow complex, since the CuCl-activity enters the equilibrium to such a high power. An adjustment of the results to a common CuCl-activity, utilizing an equilibrium constant for the simple addition complex (determined through solubility experiments) gives a linear relation between the concn. of yellow complex and acetylene pressure.

A structural similarity seems likely between these copper acetylene compounds containing the group $C_2(CuCl)_n^{2-}$ and the silver compounds[3] of the type $C_2(Ag^+)_n$. (There exists a solid nitrate where $n = 8$, $C_2Ag_3(NO_3)_6$. In both cases a large number of metal atoms — eight or less, but perhaps preferably eight — is bound to the C_2-group. The dissimilarity between the compounds may depend on the fact that the copper atoms, unlike silver, do not become coordinatively saturated, and thus every copper atom brings a chloride ion into the complex. When $n = 8$ the silver complex thus becomes a hexavalent kation but the cuprous chloride complex a bivalent anion.

In solutions containing cuprous chloride and an alkali chloride polymerization of acetylene to monovinylacetylene occurs[5]. Kinetic investigations on this process (performed as 'flow experiments' in stationary state) indicate that the catalytically active agent should be the ions of the dissolved yellow complex. At a constant acetylene pressure the polymerization rate seems approximately proportional to the concn. of the dissolved yellow complex.

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Received August 8, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 653—654

New Books

B. Eistert. *Chemismus und Konstitution.* Erster Teil. Ferdinand Enke Verlag, Stuttgart, 1948. 378 S. mit 14 Abb. und 95 Tab. 40 DM.

B. Eisterts Monographie *Tautomerie und Mesomerie* weckte, als sie vor etwa zehn Jahren erschien, allgemein Aufmerksamkeit in Europa und U. S. A. Sie war die erste moderne Monographie über die von der deutschen Schule emanierende elektronentheoretische Chemie, wie sie vor allem durch F. Arndt entwickelt wurde. Seit dem Erscheinen von Eisterts Arbeit ist die Elektronentheorie vertieft worden und hat auf experimenteller Basis festere Konturen angenommen. Es war deshalb wünschenswert, dass eine neue Auflage des Eistertschen Werkes herauskam.

Dies ist nun in Form eines kürzlich von Eistert herausgegebenen Werks, *Chemismus und Konstitution* geschehen, dessen bisher vorliegender erster Teil bald durch einen zweiten ergänzt werden soll. In seinem neuen Buch will Eistert eine breitere Darstellung der Elektronentheorie geben »und sie auf viele Konstitutions- und Reaktionsfragen anwenden, die weder mit tautomeren noch mit mesomeren Systemen zu tun haben. Der vorliegende erste Band behandelt, nach einer kurzen historischen Übersicht, solche allgemeineren Themen, zu denen aber auch die Einführung in den Mesomerie-Begriff gehört. Im zweiten Bande, der in tunlichst kurzer Zeit folgen soll, werden tautomere Systeme, andere Umlagerungen und mesomere Ionen besprochen werden».

Nach einem kurzen ersten Kapitel, das einen Überblick über die Entwicklung der Vorstellungen über das Zustandekommen chemischer Verbindungen gibt, behandelt der Verfasser im Kapitel 2 die Grundbegriffe und formalen Ausdrucksmittel der Elektronentheorie, wie sie von der deutschen Schule ausgeformt worden sind. Die Begriffe werden in strengen und klaren Definitionen festgelegt. Der dubiöse Begriff »Valenz» ist in die eindeutigen Begriffe »Ladungswert» (nach G. Schwarzenbach), »Bindigkeit» und »Zähligkeit» aufgeteilt. Anionen mit 3-bindigem Kohlenstoff werden »Carbeniat-ionen» und entsprechende Kationen »Carbenium-ionen» genannt und die Formeln mit bzw. ohne freies Elektronenpaar (Strichsymbol) geschrieben. Das freie, paramagnetische Radikal dagegen wird mit einem unkompensierten Elektron (Symbol: Kreuz) gekennzeichnet. Die wichtigsten wellenmechanischen Vorstellungen werden in anschaulicher Form beschrieben.

In den beiden folgenden Kapiteln beschreibt der Verfasser physikalische Verfahren der Strukturermittlung wie Röntgen- und Elektronenstrahl-Methoden, Infrarot- und Raman-Spektren, weiter Ionen- und Atom-Radien und ihre Abhängigkeit von der Konstitution, Bindungsenergien, Bindungswinkel, asymmetrische Zentralatome, freie und behinderte Drehbarkeit, Polarität und Polarisierbarkeit. Man könnte hier einwenden, dass sich der Verfasser gelegentlich recht kurz fasst und ziemlich grosse Vorkenntnisse voraussetzt. Anderseits gibt er aber ständig gute Hinweise auf die einschlägige Spezialliteratur.

Die drei folgenden Kapitel sind wohl die wertvollsten des Werks. Mit Eleganz und grossem pädagogischen Geschick beschreibt

Eistert die Grundgesetze für Lichtabsorption und Konjugation vom Standpunkt der Quantentheorie und der Mesomerie-Lehre. Hier werden u. a. Mono-Olefine, offenkettige und cyclische Polyene, Enine, Polyine, Kumulene, Trityl-Radikale und -Ionen behandelt. Auch die modernsten Theorien werden hier angewandt, z. B. R. S. Mullikens Prinzip der »Überkonjugation» (»hyperconjugation»), d. h. CH_3- oder R-CH_2-Gruppen als Partner in Konjugationsketten. Bekanntlich hat man ja lang nach einer plausiblen Theorie gesucht, die beispielsweise den positiven elektromeren Effekt der CH_3-Gruppe erklärt.

Im Kapitel 8 gibt der Verfasser eine Orientierung über die chemische Thermodynamik und Kinetik und im Kapitel 9 werden Acidität und Alkalinität behandelt, die Dissociationskonstante und ihre Abhängigkeit von der Konstitution. Die frühere deutsche thermodynamische Symbolik ist hier mit der rationelleren angelsächsischen ersetzt worden. Brönstedts Säure-Base-Begriff ist konsequent durchgeführt. Diese von englischer und amerikanischer Literatur stark beeinflussten Kapitel sind ausgezeichnet komponiert, stellen aber teilweise starke Ansprüche an die physikalische Schulung des Lesers.

Im 10. Kapitel beschreibt Eistert die induktiven Effekte, nämlich den allgemeinen Feldeffekt (»F-Effekt») und den alternierenden Effekt (»A-Effekt»). Letzterer ist in den letzten Jahren weiter entwickelt und vertieft und auf eine Art »Überkonjugation» zurückgeführt worden. Eistert lässt den Leser verstehen, dass nur der Initiierte mit diesem Effekt umgehen kann, der zwar unbestreitbar existiert, aber oft von anderen Effekten überdeckt wird.

Kapitel 11 ist den zwischenmolekularen Kräften und Molekülverbindungen gewidmet, wobei besondere Aufmerksamkeit der Bildung von Protonbrücken und ähnlichen »π-Komplexen», z. B. Anionbrücken, geschenkt wird.

In seinem letzten Kapitel beschreibt Eistert den allgemeinen Mechanismus bei Additions-, Substitutions- und Eliminierungs-Reaktionen. Das Kapitel ist breit und mit pädagogischem Geschick angelegt und enthält zahllose Beispiele aus verschiedenen Gebieten der organischen Chemie.

Die deutsche »elektronenchemische» Schule hat mit der Herausgabe des Eistertschen Buches ihr Schweigen während und nach dem Kriege gebrochen. Eisterts Buch zeigt, dass die Konkurrenz mit der angelsächsischen Schule gut gehalten wurde. Die letztgenannte hat früher starke Eindrücke von der Arndt-Eistertschen Betrachtungsweise empfangen, vor allem durch die klare und übersichtliche Symbolik. Der Referent ist der Ansicht, dass Eistert mit seinem Buch weit ausserhalb der Grenzen seines Landes Gehör finden wird.

Nils Löfgren

ACTA CHEMICA SCANDINAVICA 3 (1949) 655—659

Studies in the Pyrene Series[1]

V. 3-Pyrenyl-lithium

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All attempts to make 3-bromo- or 3-iodopyrene react with magnesium to form a Grignard reagent have been unsuccessful. Only the iodine compound reacts slowly, the result of this reaction being dipyrenyl [1a]. Yet, in order to utilize the great reactivity of organo-metallic compounds in pyrene chemistry, experiments were carried out to synthesize 3-pyrenyl-lithium. This compound is readily formed, when 3-bromopyrene in an ether-benzene solution is treated with phenyl-lithium. The pyrenyl-lithium partly separates from the solution as yellow crystals. Subsequent carbonation gave pyrene-3-carboxylic acid in yields indicating at least a 75 % conversion of the bromopyrene into pyrenyl-lithium.

The slight solubility of pyrenyl-lithium makes it easy to isolate this compound in a pure state, before it is brought into reaction with other substances.

The amount of pyrenyl-lithium, that separates as crystals, may be estimated as the difference between the equivalents of phenyl-lithium used and the equivalents of aryl-lithium remaining in the solution, by pouring the mother liquor into water and titrating the lithium hydroxide with hydrochloric acid (methyl red as an indicator). The results so obtained are in good accordance with the amounts of pyrene found on hydrolysis of the crystalline pyrenyl-lithium. In some cases the yield of crystalline pyrenyl-lithium were as high as 85 %.

In its reactivity and mode of reaction 3-pyrenyl-lithium behaves as an ordinary organo-lithium compound. As mentioned above, treatment with carbon dioxide yields the corresponding carboxylic acid. With ketones pyrenyl-lithium reacts readily to give carbinols. As an example 3-benzoyl-pyrene yielded 3,3′-dipyrenyl-phenyl-carbinol, which is formed, too, from dipyrenyl ketone and phenyl-lithium thus demonstrating the structure of the

dipyrenyl ketone, the synthesis of which is described, to be 3,3'-. In the same way benzophenone yielded 3-pyrenyl-diphenyl-carbinol, identical with the carbinol obtained from 3-benzoyl-pyrene and phenyl-magnesium bromide [2].

Of special interest is the reaction between pyrenyl-lithium and alkyl halides, because of the difficulty of the direct monoalkylation of pyrene in the Friedel-Crafts reaction [1b]. Both of these reactions will be the subjects of subsequent papers. Here, to show the applicability of the present method, the preparation of methyl- and ethylpyrene is described.

With mercuric chloride pyrenyl-lithium gives dipyrenyl-mercury, and with iodine iodopyrene is formed in good yield, making this route to the iodine compound an alternative to the diazotization method [1a].

EXPERIMENTAL

3-Pyrenyl-lithium

In a typical experiment 5.6 g (0.02 mole) of 3-bromopyrene, dissolved in 10—15 ml of dry benzene and diluted with 20—30 ml of dry ether, were placed in a Schlenk tube, the sidearms of which were both fitted with a stopcock. 0.022 Mole of a phenyl-lithium solution was measured from a burette flask into the tube, which was then allowed to stand for about six hours at room temperature. All operations were carried out in a nitrogen atmosphere. The 3-pyrenyl-lithium crystallized in yellow needles forming clusters adhering to the walls of the tube. The clear solution was decanted and hydrolyzed (the crystals were washed twice with dry ether, which was added to the main portion). Titration of the aqueous layer showed that 0.0112 mole of aryl-lithium had remained in solution, while 0.0108 mole had disappeared. From the ether layer 1.3 g of pyrene was isolated. The yellow crystals on hydrolysis yielded 1.6 g of pyrene corresponding to 0.008 mole of pyrenyl-lithium, in accordance with 0.0086 mole as found on titrating the aqueous layer, but only 74 % of the amount expected from the first titration. The total yield of crude pyrene corresponds to a 72 % yield of pyrenyl-lithium. The pyrene formed was identified (m. p., mixed m. p. and picrate) with an authentic sample.

If desired, the pyrenyl-lithium may be obtained as a more finely dispersed precipitate by shaking the reaction tube, when crystallization starts. This is convenient in cases, where a reagent reacts slowly with the lithium compound.

Pyrene-3-carboxylic acid

Phenyl-lithium was prepared from 0.4 g of rasped lithium and 3.45 g (0.022 mole) of bromobenzene in 50 ml of dry ether. The phenyl-lithium solution was filtered, in a nitrogen atmosphere, into a Schlenk tube and after addition of 5 g (0.018 mole) of 3-bromopyrene in 15 ml of benzene and 20 ml of ether left for 16 hours. The yellow crystals and the mother liquor were carbonated separately with solid carbon dioxide. Water was added and 2.2 g and 1.1 g, respectively, of pyrene-3-carboxylic acid were isolated from the water layers, representing a total yield of 75 %, based on bromopyrene. From the ether layers 0.2 g of pyrene and 0.8 g of impure bromopyrene, respectively, were isolated.

The pyrene undoubtedly was formed from unreacted pyrenyl-lithium, thus rising the yield of this compound to 80 %. The acid was found to be identical with that obtained in the usual way by oxydation of 3-acetyl-pyrene [3]. M. p. and mixed m. p. 273—274°; ethyl ester, m. p. and mixed m. p. 63°.

Phenyl-3,3'-dipyrenyl-carbinol

3-Pyrenyl-lithium was prepared from 10 g (0.036 mole) of 3-bromopyrene and 0.045 mole of phenyl-lithium. Decantation and titration of the mother liquor indicated a 78 % yield (0.028 mole) of crystalline pyrenyl-lithium. On addition of 7 g (0.023 mole) of 3-benzoylpyrene [3] dissolved in an ether-benzene mixture (2 : 1) the pyrenyllithium readily dissolved. The tube was left overnight at room temperature, when a yellowish crystalline complex of one mole of carbinol and one mole of ether had separated. Solution and crystals were poured into acidified water and extracted with ether. After evaporation of the ether 8 g of the complex crystallized. It could be recrystallized from benzene-ether (1 : 3). By rapid heating the m. p. is about 210°, dec.

$C_{39}H_{24}O,C_4H_{10}O$	Calc.	C 88.7	H 5.84	Mol. wt.	582.6 = 2 · 291.3
	Found	» 88.1	» 5.91	» » (benzene)	282

The ether molecule cannot be removed from the complex by heating for three hours at 100°, but heating at 140° for four hours removed it completely. 1.107 g lost 0.149 in weight, corresponding to 0.141 g as calculated for one mole of ether. The ether free carbinol has no definite m. p. but decomposes at about 270°.

$C_{39}H_{24}O$	Calc.	C 92.1	H 4.75
	Found	» 91.5	» 4.75

The same carbinol was prepared from dipyrenyl ketone (4.3 g; 0.01 mole) and phenyl-lithium (0.0125 mole). Yield 3.4 g of the carbinol-ether complex.

Found C 88.1 H 5.93

The solubility of the carbinol in glacial acetic acid is very slight and the solution is nearly colourless. On heating, however, to the boiling point the colour distinctly turns green, indicating formation of the ionized acetate. On cooling the colour disappears. Addition of concentrated mineral acids develops the usual [2] intensely green halochromic colour.

Phenyl-dipyrenyl-methyl chloride. Phenyl-dipyrenyl-carbinol (1 g of the ether complex) was dissolved in 15 ml of dry benzene and 4 ml of acetyl chloride were added. In a few minutes the chloride began to separate (0.75 g) as a yellowish green solid. It could not be recrystallized because of the poor solubility and non-stability at elevated temperatures. Drying at 100° for 2—3 hours causes blackening. By treatment of the chloride, suspended in dry benzene and with exclusion of the air, with molecular silver the phenyl-dipyrenyl-methyl radical was formed as indicated by a brownish-red colour

which, on exposure to the air, turned pale yellow. Further experiments on this free radical have not yet been made.

$C_{39}H_{23}Cl$ Calc. Cl 6.73 Found Cl 6.60

3,3'-Dipyrenyl ketone. 20.2 g (0.1 mole) of pyrene and 35 g of aluminium bromide were dissolved in 200 ml of tetrachloroethane. A solution of 7 g of carbonyl chloride in 50 ml of tetrachloroethane was added, with stirring, in the course of 20 minutes. After two hours at room temperature and one hour at 50° the reaction product was poured on ice and hydrochloric acid. The organic layer, after washing, was diluted with 400 ml of ether, when a yellow precipitate (2.7 g) appeared (I). M. p. after recrystallization from 1,2,4-trichlorobenzene 340° (355° corr.). I undoubtedly is dipyrenoyl-pyrene, $C_{16}H_9.CO.C_{16}H_8.CO.C_{16}H_9$, and probably, because of the high m. p., the 3,8-compound.

$C_{50}H_{26}O_2$	Calc.	C 91.2	H 3.98
	Found	» 90.5	» 4.01

To the filtrate from I 600 ml of ether were added, and another yellow precipitate (3.4 g) was formed (II), which proved to be the dipyrenyl ketone; m. p. upon crystallization from xylene 232°.

$C_{33}H_{18}O$	Calc.	C 92.1	H 4.19
	Found	» 91.3	» 4.16

From the filtrate from II 5 g of unchanged pyrene were isolated besides another crop of II, crude product 6.0 g, recrystallized 2.4 g, m. p. 231—232°.

Dipyrenyl ketone was also prepared from pyrene (30 g) and oxalyl chloride (15 g) in carbon disulfide (120 ml) with aluminium chloride (22 g). The mixture was refluxed for 6.5 hours and then poured on ice and hydrochloric acid. The yellow solid formed was extracted several times with carbon tetrachloride to remove unchanged pyrene (13 g), and the residue recrystallized from 300 ml of xylene. Yield 12.5 g, m. p. 231°. Repeated recrystallization yielded 10.5 g, m. p. 234—235°.

Alkylation

3-Methyl-pyrene[3]. This compound was prepared from 0.025 mole of crystalline 3-pyrenyl-lithium and 5 g (0.035 mole) of methyl iodide in 25 ml of dry ether. The pyrenyl-lithium rapidly dissolved with considerable evolution of heat. After 15 minutes the clear solution was poured into water. The aqueous layer by titration was found to contain 0.003 mole of lithium hydroxide and 0.0224 mole of lithium iodide, indicating a nearly complete reaction. The ether layer, on evaporation, left 4.0 g of methyl-pyrene, m. p. 70.5—71.5°, or 83 %, based on pyrenyl-lithium. In another experiment 0.025 mole of pyrenyl-lithium and 7.0 g (0.05 mole) of methyl iodide yielded 5 g of crude methyl-pyrene or 4.5 g recrystallized from ethanol.

3-Ethyl-pyrene[3] was prepared in the same way from 0.03 mole of pyrenyl-lithium and 7.3 g(0.047 mole) of ethyl iodide, yielding 5.1 g (77.3 %) of crude ethyl-pyrene, recrystallized from ethanol 4 g, m. p. 91°.

3-Iodopyrene

7.0 g (0.055 atom) of finely powdered iodine were added to 0.025 mole of crystalline 3-pyrenyl-lithium suspended in 25 ml of dry ether. In a vigorous reaction the pyrenyl-lithium dissolved. After 30 minutes the solution was poured into water. The ether layer was separated and washed free from iodine with a solution of sodium thiosulfate and dried over calcium chloride and decolourized with Norite. After evaporation of the solvent 4.6 g of crude iodopyrene, m. p. 81—82°, remained (56 %). Recrystallized from ethanol-ethyl acetate-glacial acetic acid (4 : 1 : $\frac{1}{2}$) it melted at 85°; mixed m. p. with iodopyrene (m.p. 87°) from 3-aminopyrene [1a], the same.

3,3′-Dipyrenyl-mercury

To 0.029 mole of pyrenyl-lithium suspended in ether were added 4 g of finely powdered mercuric chloride (0.015 mole). After the initial vigorous reaction the tube was shaken for 12 hours. The mixture was poured into water and the solid was filtered off and washed several times with water, ethanol and, finally, with ether. 7 g (80.4 %) of a greyish white powder was obtained. The dipyrenyl-mercury has no definite m. p.

$C_{32}H_{18}Hg$ Calc. Hg 33.2 Found Hg 33.3

SUMMARY

3-Pyrenyl-lithium has been prepared by the action of phenyl-lithium on 3-bromopyrene, and its characteristics as a starting-point for syntheses in various directions have been elucidated.

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Received May 12, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 660—675

The Crystal Structure of the Isomorphous Orthoborates of Cobalt and Magnesium

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Crystals of orthoborates of cobalt and magnesium have been described by Ebelmen [1], Mallard [2], le Chatelier [3], Ouvrard [4], Guertler [5] and Hofmann-Höschele [6] among others. They are reported to be isomorphous and orthorhombic with a rather perfect cleavage parallel to (110). Those of $Mg_3(BO_3)_2$ are further described to have the axial ratios 0.641 : 1 : 0.549 and to be insoluble in dilute acetic acid.

The crystals used in the present investigation were prepared by melting together in a ZrO_2-crucible 3CoO (3MgO) with 1 B_2O_3. They form thin needles parallel to the axis, which was chosen as *c*-axis. By means of crystals picked out, the crystallographic information given above could be confirmed. Of each substance one needle, suitable for taking single crystal photographs, was examined by the rotation and Weissenberg methods, and the ground crystals by the powder method. Mo-K-radiation was used for the single crystal and Cr-K-radiation for the powder exposures. The parameters of the atoms were determined by Fourier-methods [7].

THE UNIT CELL AND THE SPACE GROUP

The Laue-symmetry is D_{2h}-*mmm*. Rotation and Weissenberg photographs were taken about the *b* and *c* (needle) directions. In the first case only the 0-layer line was registered. By rotation around the needle axis, layer lines with $l = 0 - 6$ were obtained. The dimensions of the unit cell were determined by means of powder photographs from a focusing camera. A list of the observed diffraction lines is given in the Table 1.

Table 1. Powder diffraction data for the orthorhombic borates of cobalt and magnesium.

	$Co_3(BO_3)_2$			$Mg_3(BO_3)_2$		
hkl	Int.	$\sin^2\Theta_{obs}$	$\sin^2\Theta_{calc}$	Int.	$\sin^2\Theta_{obs}$	$\sin^2\Theta_{calc}$
020 α	w —	0.0719	0.0736	w	0.0731	0.0740
011 α	w —	.0803	.0823	w —	.0817	.0829
101 α	w	.1076	.1078	s	.1088	.1097
111 α	vw	.1255	.1263	m	.1273	.1283
121 α	s	.1814	.1815	vs	.1839	.1838
130 α	m	.2094	.2096	m	.2113	.2114
031 α	w	.2299	.2296	m —	.2296	.2310
201 α	m	.2402	.2397	s	.2454	.2449
220 α	w +	.2498	.2495	m —	.2544	.2540
211 α	s	.2588	.2581	vs	.2633	.2633
131 α	m	.2739	.2736	vs	.2762	.2762
102 α	w +	.2999	.2995	m —	.3039	.3041
221 α	w —	.3139	.3133	w —	.3191	.3189
112 α	w —	.3189	.3181	w —	.3228	.3225
022 α	w —	.3298	.3292	w —	.3328	.3330
141 α	w	.4024	.4024	w	.4059	.4058
231 α	w	.4056	.4053	vw	.4112	.4112
202 α	s	.4317	.4311	vs	.4389	.4390
132 α	s	.4656	.4654	vs	.4710	.4707
311 α	vw	.4776	.4775	w —	.4884	.4881
150 α	w —	.5044	.5042	w —	.5075	.5075
051 α_1	m	.5248	.5242	w +	.5270	.5272
α_2	vw	.5264	.5260	vw	.5286	.5289
321 α_1	s	.5330	.5330	m	.5436	.5436
α_2	w	.5347	.5345	w	.5451	.5454
042 α_1	w —	.5500	.5500	w	.5549	.5550
α_2				vw	.5562	.5567
330 α_1	s	.5608	.5608	vs	.5713	.5713
α_2	m	.5627	.5626	m +	.5733	.5732
013 α_1	m —	.5935	.5932	m	.6002	.6009
α_2	w —	.5955	.5952	w	.6024	.6028
331 α_1	vw	.6248	.6248	w —	.6362	.6360
α_2				vw	.6386	.6382
302 α_1	w	.6510	.6508	vw	.6636	.6638
060 α_1	m —	.6622	.6622	m	.6661	.6660
α_2	w	.6645	.6647	w	.6684	.6683
123 α_1	s	.6922	.6923	s —	.7012	.7019
α_2	m	.6946	.6948	m —	.7035	.7039
251 α_1	s	.6996	.6999	s	.7070	.7070
α_2	m	.7024	.7023	m	.7096	.7094
400 α_1	m —	.7024	.7025	m	.7196	.7193
α_2	w +	.7050	.7051	w	.7220	.7220

		$Co_3(BO_3)_2$			$Mg_3(BO_3)_2$	
hkl	Int.	$\sin^2\Theta_{obs}$	$\sin^2\Theta_{calc}$	Int.	$\sin^2\Theta_{obs}$	$\sin^2\Theta_{calc}$
033 α_1	w	0.7400	0.7403	w	0.7492	0.7498
α_2	vw	.7435	.7432	vw	.7520	.7521
203 α_1				w —	.7619	.7627
α_2				vw	.7655	.7656
401 α_1	w +	.7674	.7665	m	.7846	.7846
α_2	vw	.7700	.7692	w	.7869	.7870
420 α_1				m	.7935	.7937
α_2				w	.7958	.7961
411 α_1	m	.7850	.7848	m	.8032	.8031
α_2	w	.7857	.7878	w	.8060	.8059
332 α_1	m	.8161	.8162	w +	.8300	.8302
α_2	w	.8189	.8190	vw	.8330	.8332
260 α_1	w —	.8380	.8382	vw	.8465	.8461
421 α_1				vw	.8578	.8580
350 α_1				vw	.8647	.8648
261 α_1	w —	.9041	.9021	vw	.9104	.9104
143 α_1	w —	.9131	.9133	w	.9238	.9241
α_2				vw	.9274	.9272
233 α_1	vw	.9157	.9159	vw	.9288	.9291
062 α_1	w +	.9178	.9180			
α_2	vw	.9208	.9210			
431 α_1	m —	.9319	.9322			
α_2	w —	.9352	.9356			
170 α_1				vw	.9513	.9512
402 α_1	m	.9580	.9582	w	.9781	.9784
α_2	w —	.9615	.9618	vw	.9815	.9819

vs = very strong, s = strong, m = medium, w = weak and vw = very weak.

Measurements in the reflection region of the highest $\sin^2\Theta$ led to the following axial lengths (referred to wave lengths of Cr-Kα_1 = 2.28962 Å and Cr-Kα_2 = 2.29352 Å)

	$Co_3(BO_3)_2$	$Mg_3(BO_3)_2$
a	5.462 ± 0.002 Å	5.398 ± 0.002 Å
b	8.436 ± 0.002	8.416 ± 0.002
c	4.529 ± 0.002	4.497 ± 0.002

The values given above correspond to the axial ratios — values for the Mg-compound within parentheses — 0.648 : 1 : 0.537 (0.642 : 1 : 0.535). The unit

cell containing 2 $Me_3(BO_3)_2$ has the volume 208.7 (204.3) Å^3, and the calculated density is 4.69 (3.10) g · cm^{-3}. The observed densities are 4.66 (3.04) g · cm^{-3}.

The observed powder and Weissenberg reflections correspond to a simple orthorhombic translation lattice since general planes *hkl* show no regular absences. However, planes *hk*0 and 0*kl* reflect only if $h + k$ and $k + l$ are even. These conditions, and the fact that no piezoelectric effect could be detected, led to D_{2h}^{12} - *Pnmn* as the most probable space group.

INTENSITY MEASUREMENTS

For Mo-K-radiation the observed intensities are based on the relation $I_\beta : I_{\alpha 2} : I_{\alpha 1} = 1 : 2 : 4$ for a given reflection and the relative $|F|$-values are determined according to

$$I = C \cdot \lambda^3 \cdot \frac{1 + \cos^2 2\Theta}{\cos^2 \mu \cdot \sin v} \cdot |F|^2$$

where μ = the angle between the primary beam and the equatorial plane, and

v = the azimute of the reflection from the plane through the primary beam and the rotation axis.

In the present case, where crystals containing light atoms were exposed with Mo-K-radiation, no corrections for absorption are performed. The graphs given by Lu [8] for the inversed value of $\frac{1 + \cos^2 2\Theta}{\cos^2 \mu \cdot \sin v}$ have been used.

The Weissenberg photographs were taken with the equi-inclination method. The connection between intensities of reflections from different layer lines was obtained by changing rotation axis and by Weissenberg oscillation photographs [9].

PATTERSON-HARKER-ANALYSIS

There are about 250 reflections available for the structure determination. By means of the $|F|^2$-values from the reflections *hk*0 and 0*kl* the Patterson-projections $p(xy)$ and $p(yz)$ were calculated. In the case of $Mg_3(BO_3)_2$ the result of these calculations is given in the Figs. 1 and 2.

On account of the symmetry elements existing in the space group D_{2h}^{12}-*Pnmn* it is advisable to develop the function $p(xyz)$ in the cuts $P(xy0)$ and $P(xy\,\frac{1}{2})$ to get the sites of the different kind of atoms. In the Figs. 3 and 4 these sections are reproduced in the case of $Mg_3(BO_3)_2$.

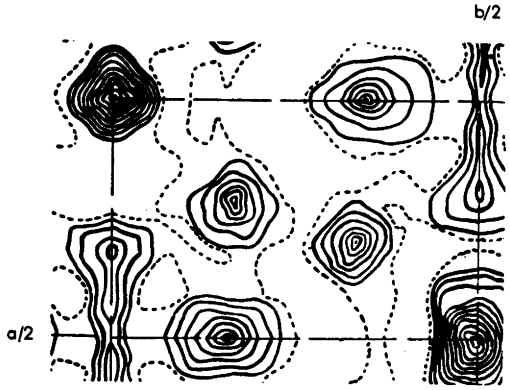

Fig. 1. Projection of the Patterson-function of $Mg_3(BO_3)_2$ on the ab-plane, p(xy). The dotted line corresponds to the 0-level.

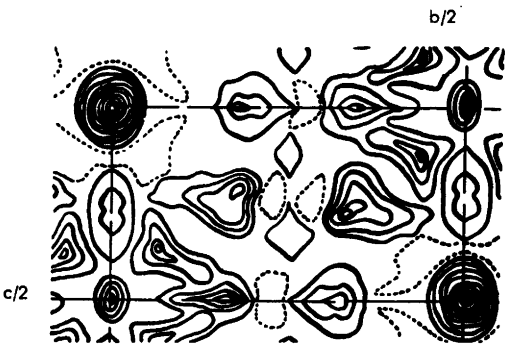

Fig. 2. Projection of the Patterson-function of $Mg_3(BO_3)_2$ on the bc-plane, p(yz).

By combining the different vectors found in the above projections and cuts the atomic arrangement given below will be the only one possible from space reasons. The (4g)-position of the boron-ions is based on the fact that it permits the placing of the boron ions in the centres of gravity of the nearly equilateral triangles formed by the oxygen ions. As will be shown below the dimensions of these triangles are nearly the same as the dimensions of $(BO)_3^{3-}$-triangles, which have been determined in other borates. The denomination is in accordance with the International tables for the determination of crystal structure, Berlin (1935).

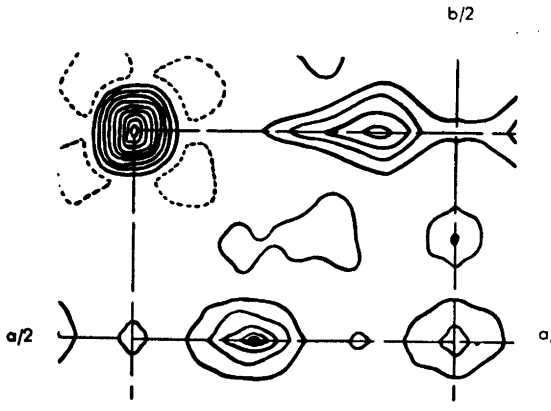

Fig. 3. The Harker-section P(xy0) of $Mg_3(BO_3)_2$.

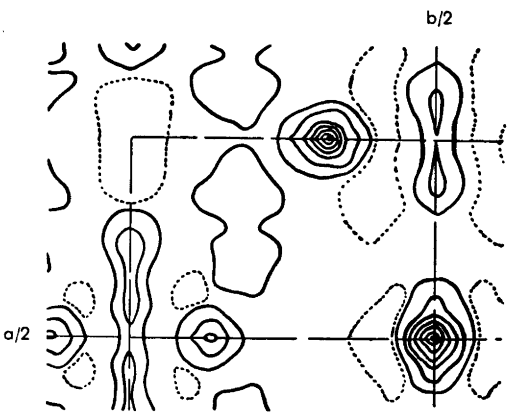

Fig. 4. The Harker-section P(xy1/2) of $Mg_3(BO_3)_2$.

2 Me^{2+} in (2a) = MeI
4 Me^{2+} » (4f) = MeII
4 B^{3+} » (4g) = B
4 O^{2-} » (4g) = OI
8 O^{2-} » (8h) = OII

The result of the above calculations are registered in Table 2 pointing out the different maxima arising from the reported vectors. The relative heights of the peaks are assigned to a common scale based on the calculated heights of the maxima in the origin. The coordinates given are not differentiated for the two compounds because of the nearly equal values found. Reference is also made to Fig. 5.

Table 2. The interatomic vectors of the function p(xyz).

Coordinates			Relative heights		Interatomic vectors
a/60	b/60	c/60	$Co_3(BO_3)_2$	$Mg_3(BO_3)_2$	
0	0	0	4 966	1 816	6 Me — Me 12 O — O 4 B — B
18	0	14	1 250	593	4 MeI — OI 8 MeII — OII 4 MeI — B 2 OI — OI 4 OII — OII 4 OI —B
12	10	14	1 386	655	4 MeI — OII 4 MeII — OI 4 MeII — OII 4 MeII — B 4 OI — OII 2 OII — OII 4 OII — B
30	10	0	3 420	1 042	4 MeI — MeII 2 MeII — MeII 8 OI — OII 4 OII — OII 8 OII — B

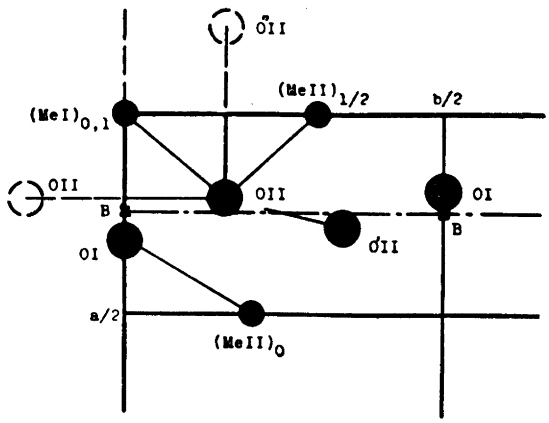

Fig. 5. Projection of the structure parallel to (001). The subscripts correspond to the z-coordinates.

The coordinates of the maxima given in Table 2 lead to the following approximate parameters.

MeII		$y = 0.327$	
OI	$x = 0.317$		$z = 0.273$
OII	$x = 0.202$	$y = 0.139$	$z = 0.702$

Thereby the parameter of the metal-ions is obtained from $Co_3(BO_3)_2$ and the oxygen-parameters from $Mg_3(BO_3)_2$. By means of these parameters the signs necessary for calculating the electron density function are known.

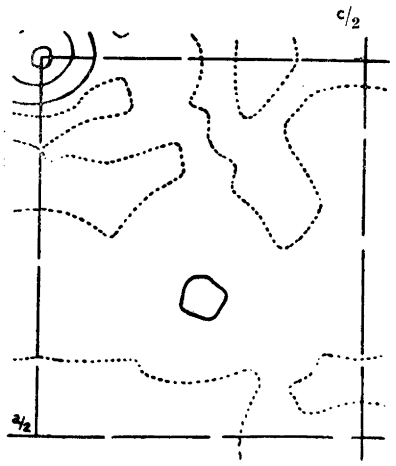

Fig. 6. The section ϱ (x0z) of the electron density of $Mg_3(BO_3)_2$. The height difference = 100. The dotted line = 0-level.

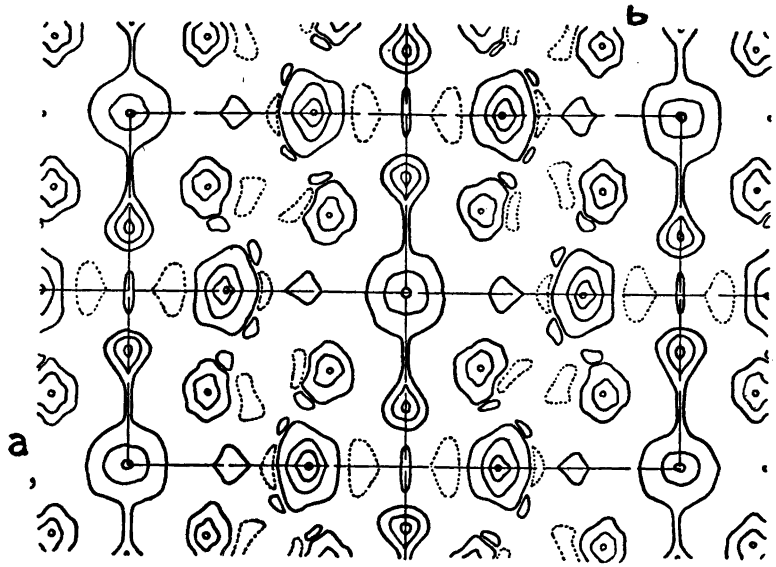

Fig. 7. $Mg_3(BO_3)_2$. *Projection of the electron density parallel to [001].*
The height-difference = 10.
The dotted line = −10-level.

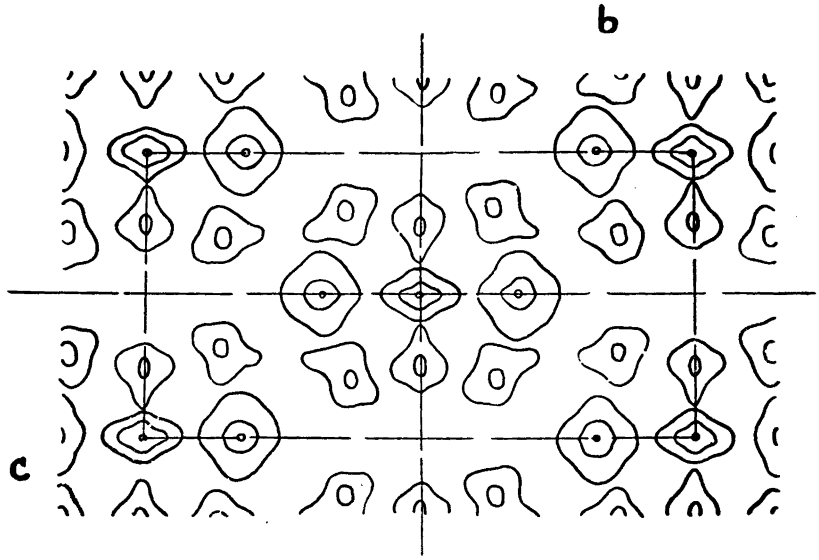

Fig. 8. $Mg_3(BO_3)_2$. *Projection of the electron density parallel to [100].*

DETERMINATION OF THE FINAL PARAMETERS

The practically equal coordinate values for the Patterson maxima of $Co_3(BO_3)_2$ and $Mg_3(BO_3)_2$ respectively, together with the fact that the ionic radii of Co^{2+} and Mg^{2+} are nearly the same — which is also evident from the close agreement between unit dimensions and axial ratios of the two compounds — makes it highly probable that the parameter values of the two cells are nearly equal. Thus, the approximate parameter values, derived from the Patterson syntheses, have been used for determining the signs of the F-values in calculating the electron density. For both compounds the electron density was calculated in the section ϱ $(x0z)$ and the projections ϱ (xy) and ϱ (yz). The results are shown for $Mg_3(BO_3)_2$ in the Figs. 6—8. The final parameter values are given below and proved to be identical for the two compounds. The boron values have been calculated on the asumption that the boron ions occupy the centres of gravity of the practically equilateral oxygen triangles.

The structure of $Co_3(BO_3)_2$ and $Mg_3(BO_3)_2$ in D_{2h}^{12} - *Pnmn* will then be characterized by the positions

2 Me^{2+} in (2a) = MeI				
4 Me^{2+} in (4f) = MeII		$y = 0.321$		
4 B^{3+} in (4g) = B	$x = 0.25$		$z = 0.56$	
4 O^{2-} in (4g) = OI	$x = 0.316$		$z = 0.258$	
8 O^{2-} in (8h) = OII	$x = 0.218$	$y = 0.139$	$z = 0.705$	

DISCUSSION OF THE STRUCTURE

The structure in question is projected in the Figures 9 and 10.

$(BO_3)^{3-}$-*triangle*. The most characteristic feature of the atomic arrangement is formed by the rather equilateral $(BO_3)^{3-}$-triangles, with their very short co-planar bonds found to be

	$Co_3(BO_3)_2$	$Mg_3(BO_3)_2$
OI — OII	2.399 Å	2.383 Å
OII — OII	2.346	2.339
B — OI	1.44	1.42
B — OII	1.35	1.34

Analogous distances within $(BO_3)^{3-}$-triangles have been proved by

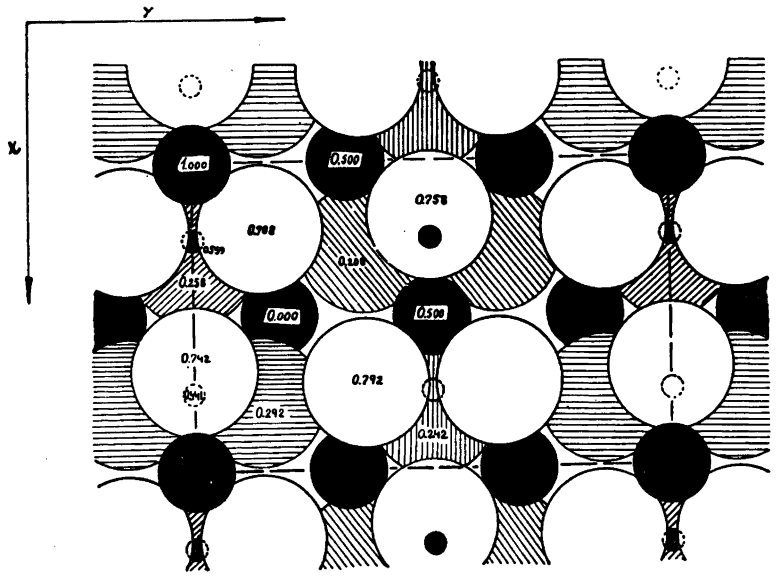

Fig. 9. Projection of the structure parallel to [001].

The large black spheres represent the metal-ions,
» small » » » » boron- »
» large white » » » oxygen- »

***Equal** heights over the planes in question are similarely marked, and the heights over the base-plane are given by numbers within the spheres in the cell.*

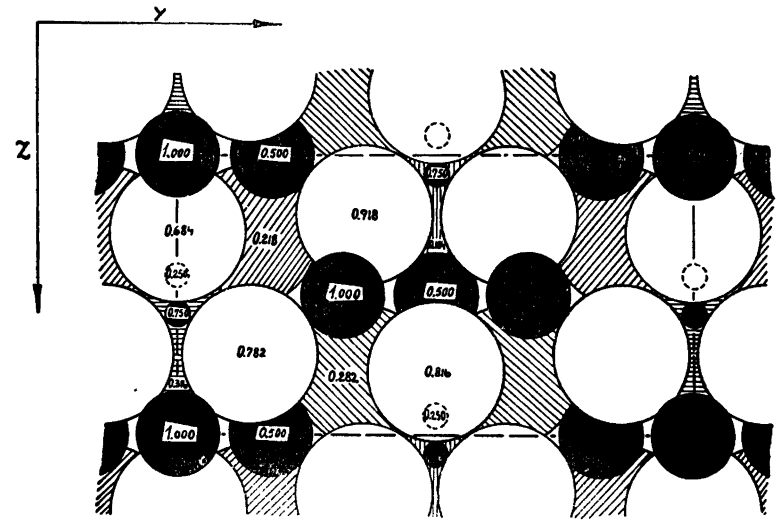

Fig. 10. Projection of the structure parallel to [100].

Author	Substance	Oxygen-oxygen-distances	Boron-oxygen-distances
Zachariasen [11]	Hambergite	2.31—2.35—2.39 Å	1.42—1.35—1.28 Å
Zachariasen and Ziegler [12]	CaB_2O_4	2.35—2.36—2.37	1.38—1.37—1.34
Fang [13]	NaB_2O_4	2.32—2.37	1.34—1.38

Wells [14] reports the boron-oxygen distance within the triangles to be 1.35 Å.

As will be seen from the projection of the structure on the *ab*-plane — Fig. 9 — the oxygen-ions form layers including the $(BO_3)^{3-}$-triangles, with the boron-ions situated in the *n*-planes at right angles to the *x*-axis.

$(MeO_6)^{10-}$-*octahedron*. Moreover, it is obvious from the Figs. 9 and 10 that the metal-ions connecting the layers have the coordination number six. Within the octahedrons formed, the following interionic distances are found

		$Co_3(BO_3)_2$	$Mg_3(BO_3)_2$
$(MeI)_1$	— OII	2.141 Å	2.108 Å
$(MeI)_0$	— OI	2.086	2.063
$(MeII)_0$	— OI	2.157	2.146
$(MeII)_{1/2}$	— OII	2.152	2.164

The remaining interionic distances in the structure are

OII	— O'II	2.958 Å	2.945 Å
OII	— O''II	3.020	2.988

These distances show that the binding within the $(BO_3)^{3-}$-triangles undoubtedly are much stronger than the other bonds. There is, in other words direct evidence for the existance of $(BO_3)^{3-}$-groups in the structure. Accordingly the chemical formula of the compounds under investigation must be written as $Me_3(BO_3)_2$.

By using the above parameters and atomic scattering factors according to the *International tables for the determination of crystal structure* (Berlin, 1935), the following agreement between calculated and observed F-values was obtained:

0k0-reflections

	$Co_3(BO_3)_2$		$Mg_3(BO_3)_2$	
hkl	$\|F\|_{obs}$	$F_{calc.}$	$\|F\|_{obs}$	$F_{calc.}$
020	15.8	10.1	23.0	13.9
040	10.1	2.5	5.5	— 7.6
060	110.0	108.7	59.5	61.7
080	—	2.0	18.8	13.8
0 10 0	20.3	28.8	12.6	7.8
0 12 0	16.3	39.2	10.9	14.3
0 14 0	—	0.6	6.1	11.8
0 16 0	15.9	40.7	9.1	17.0
		hk0-reflections		
110	—	1.7	—	— 1.5
130	114.7	98.3	36.7	34.0
150	34.8	— 27.4	20.1	— 16.1
170	33.9	28.0	16.2	11.8
190	42.2	54.8	16.8	18.9
1 11 0	12.7	— 24.3	7.3	— 11.8
1 13 0	20.2	30.8	1.9	8.8
200	62.8	62.6	16.5	— 6.6
220	28.5	— 17.4	20.1	— 11.4
240	42.3	35.4	24.0	23.8
260	53.3	62.0	10.5	21.7
280	40.5	— 36.7	31.1	— 24.8
2 10 0	38.8	35.7	14.7	16.3
2 12 0	32.2	41.5	2.9	15.4
2 14 0	8.1	— 28.9	10.1	— 18.7
310	—	7.9	—	— 5.5
330	115.2	121.4	64.1	64.1
350	6.2	— 1.4	9.2	7.4
370	33.1	23.6	12.6	8.5
390	52.8	59.8	26.3	26.6
3 11 0	5.1	— 4.1	2.7	7.2
3 13 0	21.2	34.4	6.8	12.7
400	104.8	103.1	54.9	52.7
420	10.1	— 11.5	—	— 6.8
440	1.0	1.7	5.5	— 7.3
460	65.1	70.7	35.7	30.0
480	8.4	— 10.5	—	0.8
4 10 0	13.0	20.6	—	1.8

	$Co_3(BO_3)_2$		$Mg_3(BO_3)_2$	
hkl	$\|F\|_{obs}$	$F_{calc.}$	$\|F\|_{obs}$	$F_{calc.}$
510	—	5.9	—	3.3
530	47.7	52.7	vw	8.1
550	10.9	− 24.7	19.4	− 16.0
570	26.6	27.2	17.0	13.2
590	31.7	41.9	vw	9.7
5 11 0	12.0	− 31.0	16.9	− 20.2
600	56.1	69.3	35.4	25.5
620	—	− 0.4	—	4.8
640	16.3	25.8	4.1	14.8
660	48.5	58.4	25.2	21.7
710	—	− 5.0	—	− 5.9
730	61.0	69.5	32.1	31.9
750	—	− 5.1	—	2.3
770	7.2	7.1	vw	− 4.6
800	37.2	46.3	9.2	11.8
820	vw	− 10.4	vw	− 7.5
840	8.3	14.4	—	− 2.4
860	33.8	39.3	—	2.6
910	vw	13.3	3.3	11.2
930	31.8	40.5	2.7	7.2
950	11.2	− 9.0	—	− 3.2
10 00	22.1	51.1	14.2	20.8
		0kl-reflections		
002	44.8	59.4	vw	− 5.4
004	78.4	97.2	42.5	51.0
006	40.8	52.6	5.7	13.9
008	35.3	44.4	vw	12.9
022	28.0	− 21.6	16.5	− 16.3
024	—	3.7	2.9	7.7
026	vw	− 10.6	5.8	− 7.3
031	34.6	− 28.9	17.4	− 7.8
033	55.0	− 40.7	33.0	− 24.5
035	12.6	− 10.1	—	2.9

0kl-reflections

	$Co_3(BO_3)_2$		$Mg_3(BO_3)_2$	
hkl	$\|F\|_{obs}$	$F_{calc.}$	$\|F\|_{obs}$	$F_{calc.}$
042	49.4	27.7	25.9	16.7
044	23.0	14.3	18.8	5.6
046	vw	7.0	4.6	− 0.3
051	76.3	85.3	38.0	38.1
053	61.5	64.5	26.6	24.8
055	51.5	55.4	19.4	21.4
062	53.4	57.6	13.7	12.8
064	64.5	72.6	35.2	34.0
066	22.3	42.9	10.1	9.9
071	15.9	20.0	—	5.1
073	24.1	34.9	10.3	21.1
075	—	4.1	vw	− 4.9
082	39.3	− 37.5	26.6	− 26.0
084	vw	− 4.3	—	6.2
091	10.9	− 12.6	vw	− 5.1
0 10 2	38.3	32.7	19.2	13.2
0 10 4	21.5	27.7	5.2	9.9
0 11 1	31.3	60.4	10.1	23.1

h0l-reflections

101	38.5	− 44.7	17.1	− 21.0
102	26.5	− 17.1	16.8	− 17.1
103	26.5	− 28.1	9.4	− 10.6
104	—	13.9	9.4	13.9
105	8.7	− 24.4	5.5	− 10.5
201	28.0	32.9	39.9	32.9
202	112.0	136.7	65.9	70.0
203	19.4	− 14.6	21.6	− 14.6
204	56.2	65.2	25.3	20.6
205	—	6.6	5.5	6.6
206	26.4	60.5	25.8	23.0

	$Co_3(BO_3)_2$		$Mg_3(BO_3)_2$	
hkl	$\|F\|_{obs}$	$F_{cala.}$	$\|F\|_{obs}$	$F_{calc.}$
301	27.2	− 30.0	13.3	− 18.0
302	vw	9.7	12.2	9.7
303	38.1	− 33.2	27.8	− 17.4
304	—	− 11.0	vw	− 11.0
305	vw	− 14.4	—	− 1.2
306	—	9.6	12.5	9.6
401	27.5	− 26.4	35.5	− 26.4
402	59.2	70.3	27.4	22.3
403	19.8	16.7	14.6	16.7
404	62.1	69.7	33.7	28.3
405	—	− 8.6	—	− 8.6
406	30.1	52.2	26.2	16.8
501	22.5	− 24.8	19.5	− 9.5
502	—	− 4.8	—	− 4.8
503	vw	− 14.1	—	− 0.3
504	—	7.0	vw	7.0
505	12.7	− 27.0	14.3	− 14.8
601	10.9	18.5	25.1	18.5
602	51.0	55.3	8.3	13.9
603	—	− 13.1	5.1	− 13.1
604	50.1	57.8	21.4	20.6
701	vw	− 16.4	—	− 3.4
702	—	1.1	—	1.1
801	—	− 10.5	3.4	− 10.5
802	50.7	57.3	22.9	22.2

SUMMARY

The crystal structure of the isomorphous orthoborates of cobalt and magnesium is found to be orthorhombic with $2Me_3(BO_3)_2$ in the unit cell. The dimensions of the orthorhombic unit are determined. From the interatomic bond lengths found, it is clear that the most characteristic part of the structure is the nearly equilateral $(BO_3)^{3-}$-triangles and that in consequence of this the formula must be written as $Me_3(BO_3)_2$. The oxygen lattice forms layers parallell to [100] including the triangles and connected by the metal ions with coordination number six. The parameters have been determined by means of Fourier-methods.

The present study was carried out at the Institute of Inorganic Chemistry of the University of Uppsala. To the Head of this Institute Professor G. Hägg, who has not only suggested the problem but even stimulated the interest by many discussions and helpful criticism, I wish to express my sincere gratitude.

My best thanks are also due to his co-workers, especially Dr. A. Magnéli, for many valuable conversations.

To the Head of the Institute of Silicate Research at Chalmers Techn. Univ. Professor J. Arvid Hedvall I wish to form my special gratitude for the kind interest he always has devoted to my work.

After having finished the present study a copy of a japanese paper by R. Sadanaga[15] 'The crystal structure of Kotoite, $Mg_3B_2O_6$' — up to this day not yet reported in literature — has been received in this Institute.

This structure determination which is based on the asumption of a structural analogy between Mg_2SiO_4 and $Mg_3B_2O_6$ and performed by means of trial and error methods, has resulted in a structure, which is practically identical with the above. The parameter values show slight differences but the present author is of the opinion that his values, derived by Fourier-methods, give a slightly better agreement between observed and calculated intensities. It is also worth mentioning that the dimensions of the $(BO_3)^{3-}$-triangles obtained with Sadanaga's values do not agree very well with those reported by earlier authors.

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Received May 20, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 676–696

The Effect of Metal Ions on the Rate of Decomposition of Nitroacetic Acid

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It has been shown in earlier papers [1–4] that the rate-determining step in the decomposition of nitroacetic acid into nitromethane and carbon dioxide is an apparently spontaneous cleavage of the univalent ion

$$O_2N \cdot CH_2 \cdot COO^- \rightarrow {}^-OON : CH_2 + CO_2$$

The first dissociation constant of the acid is 0.0210 (at 18° C and zero ionic strength) [2, 4], the second is about 10^{-9} (Heuberger [5]). The velocity constant for the decomposition of the univalent ion may therefore be found directly from measurements in acetate buffer solutions. A preliminary study of the effect of various salts added to the acetate buffers was made in the first paper [1]. It was found that the rate decreases considerably when certain cations (*e. g.* cupric ions) are added, while others (*e. g.* barium ions) have no, or only an insignificant effect. No satisfactory explanation of the effect could at that time be given, but it was suggested that the decrease in velocity is due to formation of complexes between the nitroacetate ion and the metal ions added. The nitroacetate ion decomposes spontaneously only when it is free. It is stable when it is bound either to a hydrogen ion or to a metal ion. The experiments in the present paper were carried out in order to test this explanation.

In the analysis of the effect of metal ions great difficulties are encountered. When the effect is studied in acetate buffer solutions it must be taken into account that most metal ions form complexes with acetate ions, and the extent of this complex formation is as a rule unknown. Only for cupric ions, fairly satisfactory data are available [6]. For this reason, only cupric ions were studied in acetate buffers. For the main part of the experiments were used unbuffered solutions of nitroacetic acid to which had been added a small

amount of nitric acid (usually 0.002 molar). In these solutions, nitroacetic acid is only partly dissociated, and the degree of dissociation increases during reaction. In the following we shall deduce formulae by means of which a kinetic analysis may be carried out for such solutions.

EQUATIONS FOR CALCULATING VELOCITY CONSTANTS

We shall first examine the simple case when no complex-forming metal ion is present. Suppose that the solution contains h molar strong acid (*e. g.* nitric acid) and, at the time t minutes after the start, x molar nitroacetic acid of which the fraction α is present as univalent ion. α approaches α_∞ when x approaches zero (t infinity). When K denotes the dissociation constant of nitroacetic acid, we have

$$K = \frac{\alpha}{1-\alpha}\,(\mathrm{H^+}) = \frac{\alpha}{1-\alpha}\,(h + \alpha x) = \frac{\alpha_\infty}{1-\alpha_\infty}\,h$$

whence

$$\frac{1}{\alpha_\infty} = 1 + \frac{h}{K} \tag{1}$$

and

$$\frac{1}{\alpha} = \frac{1}{\alpha_\infty} + \frac{\alpha x}{K} \tag{2}$$

On multiplication by αx we obtain

$$x = \frac{1}{\alpha_\infty}\,(\alpha x) + \frac{1}{K}\,(\alpha x)^2 \tag{3}$$

from which we find

$$\alpha x = \frac{K}{2\alpha_\infty}\left[\sqrt{1 + \frac{4\,\alpha_\infty{}^2}{K}\,x} - 1\right] \tag{4}$$

Differentiation of equation 3 leads to

$$\mathrm{d}x = \frac{1}{\alpha_\infty}\,\mathrm{d}(\alpha x) + \frac{2}{K}\,(\alpha x)\,\mathrm{d}(\alpha x) \tag{5}$$

We assume that the velocity is proportional to αx:

$$-\frac{\mathrm{d}x}{\mathrm{d}t} = k\,(\alpha x)$$

On introducing equation 5 we obtain

$$-k\,\mathrm{d}t = \frac{1}{a_\infty}\,\mathrm{d}\ln(ax) + \frac{2}{K}\,\mathrm{d}(ax)$$

When we integrate this equation, introduce expression 4, and change to decadic logarithms, we find

$$^*a_\infty t + \text{const.} = 0.4343\sqrt{1+\frac{4\,a_\infty{}^2}{K}x} + \log\left[\sqrt{1+\frac{4\,a_\infty{}^2}{K}x}-1\right] \qquad (6)$$

where $k^* = 0.4343\ k$.

The reaction is followed by measuring the pressure above the solution during the reaction. P, the difference between the final pressure reading and the reading at the time t, is proportional to x:

$$P = \varphi\ x \qquad (7)$$

The proportionality factor φ may be found from the initial value of P and the known initial value of x: $\varphi = P_0/x_0$. If we use the abbreviation

$$\lambda = \frac{4a_\infty{}^2}{K\varphi} \qquad (8)$$

equation 6 may be written as follows

$$-k^*a_\infty t + \text{const.} = 0.4343\sqrt{1+\lambda P} + \log\left[\sqrt{1+\lambda P}-1\right] \qquad (9)$$

By means of this equation we may find k^* when K is known. a_∞ is given by equation 1.

If no strong acid has been added ($h = 0$, $a_\infty = 1$), equation 6 reduces to

$$-k^*t + \text{const.} = 0.4343\sqrt{1+\frac{4}{K}x} + \log\left[\sqrt{1+\frac{4}{K}x}-1\right] \qquad (10)$$

We shall now consider the case when complex-forming metal ions are present in the solution. We make the assumptions that one metal ion (Me^{++}) combines with only one univalent nitroacetate ion (HN^-):

$$Me^{++} + HN^{-} \rightleftharpoons MeHN^{+}$$

and that no other complexes of the ion Me^{++} are formed. When c denotes the stoicheiometric concentration of the metal ion added, α_1 the fraction of the total amount of nitroacetic acid which is bound to the metal ion, and K_1 the complexity constant, we have

$$K_1 = \frac{\alpha_1 x}{(c - \alpha_1 x)\,\alpha x}$$

from which we obtain

$$\alpha_1 = \frac{K_1 \alpha c}{1 + K_1 \alpha x} \tag{11}$$

The dissociation constant of nitroacetic acid may be written as follows

$$K = \frac{\alpha}{1 - \alpha - \alpha_1}\,(h + \alpha x + \alpha_1 x),$$

from which we find

$$\frac{1 - \alpha - \alpha_1}{\alpha} = \frac{h}{K} + \frac{\alpha x}{K} + \frac{\alpha_1 x}{K} \tag{12}$$

When equations 11 and 12 are introduced into the identity

$$\frac{1}{\alpha} = 1 + \frac{1 - \alpha - \alpha_1}{\alpha} + \frac{\alpha_1}{\alpha}$$

we obtain

$$\frac{1}{\alpha} = 1 + \frac{h}{K} + \frac{\alpha x}{K} + \frac{K_1 \alpha x c}{K\,(1 + K_1 \alpha x)} + \frac{K_1 c}{1 + K_1 \alpha x}$$

$$= 1 + \frac{h}{K} + \frac{\alpha x}{K} + \frac{c}{K} - \frac{(1 - KK_1)\,c}{K\,(1 + K_1 \alpha x)}$$

$$= 1 + \frac{h}{K} + \frac{\alpha x}{K} + \frac{c}{K} - \frac{(1 - KK_1)\,(1 - K_1 \alpha x)\,c}{K\,(1 - K_1^2 \alpha^2 x^2)}$$

$K_1^2\alpha^2x^2$ in the denominator of the last term may be neglected since we shall apply the formula only to experiments where $x < 0.021$ and $K_1 < 3.2$. We therefore obtain

$$\frac{1}{\alpha} = 1 + \frac{h}{K} + \frac{\alpha x}{K} + \frac{c}{K} - \frac{(1 - KK_1)(1 - K_1 \alpha x)\, c}{K}$$

$$= 1 + \frac{h}{K} + K_1 c + \frac{1}{K}\left[1 + (1 - KK_1) K_1 c\right] \alpha x \qquad (13)$$

It follows from this equation that

$$\frac{1}{\alpha_\infty} = 1 + \frac{h}{K} + K_1 c \qquad (14)$$

Equation 13 may therefore be written as

$$\frac{1}{\alpha} = \frac{1}{\alpha_\infty} + \frac{1}{K}\left[1 + (1 - KK_1) K_1 c\right] \alpha x \qquad (15)$$

This equation is the same as equation 2 except that the constant factor before αx is different. On proceeding in the same way as in the simple case we therefore obtain an equation which may be written exactly as equation 9, but λ is here given by the expression

$$\lambda = \frac{4 \alpha_\infty^2}{K \varphi}\left[1 + (1 - KK_1) K_1 c\right], \qquad (16)$$

and α_∞ by equation 14.

By means of equations 9, 14, and 16, we may directly find k^* when K and K_1 are known. Our problem is, however, to find K_1 when k^* and K are known. We therefore choose a reasonable value for K_1 and calculate the corresponding values of α_∞ and λ. We then compute $k^* \alpha_\infty$ from the measurements by means of equation 9, divide it by the known value of k^*, and introduce the obtained α_∞ into equation 14. If the value of K_1 found in this way does not agree with the chosen value, the computation is repeated on the basis of the new value, and so on until agreement is obtained.

In order to facilitate the computations by means of formula 9, values of the function

$$Y = 0.4343 \sqrt{1 + X} + \log\,(\sqrt{1 + X} - 1) + 1$$

have been calculated for values of X from 2.00 to 0.01 with an interval of 0.01. They are given in Table 1.

Table 1. Values of the function

$$Y = 0.4343\sqrt{1+X} + \log(\sqrt{1+X}-1) + 1$$

X	Y	Diff.	X	Y	Diff.	X	Y	Diff.	X	Y	Diff.
2.00	1.6168		1.60	1.4874	35	1.20	1.3283	45	0.80	1.1162	64
1.99	1.6138	30	1.59	1.4838	36	1.19	1.3238	45	0.79	1.1098	64
1.98	1.6108	30	1.58	1.4802	36	1.18	1.3193	45	0.78	1.1034	64
1.97	1.6078	30	1.57	1.4766	36	1.17	1.3147	46	0.77	1.0968	66
1.96	1.6048	30	1.56	1.4730	36	1.16	1.3101	46	0.76	1.0902	66
1.95	1.6018	30	1.55	1.4694	36	1.15	1.3055	46	0.75	1.0836	66
1.94	1.5987	31	1.54	1.4658	36	1.14	1.3008	47	0.74	1.0768	68
1.93	1.5957	30	1.53	1.4621	37	1.13	1.2961	47	0.73	1.0699	69
1.92	1.5926	31	1.52	1.4584	37	1.12	1.2913	48	0.72	1.0630	69
1.91	1.5896	30	1.51	1.4547	37	1.11	1.2865	48	0.71	1.0560	70
1.90	1.5865	31	1.50	1.4510	37	1.10	1.2817	48	0.70	1.0489	71
1.89	1.5834	31	1.49	1.4472	38	1.09	1.2769	48	0.69	1.0417	72
1.88	1.5803	31	1.48	1.4434	38	1.08	1.2720	49	0.68	1.0344	73
1.87	1.5772	31	1.47	1.4396	38	1.07	1.2670	50	0.67	1.0270	74
1.86	1.5740	32	1.46	1.4358	38	1.06	1.2621	49	0.66	1.0196	74
1.85	1.5709	31	1.45	1.4320	38	1.05	1.2571	50	0.65	1.0120	76
1.84	1.5677	32	1.44	1.4282	38	1.04	1.2520	51	0.64	1.0043	77
1.83	1.5645	32	1.43	1.4243	39	1.03	1.2469	51	0.63	0.9965	78
1.82	1.5614	31	1.42	1.4204	39	1.02	1.2418	51	0.62	0.9886	79
1.81	1.5582	32	1.41	1.4165	39	1.01	1.2366	52	0.61	0.9806	80
1.80	1.5549	33	1.40	1.4125	40	1.00	1.2314	52	0.60	0.9724	82
1.79	1.5517	32	1.39	1.4086	39	0.99	1.2261	53	0.59	0.9642	82
1.78	1.5484	33	1.38	1.4046	40	0.98	1.2208	53	0.58	0.9558	84
1.77	1.5452	32	1.37	1.4006	40	0.97	1.2155	53	0.57	0.9473	85
1.76	1.5419	33	1.36	1.3965	41	0.96	1.2101	54	0.56	0.9386	87
1.75	1.5386	33	1.35	1.3925	40	0.95	1.2046	55	0.55	0.9298	88
1.74	1.5353	33	1.34	1.3884	41	0.94	1.1991	55	0.54	0.9209	89
1.73	1.5320	33	1.33	1.3842	42	0.93	1.1935	56	0.53	0.9118	91
1.72	1.5287	33	1.32	1.3801	41	0.92	1.1880	55	0.52	0.9026	92
1.71	1.5253	34	1.31	1.3760	41	0.91	1.1823	57	0.51	0.8932	94
1.70	1.5219	34	1.30	1.3718	42	0.90	1.1766	57	0.50	0.8836	96
1.69	1.5186	33	1.29	1.3675	43	0.89	1.1708	58	0.49	0.8738	98
1.68	1.5152	34	1.28	1.3633	42	0.88	1.1650	58	0.48	0.8639	99
1.67	1.5117	35	1.27	1.3590	43	0.87	1.1591	59	0.47	0.8538	101
1.66	1.5083	34	1.26	1.3547	43	0.86	1.1532	59	0.46	0.8434	104
1.65	1.5048	35	1.25	1.3504	43	0.85	1.1472	60	0.45	0.8329	105
1.64	1.5014	34	1.24	1.3460	44	0.84	1.1411	61	0.44	0.8222	107
1.63	1.4979	35	1.23	1.3417	43	0.83	1.1350	61	0.43	0.8112	110
1.62	1.4944	35	1.22	1.3372	45	0.82	1.1288	62	0.42	0.8000	112
1.61	1.4909	35	1.21	1.3328	44	0.81	1.1226	62	0.41	0.7885	115
					45			64			117

Table 1 (continued).

X	Y	Diff.	X	Y	Diff.	X	Y	Diff.	X	Y	Diff.
0.40	0.7768	117	0.30	0.6419	152	0.20	0.4555	222	0.10	0.1440	425
0.39	0.7648	120	0.29	0.6261	158	0.19	0.4322	233	0.09	0.0972	468
0.38	0.7525	123	0.28	0.6098	163	0.18	0.4077	245	0.08	0.0450	522
0.37	0.7400	125	0.27	0.5930	168	0.17	0.3818	259	0.07	0.9859−1	591
0.36	0.7271	129	0.26	0.5756	174	0.16	0.3544	274	0.06	0.9178−1	681
0.35	0.7138	133	0.25	0.5575	181	0.15	0.3253	291	0.05	0.8377−1	801
0.34	0.7002	136	0.24	0.5388	187	0.14	0.2944	309	0.04	0.7396−1	981
0.33	0.6863	139	0.23	0.5193	195	0.13	0.2611	333	0.03	0.6136−1	1260
0.32	0.6719	144	0.22	0.4990	203	0.12	0.2253	358	0.02	0.4364−1	1772
0.31	0.6571	148	0.21	0.4777	213	0.11	0.1865	388	0.01	0.1346−1	3018
		152			222			425			

PROCEDURE AND MATERIALS

The experimental procedure was the same as in earlier papers [2–4]. The temperature was 18.0° C, and the initial concentration of nitroacetic acid was between 0.018 and 0.021 molar. The velocity constants given in the following tables are always average values from two separate runs with solutions of the same composition. The nitroacetic acid used for the measurements had the melting point 91.5—92° C. Standard solutions of the other substances were prepared from the purest commercial preparations, and the concentrations were in most cases checked by analysis. The standard solutions of beryllium, cadmium, and zinc nitrate were prepared by mixing equivalent amounts of solutions of the corresponding sulphates and barium nitrate.

THE VELOCITY CONSTANTS FOR THE DECOMPOSITION OF THE NITROACETATE ION AND THE DISSOCIATION CONSTANT OF NITROACETIC ACID

Table 2 shows the velocity constant k^* for the decomposition of the nitroacetate ion determined directly from measurements in acetate buffer solutions. It is seen that addition of up to 0.2 molar barium nitrate has no or only an insignificant influence on k^*.

Table 2. Decomposition of nitroacetic acid in 0.100 molar acetic acid, 0.100 molar sodium acetate, c molar barium nitrate.

c	k^*
0.000	0.02470
0.100	0.02449
0.200	0.02449

Table 3. Decomposition of nitroacetic acid in h molar nitric acid, 0.200 molar barium nitrate ($k^* = 0.02449$).

h	k^*a_∞	a_∞	K
0.09976	0.007716	0.3151	0.0459
0.04986	0.01174	0.4794	0.0460
0.01995	0.01708	0.6974	0.0460

Results of measurements in solutions containing 0.200 molar barium nitrate and various amounts of nitric acid are given in Table 3. In these solutions, the degree of dissociation a increases a little during reaction. The velocity constant k^*a_∞ corresponding to an infinitely small concentration of nitroacetic acid has been calculated by means of a formula given in an earlier paper [2]. When the value 0.02449 for k^* found above for acetate buffers is used, we find a_∞ and K given in the last two columns of Table 3. The measurements show that the dissociation constant of nitroacetic acid in 0.200 molar barium nitrate is 0.0460. It was found earlier [3] that it is 0.0210 at the ionic strength zero.

Table 4. Decomposition of nitroacetic acid in h molar nitric acid, 0.200 molar barium nitrate ($K = 0.0460$).

h	$1 + \frac{h}{K}$	a_∞	k^*a_∞	k^*
0.00100	1.0217	0.9788	0.02380	0.02432
0.00200	1.0435	0.9583	0.02339	0.02441
0.00992	1.2157	0.8226	0.02022	0.02458
0.01994	1.4335	0.6976	0.01721	0.02467

Table 4 gives a summary of results obtained for solutions containing 0.200 molar barium nitrate and from 0.001 to 0.02 molar nitric acid. The velocity constants k^*a_∞ and k^* were calculated by means of formula 9 with $K = 0.0460$. The agreement with k^* found directly from experiments in acetate buffers (Table 2) is fairly good.

One of the runs from Table 4 has been presented in more detail in Table 5 in order to illustrate the application and validity of formula 9. From the initial values $x_0 = 0.02039$ and $P_0 = 11.00$ (cm Hg), we find $\varphi = P_0/x_0 = 539.5$. The initial and final degrees of dissociation are, according to equations 1 and 4, $a_0 = 0.731$ and $a_\infty = 0.9583$. From equation 8 we obtain $\lambda = 0.1480$. $X = \lambda P$ is computed for all the values of P given in the table, and the corresponding values of Y are taken from Table 1. When Y is plotted against t we obtain the linear equation given at the top of the table. Hence, it follows

Table 5. Decomposition of nitroacetic acid in 0.00200 molar nitric acid, 0.200 molar barium nitrate.

$x_0 = 0.02039$, $P_0 = 11.00$, $\varphi = 539.5$, $K = 0.0460$.
$a_0 = 0.731$, $a_\infty = 0.9583$, $\lambda = 0.1480$.
$Y = 1.4663 - 0.02335\ t$. $k^* a_\infty = 0.02335$, $k^* = 0.02436$.

t	P(obs.)	Y	P(calc.)	$\Delta \cdot 10^3$
1	10.54	1.4730	10.59	+ 5
2	10.15	1.4518	10.16	+ 1
3	9.75	1.4293	9.74	− 1
4	9.35	1.4061	9.34	− 1
5	8.94	1.3814	8.95	+ 1
6	8.56	1.3577	8.58	+ 2
7	8.23	1.3363	8.22	− 1
8	7.86	1.3116	7.87	+ 1
9	7.55	1.2900	7.53	− 2
10	7.18	1.2634	7.21	+ 3
11	6.88	1.2409	6.89	+ 1
12	6.58	1.2175	6.59	+ 1
13	6.31	1.1957	6.30	− 1
14	6.04	1.1731	6.03	− 1
15	5.75	1.1478	5.76	+ 1
16	5.49	1.1242	5.50	+ 1
17	5.25	1.1014	5.25	0
18	5.01	1.0778	5.02	+ 1
19	4.77	1.0529	4.79	+ 2
20	4.57	1.0317	4.57	0
21	4.35	1.0072	4.36	+ 1
23	3.95	0.9597	3.97	+ 2
25	3.58	0.9116	3.60	+ 2
27	3.27	0.8679	3.27	0
29	2.97	0.8218	2.97	0
31	2.69	0.7745	2.69	0
33	2.45	0.7305	2.44	− 1
35	2.23	0.6863	2.21	− 2
37	2.03	0.6425	2.00	− 3
39	1.84	0.5969	1.81	− 3
44	1.45	0.4875	1.40	− 5

that $k^* a_\infty = 0.02335$, and $k^* = 0.02436$. When we start from the linear equation just mentioned, and carry out the computations in the opposite order, we find the values of P given in the fourth column of the table. The differences between the calculated and observed values of P are given in the last column. The agreement is satisfactory.

THE EFFECT OF METAL IONS IN SOLUTIONS CONTAINING NITRIC ACID

The decomposition was studied in solutions of the salts: cupric, beryllium, cadmium, lead, nickel, zinc, cobaltous, magnesium, calcium, and aluminium nitrate. The solutions contained 0.001 or 0.002 molar nitric acid, except in the case of aluminium and beryllium nitrate where the concentration of nitric acid was raised to 0.01 and 0.02 in order to repress hydrolysis. The solutions contained further sufficient barium nitrate for maintaining nearly the same ionic strength (0.6) in all the solutions (calculated on the assumption of complete dissociation). A summary of the composition of the solutions and the results obtained is given in Table 6.

In the computations we have for the velocity constant of the nitroacetate ion k^* used the value found previously (Table 4) for 0.200 molar barium nitrate and the same concentration of nitric acid as in the solution considered. For the dissociation constant of nitroacetic acid K we have always taken the value 0.0460 found for 0.200 molar barium nitrate (Table 3).

Two of the runs, one with cupric and one with aluminium nitrate, are presented in more detail in Tables 7 and 8. The tables show only the last step in the computation based on the method of successive approximations. From the supposed value of K_1 given at the top of the tables, we find $1/\alpha_\infty$ and λ by means of equations 14 and 16. From the observed values of P we compute $X = \lambda P$ and find in Table 1 the corresponding values of Y. When Y for each of the experiments is plotted against t, the points fall close to a straight line of the equation given at the top of the table. Since Y expresses the right side of equation 9, the numerical value of the coefficient to t gives us $k^*\alpha_\infty$. When the known value of k^* is introduced we find $1/\alpha_\infty$. Finally, we compute K_1 by means of equation 14. In the column headed P (calc.) are given values of P calculated on the basis of the linear equations at the top of the tables. The listed values of $\Delta = P(\text{calc.}) - P(\text{obs.})$ show that the measurements agree well with equation 9.

The results for all the solutions examined are summarized in Table 6. The values found for K_1 are given in the next to the last column, while mean values of K_1 for each of the 10 ions examined are presented at the top of the table. $k^*\alpha_\infty$ calculated from the mean values of K_1 is given in the last column of the table.

It is seen from Table 6 that the concentrations of nitric acid used for repressing the hydrolysis of the beryllium and aluminium ion are sufficient. If they were not, the values of K_1 would decrease with increasing h. The tendency goes rather in the opposite direction. The hydrolysis of the other ions examined is, even at the small concentrations of nitric acid used here, so weak that it is of no importance.

Table 6. Decomposition of nitroacetic acid in c molar solutions of the salts mentioned below. The solutions contained in addition d molar barium nitrate and h molar nitric acid.

Mean values of complexity constants K_1 computed from the measurements:

Cu^{++}	Be^{++}	Cd^{++}	Pb^{++}	Ni^{++}	Zn^{++}	Co^{++}	Mg^{++}	Ca^{++}	Al^{+++}
2.77	1.82	1.54	1.39	1.14	1.08	1.00	0.65	0.50	3.05

	c	d	h	k^*a_∞	$1/a_\infty$	K_1	k^*a_∞(calc.)
$Cu(NO_3)_2$	0.1999	0.000	0.00200	0.01519	1.607	2.82	0.01528
	0.1596	0.040	0.00200	0.01632	1.496	2.83	0.01643
	0.1195	0.080	0.00200	0.01796	1.359	2.61	0.01776
	0.0798	0.120	0.00200	0.01926	1.267	2.81	0.01930
	0.0798	0.120	0.00100	0.01956	1.243	2.78	0.01955
	0.0399	0.160	0.00200	0.02118	1.152	2.73	0.02115
$Be(NO_3)_2$	0.200	0.000	0.00992	0.01555	1.581	1.82	0.01556
	0.150	0.050	0.01995	0.01442	1.711	1.85	0.01445
	0.150	0.050	0.00992	0.01654	1.486	1.80	0.01651
	0.100	0.100	0.01995	0.01518	1.625	1.92	0.01527
	0.100	0.100	0.00992	0.01770	1.389	1.73	0.01759
$Cd(NO_3)_2$	0.200	0.000	0.00200	0.01792	1.362	1.59	0.01806
	0.150	0.050	0.00200	0.01912	1.277	1.55	0.01915
	0.100	0.100	0.00200	0.02042	1.195	1.52	0.02038
	0.050	0.150	0.00200	0.02202	1.108	1.30	0.02178
$Pb(NO_3)_2$	0.200	0.000	0.00200	0.01852	1.318	1.37	0.01847
	0.150	0.050	0.00200	0.01945	1.255	1.41	0.01950
	0.100	0.100	0.00200	0.02062	1.184	1.40	0.02064
	0.050	0.150	0.00200	0.02192	1.114	1.40	0.02193
$Ni(NO_3)_2$	0.1983	0.000	0.00200	0.01914	1.275	1.17	0.01923
	0.1492	0.050	0.00200	0.02013	1.213	1.13	0.02011
	0.0992	0.100	0.00200	0.02111	1.156	1.14	0.02110
	0.0498	0.150	0.00200	0.02228	1.096	1.05	0.02218
$Zn(NO_3)_2$	0.1981	0.000	0.00200	0.01938	1.260	1.09	0.01941
	0.1486	0.050	0.00200	0.02036	1.199	1.05	0.02027
	0.0990	0.100	0.00200	0.02136	1.143	1.00	0.02122
	0.0496	0.150	0.00200	0.02206	1.107	1.27	0.02225
$Co(NO_3)_2$	0.200	0.000	0.00200	0.01954	1.249	1.03	0.01963
	0.200	0.000	0.00100	0.01992	1.221	1.00	0.01991
	0.150	0.050	0.00200	0.02035	1.200	1.04	0.02045
	0.100	0.100	0.00200	0.02132	1.145	1.01	0.02135
	0.050	0.150	0.00200	0.02250	1.085	0.83	0.02232

Table 6 (continued)

	c	d	h	k^*a_∞	$^1/a_\infty$	K_1	k^*a_∞(calc.)
$Mg(NO_3)_2$	0.200	0.000	0.00200	0.02073	1.178	0.67	0.02080
	0.100	0.100	0.00200	0.02208	1.106	0.62	0.02202
$Ca(NO_3)_2$	0.200	0.000	0.00200	0.02135	1.143	0.50	0.02131
	0.100	0.100	0.00200	0.02229	1.095	0.52	0.02230
$Al(NO_3)_3$	0.1006	0.000	0.01995	0.01408	1.752	3.16	0.01417
	0.1006	0.000	0.00992	0.01614	1.523	3.05	0.01614
	0.0503	0.100	0.01995	0.01557	1.584	3.00	0.01554
	0.0503	0.100	0.00992	0.01805	1.362	2.90	0.01795

Table 7. Decomposition of nitroacetic acid in 0.00200 molar nitric acid, 0.1999 molar cupric nitrate.

$x_0 = 0.02008$, $P_0 = 10.74$, $\varphi = 534.9$, $k^* = 0.02441$, $K = 0.0460$.
Suppose that $K_1 = 2.803$, hence $^1/a_\infty = 1.6038$, $\lambda = 0.09405$.
$Y = 1.2410 - 0.01522\ t$, $k^*a_\infty = 0.01522$, $^1/a_\infty = 1.6038$, $K_1 = 2.803$.

t	P(obs.)	P(calc.)	$\Delta\ 10^2$	t	P(obs.)	P(calc.)	$\Delta\ 10^2$
1	10.52	10.52	0	18	6.32	6.31	− 1
2	10.22	10.22	0	20	5.93	5.93	0
3	9.93	9.92	− 1	22	5.59	5.57	− 2
4	9.62	9.63	+ 1	24	5.23	5.23	0
5	9.36	9.35	− 1	26	4.91	4.91	0
6	9.08	9.08	0	28	4.62	4.61	− 1
7	8.83	8.82	− 1	30	4.32	4.32	0
8	8.56	8.56	0	32	4.04	4.05	+ 1
9	8.31	8.30	− 1	34	3.81	3.80	− 1
10	8.04	8.06	+ 2	36	3.53	3.56	+ 3
11	7.82	7.82	0	38	3.31	3.34	+ 3
12	7.60	7.59	− 1	40	3.14	3.12	− 2
13	7.36	7.36	0	42	2.92	2.93	+ 1
14	7.12	7.14	+ 2	44	2.74	2.74	0
15	6.90	6.92	+ 2	46	2.59	2.56	− 3
16	6.71	6.71	0	48	2.41	2.40	− 1
17	6.50	6.51	+ 1	50	2.24	2.24	0

In the computation of K_1 we have assumed that K, the dissociation constant of nitroacetic acid, is 0.0460 for all the solutions, the same as for a solution of barium nitrate of the same ionic strength. This assumption is to some extent justified by the good agreement with formula 9 obtained in all the experiments. The rate depends on both K and K_1, but the influence of the former decreases gradually during the reaction owing to the disappearance

Table 8. Decomposition of nitroacetic acid in 0.01995 molar nitric acid, 0.1006 molar aluminium nitrate.

$x_0 = 0.01913$, $P_0 = 10.18$, $\varphi = 532.1$, $k^* = 0.02467$, $K = 0.0460$.
Suppose that $K_1 = 3.07$, hence $^1/a_\infty = 1.7425$, $\lambda = 0.0681$.
$Y = 1.0448 - 0.01416\ t$, $k^* a_\infty = 0.01416$, $^1/a_\infty = 1.7422$, $K_1 = 3.07$.

t	P(obs.)	P(calc.)	$\Delta\ 10^2$	t	P(obs.)	P(calc.)	$\Delta\ 10^2$
1	9.82	9.91	+ 9	20	5.70	5.69	− 1
2	9.61	9.63	+ 2	22	5.37	5.36	− 1
3	9.38	9.36	− 2	24	5.04	5.04	0
4	9.10	9.10	0	26	4.76	4.75	− 1
5	8.84	8.84	0	28	4.49	4.47	− 2
6	8.60	8.59	− 1	30	4.21	4.20	− 1
7	8.33	8.34	+ 1	32	3.93	3.95	+ 2
8	8.11	8.10	− 1	34	3.72	3.72	0
9	7.89	7.87	− 2	36	3.50	3.50	0
10	7.63	7.65	+ 2	39	3.18	3.19	+ 1
11	7.41	7.42	+ 1	42	2.90	2.90	0
12	7.21	7.21	0	45	2.63	2.64	+ 1
14	6.80	6.80	0	48	2.42	2.41	− 1
16	6.42	6.41	− 1	51	2.20	2.19	− 1
18	6.02	6.04	+ 2	54	2.00	1.99	− 1

of hydrogen ions. The plot of Y against t would therefore fall on a curved line if a wrong value of K were chosen. In none of the experiments a systematic deviation from linearity was observed. The agreement with formula 9 was, as a rule, of a similar quality as that shown in Tables 7 and 8.

We shall examine the influence of the value chosen for K a little closer, and choose for that purpose the experiment presented in Table 7. It follows from the table that the measurements are in agreement with formula 9 when $K = 0.0460$ and $K_1 = 2.80$. The true values of α are therefore those calculated from equation 13 when these values for K and K_1 are introduced. They are given in Table 9 for three values of x, corresponding to the beginning, the middle, and the end of the run. If we choose another value of K we may calculate the corresponding value of K_1 from the values of α by means of equation 13. Finally, we may calculate $k^* a_\infty$ by means of equation 14 and the known value of k^*. The results of the computation for different values of K are given in Table 9. It is seen that the "velocity constant" $k^* a_\infty$ varies with x when a wrong value of K is chosen.

Summarizing, we may say that the results obtained for solutions containing nitric acid and various metal salts agree with the assumption that only the free univalent nitroacetate ion decomposes while its complexes with either

Table 9. K_1 and k^*a_∞ calculated from different values of K for the experiment presented in Table 7.

x	a	Assumed values of K:				
		0.0300	0.0400	0.0460	0.0500	0.0600
		Calculated values of K_1:				
0.0200	0.5161	2.01	2.56	2.80	2.94	3.22
0.0100	0.5602	2.27	2.65	2.80	2.89	3.02
0.0000	0.6235	2.69	2.77	2.80	2.82	2.85
		Calculated values of k^*a_∞:				
0.0200		0.01662	0.01563	0.01522	0.01499	0.01455
0.0100		0.01605	0.01544	0.01522	0.01509	0.01491
0.0000		0.01522	0.01522	0.01522	0.01522	0.01522

hydrogen or metal ions are stable. In order to explain the measurements quantitatively it is sufficient to assume that complexes of one metal ion and one nitroacetate ion are formed, and that the complexity constants K_1 have the values given at the top of Table 6 (at the ionic strength 0.6). To the list may be added the complexity constant for the hydrogen ion $K_1 = 1/0.0460 = 21.7$, and that for the barium ion $K_1 = 0$.

THE EFFECT OF CUPRIC IONS IN ACETATE BUFFER SOLUTIONS

The effects of a series of metal salts on the decomposition of nitroacetic acid in acetate buffer solutions were studied already in the paper from 1927 [1]. The quantitative results obtained are however of little interest because the extent of complex formation between the metal ions and the acetate ion is unknown, and because the effect of changes in the concentrations of the buffer constituents were not examined. Data which make it possible to estimate the extent of complex formation for cupric ions are now available [6]. New experiments on the effect of cupric ions were therefore carried out, and an attempt was made to give a quantitative interpretation of the results.

When nitroacetic acid is dissolved in an acetate buffer solution, an equivalent amount of acetate ion is transformed into acetic acid. For this reason, both the hydrogen and the cupric ion concentration decreases during reaction. We shall see that the rate depends not only on the concentration of free cupric ion, but (contrary to expectation) also on the hydrogen and acetate ion concentrations. Since all three concentrations change, the first-order law will not hold exactly. For most of the solutions examined (see Table 10a),

Table 10 a. The effect of cupric ions on the decomposition of nitroacetic acid in acetate buffer solutions. The table gives the stoicheiometric concentrations before the addition of nitroacetic acid and the first-order velocity constant k^*a.

No.	CH_3COOH	CH_3COONa	$Cu(NO_3)_2$	$Ba(NO_3)_2$	$NaNO_3$	k^*a
1	0.798	0.2983	0.09985	0.000	0.000	0.02133
2	0.3988	0.2983	0.09985	0.000	0.000	0.02030
3	0.1994	0.2983	0.09985	0.000	0.000	0.01816
4	0.0994	0.2983	0.09985	0.000	0.000	0.01556
5	0.798	0.1993	0.04990	0.050	0.100	0.02288
6	0.4990	0.1993	0.04990	0.050	0.100	0.02237
7	0.3988	0.1993	0.04990	0.050	0.100	0.02227
8	0.1994	0.1993	0.04990	0.050	0.100	0.02102
9	0.0994	0.1993	0.04990	0.050	0.100	0.01912
10	0.798	0.1993	0.09985	0.000	0.100	0.02081
11	0.3988	0.1993	0.09985	0.000	0.100	0.01990
12	0.1994	0.1993	0.09985	0.000	0.100	0.01817
13	0.0994	0.1993	0.09985	0,000	0.100	0.01598
14	0.3988	0.0996	0.04990	0.050	0.200	0.02153
15	0.1994	0.0996	0.04990	0.050	0.200	0.02139
16	0.0994	0.0996	0.04990	0.050	0.200	0.01971
17	0.1994	0.0996	0.09985	0.000	0.200	0.01843
18	0.1994	0.1993	0.07480	0.025	0.100	0.01960
19	0.1994	0.1993	0.02486	0.075	0.100	0.02284
20	0.1994	0.1993	0.00000	0.100	0.100	0.02488

the concentrations of the buffer components were so large that no systematic deviation from a straight line could be detected when log P was plotted against t, but in some cases a small but distinct curvature was seen. An average slope was found, and it was assumed that its numerical value is equal to the velocity constant k^*a corresponding to $x = 0.01$, that is, to the solution when about one half of the nitroacetic acid is decomposed. The velocity constants k^*a found in this way are given in the last column of Table 10a. The velocity constant for experiment no. 20 is equal to k^* since no cupric salt is present in the solution ($a = 1$). a for the rest of the experiments may therefore be found by dividing their velocity constants by that for experiment no. 20.

In order to compute the concentrations of free cupric and acetate ion when $x = 0.01$, we use the complexity constants K_1', K_2', and K_3' for the complexes $CuAc^+$, $CuAc_2$, and $CuAc_3^-$, respectively (where Ac^- denotes acetate ion).

Table 10 b. The effect of cupric ions on the decomposition of nitroacetic acid in acetate buffer solutions. Concentrations corresponding to $x = 0.01$ (about half decomposition). The velocity constant k^*a calculated by means of equation 21 and $k^* = 0.02488$.

No.	μ	(Cu^{++})	(Ac^-)	$\frac{(Ac^-)}{(HAc)}$	$\frac{1-a}{a\,(Cu^{++})}$	k^*a (calc.)	Percentage diff.
1	0.369	0.00802	0.1474	0.1824	20.7	0.02147	+ 0.7
2	0.369	0.00796	0.1478	0.3615	28.3	0.02027	− 0.1
3	0.369	0.00782	0.1487	0.7101	47.3	0.01833	+ 0.9
4	0.369	0.00765	0.1498	1.369	78.3	0.01556	0
5	0.491	0.00555	0.1244	0.1540	15.7	0.02280	− 0.4
6	0.491	0.00550	0.1247	0.2450	20.4	0.02238	0
7	0.491	0.00550	0.1247	0.3050	21.3	0.02209	− 0.8
8	0.491	0.00542	0.1252	0.5979	33.9	0.02085	− 0.8
9	0.491	0.00528	0.1261	1.1527	57.1	0.01889	− 1.2
10	0.408	0.01790	0.0802	0.0993	10.9	0.02091	+ 0.5
11	0.408	0.01776	0.0805	0.1969	14.1	0.01996	+ 0.3
12	0.408	0.01751	0.0810	0.3868	21.1	0.01838	+ 1.1
13	0.408	0.01721	0.0816	0.7459	32.4	0.01601	+ 0.2
14	0.522	0.01535	0.0487	0.1191	10.1	0.02195	+ 1.9
15	0.522	0.01533	0.0487	0.2326	10.6	0.02112	− 1.3
16	0.522	0.01501	0.0491	0.4488	17.5	0.01977	+ 0.3
17	0.480	0.04430	0.0289	0.1378	7.9	0.01869	+ 1.4
18	0.446	0.01043	0.1007	0.4809	25.8	0.01948	− 0.6
19	0.543	0.00208	0.1549	0.7397	43	0.02260	− 1.1

The following formulae have been found[6] to hold when the ionic strength μ is less than 0.4:

$$\begin{aligned} \log K_1' &= 2.164 - 1.992\sqrt{\mu}/(1 + 1.8\sqrt{\mu}) \\ \log K_2' &= 3.17 - 2.988\sqrt{\mu}/(1 + 2\sqrt{\mu}) \\ K_3' &= K_2' \end{aligned} \qquad (17)$$

We shall use these formulae although the ionic strength for most of the solutions exceeds 0.4. The stoicheiometric concentration of cupric ion c is first corrected for the amount bound to nitroacetate ion. If it is assumed that each stabilized nitroacetate ion binds one cupric ion we find that the concentration of cupric ion which is either free or bound to acetate ions is $c' = c - 0.01(1-a)$ when $x = 0.01$. The ionic strengths of the solutions and their

concentrations of free cupric and acetate ion, computed by means of equations 17, are given in the second, third, and fourth column of Table 10b. In the next column is presented the ratio $(Ac^-)/(HAc)$, where (HAc) has been calculated by adding 0.01 to the concentration of acetic acid before nitroacetic acid was dissolved.

We shall first examine whether the total effect may be explained alone by the formation of the complex $CuHN^+$ according to the scheme

$$Cu^{++} + HN^- \rightleftharpoons CuHN^+ \tag{18}$$

where HN^- denotes nitroacetate ion. In this case we have

$$\frac{1-\alpha}{\alpha\,(Cu^{++})} = \frac{x-(HN^-)}{(HN^-)\,(Cu^{++})} = \frac{(CuHN^+)}{(HN^-)\,(Cu^{++})} = K_1$$

In the previous section of the paper it was found that $K_1 = 2.77$ when $\mu = 0.6$. Here μ varies from 0.37 to 0.54. We should therefore expect to find $(1-\alpha)/\alpha(Cu^{++})$ equal to about 3 for all the solutions, but actually it varies from 7.9 to 78 (see the sixth column of Table 10b). Formation of the complex $CuHN^+$ is therefore far from sufficient to explain the whole effect.

The first 16 experiments in Table 10b fall in four groups with nearly constant μ, (Cu^{++}), and (Ac^-). Average values are given in Table 11. If we plot $(1-\alpha)/\alpha(Cu^{++})$ against $(Ac^-)/(HAc)$, the points fall, for each of the four groups, close to a straight line of the equation

$$\frac{1-\alpha}{\alpha\,(Cu^{++})} = A + B\,\frac{(Ac^-)}{(HAc)} \tag{19}$$

where A and B have the values given in Table 11. Since the ratio $(Ac^-)/(HAc)$ is inversely proportional to the hydrogen ion concentration, this indicates the existence of the equilibrium

$$CuHN^+ \rightleftharpoons CuN + H^+ \tag{20}$$

Formation of the two complexes $CuHN^+$ and CuN is, however, not sufficient for explaining the total effect. If this were the case, the points for all the four groups would fall close to the *same* straight line, and A would have a value of about 3. An examination shows that both A and B increase approximately linearly with the acetate ion concentration (see Table 11). As a result of the analysis we obtain the equation

Table 11. A and B of equation 19.

A(calc.) = 3.0 + 54 (Ac^-). B(calc.) = 14.3 + 232 (Ac^-).

Nos.	μ	(Cu^{++})	(Ac^-)	A	B	A (calc.)	B (calc.)
1— 4	0.369	0.00786	0.1484	11.0	49.9	11.0	48.7
5— 9	0.491	0.00545	0.1250	9.4	41.4	9.8	43.3
10—13	0.408	0.01760	0.0808	7.5	34.5	7.4	33.0
14—16	0.522	0.01523	0.0488	5.6	26.5	5.7	25.6

$$\frac{1-\alpha}{\alpha\,(Cu^{++})} = 3.0 + 54\ (Ac^-) + \left[14.3 + 232\ (Ac^-)\right] \frac{(Ac^-)}{(HAc)} \qquad (21)$$

which holds with good approximation for all the experiments. This is seen from Table 10b where $k^*\alpha$ calculated by means of formula 21 is presented in the next to the last column, while the last column shows the percentage difference between the calculated value and that found directly from the measurements.

In order to explain formula 21 we must assume that also the following two equilibria contribute to the stabilisation of the nitroacetate ion:

$$Cu^{++} + HN^- + Ac^- \rightleftharpoons CuHNAc \qquad (22)$$

$$CuHNAc \rightleftharpoons CuNAc^- + H^+ \qquad (23)$$

When K_{HAc} denotes the dissociation constant of acetic acid, and the mass action constants for the equilibria 18, 20, 22, and 23 are designated as K_1, K_2, K_3, and K_4, respectively, we obtain

$$(CuHN^+) = K_1\ (Cu^{++})\ (HN^-) \qquad (24)$$

$$(CuN) = K_2 \frac{(CuHN^+)}{(H^+)} = \frac{K_1K_2}{K_{HAc}}\ (Cu^{++})\ (HN^-) \frac{(Ac^-)}{(HAc)} \qquad (25)$$

$$(CuHNAc) = K_3\ (Cu^{++})\ (HN^-)\ (Ac^-) \qquad (26)$$

$$(CuNAc^-) = K_4 \frac{(CuHNAc)}{(H^+)} = \frac{K_3K_4}{K_{HAc}}\ (Cu^{++})\ (HN^-) \frac{(Ac^-)^2}{(HAc)} \qquad (27)$$

$$\frac{1-\alpha}{\alpha\,(Cu^{++})} = \frac{x-(HN^-)}{(HN^-)\ (Cu^{++})} = \frac{(CuHN^+) + (CuHNAc) + (CuN) + (CuNAc^-)}{(HN^-)\ (Cu^{++})}$$

$$= K_1 + K_3\,(\mathrm{Ac^-}) + \left[\frac{K_1K_2}{K_{\mathrm{HAc}}} + \frac{K_3K_4}{K_{\mathrm{HAc}}}\,(\mathrm{Ac^-})\right]\frac{(\mathrm{Ac^-})}{(\mathrm{HAc})} \qquad (28)$$

Comparison with formula 21 shows that

$$K_1 = 3.0 \qquad K_3 = 54 \qquad \frac{K_1K_2}{K_{\mathrm{HAc}}} = 14.3 \qquad \frac{K_3K_4}{K_{\mathrm{HAc}}} = 232 \qquad (29)$$

If we as an estimate of K_{HAc} at the ionic strengths concerned [6] use the value 3.5×10^{-5}, we obtain

$$K_2 = 1.7 \times 10^{-4} \text{ and } K_4 = 1.5 \times 10^{-4}$$

K_2 and K_4 are the strength constants of the acids $CuHN^+$ and CuHNAc, respectively. As we should expect, both constants are much larger than the acid strength of the free nitroacetate ion, $K_{\mathrm{HN^-}} = 10^{-9}$. It is, however, a little surprising that the difference between K_2 and K_4 is not greater.

K_1 was in experiments on solutions containing nitric acid found to have the value 2.77 at the ionic strength 0.6. Since we may expect that K_1 decreases a little with increasing μ, the value $K_1 = 3.0$ found here is reasonable. We may compare K_1 with the complexity constant K_1' of the acetato complex $CuAc^+$ which, at the same ionic strength, according to the first of the equations 17, is about 36. We see that the acetate ion is bound faster than the less basic nitroacetate ion although the ratio $K_1'/K_1 = 12$ is much smaller than the ratio of their base strengths (about 1.3×10^3).

The two balanced reactions

$$\mathrm{CuHN^+ + Ac^-} \rightleftharpoons \mathrm{CuHNAc}$$

and

$$\mathrm{CuAc^+ + Ac^-} \rightleftharpoons \mathrm{CuAc_2}$$

have the equilibrium constants $K_3/K_1 = 18$ and $K_2'/K_1' = 6$, respectively. If the tendency to bind an acetate ion were the same whether a nitroacetate ion or an acetate ion were already present in the complex, the former constant would be twice as great as the latter (owing to the statistical effect of the two acetate ions present in $CuAc_2$). Actually, it is three times as great. The nitroacetato complex has therefore a somewhat greater tendency to bind an acetate ion than has the acetato complex. This consideration shows that the value found for K_3 is not unreasonable.

The cupric ion binds the divalent nitroacetate ion with great strength. This is seen from the following rough estimate of the complexity constant for the complex CuN:

$$\frac{(CuN)}{(Cu^{++})\,(N^{--})} = \frac{K_1K_2}{K_{HN^-}} = \frac{3.0 \times 1.7 \times 10^{-4}}{10^{-9}} = 5 \times 10^5$$

It follows from equations 24—29 that we may expect that all the numerical coefficients in equation 21 decrease with increasing ionic strength. For that reason, the effect should decrease ($k^*\alpha$ increase) with increasing μ, provided that (Cu^{++}), (Ac^-), and (H^+) are the same. In the solutions examined, μ varies from 0.37 to 0.54. The accuracy of the measurements is, however, not great enough to show the influence of the ionic strength.

We have seen that the measurements in solutions containing nitric acid could be explained satisfactorily without taking into account the dissociation $CuHN^+ \rightleftharpoons CuN + H^+$. Let us consider the experiment in which c has its highest value (0.2), and which therefore is most sensitive to the effect of the acid dissociation. From the data in Table 7 we find that (H^+) during the measurements decreases from 0.018 to 0.0058. If we apply equation 21 to an acetate-free solution we must replace (Ac^-)/(HAc) by $K_{HAc}/(H^+)$. When we set $K_{HAc} = 3.5 \times 10^{-5}$, we obtain

$$\frac{1-\alpha}{\alpha\,(Cu^{++})} = 3.0 + \frac{5 \times 10^{-4}}{(H^+)} = 3.0\left(1 + \frac{1.7 \times 10^{-4}}{(H^+)}\right)$$

When we neglect the dissociation of the ion $CuHN^+$ we shall therefore in the experiment shown in Table 7 find a value of K_1 which is between 1 and 3 per cent too high, but this corresponds to less than 1 per cent in α.

SUMMARY

The rate-determining reaction in the decomposition of nitroacetic acid is a spontaneous cleavage of the free univalent nitroacetate ion. The ion is stable when it is bound either to a hydrogen or a metal ion.

Formulae for the kinetic analysis of the reaction in unbuffered solutions containing no or only a small concentration of strong acid, have been deduced. In order to facilitate the use of the formulae values of the function

$$Y = 0.4343\,\sqrt{1+X} + \log\,(\sqrt{1+X} - 1) + 1$$

have been calculated for values of X from 2.00 to 0.01 (Table 1).

The kinetic effects of the metal ions mentioned below were studied in solutions of their nitrates. The solutions contained sufficient barium nitrate to maintain an ionic strength of 0.6, and, further, 0.002 or 0.001 (in the cases of Al^{+++} and Be^{++}, 0.02 or 0.01) molar nitric acid. The decrease in velocity may be explained by assuming that complexes of one metal ion and one nitroacetate ion with the following complexity constants (K_1) are formed:

H^+	Al^{+++}	Cu^{++}	Be^{++}	Cd^{++}	Pb^{++}	Ni^{++}	Zn^{++}	Co^{++}	Mg^{++}	Ca^{++}	Ba^{++}
21.7	3.05	2.77	1.82	1.54	1.39	1.14	1.08	1.00	0.65	0.50	0

The effect of cupric ions has also been studied in acetate buffer solutions. The influence of the formation of complexes between cupric and acetate ions has been taken into account, and it has been shown that the kinetic effect may be explained by the following equilibria (HN^- and Ac^- denote nitroacetate and acetate ion, respectively):

$$Cu^{++} + HN^- \overset{K_1}{\rightleftharpoons} CuHN^+ \overset{K_2}{\rightleftharpoons} CuN + H^+$$

$$Cu^{++} + HN^- + Ac^- \overset{K_3}{\rightleftharpoons} CuHNAc \overset{K_4}{\rightleftharpoons} CuNAc^- + H^+$$

with the equilibrium constants (ionic strength about 0.45):

$$K_1 = 3.0, \quad K_2 = 1.7 \times 10^{-4}, \quad K_3 = 54, \quad \text{and} \quad K_4 = 1.5 \times 10^{-4}$$

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Received June 11, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 697–707

Investigations on Malt Amylase

II. On the Viscosimetrical Determination of α-Amylase

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In a previous paper[1] the present author deduced the following formula for the enzymatic depolymerization of polymeric homologous substrates, if all linkages between the primary molecules are broken with equal ease and if the number of linkages broken is small in comparison with the number of linkages at complete polymerization:

$$A^{t^\circ}_{e|s} = c_s^2 \cdot \frac{\mathrm{d}\,\frac{1}{\eta_{sp}}}{\mathrm{d}t} \qquad (1)$$

where

$A^{t^\circ}_{e|s}$ = the enzymic activity at t° C (units per gram) in the reaction mixture; e and s are abbreviations for the names of the enzyme and the substrate (the indices may be omitted when there is no risk of confusion),
η_{sp} = the specific viscosity,
c_s = the concentration of the substrate in grams per gram reaction mixture, and
t = the time in seconds.

This formula was based upon Staudinger's equation

$$\eta_{sp} = K_m c_{gm} M \qquad (2)$$

where

K_m = the viscosity-molecular weight constant,
c_{gm} = the concentration of the substrate in basic moles per litre, and
M = the molecular weight.

For substrates, for which this linear relationship is not valid, but for which the modified Arrhenius-Staudinger formula (η_r = the relative viscosity)

$$\ln \eta_r = K_m c_{gm} M \tag{3}$$

can be applied, another formula has recently been deduced by the present author [2]:

$$A^{t^\circ}_{e/s} = c_s^2 \cdot \frac{\mathrm{d}\,\dfrac{1}{\ln \eta_r}}{\mathrm{d}t} \tag{4}$$

Equations (1) and (4) also define a unit A for the enzyme assay. In most cases, however, the unit $\mu A = A \cdot 10^{-6}$ is more convenient [3].

CALCULATION OF THE ACTIVITY

In addition to the guidance given in the first report [4] of this investigation, the following procedure is recommended.

For the calculation of the activity of an enzyme solution we employ the following notation:

m_e = grams enzyme solution mixed with the substrate solution,
m_s = grams substrate solution mixed with the enzyme solution,
C = the concentration of the stock substrate solution (grams substrate per gram solution).

The activity of the enzyme solution in the units $\mu A/g$ is then

$$\frac{m_s^2 C^2 \cdot 10^6}{m_e(m_e + m_s)} \cdot \frac{\mathrm{d}\,\dfrac{1}{\eta_{sp}}}{\mathrm{d}t} \tag{5}$$

and

$$\frac{m_s^2 C^2 \cdot 10^6}{m_e(m_e + m_s)} \cdot \frac{\mathrm{d}\,\dfrac{1}{\ln \eta_r}}{\mathrm{d}t} \tag{6}$$

respectively.

In calculating the activity much time can be saved if one always uses substrate solutions of the same concentration and calculates once and for all the factor $m_s^2 C^2 \cdot 10^6 / m_e(m_e + m_s)$. If one aims at the highest possible accuracy, one should calculate a table of this factor as a function of m_s and C. The

amount m_e of enzyme solution may be assumed to be measured with fairly good accuracy, but the amount m_s of substrate solution depends rather on the viscosity of the substrate and the drainage time of the pipette. The concentration of the stock starch solution may differ a little from the intended value. As an example a short table of the factor is given here as Table 1.

Table 1. Short table of the factor for calculation of enzymic activity. $m_e = 3.00$.

$100\ C$ / m_s	2.496	2.498	2.500	2.502	2.504
29.91	5645	5654	5663	5672	5681
29.94	5651	5660	5669	5679	5688
29.97	5657	5667	5676	5685	5694
30.00	5664	5673	5682	5691	5700
30.03	5670	5679	5688	5697	5706

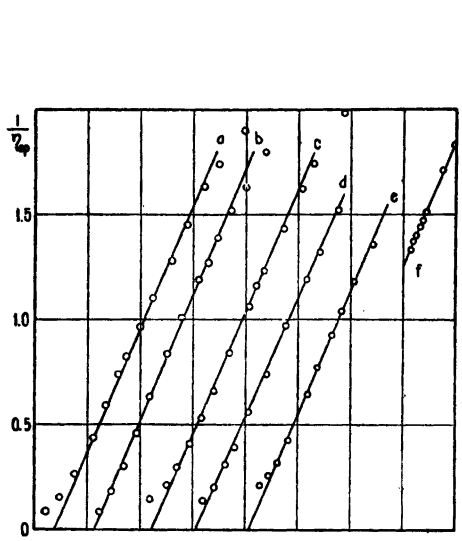
The calculation of the specific viscosities was discussed in the first report of this investigation. Tables 1 and 2 there are calculated for different concentrations of the substrate solution. Otherwise they give the same number $\tau_0 = \eta_w/dk_\tau$ *. For Table 1 only one decimal is required, but for Table 2 two decimals are often necessary. Sometimes sufficient accuracy is attained if the table is calculated so that the second decimal is an even number or even if it assumes the values 0 and 5. The relative viscosities are obtained similarly to τ/τ_0.

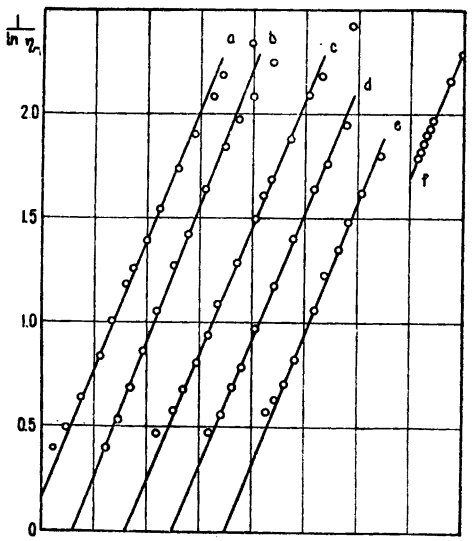
THE VISCOSITY OF THE SOLVENT

In calculations of the specific viscosities of starch solutions at different times, the solvent was referred to as water. However, distilled water is not actually used for viscosity measurements but buffer solutions; in the present investigation a buffer solution of sodium acetate and acetic acid, being 0.16 N in regard to sodium acetate and 0.04 N in regard to acetic acid. Hence, the viscosity of the buffer solution should be used in the calculations instead of the viscosity of distilled water.

The relative viscosities of sodium acetate and acetic acid solutions at 25° C were measured by Reyher [5]. On assuming the changes in the relative viscosities with the temperature to be negligible in these experiments, the relative viscosity of the present solvent in the stock solution may be calculated from

* d is here substituted for D in that paper.





Figs. 1 and 2. Graphs for amylase activity assay (see Table 2).
The distance between the lines cutting the time axis corresponds to 1000 sec. These lines are also zero lines for the various curves.

his measurements to be 1.060. In the calculations of specific viscosities, and relative viscosities of the substrate, the dilution of the stock substrate solution with the enzyme solution should be taken into account in accurate determinations.

The formula, if applicable to the break down of starch with α-amylase, should give correct values if the enzyme concentration, the substrate concentration and the polymerization degree of the substrate are changed within reasonable limits.

Blom and Bak [6], whose formula is equivalent with mine at constant substrate concentration [4], have found that the time required for the viscosity to decrease from one given value to another, is inversely proportional to the enzyme concentration. They also found that starch from various plants gave the same result on assaying the activity of equally active enzyme solutions.

The influence of the concentration of the starch solution was investigated in the first report of this investigation and the results given in Table 4 there. It was shown that good values are obtained if the concentration of the starch in the reaction mixture is not higher than about $2\frac{1}{2}$ %.

The influence of the initial degree of polymerization of the starch has been further investigated. Samples of potato starch were treated for various times

with 1.5 *N* hydrochloric acid, whereupon the starch was thoroughly washed and dried. Starch solutions were prepared in the manner previously recommended [4]. The activity of a given enzyme solution was determined with these various starch solutions. The viscosity measurements expressed as $1/\eta_{sp}$ and $1/\ln \eta_r$ are given in Figs. 1 and 2 respectively, and the results from the calculations in Table 2.

In all experiments 3 ml of enzyme solution were mixed with about 30 ml of starch solution, the accurate weight determined and used for the activity calculations. The initial specific viscosity of the starch solution was calculated from measurements using a mixture of the stock solution and water in the same proportions as in the enzyme assays.

Table 2. Viscosimetric assay of the activity of an amylase solution with potato starch.

Time for the treatment with 1.5 *N* HCl	η_{sp} (initial)	Curve	$100 \cdot c_s$	Activity (μA/g)	
				using η_{sp}	using $\ln \eta_r$
0	30	*a*	2.271	3.24	3.50
		b	2.274	3.32	3.66
5 min	10	*c*	2.271	3.36	3.47
		d	2.274	3.31	3.48
21 hours	5.5	*e*	2.275	3.42	3.57
115 hours	0.8	*f*	2.276	3.40	3.52

These experiments show that potato starch may be partially depolymerized with hydrochloric acid without any appreciable influence on the results of viscosimetric amylase assays. If the assays are performed with starch which has a very high degree of polymerization, there will be some deviations from the expected straight line, indicating that neither Staudinger's nor the modified Arrhenius-Staudinger formula is completely valid here. How far the enzymatic depolymerization will follow the linear relationship between the functions of the viscosity and the time cannot accurately be judged from these experiments, since the Oswald viscosimeter in all these experiments had a flow time of only about 10 seconds for the pure solvent. In the experiments where the starch concentration was about 2.3 %, measurements where $1/\eta_{sp}$ was below 1.5 could be taken into consideration.

DILUTION OF ENZYME SOLUTIONS TO SUITABLE STRENGTHS

It is well-known that various enzyme solutions become less stable when strongly diluted. This also holds true for malt α-amylase if diluted with water. Difficulties seldom arise if the activity is to be determined when the amylase concentration must be relatively large, but if the assay is to be performed viscosimetrically, the amylase concentration must be relatively small, and the inactivation may cause errors in the results. A fairly reliable method for obtaining stable dilute enzyme solutions is to use a boiled enzyme solution for the dilution. This method gives good result but is too enzyme-consuming to be of practical use. I have tried a 1 % egg albumine solution for the dilution and always found good stability, but these solutions are a little more difficult to treat, for they give rise very easily to disturbing bubbles in the viscosimeter, which may be very difficult to remove.

Wallerstein [7] recommended the addition of 1 g of calcium sulfate to 1000 ml of water, corresponding to a 0.012 N solution. Nakamura [8] found that an addition of calcium salts corresponding to about a 0.001 N solution was necessary to give solutions of malt amylase maximum stability. Holmbergh [9] has recommended an addition of 0.4 g of calcium acetate to 100 ml of water, corresponding to a 0.05 N solution, for the extraction of malt. Kneen, Sandstedt, and Hollenbeck [10] found that an addition of 0.004 mg calcium ion per ml solution, corresponding to a 0.0002 N solution, gave a considerably increased stability.

The following experiments give an idea of the stability of malt α-amylase solutions on dilution.

From a malt extract, in which the β-amylase had been distroyed by heating to 70° C, a dry preparation of malt amylase was made by precipitation with tannin and subsequent washing with aceton and ether, whereupon the amylase was sucked off and dried. A *dialyzed* 2 % solution of this preparation in distilled water had an activity of 540 $\mu A/g$. Samples of this solution were diluted 500 times at a favourable pH with distilled water, with calcium chloride solutions of various strengths, with boiled 2 % amylase preparation solution and with boiled 2 % amylase preparation solution with addition of potassium oxalate to a final concentration of 0.01 N. The activity of the diluted solutions immediately after the mixing and after various times was determined, and the results are given in Fig. 3.

The values in Fig. 3 indicate the importance of calcium ions for the stability of the amylase. Some stabilizing agent other than calcium ion seems, however, to be present in the boiled amylase solution.

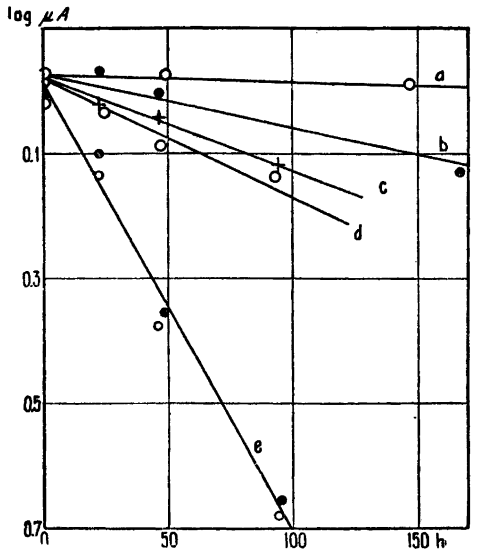


Fig. 3. The stability of diluted amylase solutions.
a (rings): boiled amylase solution, pH = 6.0
b (dots): 0.1 N $CaCl_2$ solution, pH = 6.1
c (crosses): 0.01 N $CaCl_2$ solution, pH = 6.3
d (rings): 0.0001 N $CaCl_2$ solution, pH = 6.5
e (dots): distilled water, pH = 5.9
(rings): boiled amylase solution, 0.01 N $K_2C_2O_4$, pH = 6.0

THE INFLUENCE OF THE TEMPERATURE ON THE VISCOSIMETRICAL ASSAY OF AMYLASE

It is customary to express the influence of the temperature by stating how many times faster the reaction proceeds if the temperature is raised 10° C. This can be expressed mathematically in the following way, if k_{t+10}/k_t is this factor:

$$A^{t+\Delta t^\circ} = A^{t^\circ} \cdot \left[\frac{k_{t+10}}{k_t}\right]^{\frac{\Delta t}{10}} \tag{7}$$

Investigations on the activity of amylase at various temperatures have already been performed long time ago by Roberts [11] and by Vernon [12].

Roberts investigated the activity of pancreatic diastase at temperatures from 3 to 70 °C. He found an activity maximum at 30—45°, which probably depends on the irreversible heat inactivation of the enzyme. From his activity values at 3—5, 10, 15, and 20 ° I have calculated the increase in the reaction velocity and found the factor to be 2.1 for 10 degrees.

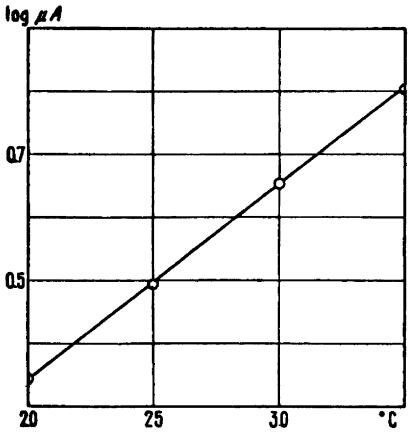


Fig. 4. The activity of an amylase solution as a function of the temperature.

Vernon gives in a figure the results of activity determinations at temperatures from 20 to 60° C of pancreatic diastase in a 0.2 % NaCl solution. If the logarithms of his activity determinations are plotted against the temperature, the points lie tolerably well on a parabola. From the inclination of its tangent at 25° C, a factor can easily be obtained, giving the increase of the reaction velocity for a temperature increase of 10° and valid at least for the first part of the curve, *i. e.* 20—30° C. In this way I have calculated the factor from Vernon's measurements and found the value 2.05.

v. Euler and Svanberg [13] also calculated their value for this factor from Vernon's measurements and gave the value 2.0.

The present author has performed some experiments in order to ascertain the influence of the temperature on the activity of amylase and the influence of errors in the temperature on the result.

The activity of an amylase solution was determined at 20, 25, 30, and 35 °C. For the calculations of the specific viscosities the respective values of the viscosity of the buffer solution were employed. The logarithms of the activity values are plotted against the temperature in Fig. 4, and from the inclination of the straight line through the points the value of the factor was calculated to be 2.03.

An estimation of the influence on the result of errors in the temperature can be obtained in the following way.

The value of the enzymic activity is proportional to the derivative $d\frac{1}{\eta_{sp}}/dt$, where

$$\frac{1}{\eta_{sp}} = \frac{\eta_{solvent}}{\eta_{solution} - \eta_{solvent}}$$

An error in the temperature determination will give raise to the use of an incorrect value for $\eta_{solvent}$ (in the calculations: an incorrect value for the corrected flow time for the pure solvent). In the case of the difference $\eta_{solution} - \eta_{solvent}$ this error may be neglectible, if the flow time of the solution is much longer than the flow time of the solvent.

If A = the activity calculated from measurements at $t + \Delta t$ °C under the assumption that the temperature was $t°$, and if the viscosity of water at $t°$ is denoted by η_t, we have

$$A^{t+\Delta t°} = A \cdot \frac{\eta_t + \Delta t}{\eta_t}$$

$$A^{t°} = A \cdot \frac{\eta_t + \Delta t}{\eta_t} \cdot \left[\frac{k_{t+10}}{k_t}\right]^{-\frac{\Delta t}{10}}$$

At 30° C and $\Delta t = 1$, we get

$$A^{30°} = A \cdot \frac{0.7840}{0.8007} \cdot 2^{-0.1} = A \cdot 0.98 \cdot 0.93 = A \cdot 0.91$$

An error of 1 degree in the temperature thus gives raise to an error in the result of about 10 %. Most of this error (about 7 %) originates from the increased velocity of the break down, and is consequently present in all methods for amylase assay. If the error originating from imperfect temperature determination must not exceed 1 %, the temperature assay should be accurate to within 0.1° C.

COMPARISON OF THE AMYLOLYTIC ACTIVITY OF α-AMYLASE AND ITS POWER TO LIBERATE REDUCING SUGARS

The activity of an amylase solution was determined viscosimetrically. Its power to liberate reducing sugars was determined by Blom and Rosted's [14] modification of Linderstrøm-Lang and Holter's [15] method. The activity was calculated in $\mu A/g$ and in mg maltose per minute and milliliter solution. The results are given in Table 3.

Table 3. The activity of an amylase solution in $\mu A/g$ and in mg maltose/min · ml.

$\mu A/g$	mg maltose / min · ml
1680	28.8
1690	29.0
1690	29.0
1620	30.2
	30.2

From this table we gather that the amylase amount 1 μA liberates reducing sugars equivalent to 0.018 mg maltose per minute.

SUMMARY

1. The calculation of enzyme activities from viscosimetric measurements are simplified by means of a specially calculated table, giving — for various concentrations and amounts of stock substrate solution — the factor, by which the derivative $d\frac{1}{\eta_{sp}}/dt$ or $d\frac{1}{\ln \eta_r}/dt$ must be multiplied to give the activity of the enzyme solution.

2. Certain deviations from the expected viscosity of starch solutions may occur in the beginning of the break down, when the degree of polymerization is still very high.

3. Enzyme solutions, which are too strong to be measured without previous dilution, should be diluted with a 0.1—0.01 N calcium salt solution.

4. The velocity of the break down increases 2.03 times if the temperature is raised 10° C.

5. In viscosimetric determinations of amylase activity, an error in the temperature assay of 0.1° C gives raise to an error in the activity of 1 %.

6. One conversion factor is calculated and tentatively suggested: the amylase activity 1 $\mu A/g$ corresponds to the power to liberate reducing sugars equivalent to 0.018 mg maltose/min · ml.

For this investigation the author received financial support from *Statens Tekniska Forskningsråd* and *Statens Naturvetenskapliga Forskningsråd*. Prof. Karl Myrbäck kindly granted me the use of his laboratories. Miss Anna Hörnfeldt, Miss Helena Bischof and Miss Margareta Kjellén assisted in performing the experiments. The English translation was revised by Mrs William Cameron. For all this help I wish to express my cordial thanks.

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Received June 13, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 708—716

Salts of Monotelluropentathionic Acid

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In an earlier paper [1] the preparation and some properties of sodium and potassium selenopentathionate were described. They were prepared by use of the Norris and Fay reaction [2] between selenious acid and thiosulphate:

$$H_2SeO_3 + 4\ S_2O_3^{--} + 4\ H^+ = S_4O_6^{--} + Se(S_2O_3)_2^{--} + 3\ H_2O$$

Norris and Fay (*l. c.*) said that 'An acid solution of tellurium dioxide is reduced by sodium thiosulphate, giving a bright yellow solution, from which sodium hydroxide precipitates tellurium. Tellurium, therefore, probably forms a compound analogous to selenopentathionate'. Later, Norris [3] made use of these reactions in order to purify tellurium for atomic weight determinations. He made no attempts, however, to isolate the telluropentathionate.

By use of the above-mentioned process, *viz.*:

$$TeOOH^+ + 4\ S_2O_3^{--} + 3\ H^+ = S_4O_6^{--} + Te(S_2O_3)_2^{--} + 2\ H_2O$$

sodium and potassium telluropentathionate have been isolated in a pure state, as the first salts of telluropentathionic acid.

We thus have the following series of analogous thiosulphate compounds:

Pentathionate	$S(S_2O_3)_2^{--}$
Selenopentathionate	$Se(S_2O_3)_2^{--}$
Telluropentathionate	$Te(S_2O_3)_2^{--}$

In reactions with nucleophilic reagents, pentathionate and selenopentathionate behave as a monosulphur di(thiosulphate) and a selenium di(thiosulphate), respectively [1, 4]. Correspondingly, telluropentathionate is a tellurium (dithiosulphate), its thiosulphate groups being displaceable by ethylxanthate, diethyldithiocarbamate, and hydroxide.

SODIUM AND POTASSIUM TELLUROPENTATHIONATE

The salts were prepared by an experimental procedure analogous to that used in the case of the selenopentathionates [1]. Since tellurium dioxide and tellurous acid are but sparingly soluble in water and in acetic acid, a mixture of hydrochloric acid and acetic acid was used as a solvent. An excess of tellurous acid was maintained at every stage of the process.

Sodium telluropentathionate forms small plates or flat needles. The salt is readily soluble in water, though less soluble than is sodium selenopentathionate. It is insoluble in ethanol and insoluble or very sparingly soluble in methanol. It crystallizes with two moles of water.

Potassium telluropentathionate forms tiny plates or flat prisms. It crystallizes rather slowly from its solutions, is less soluble in water than the sodium salt, and insoluble in methanol. The crystals contain no water.

The salts, when pure, are quite stable. The bulk of crystals is yellow with an orange tinge, single crystals yellow with a greenish tinge. Dilute, aqueous solutions are yellow, concentrated solutions are orange red. The solutions seem, from the preliminary observations made, to be at least as stable as those of pentathionate or selenopentathionate.

Telluropentathionate is readily oxidized by iodine, to give tellurous acid, and tetrathionate:

$$Te(S_2O_3)_2^{--} + 2\ J_2 + 2\ H_2O = TeOOH^+ + S_4O_6^{--} + 4\ J^- + 3\ H^+$$

The process may be utilized for the iodometric analysis of telluropentathionate. The tetrathionate formed may be estimated by means of the well-known sulphite method of Kurtenacker [5].

0.5 millimole of telluropentathionate is dissolved in 100—150 ml of water, and the solution is titrated with 0.1 *N* iodine (*a* ml). Towards the end of the titration 2 ml of 1 *M* potassium acetate may be added in order to precipitate the tellurous acid, the end point thus being reached more rapidly. To the titrated solution are added 30 ml of 0.2 *M* sodium sulphite and 10 ml of 1 *N* sodium hydroxide. After standing for 5 minutes, 5 ml of 40 % formaldehyde and 20 ml of 10 % acetic acid are added, and the solution is titrated with 0.1 *N* iodine (*b* ml).

0.5 millimole of telluropentathionate corresponds to $a = 20$ ml and $b = 5$ ml of 0.1 *N* iodine. For a mixture of telluropentathionate and tetrathionate, *b* is larger than one fourth of *a*: The amount of tetrathionate originally present together with the telluropentathionate, is equal to 0.1 $(b - \frac{a}{4})$ millimole.

The fact that telluropentathionate is not indifferent to iodine explains why tellurous acid cannot be analyzed iodometrically by a method analogous to the Norris and Fay method for selenious acid.

Telluropentathionate reacts quantitatively with ethylxanthate and diethyldithiocarbamate to give tellurium di(ethylxanthate) and tellurium *bis*-(diethyldithiocarbamate), respectively, and thiosulphate (R = ethyl):

$$Te(S_2O_3)_2^{--} + 2\ ROCS_2^- = Te(S_2COR)_2 + 2\ S_3O_3^{--}$$
$$Te(S_2O_3)_2^{--} + 2\ R_2NCS_2^- = Te(S_2CNR_2)_2 + 2\ S_2O_3^{--}$$

This is analogous to the behaviour of pentathionate [4] and selenopentathionate [1]. The thiocarbonyl anions act, with little doubt, as nucleophilic reagents, and the reactions are therefore ionic displacements on Te^{++}. Telluropentathionate is thereby characterized as being a tellurium di(thiosulphate). It has the same type of structure as pentathionate and selenopentathionate, *i. e.*, two thiosulphate groups are bonded to a tellurium atom, through the thio sulphur atoms of the thiosulphate groups.

The amount of ionic character of the covalent tellurium-sulphur bonds, in the sense:

$$O_3S_2^{--}\ Te^{++}\ S_2O_3^{--}$$

should be definitely larger than in the case of the corresponding bonds in pentathionate and selenopentathionate, the electronegativity values [6] of sulphur, selenium and tellurium being 2.5, 2.4 and 2.1, respectively.

Experimental

Sodium telluropentathionate, $Na_2Te(S_2O_3)_2 \cdot 2H_2O$. Tellurous acid was prepared from potassium tellurite as follows: 30 g potassium tellurite were dissolved in 30 ml of hot water, and 90 ml of 10 % acetic acid were added slowly with stirring. The tellurous acid was filtered off, washed with water containing a little acetic acid, and drained well.

The tellurous acid thus obtained (corresponding to 15 g of tellurium) is dissolved in 45 ml of concentrated hydrochloric acid and 75 ml of glacial acetic acid. To this solution are added dropwise, in the course of about 15 minutes, under mechanical stirring and cooling with ice-sodium chloride freezing mixture, 110 g of sodium thiosulphate pentahydrate in 60 ml of water (dissolved by heating, and cooled to room temperature). The temperature of the reaction mixture should be kept at about 0° C. To the clear, viscous, yellowish red solution of sodium tetrathionate and sodium telluropentathionate, containing a slight excess of tellurous acid, are added 150 ml of ethanol. The cooling and stirring are continued for about 15 minutes, the product is then filtered off, drained well, washed with ethanol and with ether, and dried *in vacuo* over sulphuric acid.

The product, which is quite stable *, contains about 35 g of $Na_2Te(S_2O_3)_2 \cdot 2H_2O$ with about 4 mole % of tetrathionate.

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* The amount of tellurous acid used should not be larger than described, since in that case the product becomes contaminated with this substance, which is difficult to remove, and also seems to decrease the stability of the product.

It is dissolved in 60—70 ml of 0.2 *N* hydrochloric acid at 45° C, and the solution is filtered with suction through a fine sintered glass filter. 80 ml of methanol are added, and the mixture is cooled in ice water. The product, consisting of about 15 g of pure sodium telluropentathionate dihydrate, is washed with ethanol and with ether, and dried *in vacuo* over sulphuric acid.

It was analyzed by means of the procedure described p. 709. The method gives accurate and consistent results. In control experiments (with 25 ml of 0.02 *M* potassium tellurite and 20 ml 0.1 *N* sodium thiosulphate, slightly acidified with acetic acid) tellurous acid was found to have no influence on the titration of thiosulphate with iodine, nor on the subsequent determination, by means of the sulphite method, of the tetrathionate thereby formed.

0.2327 g substance: (*a*) 21.31 ml (*b*) 5.33 ml 0.1006 *N* iodine.

$Na_2Te(S_2O_3)_2 \cdot 2H_2O$ (433.9) Calc. Te 29.41 Found Te 29.39

The tellurium content was also estimated directly. The substance was oxidized with concentrated nitric acid, the excess of nitric acid was destroyed by heating with several portions of concentrated hydrochloric acid, and the tellurium was filtered off and weighed after reduction with hypophosphorous acid according to Clauder [7].

0.4010 g substance: 0.1176 g Te. Found Te 29.33.

Potassium telluropentathionate, $K_2Te(S_2O_3)_2$. To a filtered solution of the crude sodium telluropentathionate in 80 ml of 0.2 *N* hydrochloric acid is added in portions, under mechanical stirring and cooling with ice-sodium chloride freezing mixture, a suspension of potassium acetate prepared as follows: 20 g of potassium acetate are dissolved by heating in 40 ml of ethanol, 20 ml of glacial acetic acid are added, and the mixture is cooled to room temperature. The crystals of potassium telluropentathionate are filtered off, and washed with ethanol and with ether. Yield, about 30 g of a product which contains only traces of tetrathionate. It is dissolved in a double amount of 0.2 *N* hydrochloric acid at about 30° C, and the solution is filtered with suction through a fine sintered glass filter. To the filtrate is added, with stirring, one third of its volume of warm methanol, and the mixture is cooled, finally in ice-water. Yield, 15—20 g of pure product.

0.2285 g substance: (*a*) 21.11 ml (*b*) 5.25 ml 0.1006 *N* iodine.

$K_2Te(S_2O_3)_2$ (430.1) Calc. Te 29.66 Found Te 29.64

The reactions with thiocarbonyl salts. To 2.176 g $Na_2Te(S_2O_3)_2 \cdot 2\,H_2O$ dissolved in 100 ml of water were added, with stirring, 50 ml of 0.3 *M* sodium diethyldithiocarbamate. A flesh-coloured product immediately separated out. The stirring was continued for 5 minutes, the product was then filtered off, washed with water, and dried *in vacuo* over sulphuric acid. Yield, 2.11 g; theoretically, 2.13 g $Te(S_2CN(C_2H_5)_2)_2$. In the filtrate the excess of diethyldithiocarbamate was removed by means of cadmium carbonate as described earlier [1]. Found, 19.87 ml 0.1006 *N* iodine (theoretically, 19.94 ml).

The tellurium *bis*(diethyldithiocarbamate) was recrystallized from carbon disulphide-ether. Red crystals, m. p. 164° C.

0.3208 g substance: 0.0959 g Te.

$Te(S_2CN(C_2H_5)_2)_2$ (424.2) Calc. Te 30.07 Found Te 29.89

In the same way was obtained, from 2.200 g $Na_2Te(S_2O_3)_2 \cdot 2H_2O$ and 50 ml of 0.3 *M* potassium ethylxanthate, 1.86 g of a flesh-coloured product; theoretically, 1.88 g tellurium di(ethylxanthate). It was recrystallized from benzene-ethanol. Red needles, m. p. 94° C.

0.3314 g substance: 0.1138 g Te.

$Te(S_2COC_2H_5)_2$ (370.0) Calc. Te 34.48 Found Te 34.35

The behaviour of tellurium di(ethylxanthate) towards alkalies is described p. 714-5.

These ethyl compounds are apparently the first xanthate and dithiocarbamate of divalent tellurium isolated in a pure state. The only analogous compound seems to be the tellurium di(thiolactic acid) prepared from tellurium dioxide and thiolactic acid by Bersin and Logemann [8].

The light absorption of telluropentathionate solutions was measured by means of a Beckman quartz spectrophotometer, model DU (1 cm cells). Distilled water was used as a solvent and as a blank. The solutions were not quite stable; though, when the measurements were made rapidly the change of transmittance was small. The solutions obeyed Beer's law in the range measured, *viz.*, from 0.01 *M* to 0.04 *M*. For the log ε curve, see Fig. 1.

THE ALKALINE HYDROLYSIS OF SELENOPENTATHIONATE AND TELLUROPENTATHIONATE

Telluropentathionate in aqueous solutions is rapidly destroyed by alkalies, tellurium being liberated. The sensitivity of this test for telluropentathionate is the same as that of the corresponding test for selenopentathionate [1]. A brownish-black colour is rapidly produced when 1 drop of 2 *N* sodium hydroxide is added to 10 ml of 10^{-4} *M* telluropentathionate.

With excess alkalies, selenopentathionate and telluropentathionate were found to undergo hydrolysis quantitatively as follows:

$$2\ Se(S_2O_3)_2^{--} + 6\ OH^- = 4\ S_2O_3^{--} + Se + SeO_3^{--} + 3\ H_2O$$
$$2\ Te(S_2O_3)_2^{--} + 6\ OH^- = 4\ S_2O_3^{--} + Te + TeO_3^{--} + 3\ H_2O$$

In view of the properties of selenopentathionate and telluropentathionate as a selenium di(thiosulphate) and a tellurium di(thiosulphate), respectively, it seems probable that the first step of hydrolysis involves ionic displacements of thiosulphate by hydroxide, to give selenium dihydroxide and tellurium dihydroxide, respectively:

$$Se(S_2O_3)_2^{--} + 2\ OH^- = Se(OH)_2 + 2\ S_2O_3^{--}$$
$$Te(S_2O_3)_2^{--} + 2\ OH^- = Te(OH)_2 + 2\ S_2O_3^{--}$$

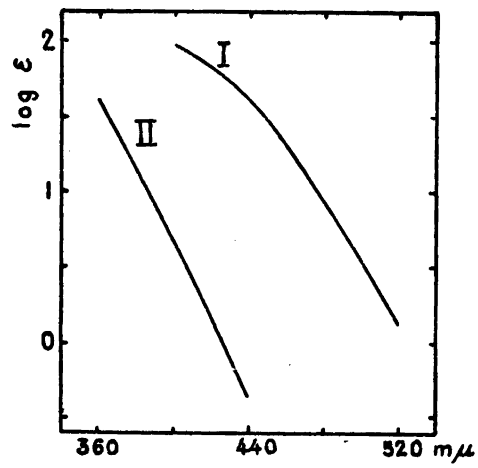

Fig. 1. Optical transmittance of

(I) telluropentathionate (in water)
(II) selenopentathionate [1] *(in 0.01 N HCl)*

The next step would be an immediate rearrangement of the hydroxides into selenium and selenite, and tellurium and tellurite:

$$2\ Se(OH)_2 + 2\ OH^- = Se + SeO_3^{--} + 3\ H_2O$$
$$2\ Te(OH)_2 + 2\ OH^- = Te + TeO_3^{--} + 3\ H_2O$$

A similar primary step probably takes place [4] in the alkaline hydrolysis of pentathionate, in analogy with the hydrolysis of other derivatives of divalent electropositive sulphur:

$$S(S_2O_3)_2^{--} + 2\ OH^- = S(OH)_2 + 2\ S_2O_3^{--}$$

It is generally assumed * that monosulphur dihydroxide in alkaline media undergoes spontaneous rearrangement to thiosulphate:

$$2\ S(OH)_2 + 2\ OH^- = S_2O_3^{--} + 3\ H_2O$$

This change is fully analogous to the rearrangements of selenium dihydroxide and tellurium dihydroxide, formulated above. Divalent sulphur dismutates into the electroneutral and the tetravalent state, followed by the union of sulphur and sulphite to give thiosulphate. No ions corresponding to thiosulphate exist in the case of selenium and tellurium.

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* For literature references, see Ref. 4. In a recent article, Goehring, Helbing and Appel [9] arrive at the same conclusions concerning monosulphur dihydroxide as an intermediate in the alkaline hydrolysis of pentathionate (and tetrathionate). They also report the reactions [4, 10, 11] of pentathionate and tetrathionate with piperidine, to give monosulphur dipiperidide, in support of this view.

Tellurium di(ethylxanthate) was found to react with alkalies in the same way as telluropentathionate:

$$2\ Te(S_2COC_2H_5)_2 + 6\ OH^- = 4\ C_2H_5OCS_2^- + Te + TeO_3^{--} + 3\ H_2O$$

The primary step in this case would be:

$$Te(S_2COC_2H_5)_2 + 2\ OH^- = Te(OH)_2 + 2\ C_2H_5OCS_2^-$$

Indications may be found in literature concerning the behaviour of oxygen compounds of divalent selenium and tellurium. No hydroxides are known to exist, neither is selenium monoxide [12]. If formed, it changes very rapidly into selenium and selenium dioxide [13]. The black solid ordinarily regarded as a tellurium monoxide [14] appears to be an equimolecular mixture of tellurium and tellurium dioxide [15].

Tellurium dichloride and dibromide are unstable in the solid state and changes gradually into tellurium and the tellurium tetrahalides [16]. In their hydrolysis reactions, they produce tellurium and tellurous acid or tellurite, *e. g.*:

$$2\ TeBr_2 + 6\ OH^- = 4\ Br^- + Te + TeO_3^{--} + 3\ H_2O$$

Damiens [16] mentioned the possibility of a transitory occurrence of an unstable tellurium monoxide in this hydrolysis.

Bersin and Logemann [8] formulated the alkaline hydrolysis of selenium di(2-oxy-3-bromo-5-methylphenylmercaptide) as involving a primary formation of selenium monoxide, which would rapidly rearrange into selenium and selenium dioxide. The intermediate occurrence of selenium monoxide in the reactions of selenium dioxide with sulphite, thiosulphate and selenosulphate, and in the equilibrium of selenosulphate with hydrogen ions, was assumed by Foerster, Lange, Drossbach and Seidel [17] and Foerster and Haufe [18].

The terms, selenium and tellurium monoxide, in these last reactions, would be synonymous with selenium and tellurium dihydroxide.

Experimental

Selenopentathionate. To approx. 2.5 millimole of the sodium salt dissolved in 25 ml of water were added 50 ml of 0.2 N sodium hydroxide. The mixture was stirred for about 5 minutes, the red selenium filtered off, washed with water, dried, and weighed. The filtrate and the washings were diluted to 250 ml, and two 100 ml samples were pipetted out for analysis.

To the first sample were added 10 ml of 6 N hydrochloric acid and 10 ml of a saturated solution of potassium bromide. A saturated solution of potassium bromate was added until a slight excess of bromine was present, and the excess was subsequently destroyed

by means of acetanilide. This procedure serves to oxydize the thiosulphate to sulphate, without affecting the selenious acid. The sample was diluted to 200 ml, and the selenious acid was determined by means of the Norris and Fay method: 25 ml of 0.1 *N* thiosulphate (consuming 24.92 ml of 0.1006 *N* iodine) was added, and the excess back-titrated with 0.1006 *N* iodine.

To the second sample were added 100 ml of water, 5 ml of 6 *N* hydrochloric acid, and 5 ml of 0.1 *N* thiosulphate (consuming 4.99 ml of 0.1006 *N* iodine). The excess of thiosulphate was back-titrated with 0.1006 *N* iodine . This method gives the amount of thiosulphate present in the sample relative to the amount of selenious acid.

(1) 1.018 g $Na_2Se(S_2O_3)_2 \cdot 3H_2O$ gave 0.0995 g Se (theoretically, 0.0996 g). Selenious acid: 24.92 ml − 4.90 ml = 20.02 ml 0.1006 *N* iodine (theoretically, 20.06 ml). Thiosulphate: 20.02 ml − 4.99 ml + 5.02 ml = 20.05 ml 0.1006 *N* iodine (theoretically, 20.06 ml).

(2) 1.062 g $Na_2Se(S_2O_3)_2 \cdot 3H_2O$ gave 0.1044 g Se (theoretically, 0.1040 g). Selenious acid: 24.92 ml − 3.96 ml = 20.96 ml 0.1006 *N* iodine (theoretically, 20.93 ml). Thiosulphate: 20.96 ml − 4.99 ml + 5.03 ml = 21.00 ml 0.1006 *N* iodine (theoretically, 20.93 ml).

Telluropentathionate. The procedure employed was the same as in the case of selenopentathionate. The tellurium was washed with water and with alcohol before drying. The filtrate and the aqueous washings were diluted to 250 ml, and one 100 ml sample was pipetted out for analysis: 5 ml of 10 % acetic was added (whereby tellurous acid settled out) and the mixture was titrated with 0.1006 *N* iodine (*a* ml). This titration gives directly the amount of thiosulphate present (tellurous acid does not interfere, *cf.* p. 711). As a check on this value, the amount of tetrathionate formed was determined by means of the sulphite method as described p. 709 (*b* ml).

(1) 1.093 g $Na_2Te(S_2O_3)_2 \cdot 2H_2O$ gave 0.1602 g Te (theoretically, 0.1607 g). Thiosulphate: (*a*) 20.00 ml (*b*) 10.00 ml 0.1006 *N* iodine (theoretically, 20.03 ml and half of this value, respectively).

(2) 1.084 g $Na_2Te(S_2O_3)_2 \cdot 2H_2O$ gave 0.1605 g Te (theoretically, 0.1593 g). Thiosulphate: (*a*) 19.84 ml (*b*) 9.92 ml 0.1006 *N* iodine (theoretically, 19.86 ml and 9.93 ml, respectively).

Tellurium di(ethylxanthate). 0.9933 g $Te(S_2COC_2H_5)_2$ was dissolved in 50 ml of warm ethanol, and 10 ml of 1 *M* sodium hydroxide were added with stirring. The mixture was cooled and diluted to about 100 ml, and the tellurium was filtered off, washed with water and with ethanol, and dried: 0.1682 g (theoretically, 0.1712 g). The filtrate and the aqueous washings were diluted to 250 ml, and one 100 ml sample was pipetted out. 10 ml of a phosphate buffer (being 0.5 *M* with respect to dihydrogen phosphate and 0.1 *M* with respect to monohydrogen phosphate) were added (whereby tellurous acid settled out). The mixture was titrated with 0.1006 *N* iodine: 21.39 ml (theoretically, for oxidation of the xanthate to dixanthyl disulphide, 21.34 ml).

SUMMARY

Sodium and potassium telluropentathionate have been isolated in a pure state, as the first salts of telluropentathionic acid. Telluropentathionate is a tellurium di(thiosulphate).

The alkaline hydrolysis reactions of selenopentathionate and telluropentathionate have been investigated, and the intermediate occurrence of selenium dihydroxide and tellurium dihydroxide, respectively, has been made probable.

The author is indebted to Prof. H. Haraldsen for a sample of potassium tellurite, and to Prof. E. Berner for the use of his Beckman spectrophotometer.

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Received June 15, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 717–747

Die Verteilung organischer Verbindungen zwischen Äther und Wasser

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Die Frage, wie die Verteilung organischer Verbindungen zwischen mit Wasser nicht misch baren organischen Lösungsmitteln und Wasser von der chemischen Konstitution der sich verteilenden Verbindung abhängt, ist bisher recht wenig untersucht worden. Overton [1, 2] hat wohl als erster planmässige Beobachtungen hierüber angestellt. Seine Ergebnisse waren aber fast ausschliesslich empirischer Natur und sind leider nur in recht summarischer Form veröffentlicht worden. Eine kleine Arbeit von Frumkin [3] aus dem Jahre 1925 stellt einen interessanten Vorstoss zur theoretischen Deutung der Verteilungserscheinungen dar, beschränkt sich aber allein auf die Frage, wie die Länge der Kohlenstoffkette die Verteilung beeinflusst. Über den Zusammenhang zwischen Molekülbau, Adsorption und Verteilung haben Meyer und Hemmi [4] beachtenswerte Gesichtspunkte vorgelegt. Ein Versuch von Smith [5], die Verteilung mit dem Molekularvolumen zu verknüpfen, kann dagegen kaum als gelungen angesehen werden. Sonst sind die Beziehungen zwischen chemischer Konstitution und Verteilung, wie es scheint, nie systematisch, auf breiter Grundlage behandelt worden.

Unter solchen Umständen dürfte die vorliegende Untersuchung über die Verteilung organischer Verbindungen im System Äthyläther/Wasser von Interesse sein, trotzdem der Verf. kein Fachmann auf dem Gebiet der physikalischen Chemie ist und er somit keine Möglichkeit hat, das hier zusammengetragene empirische Material in theoretischer Hinsicht erschöpfend zu bearbeiten.

* Bei der Ausführung der Verteilungsbestimmungen wurde Verf. von den Herren Väinö Heikinheimo, Kalervo Heinonen, Leo Lehtoranta, Sakari Piha und Hjörvard Ström unterstützt. Herr Professor Dr. E. Tommila hatte die Freundlichkeit, das Manuskript durchzusehen.

ZUSAMMENSTELLUNG DER VERTEILUNGSKOEFFIZIENTEN

Tabelle 1 enthält Daten bezüglich aller derjenigen organischen Verbindungen, deren Verteilung im System Äther/Wasser mir entweder aus der Literatur oder auf Grund eigener Bestimmungen bekannt ist. Allein die in den Arbeiten von Smith [5] enthaltenen Daten über die Verteilung zahlreicher Säuren und Basen sind weggelassen worden, da es sich zeigte, dass sie — wenigstens teilweise — wenig zuverlässig sind, und ebenso die grösstenteils recht summarischen Angaben Ruges [6] über die Verteilung von Farbstoffen. Um aber die Tabelle nicht allzu sehr anschwellen zu lassen, sind für jede Verbindung höchstens zwei verschiedene Werte des Verteilungskoeffizienten k angegeben, auch wenn zahlreichere Bestimmungen vorliegen.

Nur solche k-Werte sind berücksichtigt worden, die bei einer Temperatur von 15—25° C erhalten worden sind. Da die Temperaturabhängigkeit der Verteilung verhältnismässig gering ist, beeinträchtigen Temperaturvariationen innerhalb dieser Grenzen die Vergleichbarkeit der zusammengestellten k-Werte recht wenig.

Bedenklicher ist es, dass die Verteilung mancher Stoffe ausgesprochen konzentrationsabhängig ist. Diese Komplikation macht sich besonders bei den Säuren und Basen bemerkbar, denn da die Ionen fast unlöslich in Äther sind, fällt die Verteilung um so mehr zu Ungunsten der Ätherphase aus, je verdünnter die untersuchten Lösungen sind. Durch Vermeidung von allzu verdünnten Lösungen lässt sich diese Fehlerquelle im Falle schwacher bis mässig starker Säuren und Basen jedoch weitgehend ausschalten. Bei stärkeren Elektrolyten wiederum lässt sich der Einfluss der Dissoziation auf rechnerischem Wege eliminieren. In der Tabelle 1 ist denn auch bei denjenigen Säuren und Basen, deren Dissoziationskonstante 10^{-3} übersteigt oder die in besonders grosser Verdünnung untersucht worden sind, neben der Bruttoverteilung auch die theoretisch berechnete Verteilung der undissoziierten Moleküle (mit M bezeichnet) angegeben worden. Nur bei einigen ganz starken Elektrolyten, nämlich den aliphatischen Aminosäuren und den Tetraalkylammoniumbasen, lassen sich die Konzentrationen der undissoziierten Moleküle nicht berechnen.

Ausser durch die Dissoziation wird die Verteilung der Säuren auch dadurch kompliziert, dass sie in der Ätherphase teilweise Doppelmoleküle bilden, deren relativer Anteil mit steigender Konzentration zunimmt. Quantitativ scheint diese Komplikation jedoch nicht sehr bedeutsam zu sein, denn bei schwachen und mittelstarken Säuren bewirkt eine zehnfache Erhöhung der Konzentration höchstens nur eine etwa 10prozentige Vergrösserung der Verteilungskoeffizienten. (Vgl.[7].)

Die erste Spalte der Tabelle 1 gibt die Bruttoformel und den Namen der sich verteilenden Verbindung. Die Reihenfolge der angeführten Verbindungen wird dabei in erster Linie durch die Zahl der C-Atome im Molekül bestimmt. Zuerst kommen also sämtliche C_1-Verbindungen, dann die C_2-Verbindungen usw. Innerhalb einer jeden solchen Gruppe entscheidet zunächst die Zahl der N-Atome; es kommen also zuerst die N-freien Verbindungen, dann die mit einem N-Atom usw. An dritter Stelle entscheidend für die Reihenfolge ist die Zahl der O-Atome, an vierter die der H-Atome. Schwefelverbindungen werden angeführt unmittelbar nach den entsprechenden Sauerstoffverbindungen (also z. B. Thioharnstoff unmittelbar nach dem Harnstoff), Halogenverbindungen unmittelbar nach den entsprechenden Wasserstoffverbindungen. Eine Ziffer nach dem Namen der Verbindung weist auf die Anmerkungen am Ende der Tabelle hin.

Die zweite Spalte gibt die Temperatur in °C an. Wenn keine Angabe vorhanden ist, bezieht sich die Bestimmung auf gewöhnliche Zimmertemperatur.

In der dritten und vierten Spalte ist die Konzentration der sich verteilenden Verbindung in der Äther- bzw. der Wasserphase angegeben. Sämtliche Konzentrationen sind in Millimol pro Liter Lösung ausgedrückt.

Die fünfte Spalte enthält den Verteilungskoeffizienten $k = \frac{c_{\text{Ätherphase}}}{c_{\text{Wasserphase}}}$. (Um den Vergleich der Werte zu erleichtern, wurden die in der Literatur vorliegenden reziproken Werte immer entsprechend umgerechnet.) Auch im Text wird überall, wenn kurzhin vom Verteilungkoeffizienten die Rede ist, eben dieser Koeffizient — nie sein reziproker Wert — gemeint. Nur zwei Dezimalstellen des Verteilungskoeffizienten sind angegeben, auch wenn im Original die Zahl der Stellen grösser ist. Die mit M bezeichneten Werte beziehen sich, wie vorhin erwähnt, auf die Verteilung der undissoziierten Moleküle, alle übrige auf die Bruttoverteilung.

Die letzte Spalte gibt einen Hinweis auf den Autor der betreffenden Bestimmung. Der Kürze halber ist dabei jedoch oft lediglich auf die neueste Zusammenstellung derartiger Werte, nämlich auf das Werk von Seidell[8] hingewiesen. Die in dieser Spalte benutzten Abkürzungen sind: B = Bärlund[9], C = Collander (nicht früher veröffentlichte Werte), CB = Collander und Bärlund[10], Ch = Chandler[11], D = Denigès[12], DH = Dieckmann und Hardt[13], DMT = Dermer, Markham und Trimble[14], EP = Eisenbrandt und Picher[15], ICT = International Critical Tables[16], LB = Landolt-Börnstein[17], Pi = Pinnow[18], Po = Poijärvi[19], S = Seidell[8] (die Nummer hinter dem S bezieht sich auf die Seitenzahl), Su = Sutter[20], W = Wartiovaara[21].

Hinsichtlich der hier zum ersten Male veröffentlichten Originalbestimmungen ist folgendes zu bemerken:

Die untersuchten Präparate waren im allgemeinen von den reinsten käuflich erhältlichen Qualitäten. Von ihrer Reinigung vor dem Gebrauch wurde meistens abgesehen.

Die Versuche wurden im allgemeinen in der Weise ausgeführt, dass die zu untersuchende Substanz zuerst etwa 5 Minuten lang mit Äther und Wasser in einem Scheidetrichter kräftig geschüttelt wurde. Nach dem sich dann die beiden Phasen vollständig getrennt hatten, wurde die Konzentration der sich verteilenden Substanz in beiden Phasen analytisch bestimmt.

Bei den am wenigsten ätherlöslichen Verbindungen (Zuckern, Aminosäuren usw.) wurde so vorgegangen, dass ein kleines Volumen der ziemlich konzentrierten wässerigen Lösung zunächst mit einer grossen Menge Äther in einem Scheidetrichter geschüttelt wurde. Nachdem die Ätherphase dann absolut klar geworden war, wurde ein bestimmtes Volumen, z. B. 1000 ml, derselben in einen zweiten sauberen Scheidetrichter gebracht und hier mit einer kleinen Wassermenge, z. B. 10 ml, geschüttelt. Da die Verteilung sehr stark zugunsten der Wasserphase ausfällt, kann in diesem Falle angenommen werden, dass praktisch die ganze in den 1000 ml Ätherphase enthaltene Substanzmenge in diese zweite Wasserphase übertrat. Die Konzentration derselben wurde bestimmt. Der so erhaltene Wert ergab, durch 100 dividiert, die Konzentration der ersten Ätherphase.

Einige Basen waren nur in der Form ihrer salz- oder schwefelsauren Salze erhältlich. In solchen Fällen wurde zu den wässerigen Lösungen dieser Salze eine äquivalente Menge von Natriumhydroxyd gegeben, wonach die so erhaltene Lösung mit Äther geschüttelt wurde. Die wässerige Phase enthielt also in diesen Fällen neben der sich verteilenden Base eine gewisse (im allgemeinen jedoch recht niedrige) Konzentration an Natriumchlorid oder Natriumsulfat. Dieses Neutralsalz dürfte die Verteilung der Base nicht wesentlich beeinflusst haben.

Folgende Analysenmethoden wurden benutzt: — 1. Azidimetrische bzw. alkalimetrische Titration bei fast allen Säuren und Basen. — 2. Kjeldahl-Bestimmung bei vielen N-haltigen Verbindungen. — 3. Oxydation der organischen Substanz mit Kaliumbichromat + konz. Schwefelsäure nach Bang [22]. (Da auch der Äther reduzierend wirkt, muss er vor der Ausführung derartiger Bestimmungen vollständig entfernt werden.) — 4. Bei praktisch nicht-flüchtigen Verbindungen Eindampfen bekannter Volumina beider Phasen, wonach die Trockenrückstände gewogen werden. — 5. Stalagmometrische Bestimmung der Tropfenzahl. — 6. Spezielle Methoden, die hier nicht einzeln aufgezählt werden können.

Die Genauigkeit der ermittelten k-Werte wechselt bedeutend, ist aber durchschnittlich nicht sehr gross. Fehler von etwa 10—20 Proz. dürften nämlich häufig vorkommen, vereinzelt vielleicht sogar solche bis etwa 40 Proz. Zu den Analysenfehlern kommen noch Fehler, die davon herrühren, dass die benutzten Substanzen Verunreinigungen enthielten. — Die Ergebnisse offenbar ungenauer Bestimmungen werden in Klammern gegeben.

ANMERKUNGEN ZUR TABELLE 1

Anm. 1. Nach den Daten bei Seidell [8, S. 37 und 40] ist die Löslichkeit des Methans in wasserfreiem Äther 30mal grösser als die in ätherfreiem Wasser. Nach den Angaben in Beilstein [23] (2. Erg.-W., Bd. I, S. 7) löst sich das Methan dagegen nur etwa 7mal reichlicher in Äther als in Wasser. Der Verteilungskoeffizient des Methans ist somit wahrscheinlich von der Grössenordnung 7—30.

Tabelle 1. Zusammenstellung der Verteilungskoeffizienten Äther/Wasser.

Verbindung	°C	Konz. in Äther	Konz. in Wasser	k	Autor
CH_4 Methan (1)					
CH_3J Methyljodid	21	817	9,75	84	C
CH_2O Formaldehyd	20	6,3	58,2	0,11	S 25
CH_4O Methanol (2)	20	274	1920	0,14	C
$CO_2+H_2CO_3$ Kohlendioxyd + Kohlensäure		(37)	(5,4)	(6,8)	C
CH_2O_2 Ameisensäure	18	17,6—349	47,6—836	0,37—0,42	S 28
HCN Cyanwasserstoffsäure				2,4	D
CH_5N Methylamin	15	2,2	94	0,023	C
CH_3ON Formamid	21		(5000)	0,0014	B
CH_2N_2 Cyanamid			50	0,11	CB
CH_4ON_2 Harnstoff	21		(5000)	0,00047	CB
CH_4SN_2 Thioharnstoff	22		(1000-2000)	0,0063	B
C_2H_5J Äthyljodid	18	933	3,33	280	C
C_2H_6O Äthanol (2)	19	275	1050	0,26	C
$C_2H_4O_2$ Essigsäure	20	20—200		0,48—0,52	ICT
$C_2H_3ClO_2$ Chloressigsäure	18	57,1—795	26,0—290	2,2—2,7 (M 2,9)	S 89
$C_2H_2Cl_2O_2$ Dichloressigsäure	25	190—940		7,2—10 (M 18)	DMT
$C_2HCl_3O_2$ Trichloressigsäure		79,5—1043	18,8—105	4,2—10 (M etwa 37)	C
$C_2H_3BrO_2$ Bromessigsäure	25	80—820		3,0—4,0 (M 4,4)	DMT
$C_2H_3JO_2$ Jodessigsäure	20	608	84,5	7,2	C
$C_2H_6O_2$ Äthylenglykol			(1000)	0,0053	C
$C_2H_3Cl_3O_2$ Chloralhydrat	20	766		4,3	ICT
$C_2H_4O_3$ Glykolsäure	25	4,9—24		0,028—0,028	DMT
$C_2H_2O_4$ Oxalsäure	15	5,53—29,5	89,2—343	0,062—0,086 (M 0,12)	S 84
C_2H_3N Acetonitril	20	376	624	0,60	C
C_2H_7N Dimethylamin	15	5,0	90,9	0,055	C
» Äthylamin	18	5,2	86,0	0,060	C
C_2H_7ON Äthanolamin	19	1,2	945	0,0013	C
$C_2H_5O_2N$ Methylcarbamat	23		(400)	0,14	B
» Glykokoll (3)					
» Acetamid	22		(2000)	0,0025	B
$C_2H_4ClO_2N$ Chloracetamid	20	34,7	361	0,096	C
$C_2H_4N_2$ Dicyandiamid	22		(225)	0,0029	B
$C_2H_8N_2$ Äthylendiamin	22	0,335	1020	0,00033	C
$C_2H_6ON_2$ Methylharnstoff	23		(750)	0,0012	B
$C_2H_6O_2N_2$ Methylolharnstoff			(700)	0,00028	CB
C_3H_8 Propan (4)					
C_3H_6O Aceton	20	383	617	0,62	C
» Propionaldehyd	19	710	360	2,0	C
C_3H_8O n-Propanol	22	1770	935	1,9	C
$C_3H_4O_2$ Acrylsäure	25	57—604		2,0—2,3	DMT

Verbindung	°C	Konz. in Äther	Konz. in Wasser	k	Autor
$C_3H_6O_2$ Methylacetat	20	473	177	2,7	C
» Propionsäure	22	25,6—117	15,0—67	1,7—1,8	S 188
$C_3H_5BrO_2$ α-Brompropionsäure	19	847	65	13 (M 15)	C
$C_3H_8O_2$ 1,2-Propylenglykol	20	23,0	1280	0,018	C
» Trimethylenglykol	20	15,3	1520	0,010	C
» Glykolmonomethyläther	18		(1900)	0,15	C
$C_3H_7ClO_2$ Glycerin-α-monochlorhydrin	23		(750)	0,080	B
$C_3H_4O_3$ Brenztraubensäure (5)					
$C_3H_6O_3$ Milchsäure	20	24,9—44,6	255—491	0,098—0,091	S 194
» β-Oxypropionsäure		6,84	81,8	0,084	C
$C_3H_8O_3$ Glycerin			(1000)	0,00066	CB
$C_3H_4O_4$ Malonsäure	25	2,7—13,5	33,1—148	0,082—0,092 (M 0,10)	S 168
$C_3H_6O_4$ Glycerinsäure	19	0,84	92,1	0,0090	C
C_3H_9N n-Propylamin	21	55,8	194	0,29	C
» Trimethylamin	21	84,4	183	0,46	C
C_3H_7ON Urethan	22		(500)	0,64	B
» Propionamid	23		(500)	0,013	B
C_3H_9ON iso-Propanolamin	21	4,80	1108	0,0043	C
$C_3H_3O_2N$ Cyanessigsäure	21	30	8,3	0,36	S 164
$C_3H_7O_2N$ α-Alanin	19	0,0014	1000	0,0000014	C
» Lactamid			1000	0,0018	B
$C_3H_9O_2N$ Glykokollmethylester	23	22	299	0,073	C
$C_3H_{10}N_2$ 1,2-Propylendiamin	20	0,945	833	0,0011	C
» Trimethylendiamin (6)				(0,0007)	
$C_3H_8ON_2$ Dimethylharnstoff (sym.)	19	7,01	2260	0,0031	C
» » (asym.)	19	6,29	2168	0,0029	C
» Äthylharnstoff			(500)	0,0041	CB
$C_3H_6O_2N_2$ Malonamid			(900)	0,00030	CB
$C_3H_{10}O_2N_2$ 1,3-Diaminopropanol-2	21	0,245	1225	0,00020	C
C_4H_{10} n-Butan (7)					
$C_4H_{10}O$ n-Butanol-1	18	1860	242	7,7	C
» iso-Butanol-1	20	1786	259	6,9	C
» n-Butanol-2	20	1743	390	4,5	C
» Trimethylcarbinol	20	1364	634	2,2	C
» Äthyläther (8)	20	9580	920	(10)	
$C_4H_6O_2$ α-Crotonsäure	25	150—760		4,2—5,3	DMT

Verbindung	°C	Konz. in Äther	Konz. in Wasser	k	Autor
$C_4H_8O_2$ Äthylacetat	20	660	78	8,5	C
» *n*-Buttersäure	21	74,4—171	12,1—26,4	6,1—6,5	S 251
$C_4H_{10}O_2$ 2,3-Butylenglykol				0,029	W
$C_4H_6O_3$ α-Oxy-*n*-buttersäure	20	73,3—464	232—1176	0,32—0,39	S 259
» α-Oxy-*iso*-buttersäure	25	15—180		0,22—0,26	DMT
$C_4H_8O_3$ Methyllactat	19		(1400)	0,37	C
$C_4H_{10}O_3$ Glycerinmonomethyläther			(1000)	0,019	CB
» Diäthylenglykol			(1000)	0,0040	C
$C_4H_4O_4$ Fumarsäure	25		4,1—27,1	1,0—1,3 (*M* 1,5)	Ch
» Maleinsäure	25		10,0—99,3	0,056—0,10 (*M* 0,15)	Ch
$C_4H_6O_4$ Bernsteinsäure	20	6—96	40,5—644	0,15—0,15	S 229
$C_4H_5BrO_4$ Monobrombernsteinsäure	25		5,6—87,9	1,5—2,4 (*M* 2,9)	Ch
$C_4H_4Br_2O_4$ 1,2-Dibrombernsteinsäure	25		30,2—32,7	17 (*M* 54)	Ch
$C_4H_{10}O_4$ Erythrit			(2000)	0,00011	CB
$C_4H_6O_5$ Diglykolsäure	21	13,4	447	0,030	C
» Äpfelsäure	25	2,0—17,2	142—1179	0,014 —0,015	S 232
$C_4H_6O_6$ Weinsäure	25	0,75— 3,3		0,0038—0,0034	DMT
C_4H_7N Butyronitril	21	(630)	(65)	(10)	C
$C_4H_{11}N$ Diäthylamin	18	34,2	64,6	0,53	C
C_4H_9ON *n*-Butyramid	23		(250—500)	0,058	B
$C_4H_5O_2N$ Succinimid	23		(500)	0,031	B
$C_4H_9O_2N$ α-Amino-*n*-buttersäure	21	0,043	1640	0,0000026	C
$C_4H_{11}O_2N$ Diäthanolamin		0,50	921	0,00054	C
$C_4H_4N_2$ Äthylencyanid	18	7,41	23,2	0,32	C
$C_4H_{10}N_2$ Piperazin	20	0,41	798	0,00052	C
$C_4H_{12}N_2$ Tetramethylendiamin	23	0,595	468	0,0013	C
$C_4H_4O_3N_2$ Barbitursäure	25	0,8—1,8		0,026—0,023	DMT
$C_5H_{12}O$ *iso*-Amylalkohol	19	1830	96	19	C
$C_5H_{10}O_2$ *n*-Valeriansäure	22	67,5—416	3,2—16,4	21—25	S 301
» *iso*-Valeriansäure		108—1076	6,20—54,6	17—20	C
» Trimethylessigsäure	17	362	11,3	32	C
$C_5H_4O_3$ Furancarbonsäure	25	79—340		3,8—4,5	DMT
$C_5H_8O_3$ Lävulinsäure	20	123	478	0,26	C
$C_5H_{12}O_3$ Glycerin-α-monoäthyläther	23		(500—1000)	0,026	B
» Diäthylenglykolmonomethyläther	20	33,9	915	0,037	C
$C_5H_6O_4$ Itakonsäure	25		3,9—61,5	0,29—0,33 (*M* 0,35)	Ch
» Citrakonsäure	19	28,9	141	0,20 (*M* 0,24)	C

Verbindung	°C	Konz. in Äther	Konz. in Wasser	k	Autor
$C_5H_8O_4$ Glutarsäure	25		5,6—28,0	0,26—0,27	Ch
» Dimethylmalonsäure	25	33,9—440	26,2—268	1,3—1,6	S 239
$C_5H_{10}O_4$ Monoacetin (9)	20		(500)	0,041	B
$C_5H_{12}O_4$ Pentaerythrit	18		(370)	0,00030	C
$C_5H_{10}O_5$ Arabinose	18		(1300)	(0,000038)	C
C_5H_5N Pyridin	18	354	302	1,2	C
$C_5H_{11}N$ Piperidin	18	35,7	62,6	0,57	C
$C_5H_{13}N$ *iso*-Amylamin	19	68,5	34,2	2,0	C
$C_5H_{11}ON$ *iso*-Valeramid	24		(250—500)	0,17	B
$C_5H_6N_2$ Aminopyridin	21	26,2	34,2	0,77	C
$C_5H_{14}N_2$ Pentamethylendiamin	18	0,21	84.1	0,0025	C
$C_5H_{11}ON_2$ Diäthylharnstoff (asym.)	21		(500)	0,019	B
C_6H_6O Phenol	19	598	13,5	44	C
$C_6H_4O_2$ Chinon	19	8,93—27,1	2,92—8,41	0,33—0,31	LB
$C_6H_6O_2$ Brenzcatechin	20	881	81,8	11	C
» Resorcin				4,2	ICT
» Hydrochinon	15			2,2—3,7	S 400
$C_6H_{12}O_2$ Capronsäure		133—1254	1,66—13,5	80—93	C
$C_6H_{14}O_2$ Hexamethylenglykol	20		(900)	0,12	C
» 2-Methyl-2,4-pentandiol	20	290	571	0,51	C
» Pinakon	20		(500)	(0,43)	B
$C_6H_6O_3$ Pyrogallol	20	659	380	1,7	C
» Phloroglucin	21	84,6	188	0,45	C
$C_6H_6O_2S$ Benzolsulfonsäure (10)		0,16—1,59	199—1270	0,0008—0,0013 (M etwa 0,002)	C
$C_6H_{10}O_4$ Adipinsäure	25	27—138		0,51—0,54	DMT
» Äthylendiacetat	20	626	309	2,0	C
$C_6H_{14}O_4$ Triäthylenglykol			(1000)	0,0031	C
$C_6H_{12}O_5$ Rhamnose	19		(1000)	(0,00019)	C
$C_6H_6O_6$ Aconitsäure		24,3—236	72,9—498	0,33—0,47 (M 0,50)	C
$C_6H_8O_6$ Tricarballylsäure	19	5,3	83,5	0,060	C
$C_6H_{12}O_6$ Glucose	19		(2000)	(0,0000045)	C
$C_6H_8O_7$ Citronensäure	19	0,73—7,55	92,6—880	0,0079—0,0086	C
$C_6H_{12}O_7$ Gluconsäure (11)	20	0,5	2500	(0,0002)	C
C_6H_7N Anilin		250	18,0	14	S 416
$C_6H_{15}N$ Dipropylamin	17	97,9	14,3	6,8 (M 8,9)	C
» Triäthylamin	18	97,4	16,4	5,9	C
$C_6H_{15}ON$ Diäthyläthanolamin		82	233	0,35	C
$C_6H_{13}O_2N$ Leucin	19	0,0028	232	0,000012	C

Verbindung	°C	Konz. in Äther	Konz. in Wasser	k	Autor
$C_6H_{15}O_2N$ Di-*iso*-propanolamin	20	4,46	750	0,0059	C
$C_6H_5O_3N$ *o*-Nitrophenol (12)	20	(100)		150	C
» *m*- » (12)	20	(100)		160	C
» *p*- » (12)	20	(100)		110	C
$C_6H_{15}O_3N$ Triäthanolamin	20	0,78	707	0,0011	C
$C_6H_{13}O_5N$ Glucosamin	22		(100)	(0,0001)	P
$C_6H_3O_7N_3$ Pikrinsäure	15	224	60	3,7	S 333
$C_6H_{12}N_4$ Hexamethylentetramin			(250)	0,00026	CB
$C_6H_{18}N_4$ Triäthylentetramin	21	0,0638	933	0,000068	C
$C_7H_6O_2$ Benzoesäure		132—1109	1,9—14,2	70—78	C
$C_7H_6O_3$ Salicylsäure	20	356	2,8	127 (*M* 236)	C
» *m*-Oxybenzoesäure	19	352	17,0	21	C
» *p*- »	20	930	36,4	26	C
$C_7H_{16}O_3$ Glycerin-*αα*-diäthyläther	19		670	0,84	C
$C_7H_{12}O_4$ Pimelinsäure	25	3,80—14,1	2,84—10,0	1,3—1,4 (*M* 1,5)	S 561
» Diäthylmalonsäure	19	48,1	9,25	5,2 (*M* 11)	C
$C_7H_6O_5$ Gallussäure	21	69,7	140	0,50	C
$C_7H_{12}O_5$ Diacetin	20		(500)	0,22	B
$C_7H_{12}O_6$ Chinasäure	21	0,40	1305	0,00031	C
$C_7H_{14}O_6$ Methylglucosid			(1500)	0,00005	CB
C_7H_9N Benzylamin	18	67,6	35,9	1,9	C
$C_7H_7O_2N$ *o*-Aminobenzoe-	19	199	7,40	27	C
» *m*- » säure	18	46,0	31,4	1,5	C
» *p*- »	19	420	55,0	7,6	C
$C_7H_8ON_2$ Phenylharnstoff	18	77,5	70,4	1,1	C
$C_7H_{16}O_2N_2$ Diäthylmalonamid			(50)	0,012	CB
$C_8H_8O_2$ Phenylessigsäure		1068	28,6	37	C
$C_8H_8O_3$ Mandelsäure		74,0—745	33,2—231	2,2—3,2	C
» Vanillin				9,3	ICT
$C_8H_6O_4$ *o*-Phthalsäure	25	9,1—32,2	8,5—26,1	1,1—1,2 (*M* 1,6)	Ch
» *m*- »	25	26,6—48,5	0,25—0,398	10—12 (*M* 29)	Ch
$C_8H_{14}O_4$ Korksäure	25		0,49—9,86	3,6—4,7	Ch
$C_8H_{18}O_5$ Tetraäthylenglykol			(1000)	0,0024	W
$C_8H_{14}O_6$ Diäthyltartrat	18	704	1087	0,65	C
$C_8H_{19}N$ Di-*iso*butylamin	18	113	0,75	151	C
C_8H_9ON Acetanilid	25			3,0	ICT
$C_8H_{15}ON$ Tropin	15	4,7	88	0,053	C
$C_8H_{21}ON$ Tetraäthylammoniumhydroxyd	19	(0,003)	1700	(0,000002)	C
$C_8H_{10}O_2N_4$ Kaffein (13)	25			0,040—0,060	Pi

Verbindung	°C	Konz. in Äther	Konz. in Wasser	k	Autor
$C_9H_8O_4$ Homophthalsäure	25	7,85—30,8	3,95—13,8	2,0—2,2 (M 2,5)	DH
$C_9H_{16}O_4$ Azelainsäure	25		0,96—3,10	13—15 (M 16)	Ch
$C_9H_6O_6$ Trimesinsäure	20	57,7	5,37	11	C
$C_9H_{14}O_7$ Triacetin	18		(100)	1,4	C
$C_9H_{14}O_7$ Trimethylcitrat	22		(100)	0,43	B
$C_9H_9O_3N$ Hippursäure	17	9,33	23,7	0,39	C
$C_9H_{22}N_2$ 2-Amino-5-diäthylaminopentan	20	139	241	0,58	C
$C_9H_{20}ON_2$ Tetraäthylharnstoff	18	282	25,6	11	C
$C_{10}H_{22}O_2$ Decamethylenglykol	19	99,1	4,60	21	C
$C_{10}H_3O_3S$ Naphthalinsulfonsäure	20	3,1	608	0,0051 (M etwa 0,01?)	C
$C_{10}H_{10}O_4$ Benzylmalonsäure		110	7,2	15	C
$C_{10}H_{16}O_4$ Camphersäure	25		1,48—2,29	25—26 (M 28)	Ch
$C_{10}H_{18}O_4$ Sebacinsäure	25		0,360—0,62	43—47 (M 57)	Ch
$C_{10}H_{18}O_4$ Schleimsäurediäthylester			50	0,0087	CB
$C_{10}H_{15}ON$ Ephedrin	22	34,5	17,3	2,0	C
$C_{10}H_{10}O_2N$ β-Indolylessigsäure	20			20	Su
$C_{11}H_{18}O_7$ Triäthylcitrat	23			4,4	B
$C_{11}H_{12}ON_2$ Antipyrin	22		(100)	0,073	B
$C_{12}H_{12}O_5$ α-Keto-γ-phenyladipinsäure	25	5,44—66,3	3,32—18,0	1,6—3,7 (M 7,4)	DH
$C_{12}H_{16}O_7$ Arbutin			300	0,00074	CB
$C_{12}H_{22}O_{11}$ Saccharose	18		(780)	(0,0000011)	C
$C_{13}H_{18}O_7$ Salicin	19		(200)	0,00049	C
$C_{13}H_{20}O_8$ Pentaerythrittetraacetat	20	294	31,6	9,3	C
$C_{13}H_{20}O_2N_2$ Novocain				64	EP
$C_{13}H_{17}ON_3$ Pyramidon	20	130	205	0,63	C
$C_{15}H_{26}N_2$ Spartein	20	104	1,6	65 (M etwa 350)	C
$C_{15}H_{16}N_4$ Neutralrotbase	21	(1)		5,0	C
$C_{16}H_{22}O_{11}$ Pentaacetylglucose	20	55,2	3,36	16	C
$C_{16}H_{26}O_2N_2$ Pantocain				1106	EP
$C_{17}H_{23}O_2N$ Atropin	19	21,7	5,22	4,1	C
$C_{17}H_{19}O_3N$ Morphin	18		(1)	0,21	C
$C_{17}H_{21}ON$ Cocain	18	125	0,95	138	C
$C_{18}H_{21}O_3N$ Codein		10,2	12,7	0,80	C
$C_{19}H_{21}O_3N$ Thebain	19	13,5	0,85	16	C
$C_{20}H_{19}O_5N$ Berberin	18	$<$ 0,05	10	$<$ 0,005	C

Verbindung	°C	Konz. in Äther	Konz. in Wasser	k	Autor
$C_{20}H_{24}O_2N_2$ Chinin (14)	20	36	0,82	44	C
$C_{21}H_{22}O_2N_2$ Strychnin	20	1,1	0,51	2,2	S 812
$C_{22}H_{25}O_6N$ Colchicin	20	4,5	30	0,015	S 817
$C_{32}H_{26}O_4N_2$ Brucin	18	1,03	5,58	0,18	C
$C_{32}H_{49}O_9N$ Cevadin	18	77	0,27	280	C

Anm. 2. Die hier mitgeteilten Verteilungskoeffizienten des Methanols und des Äthanols sind zwar nicht sehr genau, dürften aber trotzdem richtiger sein als die in der Literatur [9, 10] angegebenen diesbezüglichen Werte. Sie wurden unter Benutzung der Alkoholbestimmungsmethode von Fischer und Schmidt [24] erhalten.

Anm. 3. Ein Vergleich mit den für Alanin und Aminobuttersäure erhaltenen Werte zeigt, dass der in der Literatur [14] angegebene k-Wert 1/162—1/485 auch nicht entfernt richtig sein kann. Der richtige Wert dürfte vielmehr etwa 7×10^{-7} betragen.

Anm. 4. Die Löslichkeit des Propans in (wasserfreiem) Äther ist etwa 140mal grösser als die in (ätherfreiem) Wasser. (Beilstein, Hauptw. Bd. I, S. 104.)

Anm. 5. Ein ziemlich unreines Präparat ergab den k-Wert 0,16. Der richtige Wert liegt vermutlich niedriger.

Anm. 6. Der für Trimethylendiamin hier angegebene Verteilungskoeffizient 0,0007 ist nicht auf experimentellem Wege erhalten, sondern durch Interpolation aus den Werten für Di- und Tetramethylendiamin berechnet. Trotzdem dürfte er etwas genauer sein als der früher [19] angegebene Wert 0,001.

Anm. 7. Die Löslichkeit von n-Butan in Äther ist etwa 200mal grösser als die in Wasser. (Beilstein Hauptw., Bd. 1, S. 118.)

Anm. 8. Berechnet auf Grund der gegenseitigen Löslichkeiten von Äther und Wasser.

Anm. 9. Die Möglichkeit besteht, dass das untersuchte Präparat mit kleinen Mengen von Di- und Triacetin verunreinigt war und dass der erhaltene Wert des Verteilungskoeffizienten daher allzu gross ist.

Anm. 10. Die frühere Angabe [14], wonach der Verteilungskoeffizient der Benzolsulfonsäure mit zunehmender Verdünnung wächst, konnte nicht bestätigt werden.

Anm. 11. Der Verteilungskoeffizient bezieht sich auf ein Gemisch von lactonisierter und nicht-lactonisierter Säure. Wie gross der Verteilungskoeffizient von reiner nicht-lactonisierter Säure ist, lässt sich schwerlich experimentell ermitteln.

Anm. 12. Die wässerige Phase war $N/1000$ in bezug auf Schwefelsäure.

Anm. 13. Die Wiedergabe der Befunde von Pinnow [18] in den International Critical Tables ist fehlerhaft.

Anm. 14. Treadwell [52] bestimmte bei 0° den Verteilungskoeffizienten des Chinins zu 15—24. Bei der Wiedergabe dieser Befunde sind Äther- und Wasserphase von Seidell [8] (S. 803) verwechselt worden.

BEZIEHUNGEN ZWISCHEN CHEMISCHER KONSTITUTION UND VERTEILUNG

1. Allgemeine Gesichtspunkte

Seit langem [1, 2] weiss man, dass bestimmte Substituenten, wie z. B. die OH-, NH_2- und COOH-Gruppen, die Verteilung einer organischen Verbindung im System organisches Lösungsmittel/Wasser zugunsten der Wasserphase verschieben, während die Verlängerung der Kohlenwasserstoffkette in entgegengesetzte Richtung wirkt. Man kann hiernach zwischen hydrophilen (oder »organophoben») und hydrophoben (oder »organophilen») Substituenten unterscheiden. Die Substituenten der erstgenannten Art werden oft als polar, die Kohlenwasserstoffradikale dagegen als apolar bezeichnet und die Verteilung gewissermassen als das Resultat der antagonistischen Lösungstendenzen der verschiedenen Molekülbezirke aufgefasst, indem man annimmt, dass ein Lösungsmittel diejenigen Substanzen am reichlichsten löst, die ihm bezüglich der Polarität am nächsten kommen. Es lässt sich jedoch nicht bestreiten, dass das Wort »Polarität» hierbei oft (so u. a. auch in einer vorläufigen Mitteilung des Verf. [26]) in einem viel zu unbestimmten Sinn verwendet wurde.

In den letzten Jahren hat sich aber eine neue Idee bezüglich der Natur der Lösungsphänomene Bahn gebrochen. Nach dieser Anschauung wird die Löslichkeit organischer Verbindungen in erster Linie durch die Bildung von Wasserstoffbindungen zwischen den Molekülen des Lösungsmittels und denjenigen der gelösten Substanz bedingt. (Vgl. Hildebrand [27, 28], Zellhoefer, Copley und Marvel [29], Pauling [30], Ewell, Harrison und Berg [31], Francis [32], Palit [33], Hunter [34], Mecke [35].) Wir werden im folgenden sehen, dass auch die Verteilung zwischen Äther und Wasser vorwiegend von diesem Faktor beherrscht wird.

Bekanntlich hat das Wassermolekül sowohl Donator- wie Akzeptor-Eigenschaften. Seine Donator-Eigenschaften verdankt es dem Sauerstoffatom, seine Akzeptor-Eigenschaften dagegen den beiden Wasserstoffatomen. Die Wassermoleküle sind somit durch verhältnismässig kräftige Wasserstoffbindungen aneinander gekettet. Das Äthermolekül hat dagegen ausschliesslich Donator-Eigenschaften, denn direkt an Kohlenstoffatome gebundene Wasserstoffatome kommen ja im allgemeinen für die Wasserstoffbindungen nicht in Betracht. (Übrigens sind wohl auch die Donator-Eigenschaften des Äthers wesentlich weniger ausgeprägt als diejenigen des Wassers, u. a. weil das Sauerstoffatom im Äthermolekül durch die beiden Äthylgruppen bedeutend stärker abgeschirmt wird als das Sauerstoffatom des Wassermoleküls.) Zwischen den Äthermolekülen bestehen somit gar keine direkten Wasserstoffbindungen. Doch werden wohl die in der Ätherphase vorkommenden Wassermoleküle

nicht nur miteinander, sondern auch mit Äthermolekülen verkettet sein. Immerhin ist zweifellos die durchschnittliche Verkettung der Moleküle ausserordentlich viel schwächer in der Äther- als in der Wasserphase. Hieraus folgt, dass etwa ein Kohlenwasserstoffmolekül, dem ja die Befähigung zur Bildung von Wasserstoffbindungen gänzlich abgeht, nur sehr schwer aus der Ätherin die Wasserphase übertreten kann, denn um das zu tun, müsste es die an einander geketteten Wassermoleküle auseinanderzwängen. Aus der Wasserphase wird das Kohlenwasserstoffmolekül dagegen effektiv in die Ätherphase hinübergedrückt. Die Verteilung der Kohlenwasserstoffe fällt somit ganz extrem zugunsten der Ätherphase aus. Anders verhalten sich dagegen Moleküle, die solche Gruppen enthalten, die zur Bildung von intermolekularen Wasserstoffbindungen befähigt sind (z. B. OH, NH_2, COOH). Eben infolge dieser Befähigung werden sie sich an Wassermoleküle anlagern und sich somit um so überwiegender in die Wasserphase ansammeln, je zahlreicher und je effektiver diese Gruppen sind.

Tabelle 2. Die Verteilungskoeffizienten einiger C_4-Verbindungen.

Verbindung	k	Verbindung	k
Butyljodid	rund 3000?	Bernsteinsäure	0,15
Butan	» 200?	Maleinsäure	0,15
Dibrombernsteinsäure	54	*n*-Butyramid	0,058
α-Brombuttersäure	45*	Succinimid	0,031
α-Chlorbuttersäure	35*	Diglykolsäure	0,030
Diäthyläther	(10)	2,3-Butylenglykol	0,029
Butyronitril	(10)	Dioxybuttersäure	0,027*
Äthylacetat	8,5	Barbitursäure	0,023
n-Butanol	7,4	Glycerinmonomethyläther	0,019
n-Buttersäure	6,5	Äpfelsäure	0,015
Butyraldehyd	6 *	Propylharnstoff	0,0078*
α-Crotonsäure	5,3	Oxybuttersäureamid	0,006*
Monobrombernsteinsäure	2,9	Diäthylenglykol	0,0040
Methyläthylketon	2 *	Weinsäure	0,0034
Propylcarbamat	2 *	Butylglycerin	0,002*
Fumarsäure	1,5	Tetramethylendiamin	0,0013
Cyanpropionsäure	1,1*	Succinamid	0,001*
Diäthylamin	0,53	Diäthanolamin	0,00054
Methylmalonsäure	0,40*	Piperazin	0,00052
α-Oxybuttersäure	0,39	Erythrit	0,00011
Methyllactat	0,37	*α*-Aminobuttersäure	0,0000026
Äthylencyanid	0,32	Tetramethylammonium-	
Glykokolläthylester	0,22*	hydroxyd	$\leqq$ 0,000002

2. Vorläufige Orientierung über den Einfluss verschiedener Substituenten auf die Verteilung

Um den Einfluss verschiedener Substituenten auf die Verteilung im System Äther/Wasser bequem vergleichen zu können, sind in der Tabelle 2 die in dieser Hinsicht bisher untersuchten C_4-Verbindungen nach abnehmender Grösse ihres Verteilungskoeffizienten zusammengestellt worden. Ausser direkt untersuchten Verbindungen enthält die Tabelle auch einige Substanzen, deren Verteilungskoeffizienten (mit * bezeichnet) nur indirekt, d. h. auf Grund der Verteilung anderer Glieder derselben homologen Reihe geschätzt worden ist.

Die höchsten Werte des Verteilungskoeffizienten zeigen die Kohlenwasserstoffe und die Halogenkohlenwasserstoffe (k = rund 200—3000). Bereits die einwertigen Alkohole, Monoaldehyde, Monoketone, Monoäther, Fettsäuren, Fettsäureester und Alkylcyanide haben sehr viel niedrigere Verteilungskoeffizienten (k = etwa 2—10). Noch niedriger sind die Verteilungskoeffizienten der Monoamine (etwa 0.5), der Fettsäureamide (etwa 0.06) und der Säureimide vom Typus des Succinimids (0.03). Von derselben Grössenordnung wie die Verteilungskoeffizienten der zuletztgenannten Verbindungen sind auch diejenigen der Glykole, der Dicarbonsäuren, der Monooxymonocarbonsäuren, der Alkylendicyanide und der Cyanfettsäuren (k = etwa 0.03—1). Es folgen dann Verbindungen mit drei oder vier hydrophilen Substituenten oder aber mit zwei besonders stark hydrophilen Substituenten (Amino- oder Amidogruppen). Der Verteilungskoeffizient dieser Verbindungen liegt zwischen etwa 0.0001 und 0.02. Die ganze Reihe schliesst mit den extrem ätherunlöslichen α-Aminocarbonsäuren und Tetraalkylammoniumhydroxyden ab, deren extreme Verteilung zugunsten der Wasserphase zweifellos darauf beruht, dass sie starke Elektrolyte sind. — Während alle bisher genannten Substituenten die Verteilung zugunsten der Wasserphase verschieben, wirken Halogenatome in umgekehrter Richtung. Man sieht nämlich, dass die in Tabelle 2 enthaltenen Halogenverbindungen ausnahmslos höhere k-Werte haben als die entsprechenden halogenfreien Verbindungen.

3. Länge der Kohlenstoffkette

Fig. 1 zeigt das Verhalten einiger homologen Reihen im System Äther/Wasser. (Der Übersichtlichkeit zuliebe mussten mehrere Reihen fortgelassen werden.) Die sich verteilenden Verbindungen sind von links nach rechts nach der Zahl der C-Atome im Molekül geordnet. Die Ordinate gibt den Verteilungskoeffizienten in logarithmischem Massstabe an. Die zu derselben homologen Reihe gehörenden Verbindungen sind miteinander durch Linien verbunden. Man sieht, dass diese Linien ziemlich gleichmässig von links nach rechts ansteigen.

Die Verteilung fällt also mit zunehmender Länge der Kohlenstoffkette immer mehr zugunsten der Ätherphase aus. Es liegt nahe anzunehmen, dass dies ganz einfach davon herrührt, dass, je länger die Kohlenwasserstoffkette wird, ihre hydrophoben Eigenschaften um so mehr dominieren über die entgegengesetzte Tendenz der hydrophilen Atomgruppen im Molekül. Diese Erklärung genügt aber nicht in allen Fällen, denn der Verteilungskoeffizient

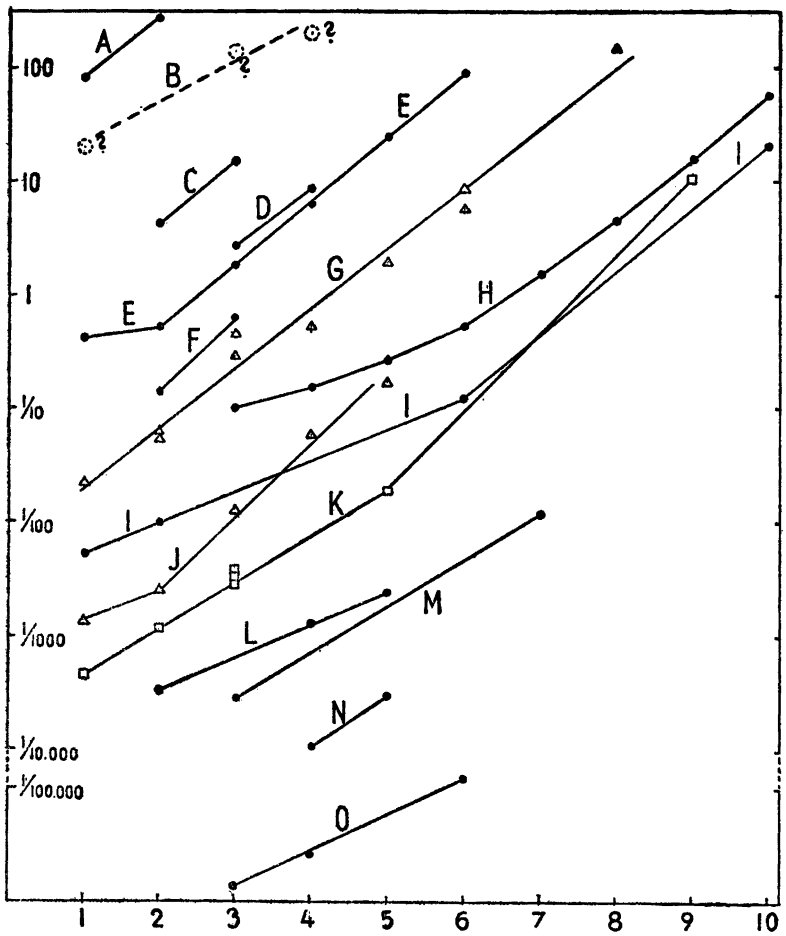

Abb. 1. Ordinate: Verteilungskoeffizient Äther/Wasser, Abszisse: Zahl der C-Atome im Molekül. A Alkyljodide, B Methankohlenwasserstoffe (sehr unsichere Werte!), C α-Bromfettsäuren, D Alkylacetate, E Fettsäuren, F Alkylcarbamate, G (△) Alkylamine, H Dicarbonsäuren, I Glykole, J (△) Fettsäureamide, K (□) (Alkyl-)Harnstoffe, L Diamine, M (Alkyl-)Malonamide, N vierwertige Alkohole, O α-Aminosäuren.

wächst mit zunehmender Länge der Kohlenstoffkette auch in der Reihe der Alkyljodide und wahrscheinlich auch in derjenigen der Methankohlenwasserstoffe, trotzdem ja in diesen Molekülen gar kein hydrophiler Teil vorhanden ist.

Die richtige Erklärung für den regelmässigen Anwachs des Verteilungskoeffizienten innerhalb homologer Reihen dürfte vielmehr die von Frumkin [3] im Jahre 1925 gegebene sein. Er sagt: »Wenn sich eine Substanz zwischen zwei

Phasen verteilt, so ist der Verteilungskoeffizient k gleich $e^{\frac{W}{RT}}$, wo W die Arbeit ist, die beim Überführen eines Mols der Substanz aus der einen Phase in die andere gewonnen wird. Bezeichnen wir mit W_{CH_2} die Arbeit, die einer CH_2-Gruppe entspricht, so muss für die Glieder einer homologen Reihe in erster Annäherung die Beziehung

$$W_n = W_1 + (n - 1)\ W_{CH_2}$$

gelten, wenn W_n die dem n:ten Gliede entsprechende Arbeit ist. Die Grösse W wird also mit der Länge der Kohlenstoffkette in arithmetrischer und die Grösse k in geometrischer Progression wachsen und zwar muss in allen homologen Reihen der Quotient dieser Progression denselben Wert haben.» — Vom modernen Standpunkt wäre wohl zu dieser Darstellung Frumkins hinzuzufügen, dass die Arbeit W im wesentlichen davon herrührt, dass bei der Überführung von Molekülen aus der einen Phase in die andere Wasserstoffbindungen teils gesprengt und teils neu geschaffen werden, wobei jeder CH_2-Gruppe eine bestimmte Anzahl solcher Bindungen entspricht.

Da das empirische Material, worauf Frumkin seine These gestützt hat, recht knapp ist, und seine Theorie, wie es scheint, auch später keiner eingehenderen Prüfung unterzogen worden ist, dürfte eine Betrachtung des jetzt hierüber vorliegenden Tatsachenmaterials am Platze sein.

Bereits ein flüchtiger Blick auf Fig. 1 zeigt, dass sich die auf die verschiedenen homologen Reihen beziehenden Linien untereinander nicht genau parallel sind und auch nicht ganz geradlinig verlaufen. Zum Teil ist dies natürlich auf Analysenfehler, auf Verunreinigungen der benutzten Präparate u. dgl. zurückzuführen. Zum Teil sind die in Rede stehenden Divergenzen jedoch auch realer Natur. So z. B. ist es ziemlich offenbar, dass die Verteilungskoeffizienten etwa der Fettsäuren, der Fettsäureamide und der Alkylmonoamine nicht unbeträchtlich schneller anwachsen als diejenigen der Dicarbonsäuren (Oxalsäure — Sebacinsäure) oder der Diamine (Äthylendiamin — Pentamethylendiamin). Auch Tabelle 3 ist in dieser Hinsicht lehrreich. Man sieht, dass q, d. h. der durchschnittliche Koeffizient der geometrischen Progression, in verschiedenen Reihen zwischen etwa 2 und 4 variiert, und zwar scheint es, dass er im allgemeinen höher ausfällt, wenn im Molekül ein deutlicher Gegensatz besteht zwischen einem ausgesprochen hydrophilen und einem deutlich hydrophoben Ende, als wenn das Molekül an beiden Enden hydrophile Gruppen trägt.

Auch innerhalb einzelner homologer Reihen kommen Variationen des q-Wertes vor, die kaum allein auf Versuchsfehler zurückgeführt werden können. Zweierlei Fälle sind dabei zu unterscheiden. — (a) In einigen Reihen scheint der q-Wert mit zunehmender Länge der Kohlenstoffkette kontinuier-

Tabelle 3. Anwachsen der Verteilungskoeffizienten in homologen Reihen.

Homologe Verbindungen	q-Wert
Arabinose — Rhamnose	(0,00019) : (0,000038) = (5,0)
Formaldehyd — Propionaldehyd	$\sqrt{2,0 : 0,11}$ = 4,3
Acetamid — *iso*-Valeramid	$\sqrt[3]{0,17 : 0,0025}$ = 4,1
Essigsäure — Capronsäure	$\sqrt[4]{93 : 0,52}$ = 3,7
Glykolsäure — α-Oxy-*n*-buttersäure	$\sqrt{0,39 : 0,028}$ = 3,7
Methylamin — Di-*iso*-butylamin	$\sqrt[7]{151 : 0,023}$ = 3,5
Harnstoff — Tetraäthylharnstoff	$\sqrt[8]{11 : 0,00047}$ = 3,5
Monobromessigsäure — Monobrompropionsäure	15 : 4,4 = 3,4
Methanol — *iso*-Amylalkohol	$\sqrt[4]{19 : 0,14}$ = 3,4
Äthanolamin — *iso*-Propanolamin	0,0043 : 0,0013 = 3,3
Methyljodid — Äthyljodid	280 : 84 = 3,3
Diäthanolamin — Di-*iso*-propanolamin	$\sqrt{0,0059 : 0,00054}$ = 3,3
Malonsäure — Diäthylmalonsäure	$\sqrt[4]{11 : 0,10}$ = 3,2
Methylacetat — Äthylacetat	8,5 : 2,7 = 3,1
Glykolsäure — α-Oxy-*iso*-buttersäure	$\sqrt{0,26 : 0,028}$ = 3,0
Äthylenglykol — Decamethylenglykol	$\sqrt[8]{21 : 0,0053}$ = 2,8
Erythrit — Pentaerythrit	0,00030 : 0,00011 = 2,7
Malonamid — Diäthylmalonamid	$\sqrt[4]{0,012 : 0,00030}$ = 2,5
Malonsäure — Sebacinsäure	$\sqrt[6]{57 : 0,10}$ = 2,3
Trimethylcitrat — Triäthylcitrat	$\sqrt[3]{4,4 : 0,43}$ = 2,2
Äthylendiamin — Pentamethylendiamin	$\sqrt[3]{0,0025 : 0,00033}$ = 2,0
α-Alanin — Leucin	$\sqrt[3]{(0,000012) : (0,0000014)}$ = (2,0)
Formamid — Acetamid	0,0025 : 0,0014 = 1,8
Maleinsäure — Citrakonsäure	0,24 : 0,15 = 1,6
o-Phthalsäure — Homophthalsäure	2,5 : 1,6 = 1,6
Ameisensäure — Essigsäure	0,52 : 0,42 = 1,2
Oxalsäure — Maleinsäure	0,10 : 0,12 = 0,8

lich zuzunehmen. Das deutlichste Beispiel hierfür bietet die verhältnismässig genau untersuchte Oxalsäurereihe. Die Verteilungskoeffizienten der undissoziierten Säuremoleküle und die zugehörigen q-Werte sind:

	k	q		k	q
Oxalsäure	0,12		Adipinsäure	0,54	
		0,8			2,8
Malonsäure	0,10		Pimelinsäure	1,5	
		1,5			3,1
Bernsteinsäure	0,15		Korksäure	4,7	
		1,8			3,4
Glutarsäure	0,27		Azelainsäure	16	
		2,0			3,6
Adipinsäure	0,54		Sebacinsäure	57	

(Der unerwartet hohe Wert des Verteilungskoeffizienten der Oxalsäure rührt vielleicht davon her, dass diese Säure in stärkerem Masse als die übrigen Glieder der Reihe zur Bildung verhältnismässig hydrophober Doppelmoleküle neigen dürfte.) — (b) In den homologen Reihen der Fettsäuren und der Fettsäureamide unterscheiden sich die zwei ersten Glieder der Reihe wesentlich weniger hinsichtlich ihrer Verteilung als die anderen. Besonders im Falle der Fettsäuren ist dies unzweideutig festgestellt. Die Verteilung gerade dieser Säuren ist nämlich von vielen Forschern untersucht worden, wobei die Differenz zwischen Ameisen- und Essigsäure immer bedeutend kleiner als die zwischen den folgenden Gliedern gefunden wurde. — Wie diese Erscheinungen zu erklären sind, mag dahingestellt bleiben.

Wie Führer [36] bemerkt hat, nimmt die Wasserlöslichkeit in homologen Reihen flüssiger organischer Verbindungen derart ab, dass jedes Glied etwa 3- bis 5mal weniger löslich als das vorhergehende ist. Man sieht hieraus, dass das entsprechende Anwachsen der Verteilungskoeffizienten im System Äther/Wasser nicht davon herrührt, dass die Ätherlöslichkeit mit zunehmender Länge der Kohlenstoffkette etwa zunähme (die Ätherlöslichkeit bleibt vielmehr hierbei ungefähr unverändert oder sinkt sogar ein wenig), sondern einzig und allein von der Abnahme der Wasserlöslichkeit.

Die Führnersche Löslichkeitsregel bezieht sich nur auf flüssige Verbindungen. Die Verteilung ist dagegen ganz unabhängig von dem Aggregatzustand der sich verteilenden Verbindung. Die Gesetze der Verteilung — oder der relativen Löslichkeit — sind daher viel einfacher als die der absoluten Löslichkeit. Ein hübsches Beispiel davon bieten die Verbindungen der Oxalsäurereihe. Wie schon längst bekannt, sind die Glieder mit einer geraden Anzahl von Kohlenstoffatomen verhältnismässig schwerlöslich, diejenige mit einer ungeraden Anzahl dagegen relativ leichtlöslich. Die absolute Löslichkeit ändert sich somit zickzackartig innerhalb dieser Reihe. Dagegen steigt die Kurve der Verteilungskoeffizienten dieser Verbindungen, wie wir gesehen haben, gleichmässig an.

4. Die alkoholische Hydroxylgruppe

Da ein an Sauerstoff gebundenes Wasserstoffatom in ausgesprochenem Masse zur Bildung von Wasserstoffbindungen befähigt ist, lässt sich voraussehen, dass eine OH-Gruppe die Affinität des Moleküls zu Wasser beträchtlich steigern wird. Diese Erwartung trifft in der Tat zu. Wie aus Tabelle 4 ersichtlich, erniedrigt nämlich jede OH-Gruppe den Verteilungskoeffizienten etwa um das 4,4- bis 190fache, d. h. etwa um ebenso viel, wie 1—4 CH_2-Gruppen ihn erhöhen. Meistens hält eine OH-Gruppe etwa 2 oder 3 CH_2-Gruppen die Waage. Daher kommt es, dass z. B. in der Reihe Methanol, Äthylenglykol, Glycerin, Erythrit, wo das Verhältnis zwischen der Zahl der C-Atome und der OH-Gruppen konstant bleibt, der Verteilungskoeffizient mit zunehmender

Tabelle 4. Einfluss einer alkoholischen Hydroxylgruppe auf den Verteilungskoeffizienten.

Verbindungen	Erniedrigung des Verteilungskoeffizienten pro OH-Gruppe
n-Propanol : Trimethylenglykol	1,9 : 0,010 = 190
n-Butanol-2 : 2,3-Butylenglykol	4,5 : 0,029 = 155
n-Propanol : 1,2-Propylenglykol	1,9 : 0,018 = 106
Diäthyläther : Diäthylenglykol	$\sqrt{(10) : 0{,}0040}$ = (50)
Äthanol : Äthylenglykol	0,26 : 0,0053 = 49
Äthylamin : Äthanolamin	0,060 : 0,0013 = 46
Diäthylamin : Diäthanolamin	$\sqrt{0{,}53 : 0{,}00054}$ = 31
Propylenglykol : Glycerin	0,018 : 0,00066 = 27
Propionsäure : β-Oxypropionsäure	1,8 : 0,084 = 21
Propionsäure : α-Oxypropionsäure	1,8 : 0,091 = 20
Diäthyläthanolamin : Triäthanolamin	$\sqrt{0{,}35 : 0{,}0011}$ = 18
Essigsäure : Glykolsäure	0,48 : 0,028 = 17
n-Buttersäure : α-Oxy-*n*-buttersäure	6,8 : 0,39 = 17
Triäthylamin : Diäthyläthanolamin	5,9 : 0,35 = 17
2,3-Butylenglykol : Erythrit	$\sqrt{0{,}029 : 0{,}00011}$ = 16
Trimethylenglykol : Glycerin	0,0010 : 0,00066 = 15
Phenylessigsäure : Mandelsäure	37 : 3,2 = 12
Bernsteinsäure : Äpfelsäure	0,15 : 0,015 = 10
Milchsäure : Glycerinsäure	0,084 : 0,0090 = 9,3
Propionamid : Lactamid	0,013 : 0,0018 = 7,2
Tricarballylsäure : Citronensäure	0,060 : 0,0086 = 7,0
Äpfelsäure : Weinsäure	0,015 : 0,0034 = 4,4
Methylharnstoff : Methylolharnstoff	0,0012 : 0,00028 = 4,3

Molekülgrösse stark abnimmt. (Die Verteilungskoeffizienten der genannten Verbindungen sind: 0,14, 0,0053, 0,00066, 0,00011.)

Dagegen mag es auf den ersten Blick etwas befremdend erscheinen, dass die den Verteilungskoeffizienten erniedrigende oder, wie wir der Kürze halber sagen wollen, dass die hydrotrope Wirkung einer OH-Gruppe so sehr verschieden ausfällt je nach der Beschaffenheit des Moleküls, in das sie eintritt. Die Erklärung hierfür liegt jedoch nahe bei der Hand. Wenn nämlich die Hydroxylgruppe an einer solchen Stelle eintritt, dass zwischen ihr und den im Molekül im voraus enthaltenen OH-, COOH- oder NH_2-Gruppen eine intramolekulare Wasserstoffbindung entsteht, so wird der hydrotrope Effekt offenbar schwächer sein — und zwar um so schwächer, je kräftiger diese intramolekulare Wasserstoffbindung ist — als wenn sie keine derartige Bindung veranlässt. Das in Tabelle 4 enthaltene Tatsachenmaterial steht, wenigstens in grossen Zügen, mit dieser Erklärung im Einklang. Jedenfalls fällt der hydro-

trope Effekt einer neu hinzutretenden Hydroxylgruppe im grossen ganzen um so schwächer aus, je zahlreichere und je kräftiger wirkende hydrophile Gruppen das Molekül von vornherein enthält.

Tabell 5. Einfluss einer Phenol-Hydroxylgruppe auf den Verteilungskoeffizienten.

Verbindungen	Erniedrigung des Verteilungskoeffizienten pro OH-Gruppe
Phenol : Hydrochinon	44 : 3,7 = 12
» : Resorcin	44 : 4,2 = 10
» : Phloroglucin	$\sqrt{44 : 0{,}45}$ = 10
Benzoesäure : Gallussäure	$\sqrt[3]{78 : 0{,}50}$ = 5,4
Phenol : Pyrogallol	$\sqrt{44 : 1{,}7}$ = 5,2
» : Brenzcatechin	44 : 11 = 4,0
Benzoesäure : *m*-Oxybenzoesäure	78 : 21 = 3,7
» : *p*- »	78 : 26 = 3,0
» : Salicylsäure	78 : 236 = 0,33

5. Die Phenol-Hydroxylgruppe

Nach dem in Tabelle 5 zusammengestellten Material zu schliessen, erniedrigt eine Phenol-Hydroxylgruppe je nach ihrer Stellung im Molekül den Verteilungskoeffizienten etwa um das 3- bis 12fache, also etwa ebenso viel oder etwas weniger als eine alkoholische Hydroxylgruppe. Eine Ausnahme macht jedoch die Salicylsäure, deren Verteilungskoeffizient nicht kleiner, sondern beträchtlich grösser als derjenige der Benzoesäure ist. Dies rührt zweifellos davon her, dass zwischen der Carboxylgruppe und der in *o*-Stellung befindlichen Hydroxylgruppe eine kräftige Wasserstoffbindung besteht, die die hydrotrope Wirkung dieser Gruppen stark herabdrückt. (Vgl. Pauling [30, S. 308]).

6. Der Carbonylsauerstoff der Aldehyde und der Ketone

Formaldehyd ($k = 0{,}11$) hat ungefähr denselben Verteilungskoeffizienten wie Methanol ($k = 0{,}14$). Ebenso verteilen sich Propionaldehyd ($k = 2{,}0$) und Aceton ($k = 0{,}6$) ungefähr wie n-Propanol ($k = 1{,}9$) bzw. *iso*-Propanol (k nicht bestimmt, aber wahrscheinlich = etwa 1). Der Verteilungskoeffizient der Lävulinsäure (d. h. der γ-Ketovaleriansäure) ist etwa 100mal kleiner als derjenige der Valeriansäure. Nach diesen Beispielen zu schliessen, beeinflusst eine Carbonylgruppe die Verteilung etwa ebenso stark wie eine Hydroxyl-

gruppe. Dies mag zunächst befremdend erscheinen, da doch das Hydroxyl sich effektiver als die Carbonylgruppe an die Wassermoleküle durch Vermittlung von Wasserstoffbindungen anlagern wird. Auf der anderen Seite ist aber zu beachten, dass die Hydroxylgruppe Wasserstoffbindungen auch mit dem Äthersauerstoff ermöglicht, wogegen zwischen Aldehyden und Ketonen einerseits und dem Äther anderseits überhaupt keine Wasserstoffbindungen entstehen können.

7. Der Äthersauerstoff

Für die Äther gilt eine ganz analoge Überlegung wie für die Aldehyde und Ketone. Die Neigung zur Anlagerung an Wassermoleküle wird bei den Äthern etwas schwächer sein als etwa bei den Alkoholen mit gleicher C-Atomenzahl. Aber anderseits fehlt den reinen Äthern jede Möglichkeit zur Bildung von Wasserstoffbindungen mit dem als Lösungsmittel dienenden Äthyläther. Es scheint daher a priori nicht unwahrscheinlich, dass die Verteilung der Äther in dem hier betrachteten System ungefähr gleich ausfallen wird wie die der entsprechenden Alkohole.

Tabelle 6. Einfluss einer Ätherbrücke auf den Verteilungskoeffizienten.

Verbindungen	Erniedrigung des Verteilungskoeffizienten pro Ätherbrücke
n-Amylalkohol [1] : Diäthylenglykolmonomethyläther	$\sqrt{21 : 0{,}037} = 24$
n-Propanol : Äthylenglykolmonomethyläther	$1{,}9 : 0{,}15 = 13$
Octamethylenglykol [2] : Tetraäthylenglykol	$\sqrt[3]{1{,}5 : 0{,}0024} = 8{,}6$
Tetramethylenglykol [3] : Diäthylenglykol	$0{,}027 : 0{,}0040 = 6{,}8$
Hexamethylenglykol : Triäthylenglykol	$\sqrt{0{,}12 : 0{,}0031} = 6{,}2$
Bernsteinsäure : Diglykolsäure	$0{,}15 : 0{,}030 = 5{,}0$

In der Tat zeigt Tabelle 6, dass die Einführung einer —O—Brücke zwischen zwei Kohlenstoffatomen den Verteilungskoeffizienten etwa 5- bis 13mal (bisweilen bis 24mal) kleiner macht. Ein Äthersauerstoffatom würde hiernach den die Verteilung beeinflussenden Effekt von etwa zwei bis drei CH_2-Gruppen kompensieren können. Dementsprechend zeigt die Reihe der Polyäthylenglykole, in der sich jedes Glied von dem vorhergehenden durch den Eintritt von zwei CH_2-Gruppen und einem Äthersauerstoffatom unterscheidet, dass der Verteilungskoeffizient sich mit zunehmender Verlängerung der Kette ganz langsam vermindert. (Die betreffenden Verteilungskoeffizienten sind:

[1] Verteilungskoeffizient berechnet auf Grund desjenigen des *iso*-Amylalkohols.

[2] Verteilungskoeffizient erhalten durch Interpolation.

[3] Verteilungskoeffizient berechnet auf Grund desjenigen des Trimethylenglykols.

Äthylenglykol 0,0053, Diäthylenglykol 0,0040, Triäthylenglykol 0,0031, Tetraäthylenglykol 0,0024.)

Einen Vergleich des hydrotropen Effekts der Methoxylgruppe mit dem der Hydroxylgruppe ermöglicht Tabelle 7. Man sieht, dass die Methylierung eines alkoholischen Hydroxyls den Verteilungskoeffizienten etwa 9- bis 28mal vergrössert. Nach dem Substanzpaar Morphin-Codein zu schliessen ist der Effekt der Methylierung einer Phenolhydroxylgruppe geringer.

Tabelle 7. Einfluss der Methylierung einer Hydroxylgruppe auf den Verteilungskoeffizienten.

Verbindungen	Veränderung des Verteilungskoeffizienten pro methylierte Hydroxylgruppe
Glycerinmonomethyläther : Glycerin	0,019 : 0,00066 = 29
Äthylenglykolmonomethyläther : Äthylenglykol	0,15 : 0,0053 = 28
Methoxyessigsäure : Glykolsäure	0,18 : 0,028 = 6,4
Diäthylenglykolmonomethyläther : Diäthylenglykol	0,037 : 0,0040 = 9,3
Codein : Morphin	0,80 : 0,21 = 3,8

8. Die Carboxylgruppe

Wenn in einem Molekül eine CH_3-Gruppe durch eine COOH-Gruppe ersetzt wird, so erniedrigt sich der Verteilungskoeffizient, wie aus Tabelle 8 ersichtlich, etwa 2- bis 170 mal, also etwa ebenso viel wie bei der Einführung einer OH-Gruppe. Die Tabelle zeigt deutlich, dass der in hohem Grade variierende Einfluss des Carboxyls im grossen ganzen um so schwächer ausfällt, je hydrophiler das Molekül im voraus ist. Die Erklärung hierfür liegt zweifellos — ähnlich wie im Falle der OH-Gruppe — wenigstens teilweise in der Bildung von intramolekularen Wasserstoffbindungen, die die Hydrophilie abschwächen.

Tabelle 8. Einfluss des Ersatzes einer CH_3-Gruppe durch eine COOH-Gruppe auf den Verteilungskoeffizienten.

Verbindungen	Erniedrigung des Verteilungskoeffizienten pro COOH-Gruppe
Capronsäure : Adipinsäure	93 : 0,54 = 172
n-Valeriansäure : Glutarsäure	25 : 0,28 = 90
Äthyljodid : Monojodessigsäure	340 : 7,2 = 47
n-Buttersäure : Bernsteinsäure	6,8 : 0,15 = 45
n-Propanol : β-Oxypropionsäure	1,9 : 0,084 = 23
Propionsäure : Malonsäure	1,8 : 0,10 = 18
Äthanol : Glykolsäure	0,26 : 0,028 = 9,3
Essigsäure : Oxalsäure	0,48 : 0,11 = 4,4
2,3-Butylenglykol : Weinsäure	$\sqrt{0,029 : 0,0043}$ = 2,6
Propylenglykol : Glycerinsäure	0,018 : 0,0090 = 2,0

Einen Fall für sich bildet der Eintritt eines Carboxyls in das Molekül eines Alkylamins. Da hierdurch ein schwacher Elektrolyt in einen starken verwandelt wird, versteht man, dass der Verteilungskoeffizient gewaltig herabfällt (z. B. beim Übergang Äthylamin → Glykokoll etwa um das 100000-fache).

9. Die Esterbindung

Aus Tabelle 9 sieht man, dass, wenn eine Säure mit Methylalkohol verestert wird, der Verteilungskoeffizient 3,7- bis 5,2mal vergrössert wird pro veresterte Carboxylgruppe. Die Verteilung verändert sich also in den betrachteten Fällen kaum mehr als wenn die Kohlenstoffkette der Säure um eine CH_2-Gruppe verlängert worden wäre. Die Erklärung dafür, dass der Effekt der Veresterung nicht grösser ausfällt, liegt vielleicht darin, dass die Ester ganz analog den Äthern, Aldehyden und Ketonen keine Wasserstoffbindungen mit den Äthermolekülen, wohl aber mit den Wassermolekülen bilden können.

Tabelle 9. Vergleich zwischen den Verteilungskoeffizienten von Säuren und ihren Methylestern.

Ester	Säure	Zunahme des Verteilungskoeffizienten pro veresterte Carboxylgruppe
Glykokollmethylester	Glykokoll	0,073 : (0,0000007) = (10^5)
Methylacetat	Essigsäure	2,7 : 0,52 = 5,2
Methyllactat	Milchsäure	0,37 : 0,091 = 4,1
Dimethyltartrat [1]	Weinsäure	$\sqrt{0{,}046 : 0{,}0034}$ = 3,7
Trimethylcitrat	Citronensäure	$\sqrt[3]{0{,}43 : 0{,}0086}$ = 3,7

Wenn dagegen eine aliphatische Aminosäure verestert wird, so steigt der Verteilungskoeffizient rund um das Hunderttausendfache, was ja eine leicht begreifliche Folge davon ist, dass dabei der als Zwitterion vorhandene starke Elektrolyt in eine schwache Base übergeht.

Wird ein Alkohol mit Essigsäure verestert, so vergrössert sich sein Verteilungskoeffizient etwa um das 6- bis 30fache (möglicherweise bis um das 60fache) pro veresterte Hydroxylgruppe (Tab. 10), d. h. etwa ebenso viel, wie wenn seine Kohlenstoffkette um etwa zwei bis drei CH_2-Gruppen verlängert worden wäre.

[1]) Die Verteilung berechnet auf Grund derjenigen des Diäthyltartrats.

Tabelle 10. Vergleich zwischen den Verteilungskoeffizienten von Alkoholen und ihren Essigsäureestern.

Verbindungen	Zunahme des Verteilungskoeffizienten pro veresterte Hydroxylgruppe		
Monoacetin : Glycerin	0,041 (?)	: 0,00066	= 62[1]
Äthylacetat : Äthanol	8,5	: 0,26	= 33
Pentaacetylglucose : Glucose	$\sqrt[5]{16}$	: (0,0000045)	= (20)
Äthylendiacetat : Äthylenglykol	$\sqrt{2,0}$	: 0,00053	= 19
Pentaerythrittetraacetat : Pentaerythrit	$\sqrt[4]{9,3}$	: 0,00011	= 17
Triacetin : Diacetin	1,4	: 0,22	= 6,4
Diacetin : Monoacetin	0,22	: 0,041 (?)	= 5,4[1]

10. Die Aminogruppe (einschliesslich = NH und ≡ N)

Da die Wasserstoffbindungen zwischen H und N wesentlich schwächer sind als diejenigen zwischen H und O, könnte man wohl a priori erwarten, dass der hydrotrope Effekt der Aminogruppe entsprechend schwächer wäre als derjenige der Hydroxylgruppe[Vgl. 32]. Tatsächlich wirkt aber, jedenfalls in der aliphatischen Reihe, eine Aminogruppe deutlich stärker auf die Verteilung im System Äther/Wasser als eine OH-Gruppe. Man vergleiche in dieser Beziehung die Tabellen 4 und 11 miteinander. Einen weiteren Hinweis in derselben Richtung geben die Verteilungskoeffizienten etwa der folgenden Ver-

Tabelle 11. Einfluss einer Amino- oder Iminogruppe auf den Verteilungskoeffizienten.

Verbindungen	Erniedrigung des Verteilungskoeffizienten pro NH_2- oder NH-Gruppe	
Capronsäure : Leucin	93 : 0,000012	= $7,8 \times 10^6$
Buttersäure : *a*-Aminobuttersäure	6,5 : 0,0000026	= $2,5 \times 10^6$
Propionsäure : *a*-Alanin	1,8 : 0,0000014	= $1,3 \times 10^6$
Diäthylamin : Piperazin	0,53 : 0,00052	= 1000
Amylamin : Pentamethylendiamin	2,0 : 0,0025	= 800
Propanol : Propanolamin	1,9 : 0,0043	= 442
Propylamin : Propylendiamin	0,29 : 0,0011	= 264
Äthanol : Äthanolamin	0,26 : 0,0013	= 200
Äthylamin : Äthylendiamin	0,060 : 0,00033	= 182
Benzoesäure : *m*-Aminobenzoesäure	78 : 1,5	= 52
Propanolamin : Diaminopropanol	0,0043 : 0,00020	= 22
Benzoesäure : *p*-Aminobenzoesäure	78 : 7,6	= 10
» : *o*- »	78 : 27	= 2,9
Pyridin : Aminopyridin	1,2 : 0,77	= 1,6

[1] Unsicherer Wert. Vgl. Tab. 1, Anm. 9.

bindungen: Äthylenglykol 0,0053, Äthanolamin 0,0013, Äthylendiamin 0,00033. (Mehrere analoge Fälle sind bekannt.) Die Frage, wie der unerwartet grosse Effekt der Aminogruppe zu erklären ist, mag offen gelassen werden.

Wird eine Aminogruppe in das Molekül einer Fettsäure eingeführt, so erniedrigt sich der Verteilungskoeffizient etwa um das Millionenfache. Dies rührt natürlich davon her, dass hierbei ein starker Elektrolyt (ein Zwitterion) entsteht.

Auch die Tetraalkylammoniumhydroxyde (in unserem Material durch das Tetraäthylammoniumhydroxyd repräsentiert) haben sehr niedrige Verteilungskoeffizienten, was natürlich gleichfalls mit der Stärke ihrer Dissoziation zusammenhängt.

Das Harnstoffmolekül enthält ja, ähnlich wie etwa das Äthylendiaminmolekül, zwei Aminogruppen, ausserdem aber ein Carbonylsauerstoffatom. Wenn man dazu noch berücksichtigt, dass das Molekül des Äthylendiamins ein C-Atom mehr enthält als das des Harnstoffs, so scheint a priori alles dafür zu sprechen, dass der Verteilungskoeffizient des Harnstoffs bedeutend niedriger sein müsste als der des Äthylendiamins. Tatsächlich ist aber der Koeffizient des Harnstoffs (0,00047) im Gegenteil ein klein wenig grösser als der des Äthylendiamins (0,00033).

Aromatische Aminogruppen scheinen einen schwächeren Einfluss auf die Verteilung zu haben als die aliphatischen. Wenigstens in den in der Tabelle 11 verzeichneten Fällen verkleinern sie den Koeffizienten nur etwa um das 2- bis 50fache. Aromatische Aminosäuren von dem Typus der Aminobenzoesäure sind schwache Elektrolyte. Es ist somit verständlich, dass die Verteilungskoeffizienten dieser Verbindungen von einer ganz anderen Grössenordnung sind als die der aliphatischen Aminosäuren.

Tabelle 12. Einfluss der Substitution einer COOH-Gruppe durch eine $CONH_2$-Gruppe.

Verbindungen	Erniedrigung des Verteilungskoeffizienten pro Amidogruppe	
Ameisensäure : Formamid	0,42 : 0,0014	= 300
Essigsäure : Acetamid	0,52 : 0,0025	= 210
Propionsäure : Propionamid	1,8 : 0,013	= 140
n-Buttersäure : *n*-Butyramid	6,5 : 0,058	= 110
iso-Valeriansäure : *iso*-Valeramid	20 : 0,17	= 110
Milchsäure : Lactamid	0,091 : 0,0018	= 51
Diäthylmalonsäure : Diäthylmalonamid	$\sqrt{11 : 0{,}012}$	= 30
Malonsäure : Malonamid	$\sqrt{0{,}10 : 0{,}00030}$	= 18

11. Die Säureamidogruppe

Die Säureamidogruppe hat eine sehr kräftig hydrotrope Wirkung. So ist z. B. der Verteilungskoeffizient des Propionamids rund 10,000mal kleiner als der des Propans. Wenn eine COOH-Gruppe durch eine $CONH_2$-Gruppe ersetzt wird, vermindert sich der Verteilungskoeffizient, wie aus Tabelle 12 ersichtlich, etwa um das 20- bis 300fache. Auch die Substitution von NH_2 durch $CONH_2$ macht den Verteilungskoeffizienten mehr als 10mal kleiner.

12. Die Cyangruppe

	k		k		k
Acetonitril	0,60	Cyanessigsäure	0,36	Äthylencyanid	0,32
Essigsäure	0,52	Malonsäure	0,10	Bernsteinsäure	0,15

Aus der obigen Zusammenstellung geht hervor, dass der Verteilungskoeffizient etwas (aber manchmal recht wenig) erniedrigt wird, wenn ein Nitril zu der entsprechenden Carbonsäure verseift wird.

13. Die Nitrogruppe

In Gegensatz zu den bisher besprochenen Substituenten bewirkt die Nitrogruppe wenigstens in einigen Fällen eine Erhöhung des Verteilungskoeffizienten. Die Verteilung sämtlicher Mononitrophenole fällt nämlich stärker zugunsten der Ätherphase aus als diejenige des unsubstituierten Phenols. Andere Nitroverbindungen sind nicht untersucht worden.

Tabelle 13. Einfluss eines Halogenatoms auf den Verteilungskoeffizienten.

Verbindungen	Vergrösserung des Verteilungskoeffizienten pro Halogenatom	
Chloracetamid : Acetamid	0,096 : 0,0025	= 38
Dichloressigsäure : Monochloressigsäure	18 : 2,9	= 6,2
Monochloressigsäure : Essigsäure	2,9 : 0,52	= 5,6
Glycerinmonochlorhydrin : Propylenglykol	0,080 : 0,018	= 4,4
Trichloressigsäure : Dichloressigsäure	(37) : 18	= (2,1)
Monobrombernsteinsäure : Bernsteinsäure	2,9 : 0,15	= 19
Dibrombernsteinsäure : Monobrombernsteinsäure	54 : 2,9	= 19
Monobromessigsäure : Essigsäure	4,4 : 0,52	= 8,5
α-Brompropionsäure : Propionsäure	15 : 1,8	= 8,3
Monojodessigsäure : Essigsäure	7,2 : 0,52	= 14

14. Halogen

Aus der Tabelle 13 geht hervor, dass Halogenatome in allen untersuchten Fällen die Verteilung zugunsten der Ätherphase verschieben, und zwar vergrössert ein Cl-Atom den Verteilungskoeffizienten in den untersuchten Fällen meistens etwa um das 4- bis 6- fache, ein Br-Atom etwa um das 8- bis 19- fache, ein J-Atom in dem einzigen untersuchten Fall um das 14-fache. Dies ist vermutlich auf wenigstens zwei verschiedene Umstände zurückzuführen. Erstens bewirkt ja die Substitution von Wasserstoff durch Halogen eine Vergrösserung des Molekularvolumens. (Ein Cl-Atom ist in dieser Beziehung etwas einer CH_2-Gruppe, ein J-Atom dagegen etwa 1 3/4 CH_2-Gruppen gleich.) Dazu kommt aber vermutlich noch als zweiter Faktor hinzu, dass, wie Marvel, Dietz und Copley [37] wahrscheinlich gemacht haben, Wasserstoffbindungen vom Typus C—H ← O beim Auflösen von teilweise halogenisierten Kohlenwasserstoffen in Äther (und anderen »donor solvents») gebildet werden. Mit dieser zweiten Erklärung stimmt es überein, dass der Übergang von Di- zu Trichloressigsäure eine wesentlich geringere Erhöhung des Verteilungskoeffizienten bewirkt als der Übergang von Essigsäure zu Monochloressigsäure oder von dieser zu Dichloressigsäure. Nach Marvel, Copley und Ginsberg [38] nimmt die Aktivierung der Wasserstoffatome durch Halogen in der Reihe Cl > Br > J ab. Da trotzdem die Beeinflussung des Verteilungskoeffizienten die umgekehrte Reihenfolge aufweist, so liegt dies wohl wenigstens teilweise daran, dass das J-Atom das Molekularvolumen stärker beeinflusst als das Cl-Atom.

15. Substitution von Sauerstoff durch Schwefel

Thioharnstoff hat einen 13mal höheren Wert des Verteilungskoeffizienten als Harnstoff. Nach Overton fällt auch die Verteilung der Mercaptane und der Mercaptide stärker als die der entsprechenden Alkohole und Alkyloxyde zugunsten des Äthers aus. Dies ist ohne weiteres verständlich, da ja an Schwefel gebundene Wasserstoffatome nicht oder kaum zur Bildung von Wasserstoffbindungen befähigt sind.

16. Doppelbindung

Aus der Tabelle 14 geht hervor, dass, wenn eine Äthylenbindung zwischen zwei C-Atomen unter Wegfall von zwei H-Atomen zustandekommt, der Verteilungskoeffizient manchmal fast unverändert bleibt, während er in anderen Fällen bis etwa um das 10fache vergrössert wird.

Tabelle 14. Einfluss einer Doppelbindung auf den Verteilungskoeffizienten.

Verbindungen	Vergrösserung des Verteilungskoeffizienten
Fumarsäure : Bernsteinsäure	1,5 : 0,15 = 10
Aconitsäure : Tricarballylsäure	0,50 : 0,060 = 8,3
Acrylsäure : Propionsäure	2,3 : 1,8 = 1,3
Maleinsäure : Bernsteinsäure	0,15 : 0,15 = 1,0
α-Crotonsäure : Buttersäure	5,3 : 6,5 = 0,8

17. Isomerie

Der Einfluss der Isomerie ist nur stichprobenweise untersucht worden.

a) *Verzweigung der Kohlenstoffkette und Ortsisomerie aliphatischer Verbindungen.* Bei den vier Butanolen fällt der Verteilungskoeffizient in folgender Reihenfolge ab: *n*-Butanol-1 (7,7) > *iso*-Butanol-1 (6,9) > *n*-Butanol-2 (4,5) > Trimethylcarbinol (2,2). Hiernach zu schliessen fällt die Verteilung umso mehr zugunsten der Wasserphase aus, je mehr die C-Kette verzweigt ist und je zentraler die hydrophile Gruppe im Molekül gelegen ist. Vgl. [33]. Ähnlich verhalten sich die untersuchten α-Oxybuttersäuren (*k* der *n*-Verbindung 0,39, der *iso*-Verbindung 0,26). Die Valeriansäuren (*k* der *n*-Verbindung 25, der *iso*-Verbindung 20, der Trimethylessigsäure 35) verhalten sich dagegen weniger regelmässig.

Die Alkylmalonsäuren haben einen wesentlich grösseren Verteilungskoeffizienten als die isomeren Dicarbonsäuren mit unverzweigter Kohlenstoffkette:

Dimethylmalonsäure	1,6	Diäthylmalonsäure	11
Glutarsäure	0,28	Pimelinsäure	1,5

Es liegt nahe anzunehmen, dass die verhältnismässig grosse Ätherlöslichkeit der Malonsäuren dadurch bedingt ist, dass die beiden Carboxyle einander so nahe gelegen sind, dass die Bildung intramolekularer Wasserstoffbindungen ermöglicht ist. Analog ist es wohl zu erklären, dass die Verteilung sowohl des Pinakons wie des 2-Methyl-2,4-pentandiols mehr zugunsten der Ätherphase ausfällt als die des Hexamethylenglykols. Weitere Beispiele dafür, dass die Verteilung bei zunehmender Entfernung der hydrophilen Gruppen zugunsten der Wasserphase verschoben wird, bieten folgende Stoffpaare:

	k		*k*		*k*
1,2-Propylenglykol	0,018	1,2-Propylendiamin	0,0011	α-Oxypropionsäure	0,091
Trimethylenglykol	0,010	Trimethylendiamin	0,0007	β-Oxypropionsäure	0,084

Nach dem bisher vorliegenden, allerdings viel zu knappen Beobachtungsmaterial zu schliessen besteht hinsichtlich der Verteilung kein gesetzmässiger Unterschied zwischen primären, sekundären und tertiären Aminen:

	k		*k*		*k*
Äthylamin	0,060	n-Propylamin	0,29	Dipropylamin	8,9
Dimethylamin	0,055	Trimethylamin	0,46	Triäthylamin	5,9

Auch isomere Alkylharnstoffe verschiedenen Symmetriegrades (sym. und asym. Dimethylharnstoff sowie Äthylharnstoff) scheinen sich bezüglich der Verteilung recht ähnlich zu verhalten.

b) *Ortsisomerie der Benzolverbindungen.*

	k		*k*		*k*
Brenzcatechin	11	*o*-Oxybenzoesäure	236	*o*-Aminobenzoesäure	27
Resorcin	4,2	*m*- »	21	*m*- »	1,5
Hydrochinon	3,7	*p*- »	26	*p*- »	7,6
		o-Nitrophenol	150		
Pyrogallol	1,7	*m*- »	160	*o*-Phthalsäure	1,6
Phloroglucin	0,45	*p*- »	110	*m*- »	28

Bei den Dioxybenzolen, Oxybenzoesäuren und Aminobenzoesäuren ist, wie ersichtlich, die relative Ätherlöslichkeit am grössten, wenn die Substituenten in *o*-Stellung zueinander stehen, was ungezwungen darauf zurückgeführt werden kann, dass gerade diese Isomeren durch kräftige intramolekulare Wasserstoffbindungen ausgezeichnet sind. Analog hiermit fällt die Verteilung des 1,2,3-Trioxybenzols mehr zugunsten der Ätherphase aus als die der entsprechenden 1,3,5-Verbindung. Es liesse sich denken, dass sich ähnliches bei den Nitrophenolen wiederholen würde, wie ersichtlich war dies aber nicht der Fall. Auch bei den Phthalsäuren scheint die *m*-Stellung einen grösseren Verteilungskoeffizienten als die *o*-Stellung zu bedingen.

c) *Stereoisomerie.* Da die frei drehbaren Carboxylgruppen im Bernsteinsäuremolekül infolge gegenseitiger Abstossung überwiegend in trans-Stellung stehen (wenigstens in wässeriger Lösung), würde es wohl nahe liegen, anzunehmen, dass sich die Fumarsäure, wo die *trans*-Stellung fixiert ist, ähnlich verhalten würde. Tatsächlich liegt die Sache jedoch umgekehrt: der Verteilungskoeffizient der Maleinsäure (also der *cis*-Verbindung) stimmt genau mit demjenigen der Bernsteinsäure überein, wogegen derjenige der Fumarsäure 10mal grösser ist.

SUMMARY

The distribution coefficients of some 200 organic compounds in the system ethyl ether/water are given in Table 1. A contemplation of these coefficients reveals, among other things, the following facts:

1. In each homologous series the distribution coefficient $\frac{c_{ether}}{c_{water}}$, with a few exceptions, increases by 2—4 times for every new CH_2 group incorporated in the molecule (Table 3).

2. An alcoholic hydroxyl group reduces the distribution coefficient by about 5—150 times. The more numerous and the more effective the hydrophilic groups that the molecule initially containes, the smaller is the effect (Table 4).

3. The distribution of the aldehydes and ketones is about the same as that of the corresponding alcohols.

4. An ether bridge reduces the distribution coefficient by about 5—20 times (Table 6).

5. If a CH_3 group is substituted by a COOH group this makes the distribution coefficient about 2—170 times smaller. In this case also the more hydrophilic the molecule is originally the smaller is the effect (Table 8).

6. If a COOH group is esterified with methyl alcohol, the distribution coefficient is not much more affected than if the carbon chain of the acid had been lengthened by a CH_2 group (Table 9).

7. An aliphatic amino group makes the distribution coefficient about 20—1000 times smaller (Table 11).

8. If a COOH group is substituted by a $CONH_2$ group the distribution coefficient becomes about 20—300 times smaller (Table 12).

9. A halogen atom usually makes the distribution coefficient 4—40 times greater (Table 13).

10. If two hydrophilic groups are in the α- or ortho-position the distribution coefficient is greater than if the groups are more remote.

11. The distribution of organic compounds between ether and water is largely understandable if we assume that it is principally due to the formation of hydrogen bonds between solute and solvent molecules.

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Eingegangen am 24. April 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 748–754

Retene investigations

I. Acetyl retene

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Retene, 1-methyl-7-isopropyl phenanthrene, which occurs in considerable quantities in pine wood tar, has in later years attracted the attention of several chemists, not only with regard to its industrial possibilities but also because of its peculiar chemical properties.

The investigation of retene substitution products is complicated by the fact that a great number of isomers may be formed simultaneously and may be extremely difficult to separate because many retene derivatives are very slowly crystallizing substances or, while in mixture with closely related compounds, completely unable to crystallize.

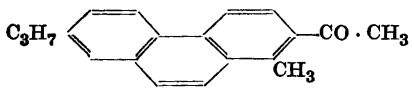
The introduction of an acetyl group by means of the Friedel-Crafts reaction has been studied by several authors [1-4]. All of them have used acetyl chloride in either carbon disulfide or nitrobenzene for the reaction. Nyman (*l. c.*) reports a total yield of 60 % without stating the amount of pure compound obtained. All the said investigators have isolated only one acetyl retene, *viz.* 3-acetyl retene, m. p. 99°, the yield of this compound varying but never exceeding 45 %.

In this laboratory acetic anhydride has been used instead of acetyl chloride. It hardly makes any difference as to the products obtained, but the low vapour pressure of the anhydride as compared with the acid chloride is a definite advantage. Various solvents have been used, in addition to the aforementioned: tetrachloro ethane and trichloro ethylene. The total yield of acetyl retenes is practically independent of the solvent chosen; but the yields of the different isomers vary considerably with the nature of the solvent, nitrobenzene being the most favorable for the formation of the 3-substitute.

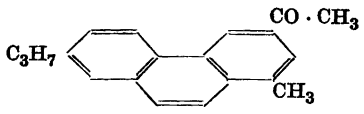
In our experiments we, rather surprisingly, have found a new isomer which is less soluble and more readily crystallizing than 3-acetyl retene. The reason

why it has escaped the attention of the earlier workes may be that in carbon disulfide relatively small amounts are formed. Trichloro ethylene seems to be most favorable for the new compound. While only 5—8 % are formed in carbon disulfide and nitrobenzene some 15 % can be isolated from the reaction mixture when trichloro ethylene is used.

The new acetyl retene has m. p. 156—57° and is very slightly soluble in alcohol, ether, and glacial acetic acid, more soluble in benzene. The position of the acetyl group has been determined in the following way. The substance is oxidized with chromic acid in acetic acid to a quinone with the same melting point as an acetyl retene quinone which has been obtained by Nyman [5] by oxidation of 2-acetyl-9,10-dihydroretene the constitution of which has been established by him. In order to secure the identity of these quinones the new one was oxidized with hydrogen peroxide and gave the same diphenic acid and its anhydride as those described by Nyman. The 2-position of the acetyl group is thus firmly established.



2-Acetyl retene



3-Acetyl retene

In a recent paper [6] Buu-Hoi and co-workers claim to have isolated a diacetyl retene, m. p. 157°, by direct acetylation of retene in carbon disulfide, and to have prepared 2-acetyl retene, m. p. 151°, by dehydrogenation of 2-acetyl dihydroretene with selenium. They state that the latter is very soluble in hot alcohol which, however, is not the case with our compound. There can hardly be any doubt about the identity of their 'diacetyl retene' with our 2-acetyl retene. As we have prepared and analyzed several derivatives of this substance we believe that an analytical error has misled the French authors. The purpose of their experiment: to prove that 2-substitution can occur in retene, is, however, fulfilled through our results.

When 2- and 3-acetyl retenes are removed in crystalline form from the reaction mixture (see experimental part) the remaining oil is distilled under reduced pressure and a very viscous light yellow oil is obtained from which no crystalline material has hitherto been isolated. Practically the entire distillate passes over within 2—3 degrees when heating and pressure are kept constant. It is, therefore, hopeless to attempt a separation of the components through fractional distillation.

The mixture still contains some 2- and 3-acetyl retene, but the bulk of the oil consists of two or more hitherto unknown acetyl retenes which have with-

stood all efforts of isolation. The content of 3-acetyl retene can be estimated by addition of dinitrophenyl hydrazine to a hot solution of the oil in glacial acetic acid, the dinitrophenyl hydrazone of 3-acetyl retene being nearly insoluble in the said solvent while the other hydrazones are rather soluble. 2-Acetyl retene reveals its presence when the oil is oxidized with sodium hypochlorite. Of the resulting retene carboxylic acids — which are being investigated at present and shall be dealt with in another paper — the 2-acid forms an extremely slightly soluble sodium salt crystallizing out in the oxidation mixture.

Attempts to isolate the unknown acetyl retenes as picrates have been unsuccessful, these picrates being very soluble in alcohol, benzene, and ether at room temperature. On cooling an alcoholic solution of the picrates to 0° or below a yellow oil separates which on long standing solidifies, but at room temperature the mass liquefies completely again and so far no solvent has been found by means of which a fractional crystallization has been possible.

Separation through phenyl hydrazones, too, has been tried in vain.

A number of derivatives of 2-acetyl retene are described.

EXPERIMENTAL PART

1. Preparation of acetyl retenes

Retene * (0.5 mole, 120 g), dissolved in trichloro ethylene (500 ml), and acetic anhydride (0.55 mole, 56 g) are placed in a flask provided with an efficient stirrer and cooled with running water. In the course of 30—40 minutes finely powdered aluminium chloride (1 mole, 135 g) is added in 7—8 portions and stirring is continued for 2—3 hours. In some instances the aluminium chloride-ketone complexes formed crystallize out suddenly making stirring temporarily inefficient. By means of a spatula the paste formed is broken and becomes again mobile.

The product is decomposed with ice and hydrochloric acid and the light yellow solution is separated, washed with water, and dried with calcium chloride. The solvent is removed by distillation from a water bath at reduced pressure. The remaining oil is cooled and diluted with ether (300 ml). On short standing — sometimes immediately — a crystallization sets in and after 2—3 hours at room temperature the crystals are filtered off with suction and washed with ether. The crystalline product is almost pure 2-acetyl retene the yield of which is about 15 g. It may be recrystallized from a large volume of alcohol or from glacial acetic acid.

The filtrate from 2-acetyl retene then is placed in a refrigerator at 0° for 24 hours when a few grams (3—5) of 3-acetyl retene have separated and are filtered off. Concentration of the filtrate and renewed standing at 0° in 2 days may yield another small

* Thanks are due to Dr. K. J. Karrman, Lund, Sweden, who has kindly supplied the crude retene used in this investigation. The product was distilled under reduced pressure (0.5—1 mm) before use, m. p. 95—96°.

amount of 3-acetyl retene. The rather viscous mother liquor is freed from ether and trichloro ethylene by heating on the steam bath and suction with the water pump and is then distilled at 0.1—0.2 mm mercury. When the heating and the pressure is kept as constant as possible the distillation proceeds at practically constant temperature (about 190°) during nearly the entire distillation. Toward the end the temperature of the bath is raised some twenty degrees. In the flask a dark mass remains which solidifies on cooling but yields no crystalline products. It is soluble in benzene and in trichloro ethylene and consists probably mainly of diacetyl retenes. — Total yield of crystallized and distilled acetyl retenes 110—120 g. An elementary analysis of the distilled oil — which is very viscous at room temperature — shows it to be a mixture of pure monoacetyl retenes. It has a light yellow colour.

When tetrachloro ethane is used as a solvent it is removed (after decomposition of the aluminium chloride complex) by steam distillation. When the tough ketone mixture is taken up in ether the 2-acetyl compound usually crystallizes out immediately. The yield of this substance is somewhat lower than from trichloro ethylene while a higher yield of 3-acetyl retene is obtained.

2. 2-Acetyl retene. $C_{18}H_{17}COCH_3$

Recrystallized from alcohol or glacial acetic acid the new compound melts at 156—57°. Slightly soluble in all common solvents at room temperature. It dissolves in conc. sulphuric acid with an orange-yellow colour very similar to that of 3-acetyl retene in the same solvent. (comp. the corresponding cinnamalaceto retenes below).

$C_{20}H_{20}O$	Found	C 86.9	H 6.99
	Calc.	» 86.95	» 7.25

a. *Semicarbazone*, from alcohol, m. p. 264—68° (decomposition).

$C_{21}H_{24}ON_3$ Found N 12.46 Calc. N 12.57

b. *Oxime* 'from alcohol' m. p. 214—17° with decomp.

$C_{20}H_{21}ON$ Found N 4.81 Calc. N 4.80

c. *Phenylhydrazone.* To a hot solution of 2-acetyl retene (1 g) in glacial acetic acid (15 ml) is added phenyl hydrazine (0.6 g) in 50 % acetic acid (2 ml) and the mixture is boiled for 10 minutes. On cooling, the phenyl hydrazone separates as an oil which on scratching soon solidifies. Recrystallized from 90 % acetic acid or from alcohol it melts at 119°.

$C_{26}H_{26}N_2$ Found N 7.17 Calc. N 7.10

d. *Dinitrophenyl hydrazone.* On boiling a solution of equimolar amounts of the ketone and dinitrophenyl hydrazine in glacial acetic acid for ten minutes and cooling, the dinitrophenyl hydrazone crystallizes out, m. p. after recrystallization from acetic acid 176—77°.

$C_{26}H_{24}O_4N_4$ Found N 12.5 Calc. N 12.3

The isomeric *3-acetyl retene dinitrophenyl hydrazone* has not been described before. It is rapidly formed when the two reactants are brought together in boiling glacial acetic acid solutions. It is very sparingly soluble in acetic acid, very much more so than the corresponding derivatives of the other acetyl retenes. It is peculiar that its m. p. (297—99°) is 100° higher than that of the 2-isomer while the ketone proper melts considerably lower (at 99°) than the 2-ketone. As mentioned in the theoretical part, the precipitation of 3-acetyl retene dinitrophenyl hydrazone on treatment of the distilled ketone mixture with dinitrophenyl hydrazine shows the presence of the 3-isomer in the mixture and by weighing the precipitate the content of this compound was found to be 20—30 %.

$C_{26}H_{24}O_4N_4$ Found N 12.35 Calc. N 12.3

e. *2-Acetyl retene quinone.* 2-Acetyl retene (2 g) is dissolved in hot glacial acetic acid (30 ml). At about 70° a solution of chromic acid anhydride (3 g) in water (5 ml) is added dropwise with shaking. After 10 minutes at 70° water (10 ml) is added. On cooling, the quinone separates, is filtered off, washed with dilute acetic acid and recrystallized from 90 % acetic acid. Very beautiful orange red crystals which are very sensitive to light. M. p. 197—98°.

$C_{20}H_{18}O_3$	Found	C	78.0	H	5.88
	Calc.	»	78.4	»	5.93

The quinone was oxidized with hydrogen peroxide in glacial acetic acid as described by Nyman [5]. The same 3-methyl-4′-isopropyl-4-acetyl diphenic acid, m. p. 207°, and its anhydride, m. p. 155°, were obtained.

3. Retene-2-carboxylic acid. $C_{18}H_{17}COOH$

2-Acetyl retene (0.01 mole, 2.76 g) is dissolved in dioxane (20 ml) and heated to 95°. Sodium hypochlorite (about 50 % excess), made from sodium hydroxide and chlorine in the usual manner and titrated, is slowly added with efficient stirring while the temperature is kept between 90 and 100°. The slightly soluble sodium salt of the acid crystallizes out during the reaction. After cooling the mixture the salt is filtered with suction and washed with cold water. It is boiled with about 700 ml water to which is added a little ammonia, filtered from a small amount of unchanged acetyl retene and acidified with hydrochloric acid. The dried product is recrystallized from xylene. M. p. 275—78° after sintering at about 260°. It sublimes noticeably at the melting point.

$C_{19}H_{18}O_2$	Found	C	81.8	H	6.40
	Calc.	»	82.0	»	6.47

4. 2-Cinnamoyl retene (benzalaceto retene). $C_{18}H_{17}COCH{:}CHC_6H_5$

2-Acetyl retene (2 g) is dissolved in boiling alcohol (200 ml), benzaldehyde (1 g) is added and the solution is cooled with shaking to about 60° when the acetyl retene has partly separated in small crystals. Then a solution of potassium hydroxide (1 g) in alcohol (10 ml) is added and the mixture shaken with hand. The crystals soon disappear whereupon the condensation product begins to crystallize in beautiful light yellow plates. After

standing half an hour the crystals are filtered off and recrystallized from alcohol. M. p. 147—48°.

$C_{27}H_{24}O$	Found	C	89.2	H	6.64
	Calc.	»	89.0	»	6.64

Similarly *2-cinnamalaceto retene*, $C_{18}H_{17}COCH{:}CHCH{:}CHC_6H_5$ is formed by condensation of cinnamic aldehyde with 2-acetyl retene. Yellow crystals from benzene, m. p. 189°. Yield almost quantitative.

$C_{29}H_{26}O$	Found	C	89.7	H	6.71
	Calc.	»	89.2	»	6.67

2-Cinnamalaceto retene dissolves in conc. sulphuric acid with a pure red colour while the halochromic colour of the isomer 3-compound resembles the colour of a permanganate solution.

5. 2-(α-Hydroxyethyl)-retene. $C_{18}H_{17}CHOHCH_3$

2-Acetyl retene (5.8 g) is reduced with aluminium-isopropoxide (40 ml of a molar solution in isopropyl alcohol + 60 ml isopropyl alcohol) in the usual manner [7]. The reduction is complete in about 90 minutes. An excess of cold dilute hydrochloric acid is added with stirring to the cooled reaction mixture. The precipitate is filtered off and washed with dilute alcohol. Yield almost quantitative. Recrystallized from alcohol the compound melts at 171—73°.

$C_{20}H_{22}O$	Found	C	85.9	H	8.05
	Calc.	»	86.3	»	7.95

6. 3-(α-Hydroxyethyl)-retene. $C_{18}H_{17}CHOHCH_3$

This compound is made from 3-acetyl retene in the same way. On addition of hydrochloric acid to the reaction mixture the carbinol separates as an oil which soon solidifies. This carbinol is considerably more soluble and more slowly crystallizing than the 2-isomer. Yield nearly quantitative. Recrystallized from alcohol its m. p. is 115—116°.

$C_{20}H_{22}O$	Found	C	86.0	H	7.93
	Calc.	»	86.3	»	7.95

SUMMARY

Introduction of an acetyl group into retene by means of the Friedel-Crafts reaction leads to a number of isomers. In addition to the previously known 3-acetyl retene a new isomer has been isolated and identified as 2-acetyl retene, but at least two other isomers are formed in considerable amounts. The relative amounts of the different components of the reaction product depend upon the solvent chosen for the reaction. Trichloro ethylene is shown to be an excellent solvent for the Friedel-Crafts reaction.

A number of compounds prepared from 2- and 3-acetyl retene are described: besides some functional derivatives, retene-2-carboxylic acid and the two secondary alcohols corresponding to the ketones.

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Received June 2, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 755–758

Constituents of Pine Heartwood

IX.* The Heartwood of *Pinus montana* Mill

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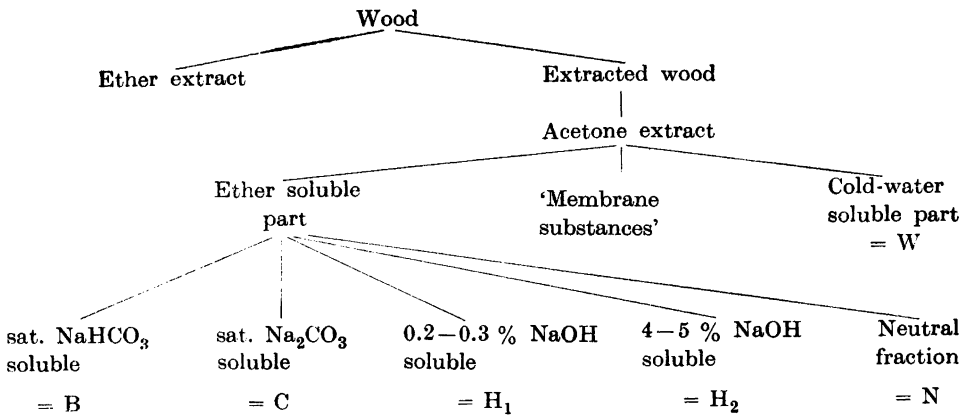
Some years ago, an investigation of the heartwood constituents of a few *Pinus* species was carried out by Erdtman [1]. All these pines were found to contain pinosylvin (3,5-dihydroxistilbene) or its monomethyl ether. Most of them also contained flavone or flavanone derivatives, and in *P. cembra* and *P. strobus*, both belonging to the section *Haploxylon*, the heartwood yielded pinitol in addition to phenolic derivatives. The presence of phenols in the heartwood can be demonstrated by staining a freshly-cut section through the stem with diazotised benzidine solution. In a few seconds the heartwood turns more or less dark red (formation of azo dyes), whereas the sapwood, containing little or no phenolic substances, shows only a slight yellowish colour.

Although the phenols are ether-soluble, they cannot be extracted from the wood with ether. They are, however, easily extracted with acetone or alcohol. This is due to the presence of ether-insoluble 'membranes' which are supposed to surround the phenols in the wood, and thus prevent them from being extracted with ether [2]. The 'membrane substances' can be precipitated from the acetone extract by ether. If the acetone extraction of the wood is preceded by an ether extraction, the greatest part of the resinous substances is removed by the ether, and isolation of crystalline products from the acetone extract is facilitated. *P. strobus*, however, behaves differently [1a]. Most of its phenolic heartwood constituents were found in the ether extract, and the quantity of 'membrane substances' was relatively small.

An investigation of pine heartwoods is now being continued by the writer, and this is the first in a series of papers describing the heartwood constituents of several *Pinus* species from both the *Haploxylon* and the *Diploxylon* sections.

The extractions were carried out according to the general scheme given below:

* VIII. Erdtman, H. *Svensk Kem. Tid.* 56 (1944) 134.



The heartwood of *P. montana* (European pine, section *Diploxylon*) has already been briefly investigated by Erdtman [1c], who isolated pinocembrin (5,7-dihydroxiflavanone) and pinosylvin monomethyl ether from the acetone extract. In addition to these substances, pinosylvin was found in the present investigation.

The ether extract always contains small amounts of phenolic substances which have been extracted because some of the »membranes» were destroyed during the grinding [2]. The phenols in question are almost insoluble in light petroleum, but most of them can be dissolved in boiling water. A rough estimation of the amount of phenols in the ether extract can be made by precipitating with light petroleum and extraction of the precipitate with boiling water. Thus the ether extract from *P. montana* yielded 0.5 % of a phenolic fraction. This was not investigated any further. The study of the resin acids and other constituents of the ether extract lies beyond the scope of this investigation.

The sodium carbonate fraction of the acetone extract yielded pinocembrin, m. p. 194—195° *, $[\alpha]_D^{20}$ — 54.5° (in methanol). (Erdtman reported —45.5° and a few lower values [1b].) The pinocembrin isolated during the present investigation was purified only by recrystallisation or adsorption, whereas earlier preparations of pinocembrin were purified also by vacuum sublimation. Control experiments have shown that vacuum-sublimated pinocembrin may have any specific rotation between zero and —50° and a m. p. between 195 and 200°. It is evident that the low rotation previously reported for pinocembrin is due to partial racemisation during the sublimation. The pinocem-

* All melting points uncorrected.

brin isolated from about ten different pine species had specific rotations between —54 and —57°. (See forthcoming papers.) It is probable, therefore, that this pinocembrin represents pure laevorotary 5,7-dihydroxiflavanone.

From the 0.3 % sodium hydroxide fraction of the acetone extract, pinosylvin was isolated, and the 5 % sodium hydroxide extract yielded pinosylvin monomethyl ether.

3.0 kg of heartwood yielded 270 g of ether extract (9 %), 2.4 g of pinocembrin (2.0 g of which were obtained in a pure state) (0.08 %), 1.4 g of pinosylvin (0.05 %) and 3.1 g of pinosylvin monomethyl ether (0.1 %).

The content of pinosylvin phenols in the heartwood of this specimen of *P. montana* seems to be low. *P. sylvestris* often contains one per cent or more of pinosylvin and its monomethyl ether. Too much stress, however, should not be laid on the quantitative yields in this investigation, since only one tree of each species has been used. An investigation of several hundred specimens of *P. sylvestris* seems to indicate that the phenol content shows great individual variations (Erdtman and Frank, to be published).

EXPERIMENTAL

The pine used for the investigation was from Omberg, Sweden. The extraction was started about three weeks after the tree had been cut down. The heartwood was stained dark red when treated with diazotised benzidine solution.

The heartwood was cut to pieces of 3—4 cm length and about 5 mm diameter, allowed to dry in the air for some days, and then ground to pieces of 2—3 mm size in a Wiley mill (weight of air-dried wood 3 kg) and extracted with ether in a stainless-steel percolator for 24 hours. The ether extract was concentrated to a yellowish-brown syrup (270 g), which crystallised entirely in a few days. 3.44 g of the ether extract were treated with 50 ml of light petroleum, which left 0.53 g undissolved. This residue was a colourless solid, melting gradually at 90—100°. 3.0 % of this substance could be extracted with boiling water. The aqueous extract deposited a yellow resinous precipitate when cooled. The alcoholic solution of this precipitate gave a very faint yellowish-brown colour with ferric chloride.

After this extraction, the ether adsorbed on the wood meal was evaporated by indirect heating with steam, and the wood extracted with acetone for about 60 hours. The acetone extract was concentrated to a dark brown syrup, and treated with 400 ml of ether. A sticky precipitate of ether-insoluble 'membrane substances' was filtered off. The filtrate was shaken four times with about 200 ml of saturated sodium bicarbonate solution and then with saturated sodium carbonate solution (4 × 200 ml). Bicarbonate extract = B, carbonate extract = C.

The ether solution was then diluted to 600 ml and shaken twice with 500 ml of 0.3 % sodium hydroxide solution (= H_1) and, finally, twice with 200 ml of 5 % sodium hydroxide (= H_2). Remaining ether solution = N.

B yielded a very small amount of sticky precipitate when acidified. This fraction was discarded.

C was acidified with dilute sulphuric acid (brown, sticky precipitate) and extracted with ether. The yellow ether solution was dried over sodium sulphate and filtered through aluminium oxide. A dark brown zone was formed in the upper part of the column, but the filtrate was still yellow. It was evaporated to dryness. A brown crystalline cake was formed, which was recrystallised from toluene, yielding pale yellow crystals melting at 183—188° and a brown oil, from which crystals melting at 191—192° could be obtained by extraction with boiling water (3 l). These crystals were combined and recrystallised twice from 50 % acetic acid, yielding colourless needles, m. p. 194—195°, no melting-point depression with pinocembrin from *P. Banksiana*. Yield 2.0 g. $[\alpha]_D^{20} - 54.5° \pm 0.5°$. (Methanol, $c = 4.0$.)

From the mother liquors 0.4 g of less pure pinocembrin was obtained.

H_1 was acidified and shaken with ether. The ether solution was dried over sodium sulphate and the ether evaporated. The remaining reddish-brown oil was left standing overnight with a small amount of benzene. The next day light brown crystals had formed. They were separated, dissolved in ether and filtered through aluminium oxide, which removed most of the colour of the solution. The filtrate was concentrated to an almost colourless crystalline cake, from which pure pinosylvin was obtained after vacuum sublimation and two recrystallisations from xylene. Yield 1.4 g, m. p. 155—156°. A mixture with pinosylvin from *P. sylvestris* melted at the same temperature.

H_2 was acidified with dilute sulphuric acid, extracted with ether, and the ether solution filtered through aluminium oxide after being concentrated to 100 ml. This treatment removed most of the colour of the solution. On evaporation, a yellow crystalline cake of crude pinosylvin monomethyl ether was obtained, which was purified by two recrystallisations from 50 % acetic acid. Yield 3.1 g, m. p. 120—121°.

N was concentrated to a brown turpentine-smelling oil (2 g). No pinosylvin dimethyl ether could be found in this fraction by treatment with 1,3,5-trinitrobenzene in methanol solution [1c].

SUMMARY

Pinocembrin (5,7-dihydroxiflavanone), pinosylvin (3,5-dihydroxistilbene), and pinosylvin monomethyl ether have been isolated from the heartwood of *Pinus montana* Mill.

The author is indebted to Professor H. Erdtman for the proposal of the subject of this investigation and for collecting the wood.

The investigation was facilitated by a grant from *Statens Tekniska Forskningsråd*.

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Received June 22, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 759–762

Constituents of Pine Heartwood

X. The Heartwood of *Pinus contorta* var. *latifolia* S. Wats

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Pinus contorta Dougl., a *Diploxylon* pine which grows in Western North America, occurs in two varieties, 'shore pine' and 'lodgepole pine'[1]. 'Shore pine' is the coast form, and 'lodgepole pine' grows in the mountains. The latter has sometimes been considered to be a separate species (*P. Murrayana* Balf.). The wood used in this investigation came from a cultivated tree (lodgepole pine), grown in the neighbourhood of Nyköbing, Denmark.

The extraction was carried out according to the general scheme given in Part IX. The ether extract (3.8 % of the heartwood) was crystalline. A phenolic fraction, amounting to only 0.1 % of the whole extract, could be prepared from it, using the method given in the preceding paper.

The acetone extract was divided into fractions in the usual manner. The sodium carbonate fraction contained a mixture of pinocembrin (5,7-dihydroxiflavanone) and pinobanksin. The last-mentioned substance was first isolated by Erdtman from *P. Banksiana* [2], and shown to contain one hydroxyl group more than pinocembrin. He assumed it to be 3,5,7-trihydroxiflavanone, and this structure seems to be correct. Studies on the structure of pinobanksin are in progress and will be published in forthcoming papers. The pinobanksin from *P. contorta* melted at 174—176°* and had $[\alpha]_D^{20}$ + 16° (in methanol). (The value $[\alpha]_D^{20}$ + 1.5° reported by Erdtman [2] is due to a misprint. Should be + 15°.) The pinocembrin had m. p. 194—195°, $[\alpha]_D^{20}$ —56° (in methanol). Both substances were obtained in very small quantities.

The 0.3 % sodium hydroxide extract yielded pinosylvin and an additional quantity of pinocembrin. From the 4 % sodium hydroxide extract a very small quantity of pinosylvin monomethyl ether could be isolated.

* All melting points uncorrected.

The water-soluble part of the acetone extract was not investigated. Later, when arabinose had been isolated from several other pines, a second extraction of the heartwood of an American lodgepole pine was carried out. The water-soluble part of its acetone extract yielded *l*-arabinose.

The following products were isolated from 2.5 kg of air-dry heartwood:

Ether extract	95 g	(3.8 %)
'Membrane substances'	2.1 g	(0.08 %)
Pinocembrin	0.47 g	(0.02 %)
Pinobanksin	0.08 g	(0.003 %)
Pinosylvin	0.30 g	(0.01 %)
Pinosylvin monomethyl ether	0.10 g	(0.004 %)
Neutral fraction of acetone extract	2.0 g	(0.08 %)

1.7 kg of heartwood yielded 1.0 g of *l*-arabinose (0.06 %).

The yields of crystalline compounds were extremely low. It is especially remarkable that the quantity of pinosylvin monomethyl ether was inferior to that of the pinosylvin.

EXPERIMENTAL

2.5 kg of heartwood were extracted with ether and acetone in the manner described for *P. montana*[3]. The ether extract was concentrated to a pale yellow syrup, which soon crystallised. Yield, 95 g or 3.8 % of the heartwood. 10.1 g of this extract were treated with 150 ml of light petroleum. A brown sticky precipitate remained undissolved. The solution was decanted, and the residue boiled with 200 ml of water. The aqueous extract was filtered, cooled and extracted with ether. The ether was dried with sodium sulphate and evaporated to dryness. The residue (0.024 g) was a yellow resin. Its alcoholic solution gave a dark violet colour with ferric chloride.

From the acetone extract, »membrane substances» (2.1 g) were precipitated with ether (600 ml). The filtrate was extracted with saturated sodium bicarbonate (3 × 200 ml, extract = B), saturated sodium carbonate (3 × 200 ml, extract = C), 0.3 % sodium hydroxide (3 × 150 ml, extract = H_1) and 4 % sodium hydroxide (2 × 100 ml, extract = H_2). The remaining »neutral fraction» was concentrated. A viscous brown oil (2 g) was obtained.

B was discarded.

C was acidified and extracted with ether. The ether solution was dried over sodium sulphate and the ether evaporated. The residue, a yellowish-brown solid, was difficult to purify by simple recrystallisations. Extraction with boiling water yielded an aqueous solution, from which a crystalline precipitate was obtained on cooling. The crystals (1.7 g) melted between 165 and 180°. After several recrystallisations from toluene and from 50 % acetic acid, 0.47 g of pinocembrin could be isolated. M. p. 194—195°, mixed m. p. with pinocembrin from *P. montana* 193—194°. $[\alpha]_D^{20}$ −56° ±1° (methanol, c = 2.7).

From the mother liquors a small quantity of crude pinobanksin, m. p. 170—173°, could be isolated after a very tedious process of separation.

After removing the crystalline precipitate from the aqueous extract of C, the filtrate was extracted with ether, the yellow ether solution dried over sodium sulphate and filtered through aluminium oxide (most of the colour removed) and evaporated. A brown syrup remained, which was dissolved in hot toluene. On cooling, crystals were obtained which were recrystallised from 50 % acetic acid and, finally, twice from toluene. Thus a small quantity of pinobanksin (83 mg) was obtained, m. p. 174—176°. The m. p. was not depressed on admixture with pure pinobanksin from *P. Banksiana*. $[\alpha]_D^{20}$ + 16° ± 1° (methanol, c = 2.5).

H_1 was acidified, extracted with ether, and the ether extract concentrated. A brown oil was obtained, which partly crystallised. After treatment with benzene (1—2 ml), a light brown crystalline powder was obtained (3.4 g), melting between 110 and 145°. It was dissolved in 1.5 l of ether and shaken with 175 ml of 0.6 % sodium hydroxide.

From the alkali phase a small quantity of partly racemised pinocembrin was isolated by vacuum sublimation and several recrystallisations from dilute acetic acid. M. p. 194—196°. One of the mother liquors contained a small amount of crude pinosylvin (m. p. 151—153°). An additional quantity of pinosylvin was isolated from the ether phase after concentrating, vacuum distillation of the residue, filtration through aluminium oxide (coloured impurities removed), and three recrystallisations from benzene. Yield, 0.30 g. M. p. 156—158°, no m. p. depression when mixed with an authentic specimen of pinosylvin.

H_2 was also acidified and extracted with ether. The ether solution was filtered through aluminium oxide, and the filtrate evaporated. A brown oil was obtained, which was distilled *in vacuo*. On cooling, the distillate immediately crystallised. After three recrystallisations from 50 % acetic acid, the substance melted at 120—121° and gave no m. p. depression when mixed with pinosylvin monomethyl ether. Yield, 0.10 g. The first mother liquor was precipitated with water and the precipitate dried and recrystallised from benzene. A small amount of pinosylvin, m. p. 154—155°, was obtained.

Isolation of *l*-arabinose from American lodgepole pine

Fine-ground heartwood (1.7 kg) was extracted with ether for 24 hours and then with acetone for 60 hours. The acetone extract was evaporated, yielding a brown resinous product and about 150 ml of an aqueous solution. The solution was decanted and the resinous product treated with ether to precipitate »membrane substances». The latter were separated, dried and stirred with 100 ml of cold water. The suspension was filtered and the filtrate combined with the main water solution. The water was removed by vacuum distillation, and the remaining syrup dissolved in hot ethanol. The alcoholic solution was filtered and concentrated to a small volume. On cooling, colourless crystals were precipitated. After three recrystallisations from ethanol, a fraction (0.3 g) melting at 156—157° was obtained. $[\alpha]_D^{20}$ + 104.5° ± 0.5° (equilibrium rotation in water, c = 2.8). Reported for *l*-arabinose + 105.5°. *p*-Bromophenylhydrazine yielded a colourless crystalline precipitate in cold acetic acid solution. A strong red pentose colour reaction was obtained with phloroglucinol and hydrochloric acid. Less pure arabinose was isolated from the mother liquors (m. p. 151—154°). Total yield of *l*-arabinose, 1.0 g.

SUMMARY

l-Arabinose, pinobanksin (probably 3,5,7-trihydroxiflavanone), pinocembrin, pinosylvin and its monomethyl ether have been isolated from the heartwood of *Pinus contorta* var. *latifolia* S. Wats.

The author is indebted to Statsskovrider Einar Bentzen, Nyköbing, Denmark, for collecting the wood. My thanks are also due to Professor N. Sylvén, Ekebo, for the identification of the wood sample. This investigation was facilitated by a grant from *Statens Tekniska Forskningsråd.*

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Received June 22, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 763–766

Constituents of Pine Heartwood

XI. The Heartwood of *Pinus radiata* D.Don

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Monterey pine, *Pinus radiata* D. Don, is a pine of the *Diploxylon* section growing on the Pacific coast of North America. The heartwood used at the present investigation came from a cultivated tree grown at Farnham, Surrey, England.

The extractions were carried out according to the general scheme given in Part IX [1]. The ether extract (8 % of air-dry heartwood) crystallised partly. A phenolic fraction (1 % of the ether extract) could be prepared from it. This was not investigated any further.

A small quantity of *l*-arabinose was isolated from the aqueous residue after the evaporation of the acetone from the acetone extract. This is in good agreement with an observation by Erdtman [2], who found *l*-arabinose along with the 'membrane substances' of *P. sylvestris*.

The sodium carbonate fraction of the acetone extract contained a precipitate, which partly consisted of the sodium salt of pinobanksin. The pinobanksin crystallised from toluene in large thick needles, m. p. 176—178° *, containing crystal toluene. After drying at 115° the m. p. was almost unchanged, 177—178°. $[\alpha]_D^{20}$ + 14.5° (in methanol). The loss in weight during the drying corresponds to half a mole of crystal toluene for each mole of pinobanksin.

Pinocembrin was obtained from the soluble part of the sodium carbonate extract and from the 0.2 % sodium hydroxide extract. M. p. 193—194°; $[\alpha]_D^{20}$—57°. This specific rotation is in relatively good agreement with that found for pinocembrin from *P. montana* [1] and *P. contorta* [3].

The 4 % sodium hydroxide extract yielded pinosylvin monomethyl ether

* All melting points uncorrected.

The following fractions were isolated from 2.5 kg of air-dry heartwood.

Ether extract	200	g	(8 %)
'Membrane substances'	1.5	g	(0.06 %)
l-Arabinose	about 0.1	g	
Pinobanksin	2.0	g	(0.08 %)
Pinocembrin	2.0	g	(0.08 %)
Pinosylvin monomethyl ether	2.0	g	(0.08 %)
Neutral fraction of acetone extract	10	g	(0.4 %)

The specimen of *P. radiata* investigated here had a relatively small amount of phenolic substances in the heartwood. Pinosylvin has not been isolated, but of course there may be small quantities of that substance in the non-crystalline resins which always accompany the crystalline substances.

EXPERIMENTAL

The wood sample came from Forest Research Station, Alice Holt Lodge, Farnham, Surrey, England.

2.5 kg of heartwood were extracted first with ether and then with acetone in the same way as described for *Pinus montana* [1].

After the ether extract had been evaporated, a dark brown syrup remained, which crystallised to a great extent in a few weeks. Yield, 200 g. 6.02 g of the ether extract were treated with 120 ml of light petroleum. A brownish-yellow precipitate was filtered off, washed and dried, yielding 0.55 g. This was boiled with 150 ml of water, and the water solution filtered and cooled. It was then extracted with ether, and the ether solution dried over sodium sulphate and evaporated to dryness, when 0.06 g of a brown resinous substance was obtained. It gave a brownish-violet colour with ferric chloride in alcoholic solution.

The acetone extract was evaporated. The residue consisted of a small volume of water and a dark brown resin. The water (= W) was separated from the resin by decantation. It reduced Fehling's solution and had a positive rotation. The resin was treated with 600 ml of ether, and the undissolved sticky brown »membrane substances» filtered off. Yield, 1.5 g. The filtrate was then shaken with 100 ml of water, which was combined with W.

The ether solution was next shaken with saturated sodium bicarbonate solution (3 × 500 ml, extract = B) and with saturated sodium carbonate (4 × 200 ml). From the last-mentioned extract a brown precipitate was separated (= C_1). Aqueous filtrate = C_2.

After the carbonate extraction, the ether solution was diluted to 800 ml and first shaken with 0.2 % sodium hydroxide (3 × 250 ml, extract = H_1) and then with 4 % sodium hydroxide (250 + 150 ml, extract = H_2). The residue was dried over sodium sulphate and evaporated. The remaining reddish-brown oil (10 g) had a strong fluorescence and a turpentine-like odour.

W was concentrated *in vacuo* to a brown syrup. The syrup was extracted with hot ethanol, the extract evaporated to dryness and the residue recrystallised twice from ethanol. A small amount of a white crystalline powder, melting at 155—158° was obtained

(about 100 mg). This product gave a precipitate with *p*-bromophenylhydrazine in acetic acid solution and a strong pentose colour reaction with phloroglucinol and hydrochloric acid. When mixed with an equal amount of *l*-arabinose, its melting point was unchanged. $[\alpha]_D^{20}$ + 105° ± 1° (equilibrium rotation in 2.4 % aqueous solution). Reported for *l*-arabinose: + 105.5°.

B, when acidified, formed a brown sticky precipitate (about 1 g), which was not investigated any further.

C_1 was stirred with dilute sulphuric acid, washed with water and dried in the air. This treatment yielded a brown solid (12.9 g). It could not be recrystallised directly, but when extracted with ether for four hours in a Soxhlet apparatus, most of it dissolved. When evaporated, the ether extract yielded a crystalline cake, melting gradually at 145—165°. Recrystallisation from toluene also yielded an impure product, which was dissolved in ether and shaken with saturated sodium carbonate. A crystalline yellow precipitate was formed in the aqueous phase. It was separated from the solution and acidified. After four recrystallisations from toluene, pure pinobanksin (2.1 g) was obtained, melting at 176—178°. When dried at 115°, the crystals lost 0.3 g in weight and melted at 177—178°. $[\alpha]_{D_i}^{20}$ + 14.5° ± 0.5° (methanol, c = 6.0).

The toluene mother liquors were evaporated and the residues recrystallised from 50 % acetic acid. Crude pinocembrin was obtained and combined with pinocembrin found in the C_2 fraction. After filtering through aluminium oxide in ether solution and recrystallisation from 40 % acetic acid, 1.8 g of pure pinocembrin (m. p. 193—194°) could be separated. The total yield of pinocembrin from the C and H fractions was 2.0 g. $[\alpha]_D^{20}$ − 57° ± 1° (methanol, c = 2.3).

The C_2 fraction was acidified and the resulting brown precipitate separated from the solution. It was extracted with ether like C_1, but the ether extract could not be crystallised from organic solvents. Several extractions with boiling water yielded crystalline colourless precipitates on cooling, the first melting about 140° and the last about 190°. From the first precipitates 0.15 g of pinobanksin (m. p. 177—178°) was isolated by precipitation as sodium salt and recrystallisation from toluene. The last extracts consisted of crude pinocembrin and were combined with the pinocembrin found in the C_1 fraction.

H_1 was acidified and extracted with ether, and the ether extract washed with a small volume of saturated sodium carbonate solution and then concentrated to a yellow oil. From this a small quantity of pinocembrin could be isolated after water extraction and recrystallisation from dilute acetic acid. M. p. 195—197°.

H_2 was acidified, extracted with ether and the ether evaporated to dryness. A brown, semi-crystalline mass was obtained, which was distilled *in vacuo* and then recrystallised from 50 % acetic acid. 2.0 g of pinosylvin monomethyl ether, m. p. 119—121°, were obtained.

SUMMARY

Pinobanksin, pinocembrin, pinosylvin monomethyl ether and a small quantity of *l*-arabinose have been isolated from the heartwood of *Pinus radiata* D. Don.

The author is indebted to Mrs. B. Strömgren for skilful assistance during the experimental work, and to Mr. M. V. Laurie, Alice Holt Lodge, Farnham, Surrey, England, for

the supply of the wood. The investigation was facilitated by a grant from *Statens Tekniska Forskningsråd*.

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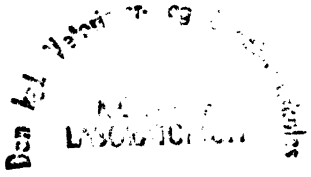
Received June 22, 1949.



ACTA CHEMICA SCANDINAVICA 3 (1949) 767–769

Constituents of Pine Heartwood

XII. The Heartwood of *Pinus ponderosa* Dougl.

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Pinus ponderosa, 'Western yellow pine', is endemic to Western North America and is one of the largest and commercially most important of the American pines. It belongs to the section *Diploxylon*. The wood used in this investigation came from Oregon, U. S. A.

The heartwood was extracted with ether and acetone as described for *P. montana* [1]. The ether extract (2.7 % of the heartwood) did not crystallise. A phenolic fraction could be prepared from it in the usual way. Its weight (1 % of the ether extract) was small when compared to the content of pinosylvin phenols in the acetone extract.

From the water-soluble part of the acetone extract, a small amount of *l*-arabinose was isolated. The 0.2 % sodium hydroxide extract yielded a small amount of pinocembrin. The 5 % sodium hydroxide extract contained pinosylvin and a large quantity of its monomethyl ether.

A parallel run was made on an acetone extract from 4 kg of heartwood prepared by Dr. A.B. Anderson, Portland, Oregon, U.S.A. From this extract a very small quantity of pinobanksin was isolated, but no pinocembrin was found. Pinosylvin and its monomethyl ether were isolated as in the first extraction.

The following products were isolated from 5.4 kg of air-dry heartwood:

Ether extract	147 g (2.7 %)	
'Membrane substances'	4.5 g (0.08 %)	
l-Arabinose	0.6 g (0.01 %)	
Pinocembrin	0.1 g (0.002 %)	(Parallel run 0.002 % of pinobanksin)
Pinosylvin	0.6 g (0.01 %)	(Parallel run 0.03 %)
Pinosylvin monomethyl ether	21.8 g (0.4 %)	(Parallel run 0.4 %)
Neutral fraction of acetone extract	5.5 g (0.1 %)	

The two specimens of *P. ponderosa* investigated here were both relatively rich in pinosylvin phenols, but the yields of pinocembrin and pinobanksin were extremely low.

EXPERIMENTAL

The wood was provided by Dr. A. B. Anderson, Portland, Oreg., U. S. A. 5.4 kg from the periphery of the heartwood of a large tree were used for the extraction. The heartwood gave a strong red colour when stained with diazotised benzidine solution. It was extracted with ether and acetone in the usual way [1]. The weight of the ether extract was 147 g. 11.8 g of the ether extract were treated with 200 ml of light petroleum. The solution was decanted from the sticky brown residue, and the latter boiled with 300 ml of water. The water solution was filtered, cooled, and extracted with ether. After evaporation, the ether solution yielded 0.10 g of a yellowish-brown syrup. Its alcoholic solution gave a brown violet colour with ferric chloride.

On evaporation, the acetone extract yielded a brown resinous residue and about 100 ml of a water solution (= W), which was decanted from the resin. The resin was treated with 400 ml of ether, the undissolved 'membrane substances' (4.5 g after drying in the air) separated from the solution, and the latter was shaken with 100 ml of water which was combined with W. The 'membrane substances' were also stirred with about 100 ml of cold water which was added to W. W was then shaken with about 50 ml of ether which was combined with the ether solution of the resin.

The ether solution was then shaken with saturated sodium bicarbonate (total volume 1 l, extract = B) and with saturated sodium carbonate (total volume 1.8 l). The sodium carbonate was first shaken with ether (ether phase = C_2) and then acidified and extracted with ether (C_3).

After the sodium carbonate extraction, the ether solution was diluted to about 600 ml and shaken with 0.2 % sodium hydroxide. Alkali phase = H_1. Finally, the ether phase was shaken with 5 % sodium hydroxide. Alkali phase = H_2. The remaining ether phase was concentrated to a reddish-brown, turpentine-smelling oil (5.5 g).

W: The water was removed from the solution by vacuum distillation. The residue, a brown syrup, was extracted with hot ethanol. When the ethanol solution was concentrated to a small volume, colourless crystals precipitated which were recrystallised twice from ethanol. *l*-Arabinose (0.47 g) melting at 158—159° and 0.13 g of less pure material melting at 155—157° were obtained. $[\alpha]_D^{20}$ + 104.0° ± 0.5°. (Equilibrium rotation in 2.5 % aqueous solution.) The sugar gave no melting point depression with *l*-arabinose and a strong pentose colour reaction with phloroglucinol-hydrochloric acid.

C_2 was concentrated to a brown semi-solid oil, which showed a small tendency to crystallise. When the oil was stirred with a little ether, a crystalline precipitate was formed, from which a small quantity of pinosylvin monomethyl ether (m. p. 120—121°) could be obtained by recrystallisation from chloroform-light petroleum.

C_3 yielded a reddish-brown syrup, from which a few mg of a crystalline solid melting at 152—158° were obtained. A mixture with pinosylvin gave a large m. p. depression. The crystals might perhaps be a mixture of pinocembrin and pinobanksin.

H_1 was acidified, extracted with ether and the ether solution evaporated. A reddish-brown viscous oil, which did not crystallise, remained. It was distilled in a high vacuum, and part of the distillate crystallised. The crystals were recrystallised from benzene and

from 50 % acetic acid. 0.12 g of a crystalline substance melting at 197—198° was obtained. The mixed melting point with pinocembrin (m. p. 193—195°) was 193—197°. The high melting point indicates that the pinocembrin had become partly racemised during the distillation.

H_2 was acidified, extracted with ether, and the ether solution concentrated to a reddish-brown, semi-crystalline mass. Vacuum distillation yielded a reddish distillate, which partly crystallised. After two recrystallisations from 50 % acetic acid, pinosylvin monomethyl ether (21.8 g), m. p. 121—122°, was obtained.

The first mother liquour was precipitated with water, and the sticky reddish-brown precipitate dissolved in ether and filtered through aluminium oxide. Most of the colour of the solution was removed. The filtrate was concentrated to a brown oil, which was extracted by boiling water. When cooled, the extract yielded crystals, which were recrystallised from benzene. Yield, 0.55 g of crystalline leaflets, m. p. 154—155°, which showed no m. p. depression with pinosylvin.

The second mother liquour contained a small amount of crude pinosylvin monomethyl ether, m. p. 114—119° after one recrystallisation from 50 % acetic acid.

A second investigation was made on an acetone extract of 4 kg of heartwood which had been prepared by Dr. A. B. Anderson, Portland, Oreg., U. S. A. The result was similar to that stated above except that no pinocembrin could be isolated and that 0.09 g of pinobanksin was isolated from the C fractions. This pinobanksin had m. p. 176—177° and showed no m. p. depression with pure pinobanksin. $[\alpha]_D^{20}$ + 13° ± 1°. (2.4 % in methanol.) From the same extract were isolated 1.1 g of pinosylvin and 16.5 g of pinosylvin monomethyl ether.

SUMMARY

Pinosylvin and its monomethyl ether, *l*-arabinose and, in very small amounts, pinocembrin and pinobanksin have been isolated from the heartwood of *Pinus ponderosa* Dougl.

The author wishes to thank Mrs. B. Strömgren for skilful experimental assistance, and dr. A. B. Anderson, Portland, Oregon, who has kindly supplied the wood and prepared an acetone extract in his laboratory. The investigation was facilitated by a grant from *Statens Tekniska Forskningsråd.*

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Received June 22, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 770–772

Constituents of Pine Heartwood

XIII. The Heartwood of *Pinus Jeffreyi* Balf

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Pinus Jeffreyi (Jeffrey pine) is a *Diploxylon* pine growing in the western part of North America. It is very closely related to *P. ponderosa*, and some botanists consider it to be a variety of this species [1]. The wood used for the present investigation came from California, U. S. A.

The heartwood was extracted with ether and acetone in the way described for *P. montana* [2]. The ether extract (1.7 % of heartwood) did not crystallise. A phenolic fraction, amounting to only 0.2 % of the ether extract, could be prepared from it.

A fair amount of *l*-arabinose was isolated from the water-soluble part of the acetone extract. The 0.2 % sodium hydroxide fraction contained pinocembrin, and the 4 % sodium hydroxide fraction yielded pinosylvin monomethyl ether, but no pinosylvin.

The following products were obtained from 6.4 kg of air-dry heartwood:

Ether extract	111 g	(1.7 %)
'Membrane substances'	2.9 g	(0.05 %)
l-Arabinose	7.0 g	(0.1 %)
Pinocembrin	2.1 g	(0.03 %)
Pinosylvin monomethyl ether	3.3 g	(0.05 %)
Neutral fraction of acetone extract	3.2 g	(0.05 %)

The specimen of *P. Jeffreyi* investigated here resembled *P. ponderosa* in that it yielded a rather small amount of ether extract [4]. There were, however, differences in the chemical composition of their heartwoods. Pinobanksin was isolated in a very small amount from *P. ponderosa* but has not been found at all in *P. Jeffreyi*. *P. ponderosa* contained about ten times as much pinosylvin phenols as this specimen of *P. Jeffreyi*, but the latter had more pinocembrin

in its heartwood. *P. Jeffreyi* differs from most other pines in that its turpentine contains no pinene but instead saturated aliphatic hydrocarbons, such as *n*-heptane [3]. Its heartwood constituents, however, seem to be the same as in the other *Diploxylon* pines.

EXPERIMENTAL

The wood used for the investigation was supplied by Dr. N. T. Mirov, California Forest and Range Experiment Station, Placerville, California, U. S. A. Its colour reaction with diazotised benzidine solution was not very strong.

Air-dried, fine-ground heartwood (6.4 kg) was extracted with ether for 24 hours, and then with acetone for 48 hours in the usual way [2]. Weight of ether extract was 111 g. It consisted of a dark viscous oil, which had not crystallised after two months. 8.70 g of the ether extract were treated with 150 ml of light petroleum. The solution was decanted, and the brown sticky residue boiled with 200 ml of water. The water was filtered, cooled, and extracted with ether. The ether was dried over sodium sulphate and evaporated, leaving 0.15 g of a brown resinous substance. Its alcoholic solution gave a dark violet colour reaction with ferric chloride.

The acetone extract was concentrated to a dark brown resinous substance and a small volume of a water solution, which was separated from the resin. Aqueous solution = W.

The resinous substance was treated with ether to precipitate the 'membrane substances'. The filtrate was shaken with a little water which was combined with W. The latter solution was then shaken with ether which was added to the first ether solution.

The ether solution was then extracted with saturated sodium bicarbonate (3 × 200 ml, extract = B), saturated sodium carbonate (5 × 140 ml, extract = C), 0.2 % sodium hydroxide (4 × 250 ml, extract = H_1), and 4 % sodium hydroxide (200 + 100 ml, extract = H_2). Each extract was acidified with dilute sulphuric acid and extracted with ether. The ether solutions were dried with sodium sulphate and the ether evaporated.

The 'membrane substances' were stirred with 100 ml of cold water, the suspension filtered and the filtrate combined with W. The remaining 'membrane substances', a light brown powder, weighed 2.0 g after drying in the air.

W was concentrated *in vacuo* to a brown syrup, which was dissolved in hot ethanol, the solution filtered and concentrated to a small volume. After cooling, a colourless crystalline precipitate was obtained. After this precipitate had been recrystallised twice from ethanol, 7.0 g of *l*-arabinose was obtained. M. p. 156—158°. $[\alpha]_D^{20}$ + 104.0° ± 0.5° (equilibrium rotation in water, c = 2.6). It gave no melting point depression with pure *l*-arabinose.

B yielded a brown oil (1 g) which did not crystallise.

C was concentrated to a brown oil (less than 1 g). Extraction with boiling water yielded a brown solid melting at 95—120°. No pure products could be isolated from this fraction.

From H_1 was obtained a brown sticky solid which partly crystallised. It was stirred with a little ether and the undissolved crystalline powder collected. The filtrate was evaporated and the ether treatment repeated, when a second crop of crystals remained undissolved. The crystals were combined (m. p. 188—194°), dissolved in ether and the

yellow ether solution filtered through aluminium oxide. Most of the colour of the solution was removed. The filtrate was evaporated and the residue recrystallised twice from 50 % acetic acid. Yield of pinocembrin, 1.2 g, m. p. 194—195°, no depression when mixed with pinocembrin from *P. Banksiana*. $[\alpha]_D^{20}$ — 55° ± 1° (methanol, c = 3.0).

The ether filtrate was concentrated, the residue distilled *in vacuo* and the distillate (partly crystalline) recrystallised from 50 % acetic acid. 1.0 g of pinocembrin, m. p. 194—195° was obtained. No crystalline products could be isolated from the mother liquor.

H_2: When the ether solution was evaporated, a brown semi-crystalline solid remained. Two recrystallisations from 50 % acetic acid yielded a light brown substance, m. p. 115—118° (4.0 g). It was distilled *in vacuo* and recrystallised again. Pinosylvin monomethyl ether (3.3 g), m. p. 120—121°, was obtained.

The neutral fraction of the acetone extract was a brown oil (3.2 g), which deposited a small quantity of crystals. It was not investigated any further.

SUMMARY

The heartwood of *Pinus Jeffreyi* Balf. has been investigated. *l*-Arabinose, pinocembrin and pinosylvin monomethyl ether were isolated.

The author is indebted to Mrs. B. Strömgren for skilful experimental assistance, and to Dr. N. T. Mirov, Placerville, California, U.S.A., for the wood samples. The investigation has been facilitated by grants from *Statens Tekniska Forskningsråd* and from *Fonden för Skoglig Forskning*.

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Received June 22, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 773–777

Determination of Sulphate by Reduction with Stannous Chloride

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Methods for the determination of sulphate by reduction to hydrogen sulphide have previously been worked out. These methods have been substantially based on dry heating or fusion of the sulphate containing substance with adequate reductants. Kurtenacker [1] gives a survey of these methods, and later sodium as well as potassium has been used as a reductant in a micromethod by Bürger [2].

In the following a method will be described in which the sulphate is reduced in solution. The principle of the method is as follows: The wellknown fact that hot concentrated sulphuric acid is a rather powerful oxidant is utilized by adding the sulphate containing solution to an acid of a high boiling point, in this case phosphoric acid, and boiling down after further addition af an adequate reductant. Investigation of diverse reductants is now beeing carried out, and in the present paper stannous chloride as a reductant will be dealt with. Experiments have shown that, when a solution of sodium sulphate, stannous chloride and phosphoric acid is boiled down, all sulphate present will be reduced. Most of the sulphate will be reduced to hydrogen sulphide, but a small percentage will only be reduced to sulphur dioxide. If the hydrogen sulphide and sulphur dioxide formed are absorbed (in carbon dioxide atmosphere) in iodine solution, the following reactions will take place:

$$H_2S + I_2 \rightarrow S + 2I^- + 2\ H^+$$

and

$$SO_2 + I_2 + 2\ H_2O \rightarrow SO_4^{--} + 2\ I^- + 4\ H^+$$

i. e. that each sulphur atom requires two iodine atoms, and the iodine in excess can be titrated with standard thiosulphate. (In some experiments with absorption of the reduced sulphur compounds in a cadmium acetate buffer, in

which only the hydrogen sulphide was absorbed quantitatively, the results found were 2—3 % too low.)

When a quantitative reduction of the sulphate shall take place, a rather large excess of stannous chloride must be used. When up to 0.1 g SO_4 is present, about 4 g of cryst. stannous chloride is used.

When the absorption of hydrogen sulphide in iodine solution takes place, a) the hydrogen sulphide must be absorbed quantitatively, and b) the iodine must be prevented from escaping together with the carbon dioxide current.

a) The quantitative absorption is obtained in an absorption tube provided with ten bulbs (Fig. 1). Into this tube 1) 25 ml standard potassium iodate solution (about 0.1 *N*) is pipetted off; furthermore 2) 2 g cryst. potassium iodide, 3) 10 ml 2 % cadmium sulphate solution, and 4) 10 ml 2 *M* sodium hydroxide solution are added. When boiling down the solution of sodium sulphate, stannous chloride and phosphoric acid about 0.035 mole hydrochloric acid is expelled from 4 g stannous chloride. This hydrochloric acid imparts to the basic iodate-iodide solution a suitable acidity, so that the later formed hydrogen sulphide is absorbed quantitatively, when cadmium salt is present. Without cadmium sulphate present in the absorbing solution or when this solution was considerably more acid, it was ascertained, that some hydrogen sulphide escaped. As an extra measure of precaution the carbon dioxide, which had passed the absorbing solution, was allowed to bubble through a solution, contained in a glass cylinder, and consisting of 10 ml 2 % cadmium sulphate solution, 1 g potassium iodide and 15 ml water, but in none of the later described experiments did hydrogen sulphide reach the cylinder. The addition of cadmium sulphate to the solution in the cylinder is consequently unnecessary.

b) To prevent a loss of iodine from the absorbing solution — due to the carbon dioxide current — a rather large excess of potassium iodide was added to that solution, and further the absorption tube was water-cooled. As already mentioned the carbon dioxide was finally allowed to bubble through a solution of 1 g potassium iodide in 25 ml water in a cylinder. In the experiments a slight amount of iodine was found in the cylinder (corresponding to about 0.05 ml 0.1 *N* iodine solution), and blank tests carried out later (see below) seem to indicate that about just as much iodine nevertheless escaped.

The experimental set-up appears from Fig. 1. The stoppers used were made of rubber. The absorption tube is protected against heat from the burner by means of a sheet of asbestos board.

The reduction of the sulphate was carried out in a 100 ml longnecked Kjeldahl flask. In this flask was placed 1) a small shard of porous porcelain, 2) 4 g stannous chloride ($SnCl_2,2H_2O$), 3) the sample (in the experiments mentioned a solution of sodium sulphate) washed down with water (the volume of sample plus water was about 20 ml), and 4)

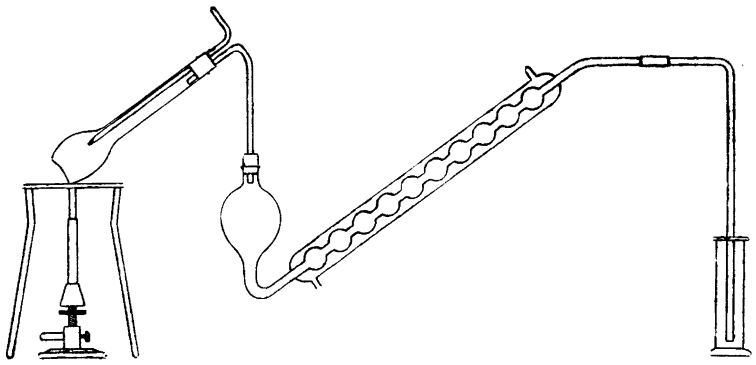

Fig. 1. Experimental set-up for reduction of sulphate with stannous chloride and following absorption of the reduced sulphur products.

10 ml conc. phosphoric acid (85 %). (After completing the experiment the flask is rinsed with cold water and boiled out with water; finally some sodium hydroxide solution is boiled in the flask.)

Into the absorption tube is 1) standard potassium iodate solution (about 0.1 *N*) pipetted off; further 2) 2 g potassium iodide, 3) 10 ml 2 % cadmium sulphate ($CdSO_4$, $\frac{8}{3}H_2O$), and 4) 10 ml 2 *M* sodium hydroxide are added.

In the cylinder was placed 1) 10 ml 2 % cadmium sulphate, 2) 1 g potassium iodide, and 3) 15 ml water.

The current of carbon dioxide was produced by a carbon dioxide pressure cylinder and the rate was 60—80 ml per min.

The heating of the Kjeldahl flask was done by a heavy duty Bunsen burner.

An experiment progresses as follows: After a little less than 3 min the mixture in the flask begins boiling. Before starting the experiment a white precipitate of cadmium hydroxide in a colourless liquid is seen in the absorption tube. Gradually as the carbon dioxide bubbles through this suspension, the liquid will assume a yellowish colour. About 10 min after the boiling has begun, considerable amounts of hydrochloric acid distills over in the absorption tube, so that the liquid becomes redbrown due to triiodide ions, coincident with the precipitate starting to dissolve. After the elapse of a further 4—5 min the beginning of a precipitate of sulphur is seen in the absorption tube, and from now on heating is continued 4—5 min until the mixture in the Kjeldahl flask is quite opaque due to a white precipitate. (This precipitate is not formed — or at least only to a slight degree — in the blank tests later mentioned, or if the analysis contains only small amounts of sulphate.)

A little more than 20 min have now elapsed since the Bunsen burner was lighted, and the gas is at this point turned off, whereas the carbon dioxide current is continued for 20 min. Hereafter the contents of the absorption tube and the cylinder are washed quantitatively over into a 500 ml Erlenmeyer flask, and the excess of iodine is titrated with standard sodium thiosulphate.

The whole determination can — when the required solutions are at hand — be carried out in one hour.

In the experiments carried out in this work a solution of sodium sulphate, containing 1.349 % SO_4 is used as sample. This solution is controlled gravimetrically as barium sulphate and by evaporation as sodium sulphate.

The 25 ml potassium iodate solution used in each experiment required, by direct titration, 24.92 ml 0.1008 *N* sodium thiosulphate. Further some blank tests are run by adding 20 ml water in stead of the sample plus washing water. In these blank tests titration of the 25 ml potassium iodate required on an average 24.82 ml sodium thiosulphate. However it is worth mentioning that while it was impossible in a real analysis to detect loss of iodine by placing a piece of potassium iodide-starch filter-paper above the mouth of the cylinder, a faint blue colour could be seen when carrying out the blank tests. This seems to indicate that the blank value 0.10 ml thiosulphate is too large, which is also rather likely, because a carbon dioxide current was bubbled through a solution containing 25 ml 0.1 *N* iodine for about 30 min, while — in a real experiment — the 25 ml 0.1 *N* iodine was only present for about 5 min, and about 5 ml 0.1 *N* iodine was present for about 25 min.

The experimental results are given in Table 1. In the last few experiments the error of the determination is of course increased due to the smaller amount of sodium sulphate solution used.

Table 1. Determination — by reduction with stannous chloride — of sulphate in a sodium sulphate solution which contains 1.349 % SO_4. The standard thiosulphate used is 0.1008 N. (A) are values without regard to the blank value; (B) are the values corrected for the blank value.

Sample g	Titration of the excess of iodine Thiosulphate used, ml	Consumption of KIO_3 recalculated to ml thiosulphate (A)	(B)	% SO_4 found (A)	(B)
7.0377	5.26	19.66	19.56	1.353	1.346
7.0052	5.37	19.55	19.45	1.352	1.344
7.0779	5.14	19.78	19.68	1.353	1.346
7.1805	4.88	20.04	19.94	1.351	1.344
7.2418	4.68	20.24	20.14	1.353	1.346
7.9921	2.59	22.33	22.23	1.353	1.346
6.0305	8.02	16.90	16.80	1.357	1.348
5.0050	10.86	14.06	13.96	1.360	1.351
4.0766	13.44	11.48	11.38	1.363	1.352
3.0632	16.25	8.67	8.57	1.370	1.355

The mean values of the first six experiments, in which 7—8 g sodium sulphate solution is used, are respectively (A) 1.353 % SO_4 and (B) 1.345 % SO_4. If we assume a blank value half of that found in the blank tests (see above), the results would agree perfectly with the value 1.349 % SO_4, found by other methods. If we do not make this assumption the results are 3 ‰ too high without taking the blank value into consideration, and 3 ‰ too low when we do take the blank value into consideration.

In the experiments mentioned only pure sodium sulphate solutions have been analyzed. It is likely that the presence of diverse compounds will complicate or even forbid the use of the method here described. Investigations of these problems will be continued. The possibility of using this method for sulphur determination in other sulphur compounds will also be investigated.

SUMMARY

A determination of sulphate in a sodium sulphate solution is carried out by reduction with stannous chloride in a solution acidified with phosphoric acid — and a following iodometric determination.

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Received June 2, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 778–782

Short Communications

Temperature Effect on the Absorption Spectrum of Cycloöctatetraene

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Pink and Ubbelohde [1] have recently reported that the yellow colour exhibited by cycloöctatetraene at ordinary temperatures more or less disappears in the neighbourhood of the boiling point of liquid oxygen, but reappears reversibly at rising temperatures. In the course of investigations of cycloöctatetraene carried out in this laboratory we have independently noticed a marked change in colour with temperature. In order to verify this visual observation we have measured the absorption of pure cycloöctatetraene quantitatively in the visible spectrum at different temperatures.

A Beckmann spectrophotometer was used for the measurements. The cycloöctatetraene was filled into a 1 cm cell which could be electrically heated, the temperature being controlled by means of a thermocouple. Care was taken to exclude the atmospheric oxygen from the sample. The absorption was measured in the region 4600–5600 Å at three different temperatures, *viz.* 25, 58 and 90° C. The results for the two higher temperatures were corrected for thermal expansion. In Fig. 1 the found log ε (in arbitrary units) are plotted against the wave numbers. It will be seen that there is a marked increase in absorption with rising temperature. Our experiments have shown that this change is reversible.

Theoretically such an increase should be expected because the long-wave end of the absorption spectrum is due to transitions from thermically excited vibrational levels of the molecule in its electronic ground state. At rising temperatures the population in these levels is increasing and hence an increasing absorption in this region will occur.

As a crude approximation the distribution of molecules in the different vibrational states may be assumed to be classical and the transition probability to be constant. On this basis one gets:

$$\varepsilon = \text{Const.} \int_{\nu_1 = \nu_0 - \nu}^{\infty} \frac{e^{-h\nu_1/kT}}{\int_0^{\infty} e^{-h\nu_1/kT}\,d\nu_1}\,d\nu_1$$

$$= \text{Const.}\; e^{-h(\nu_0 - \nu)/kT} \qquad (1)$$

where ν is the frequency of the absorbed light, ν_0 the frequency of the $0 \rightarrow 0$ transition and ν_2 the vibrational frequency of the molecule in its electronic ground state. This gives

$$\log \varepsilon = \text{Const.} + \frac{0.4343h}{kT}(\nu - \nu_0) \qquad (2)$$

According to this, the difference in log ε ($= \Delta \log \varepsilon$) for two different temperatures (T_1 and T_2) should be

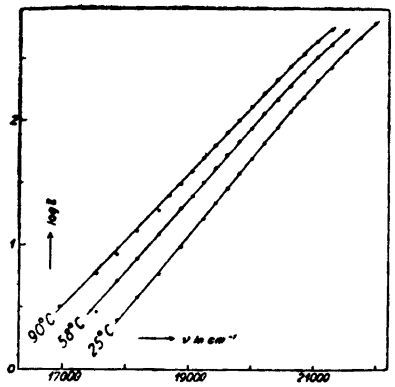

Fig. 1. The long-wave end of the absorption spectrum of cycloöctatetraene at different temperatures.

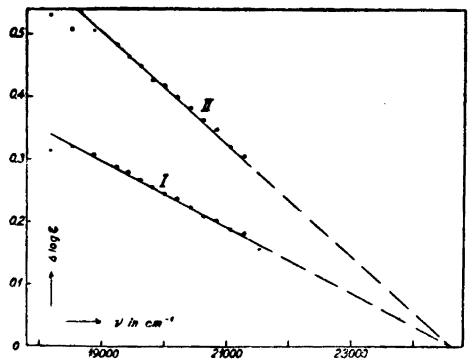

Fig. 2. Δ log ε (see text) for cycloöctatetraene. Curve I: Δ T = 58 — 15°C and curve II: Δ T = 90 — 25°C.

$$\Delta \log \varepsilon = \frac{0.4343h}{k} \times \frac{T_1 - T_2}{T_1\, T_2} (\nu - \nu_0) \quad (3)$$

By plotting Δ log ε against wave number one should get straight lines with increasing slope for increasing temperatur intervals. Furthermore, all these lines should pass through ν_0 (*i. e.* the 0 → 0 frequency of the absorption band system) for Δ log ε = 0.

Fig. 2 shows the results found for cycloöctatetraene. Curve I represents Δ log ε for ΔT = 58—25° C and curve II for 90—25° C. It will be seen that the measured points fall on straight lines passing through the same point (ν = 24600 cm^{-1}) on the axis corresponding to Δ log ε = 0. According to the theory this should be the 0 → 0 frequency of the absorption system. However, the slopes of the lines agree badly with the values computed from equation (3):

	Slopes computed	(in 10^{-3} cm) found
I 58—25° C	0.21	0.05
II 90—25° C	0.37	0.09

The reason for this bad agreement may be the very crude approximations in the theoretical considerations given above and may also be caused by impurities in the cycloöctatetrene.

We are continuing the investigations with more suitable substances in order to see if this method can be used to an approximate localization of the 0 → 0 frequency for more or less continuous absorption spectra.

However, our preliminary measurements of cycloöctatetraene show that the visually observed change in colour with temperature is real and may be explained as an effect which generally should be found for the long-wave end of all absorption spectra. Therefore it seems unnecessary to assume the presence of a small amount of a biradical form of cycloöctatetraene, as proposed by Pink and Ubbelohde [1], in order to explain the phenomenon.

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Received August 27, 1949.

Hydrolysis and Fine Structures of Cotton and Wood Pulp Fibers

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The hydrolysis of cellulose fibers has been used repeatedly in recent years to obtain information about the fine structure of fibers. (Nickerson *et. al.*[1], Conrad and Scroggie [2], Philip [3], Conrad and Nelson[4]). The loss of material during the hydrolysis has been used as a measure of the degree of lateral order (crystallinity), and the constant value for the degree of polymerization (limit DP) obtained after a certain time of hydrolysis has been taken as a measure of the size, *i. e.* length, of the regions with high lateral order (crystallite or micelle size). Lately it has been shown that a certain increase in the crystallinity occurs during the hydrolysis (Mark *et. al* [5], Howsmon [6], Hermans [7]). This increase, or recrystallization, is assumed to take place in the transitional areas of the crystallites into the disordered regions, the so-called mesomorphous areas. The length of the crystallite measured by the limit DP will, therefore, not correspond to the original length in the untreated fibers. It will, however, bear a certain relation to the original size in the regions with lateral order, and approximate the size of the crystallites with the addition of the transitional areas.

In order to explain some phenomena encountered during comparative hydrolysis experiments on cotton linters and sulfite pulps, we recently [8] put forth the hypothesis that the regions of high lateral order in the wood fibers are more irregular, not only in size but especially in their dimensions being longer and flatter than the crystalline regions in the cotton linters. We will report here some experiments which strongly indicate that the main difference in the fine structures of cotton and wood pulp fibers is in the extension of the mesomorphous areas in the latter, these being much larger in the pulp than in the cotton fibers. More extensive investigations are in progress and the results will be published later.

The fibers used in the experiments were bleached cotton and sulfite pulps. The pulps had been cooked to varying viscosities followed by a chlorine bleach. The fibers were swelled for 2 hours in sodium hydroxide solutions of different strength at 5° C, carefully washed with icewater, and dried in vacuum oven at 60° C. The water regains were determined at 55 % RH, and the samples were hydrolyzed with 2.5 N H_2SO_4 at 97° C for 6 hours. The average viscosity DP of the hydrolyzed material was found by converting to cellulose nitrate, determining the intrinsic viscosities in acetone solution, and using Staudinger's eq., with a K_m-constant of 10^{-3} (c in g/l), for conversion of the viscosities to DP's.

It can be seen from Table 1, column four, that the limit DP of the cotton remains constant until the alkaline solution is strong enough to cause intramicellar swelling (10 %). At this point a sudden drop in the limit DP, from 140 to 95, takes place, showing that the transformation from Cellulose I (native) to Cellulose II (hydrate) has changed the crystallite size. No change in the size had, however, occured prior to this transformation. The pulps exhibit quite a different behavior. The limit DP for all these samples decreased gradually as the strength of the alkaline solution increased, until at eight per cent concentration a sharp drop occured. No decrease, or only a very small one, resulted when the concentration was increased to the mercerizing strength.

Passing from the disordered to the crystalline regions, the lateral order of the transitional area will increase. Alkaline solutions of increasing concentration will

Table 1. Hydrolysis and water regain data for fibers swelled in alkaline solutions of varying concentrations.

Material	Swelled in NaOH sol. conc. in weight %	Water regain, relative values (regain with water swelling = 1.0)	Limit DP
Bleached cotton	0	1.0	140
	2	1.0	140
	4	1.05	140
	6	1.05	140
	8	1.08	140
	10	1.44	95—100
Pulp of high viscosity	0	1.0	400
	2	1.0	330
	4	1.0	275
	6	1.03	170
	8	1.16	60
	10	1.21	60
Pulp of medium viscosity	0	1.0	300
	2	1.0	275
	4	1.0	250
	6	1.0	180
	8	1.21	65
	10	1.20	55
Pulp of low viscosity	0	1.0	195
	2	1.0	190
	4	1.0	175
	6	1.07	125
	8	1.36	55—60
	10	1.36	45—40

swell and cause disorder to a larger and larger part of these regions until, at last, the crystallite themselves swell and are partly destroyed. The constant limit DP of the cotton shows that the mesomorphous region is negligible in this material, or is of such a low order that even water will penetrate it. In the pulp fibers, this area is of considerable size and contains probably a large variation of order, shown by the gradual decrease in limit DP. The length of the transitional area is several times that of the crystallite, which probably should be regarded only as a nucleus from which the large mesomorphous regions extend. It is interesting to note, that the length of the wellarranged crystallites of Cellulose I (after swelling in 8 % NaOH) is almost the same as those of Cellulose II (after swelling in 10 % NaOH), in contrast to the findings for the cotton fibers.

Table 2. Recalculated water regains for the low viscosity pulp.

Swelled in % NaOH	Material dissolved Weight %	Recalculated relative water regain: 55% RH
0	0	1.0
2	2.0	1.04
4	3.5	1.08
6	6.5	1.19
8	10.0	1.53
10	13.5	1.57

The relative water regains of the cotton follow the same course as previously found by Urquhart and Williams [9], *i. e.* almost constant regain until swelled in 10 % NaOH, when a sudden increase occurs. This is in agreement with the hydrolysis data, namely, that no large change takes place in the fine structure until swelled in 10 % NaOH. The regains of the pulps, as presented in Table 1, are confusing and not as was to be expected from the hydrolysis data. However, we have to take into account that two effects may counteract each other. The swelling and destruction of the mesomorphous regions will tend to increase the water absorption. At the same time, the loss of materials, which occurs in pulp in contrast to cotton, will cause the absorption to diminish. More correct values might be arrived at by assuming that the part which was brought in solution belonged to the disordered regions and formed Cellulose-hydrate II ($C_6H_{10}O_5 \times 1\,^1/_2\ H_2O$) at 55 % RH. On this assumption the regains in Table 2 are recalculated for the low viscosity pulp. It is now seen that the regains follow the course which might be expected, namely a gradually increase in swelling up to 6 % NaOH, followed by a sharp jump at 8 % NaOH, and very little further change when the strength is increased to 10 %.

The Constituents of the Wood of *Thuja occidentalis* L.

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The occurence of three isomeric thujaplicins and thujic acid in the heart-wood of *Thuja plicata* has been announced earlier [1, 2]. The three thujaplicins are highly toxic towards wood destroying fungi and are mainly responsible for the high durability of this wood. It was therefore of interest to investigate whether other species of the same genus also contain these substances. Such an investigation has now been carried out on the wood of *Thuja occidentalis*. This species is endemic to eastern North America and is grown in Northern Europe as an ornamental tree in parks and churchyards. It is known for the great age it can attain. The samples of the wood used were kindly supplied by Mr. K. G. Karlsson from Svartå gård in Southern Finland.

Extraction of the finely-divided wood with acetone and treatment of the extract in the manner already described for *Thuja plicata* [1], yielded α- and γ-thujaplicin in 0.08 and 0.008 percent yield respectively, whereas thujic acid was found only in traces. The substances were identified by mixed melting point determinations with authentic samples obtained from *Thuja plicata*. No β-thujaplicin could be detected, but its presence in small amounts cannot be excluded due to the difficulty in separating the thujaplicins.

The high percentage of α-thujaplicin and the relatively low percentage of γ-thujaplicin is remarkable when compared to *Thuja plicata*, where they occur in approx. equal amounts. It is also striking that *Thuja occidentalis* contains very little thujic acid, which is the main constituent of the heart-wood of *Thuja plicata*.

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Received September 22, 1949.

It is seen from the steady decrease in limit DP for pulps cooked to decreasing viscosity, that part of the mesomorphous areas is also attacked by the action of acids, at least at higher temperatures.

It is obvious that the proposed variations in lateral order and the large extension of the transitional or mesomorphous areas of wood pulp fibers will greatly influence the behaviour of these fibers in acid hydrolysis and in other chemical reactions, which involve no or little swelling of the material. It also points to the importance of defining the prehistory and refining procedures used for pulp fibers which are to undergo such reactions.

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Received September 28, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 783—808

On the Complex Chemistry of the Uranyl Ion

II. The Complexity of Uranyl Monochloroacetate. A Comparative Potentiometric and Extinctiometric Investigation

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A great number of methods: potentiometric, extinctiometric, polarimetric, conductometric, cryoskopic, ebullioscopic and several others have been used to investigate complex systems in solution. However, only the first three of the methods mentioned give any substantial quantitative results, for only in these cases does one have the possibility to make measurements in an approximately constant salt medium of high ionic strength thus securing fairly constant conditions of activity. Only in this way can a known connection between the measured quantity and the complex concentrations be obtained.

In my first paper concerning these uranyl investigations (Ahrland [1], in the following referred to as I) it was established that the uranyl ion UO_2^{2+} is to be considered as a central group which may give complexes with the anions of its salt solutions. The ion is easily hydrolysed, it is true, but stable at $p[H^+]$ * < 3. The present experiments aim to investigate such a complex system of uranyl according to two of the best methods mentioned above, namely potentiometrically and extinctiometrically. With the uranyl ion, such a comparison is especially valuable, as in this case it is very often impossible to apply the potentiometric method which is the most used and proved. This is because one is not at all able to measure $[UO_2^{2+}]$ (I p. 382). In such cases, however, it may be possible to carry out an extinctiometric determination. It is then desirable to show that both methodes give consistent results in one system at least.

* $p[H^+] = -\log [H^+]$ (see I).

It must be said that the extinctiometric method, contrary to the potentiometric, does not give directly a certain ion concentration of the solution. In practice one always measures a sum of extinctions of several components. Further, the molar extinctions are unknown quantities, thus we have here twice as many unknown constants as in a potentiometric measurement. These circumstances render the computations more difficult, and also the results more uncertain.

As in I, the numerical values obtained refer to the special medium used, *i. e.* with the ionic strength = 1 by the aid of $NaClO_4$, and the temperature 20° C.

CHOICE OF MONOCHLORACETATE AS THE MOST SUITABLE LIGAND

In the choice of a ligand, the following views should be considered:

a. The potentiometric measurement must be done as a ligand measurement, as $[UO_2^{2+}]$ cannot be determined.

b. To be measured in this way, the complexity must not be too weak (see *e. g.* Leden[2, p. 21]).

c. The measurements should be done at $p[H^+] < 3$, as the uranyl solutions are too readily hydrolysed at higher $p[H^+]$ (see I). The hydrolysis affects both methods, but in a manner most difficult to calculate in the case of the extinctiometric one.

d. The method of calculation for the extinctiometric measurements requires that no polynuclear complexes are formed in appreciable amounts (Olerup[3, p. 70], Bjerrum[4 p. 7], Fronaeus[5, pp. 89–90]. The course of the potentiometric determination will determine whether or not this qualification is fulfilled.

e. The complexes formed must at some wave-length have an extinction differing to a large extent from that of UO_2^{2+}. This is established and a suitable wave-length is choosen by photographing the whole extinction curve of the complex solution.

f. The system used must not be too sensitive to light. According to Gmelin [6] all organic salts of UO_2^{2+} are sensitive, but to a very different degree.

The most suitable method of ligand measurement here seems to be $p[H^+]$ measurements in a buffer of the ligand ion and the corresponding acid, as both quinhydrone and glass electrodes may be used in uranyl solutions (see I). From such measurements, [A] may be calculated. In order to give the solutions the low $p[H^+]$, required according to the condition c. above, it is necessary, however, to choose a rather strong acid, with pK_c* 3 to 4. On the other hand, the acid must not be too strong.

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* $pK_c = -\log K_c$; K_c is the dissociation constant in the medium used.

By using p[H^+] measurements, electrodes of the second order are avoided. This is well, because it would certainly not be easy to find such an electrode with a good function, on account of their reducing properties.

So we see our choice limited to anions of some acids of medium strength with pK_c in the neighbourhood of 3. The number is further decreased by the demand that they must be well soluble in water.

As the acid most probable to fulfil the conditions, the monochloroacetic acid has been selected. From an early conductometric investigation by Dittrich [7] one may conclude that its uranyl salt is rather strongly complex. During the course of the present investigation, it has really proved to be practicable in all other respects too.

CALCULATION OF THE COMPLEXITY CONSTANTS AND THE COMPOSITION OF THE SYSTEM WHEN THE FREE LIGAND CONCENTRATION HAS BEEN MEASURED

Both the methods used give [A], the free ligand concentration of the solutions. As stated in I p. 378 the calculation of the constants then is done via the complex formation equation *

$$\bar{n} = \frac{C_A - [A]}{C_M} = f([A]) \tag{1}$$

the general formula of which was deduced ((8) of I). From this equation it was evident, how it could be decided, whether polynuclear complexes are involved in the complex formation or not. In the last case, the course of the complex formation curve $\bar{n} = f([A])$ should namely be independent of [M], *i. e.* C_M. As this proves to be the case for the chloroacetate system (see below), the formation curve here adopts the simple form:

$$\bar{n} = \frac{\beta_1[A] + 2\beta_2[A]^2 + 3\beta_3[A]^3 + 4\beta_4[A]^4 + \ldots.}{1 + \beta_1[A] + \beta_2[A]^2 + \beta_3[A]^3 + \beta_4[A]^4 + \ldots.} \tag{2}$$

From (2), the constants and the composition of the system will be calculated according to the method of Fronaeus [5, pp. 13—14, 28—30 and 110]. We define:

$$X([A]) = 1 + \beta_1[A] + \beta_2[A]^2 + \beta_3[A]^3 + \ldots \tag{3}$$

* The symbols refer to the same quantities as in I.

and form the function $\bar{n}/[A]$ which then becomes:

$$\frac{\bar{n}}{[A]} = \frac{\frac{dX([A])}{d[A]}}{X([A])} \tag{4}$$

Hence

$$\ln \frac{X([A]_1)}{X([A]_2)} = \int_{[A]_2}^{[A]_1} \frac{\bar{n}}{[A]}\, d[A] \tag{5}$$

The integration of the experimentally known function is made graphically.

If the experiments are able to extend to sufficient low [A], the $\bar{n}/[A]$ function is extrapolated to $[A] = 0$, and $[A]_2 = 0$ is chosen as the lower limit of integration. Then we have, as $X(0) = 1$, (3):

$$\ln X([A]) = \int_{0}^{[A]} \frac{\bar{n}}{[A]}\, d[A] \tag{5a}$$

Hence we know corresponding values of [A] and $X([A])$.

If, on the contrary, no [A] values are measured in the close neighbourhood of the $\bar{n}/[A]$-axis, the extrapolation to $[A] = 0$ cannot be done with any certainty. We then choose the constant lower limit $[A]_2 = [A]_0$, where $[A]_0$ is a value immediately under the lowest [A] measured. Thus

$$\ln \frac{X([A])}{X([A]_0)} = \int_{[A]_0}^{[A]} \frac{\bar{n}}{[A]}\, d[A] \tag{5b}$$

The function $\frac{X([A])}{X([A]_0)}$ obtained by this integration, proves to have such a course that an extrapolation to $[A] = 0$ is easy. We then obtain

$$\lim_{[A]\to 0} \frac{X([A])}{X([A]_0)} = \frac{1}{X([A]_0)} \tag{6}$$

In this manner the constant quantity $X([A]_0)$ is obtained, and hence $X([A])$, too; and we have again the corresponding values of [A] and $X([A])$ searched for.

After having determined these values, we are able to calculate the complexity constants from (3). By a transformation we form a new function

$$X_1([A]) = \frac{X([A]) - 1}{[A]} = \beta_1 + \beta_2[A] + \beta_3[A]^2 + \ldots \tag{7a}$$

By the easily realizable extrapolation to $[A] = 0$, the interception on the axis $= \beta_1$, and the slope in the point of interception $= \beta_2$. The latter constant is, however, more accurately determined by the formation of

$$X_2([A]) = \frac{X_1([A]) - \beta_1}{[A]} = \beta_2 + \beta_3[A] + \beta_4[A]^2 + \ldots \tag{7b}$$

which extrapolation gives β_2 as the interception on the axis. By successive formation of analogous functions $X_n([A])$, all the constants in question may be calculated. How many complexes we have to calculate with in a solution is *a priori* known from the value of $\bar{n}$ attained.

When now the constants are obtained, the composition of the mononuclear complex system in question (*i. e.* the mol ratios α_n of the particular complexes) may be computed as a function of [A] by the aid of the equations (4) and (6) of I, p. 378. (*cf.* Fronaeus [5, p. 29]). Hence we have

$$\alpha_0 = \frac{[M]}{C_M} = \frac{1}{X([A])} \tag{8a}$$

and

$$\alpha_n = \frac{[MA_n]}{C_M} = \frac{\beta_n[A]^n}{X([A])} \tag{8b}$$

To check the calculations, $\bar{n}$ is calculated from (2) by use of the constants found. The complex formation curve so obtained must coincide with the experimentally determined points $(\bar{n}, [A])$.

CHEMICALS USED

Monochloroacetic acid, pur., was distilled twice, b. p. 186—188° C. The resulting acid was quite colourless and gave, after drying *in vacuo*, the right equivalent weight (calc. and found: 94.5). Though very hygroscopic, it may be weighed in the air.

The buffer used had the composition 1000 mC NaA, 1000 mC HA; it is a 1 : 1 buffer of the approximate ionic strength $I = 1$. It was prepared by halfneutralizing a weighed

quantity of dry acid with NaOH and then diluting to the right volume. Immediately after preparation, the buffer is free from Cl^-, but after a day a feeble Cl^--reaction can be detected which indicates the progressing hydrolysis to glycolate. But no differences arise in the measured p[H^+], if a new or two day old buffer is used. Still older buffers have not been used, however.

The *other chemicals* are the same as in I.

THE POTENTIOMETRIC MEASUREMENT

The experiments are determinations of [H^+], carried out by quinhydrone electrode. The procedure was the potentiometric titration mentioned in I p. 383: a known volume of a solution of known concentration of uranyl perchlorate was in the electrode vessel; to this the 1 : 1 chloroacetate buffer was added. The same reference quinhydrone electrode, RE, as before was used.

The potentials adjusted themselves very quick to their proper values which were reproducible within 0.2 mV. The values attained were very stable. No effect of diffuse daylight could be detected. The great sensitiveness to light of certain wavelengths which later is proved extinctiometrically for the uranyl chloroacetate system seems not at all to affect the [H^+] of the solutions during the existing conditions.

As was established in I p. 385, the quinhydrone electrode is fairly well applicable to [H^+]-measurements in uranyl solutions. Small deviations ΔE_q are, however, distinctly perceptible. A thorough discussion indicated that they were mainly due to a weak complex formation between the uranyl ion and the hydroquinone component, and so dependent on [UO_2^{2+}]. The error of [H^+] caused by these deviations thus decreases with increasing complex formation.

From the figures of I, one can estimate the effect of this error on the quantity $\bar{n}/[A]$ which is used in the final calculation of the complexity constants. One finds that the true values are lower than the calculated; at the lowest C'_A used $\approx$ 7 % at $C'_M = 100$ mC and $\approx$ 3 % at $C'_M = 25$ mC. The error falls off rapidly and is of the same order of magnitude as the accidental error when $\bar{n} \approx 1$. Thus ΔE_q does not seriously affect the measurements.

From the [H^+] measured, [A] (and hence $\bar{n}$, (1)) is calculated. For that purpose, the dissociation constant K_c of monochloroacetic acid in the medium used must be known. It is determined by separate titrations with solutions which are free from uranyl. The quantities of these solutions are indicated below with′. It is valid:

$$\frac{[H^+]' \, [A]'}{[HA]'} = K_c \qquad (9)$$

Table 1. Determination of E' as a function of C'_A, when perchlorate is exchanged for chloroacetate buffer. — Calculation of K_c in the medium used.

C'_A mC	E' mV	(16) $[H^+]'$ mC	(10) $K_c \cdot 10^3$ C	C'_A mC	E' mV
19.6	43.0	1.84	2.22	231	39.9
26.0	42.1	1.91	2.21	250	39.9
29.1	41.6	1.95	2.23	286	40.0
38.5	40.9	2.00	2.22	333	40.2
47.7	40.4	2.04	2.22	348	40.3
50.7	40.4	2.04	2.21	375	40.5
62.6	40.0	2.07	2.21	400	40.6
69.8	39.8	2.10	2.23	412	40.7
90.9	39.7	2.11	2.21	444	40.9
117.7	39.5	2.12	2.20	473	41.1
130.5	39.6	2.11	2.18	483	41.2
167	39.6	2.11	2.16	500	41.3
200	39.8	2.10	2.14	517	41.4
211	39.8	2.10	2.14	546	41.6
				572	41.8

and as the stoicheiometric concentrations of acid and salt are the same $= C'_A$

$$\frac{[H^+]' \ (C'_A + [H^+]')}{C'_A - [H^+]'} = K_c \tag{10}$$

These titrations also show the effect of diffusion potentials and qualitative changes of medium which arise when perchlorate is gradually exchanged for chloroacetate buffer. As long as the medium may be considered as constant, K_c of (10) will namely remain a constant. So is also the case up to $C'_A \approx 150$ mC, Table 1, where we find $K_c = 2.22 \cdot 10^{-3}$ C *. At higher C'_A, however, K_c is no more a constant; but the mode of calculation used below eliminates the deviations.

For a solution which contains the total uranyl concentration C_M, in addition to the same buffer concentration C'_A as above, one has:

$$\frac{[H^+] \ [A]}{[HA]} = K_i \tag{11}$$

* The thermodynamic constant $K_a = 1.4 \cdot 10^{-3}$ (Landolt-Börnstein).

Table 2. Determination of corresponding values of $\bar{n}$ and [A] at different C'_M, to form the complex formation function and the function $\bar{n}/[A] = f([A])$.

*Table 2 A. $C'_M = 25$ mC ×**

C_M mC	C'_A mC	E mV	(18) E_A mV	(18) $\frac{[H^+]'}{[H^+]}$	(17) $[H^+]$ mC	(13) [A] mC	(15) $\bar{n}$	$\frac{n}{[A]}$ C^{-1}
25.0	0	58.9			1.0			
24.5	19.6	33.6	9.4	0.689	2.65	14.2	0.33	23.3
24.3	29.1	32.2	9.4	0.689	2.8	20.6	0.465	22.6
24.0	38.5	31.7	9.2	0.695	2.9	27.5	0.58	21.1
23.8	47.7	31.6	8.8	0.709	2.9	34.6	0.67	19.4
23.6	69.8	31.9	7.9	0.731	2.8	52.0	0.87	16.7
22.7	90.9	32.2	7.5	0.743	2.8	68.5	1.11	16.2
22.2	111.2	32.8	6.8	0.764	2.7	86.1	1.25	14.5
21.7	130.5	33.2	6.4	0.776	2.7	102.3	1.42	13.9
20.8	167	34.1	5.5	0.805	2.6	136	1.62	11.9
20.0	200	34.8	5.0	0.820	2.5	166	1.80	10.9
19.2	231	35.5	4.4	0.840	2	196	1.93	9.9
18.5	259	36.1	3.8	0.860	»	224	2.00	8.9
17.9	286	36.6	3.4	0.874	»	252	2.01	8.0
17.3	310	36.9	3.2	0.881	»	275	2.14	7.8
16.7	333	37.3	2.9	0.891	»	298	2.22	7.5
15.6	375	38.0	2.5	0.906	»	340	2.37	7.0
14.7	412	38.5	2.2	0.916	»	380	2.31	6.1
13.9	444	38.9	2.0	0.924	»	412	2.45	6.0
13.15	473	39.4	1.7	0.935	»	444	2.36	5.3
12.5	500	39.7	1.6	0.939	»	471	2.48	5.3

where K_c is the same as before, if we neglect the effect of C_M, appearing in ΔE_q. [A] is the quantity searched for, and for [HA] it is valid:

$$[HA] = C'_A - [H^+] + C^0_H - C_s \tag{12}$$

Here C^0_H = the hydrogen ion concentration of uranyl solution before addition of buffer, and C_s = the hydrogen ion concentration which is consumed by pressing back the hydrolysis of uranyl, when the solution by addition of buffer and complex formation grows more acid.

The existence of the terms C^0_H and C_s is thus due to the fact that the solution besides the chloroacetate contains uranyl as another buffering system.

* These signs are used to indicate the respective C'_M in the Figs. 1 and 2.

Table 2 B. $C'_M = 50$ *mC.* ○

C_M mC	C'_A mC	E mV	(18) E_A mV	(18) $\frac{[H^+]'}{[H^+]}$	(17) $[H^+]$ mC	(13) [A] mC	(15) $\bar{n}$	$\frac{\bar{n}}{[A]}$ C^{-1}
50	0	46.7			1.6			
48.7	26.0	25.9	16.3	0.524	3.6	13.6	0.33	24.3
48.1	38.5	25.1	15.8	0.535	3.7	20.7	0.45	21.8
47.5	50.7	24.9	15.5	0.542	3.7	27.5	0.565	20.5
46.9	62.6	25.2	14.8	0.558	3.7	35.2	0.66	18.8
45.5	90.9	26.2	13.5	0.586	3.6	53.6	0.90	16.8
44.2	117.7	27.2	12.3	0.613	3.4	72.7	1.10	15.1
42.9	142.9	28.1	11.5	0.632	3.3	90.8	1.29	14.2
41.7	167	29.3	10.3	0.667	3	112	1.39	12.4
39.5	211	30.9	8.9	0.702	»	149	1.65	11.1
37.5	250	32.1	7.8	0.735	»	184	1.84	10.0
35.7	286	33.2	6.8	0.764	»	219	1.96	9.0
34.1	318	34.1	6.1	0.785	»	250	2.08	8.3
32.6	348	34.9	5.4	0.807	»	282	2.12	7.5
31.3	375	35.5	5.0	0.820	2	309	2.18	7.1
30.0	400	36.1	4.5	0.837	»	336	2.20	6.5
27.9	444	37.0	3.9	0.855	»	381	2.33	6.1
25.9	483	37.7	3.5	0.870	»	422	2.44	5.8
24.2	517	38.4	3.0	0.886	»	460	2.44	5.3
22.7	546	39.0	2.6	0.900	»	493	2.42	4.9
21.4	572	39.4	2.4	0.910	»	522	2.43	4.7

But as was mentioned above, the p[H$^+$] of the measurement has been choosen so that the buffering capacity of the uranyl system is very low (the uranyl being only slightly hydrolysed) while that of the chloroacetate system is in the neighbourhood of its maximum (1 : 1 buffer). So C_s may be treated as a correction term, even if C'_A is rather low. This is necessary, because an exact calculation presupposes a full information of the course of hydrolysis in the solutions measured which we have not. From the measurements of non-complex uranyl perchlorate solutions in I, however, we are able to do the estimation that C_s is of the same order of magnitude as the C_H^0 found, only a little smaller. We so introduce the fairly good approximation $C_H^0 = C_s$. The $\bar{n}/[A]$, calculated according to this, become too high. The error may reach 5—8 % at the lowest C'_A used, but it falls off very rapidly, when C'_A increases. Already at $\bar{n} = 0.5$—1 it may be quite neglected.

From (10), (11) and (12) we now obtain our unknown quantity [A] according to:

Table 2 C. $C'_M = 100$ *mC.* □

C_M mC	C'_A mC	E mV	(18) E_A mV	(18) $\frac{[H^+]'}{[H^+]}$	(17) $[H^+]$ mC	(13) [A] mC	(15) $\bar{n}$	$\frac{\bar{n}}{[A]}$ C^{-1}
100	0	34.4			2.6			
96.2	38.5	15.1	25.8	0.360	5.5	13.2	0.32	24.2
95.0	50.7	14.7	25.7	0.361	5.6	17.6	0.41	23.3
93.8	62.6	14.8	25.2	0.369	5.6	22.5	0.485	21.6
90.9	90.9	15.7	24.0	0.387	5.4	35.0	0.675	19.3
88.3	117.7	17.0	22.5	0.410	5.1	47.9	0.85	17.8
85.8	142.9	18.5	21.1	0.433	4.8	61.5	1.01	16.4
83.3	166.7	19.9	19.7	0.458	4.5	76.2	1.14	15.0
78.9	211	22.4	17.4	0.503	4	106	1.38	13.0
75.0	250	24.7	15.2	0.548	»	137	1.56	11.4
71.4	286	26.6	13.4	0.588	»	169	1.69	10.0
68.2	318	28.0	12.2	0.617	3	197	1.82	9.2
65.2	348	29.2	11.1	0.645	»	225	1.93	8.6
62.6	375	30.4	10.1	0.668	»	252	2.01	8.0
60.0	400	31.4	9.2	0.695	»	279	2.07	7.4
55.7	444	33.0	7.9	0.731	»	325	2.19	6.7
51.8	483	34.2	7.0	0.758	»	368	2.28	6.2
48.4	517	35.2	6.2	0.783	2	407	2.32	5.7
45.4	546	36.2	5.4	0.807	»	442	2.34	5.3
42.8	572	36.9	4.9	0.825	»	473	2.36	5.0

$$[A] = \frac{[H^+]'}{[H^+]} \cdot \frac{(C'_A + [H^+]')\ (C'_A - [H^+])}{C'_A - [H^+]'} \qquad (13)$$

For the following calculation of $\bar{n}$ according to (1), one must further know the total ligand concentration C_A and the total uranyl concentration C_M. For C_A one has:

$$C_A = C'_A + [H^+] - C^0_H + C_s \qquad (14)$$

where the approximation $C^0_H = C_s$ also may be introduced. C_M of (1), finally, is the total uranyl concentration taking part in formation of chloroacetate complexes. Thus the quantity bound as hydrolysed complexes should be subtracted from the stoicheiometric uranyl concentration. However, p[H^+] has been choosen so that this quantity is small; from the measurements of hydrolysis in I, it may be estimated to be 3—5 % of the total amount. This error is involved in the $\bar{n}$/[A] calculated below.

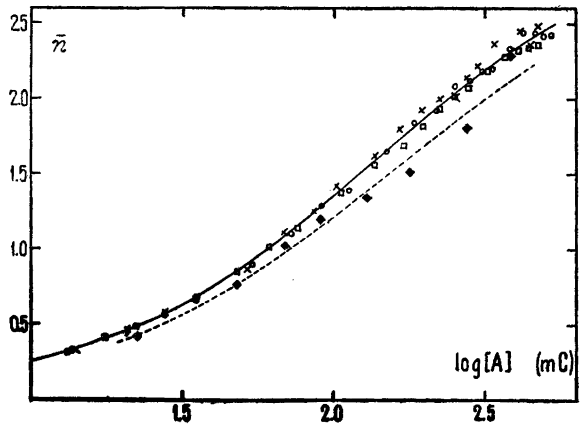

Fig. 1. The complex formation curve. ×, ○ *and* □*: potentiometrically determined values at* $C'_M = 25$*, 50 and 100 mC respectively.* — ◆*: extinctiometrically determined values.* — ***Fulldrawn*** *curve obtained from the potentiometrically and dashed curve from the extinctiometrically calculated complexity constants.*

Inserting the approximated values of [A], C_A and C_M in (1) we obtain:

$$\bar{n} = \frac{C'_A + [H^+] - \frac{[H^+]'}{[H^+]} \cdot \frac{(C'_A + [H^+]')\ (C'_A - [H^+])}{C'_A - [H^+]'}}{C_M} \tag{15}$$

The equations (15) and (13) now obtained give the corresponding values of $\bar{n}$ and [A] searched for, *i. e.* the complex formation curve, from which the function $\bar{n}/[A]$ is obtained.

In (13) and (15) C'_A and C_M are known stoicheiometric concentrations, while $[H^+]'$ and $[H^+]$ are the hydrogen ion concentrations of solutions of the same C'_A, the first without uranyl, the second with the uranyl concentration $= C_M$. $[H^+]'$ and $[H^+]$ are calculated from the emf E' and E of the measured cells. If $[H^+]_0$ is the known hydrogen ion concentration of RE (= 10.10 mC), it is valid for these cells:

$$E' = 58.2 \log \frac{[H^+]_0}{[H^+]'} \tag{16}$$

$$E = 58.2 \log \frac{[H^+]_0}{[H^+]} \tag{17}$$

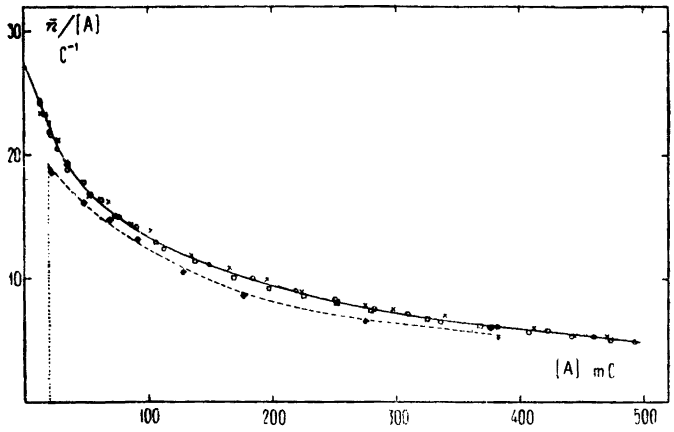

Fig. 2. $\bar{n}/[A]$ *as a function of* $[A]$ *the integration of which gives the X-functions (Tab. 3 and 8). — The signs relates to the same measurings as in Fig. 1; but the curves are here drawn according to the experimental points.*

Hence
$$E' - E = E_A = 58.2 \log \frac{[H^+]}{[H^+]'} \qquad (18)$$

From (18) the factor $\frac{[H^+]}{[H^+]'}$ of (13) and (15) is obtained independent of diffusion potentials and medium changes which arise when C'_A increases, as it was shown above. For in the (hypothetic) cell with emf E_A, $[H^+]'$ and $[H^+]$ are measured at the same C'_A. $[H^+]'$ and $[H^+]$, on the other hand, which stand at the side of C'_A in (13) and (15) are obtained from (16) and (17); at $C'_A < 150$ mC this is correct, as the medium is not yet perceptibly changed, at $C'_A >$ 150 mC $[H^+]'$ and $[H^+]$ are so small in comparison with C'_A, that the deviations there met with are of no importance.

To sum up the effects of the different systematic errors, discussed above, one may say that (13) and (15) are simplified equations which do not take into account 1. ΔE_q 2. C_s and 3. the wrong value of C_M on account of hydrolysis.

The error 1. and 2. cause small $\bar{n}$ to be calculated too high, while 3. acts in opposite direction. The total result is, perhaps, 5—10 % too high values of $\bar{n}/[A]$ at the lowest [A]. The lower limit of C'_A has, in fact, been determined so that these errors must not be too great. All errors mentioned diminish rapidly with increasing $\bar{n}$. Already at [A] = 60 mC, $\bar{n} = 1$, their total effect certainly is quite negligible.

Table 3. $X([A])$, $X_1([A])$, $X_2([A])$ and $X_3([A])$ for given $[A]$, as obtained by numerical integration of the potentiometrically determined function of Fig. 2.

[A] mC	(5a) ln $X([A])$	$X([A])$	(7a) $X_1([A])$ C^{-1}	(7b) $X_2([A])$ C^{-2}	$X_3([A])$ C^{-3}
0			*27.4*	*193*	*625*
10	0.2608	1.298	29.8		
20	0.4964	1.643	32.2		
30	0.7079	2.030	34.3		
40	0.9007	2.462	36.6	230	
50	1.0799	2.945	38.9	230	
60	1.2483	3.48	41.4	233	
80	1.5583	4.75	46.9	244	
100	1.8385	6.29	52.9	255	620
120	2.0958	8.13	59.4	267	617
140	2.3340	10.32	66.6	280	622
160	2.5552	12.87	74.2	293	625
180	2.7611	15.81	82.3	305	622
200	2.9540	19.18	90.9	318	625
250	3.3903	29.67	114.7	349	624
300	3.7738	43.6	142	381	627
350	4.1166	61.3	172	414	632
400	4.4292	83.8	207	449	640
450	4.7175	111.9	246	487	653

Table 4. The ligand number and the composition of the system as calculated for some round $[A]$ with the constants obtained.

$\beta_1 = 27.5 \pm 0.5\ C^{-1}$ $\beta_2 = 195 \pm 20\ C^{-2}$ $\beta_3 = 625 \pm 150\ C^{-3}$

[A] mC	(2) $\bar{n}$	(8a) α_0 %	(8b) α_1 %	(8b) α_2 %	(8b) α_3 %
10	0.245	77	21.5	1.5	0
30	0.605	49	41	8.5	1.5
50	0.875	34	47	16.5	2.5
100	1.345	16	43.5	30.5	10
200	1.87	5.5	28.5	40	26
300	2.16	2	19	40	39
400	2.33	1	13.5	37	48.5

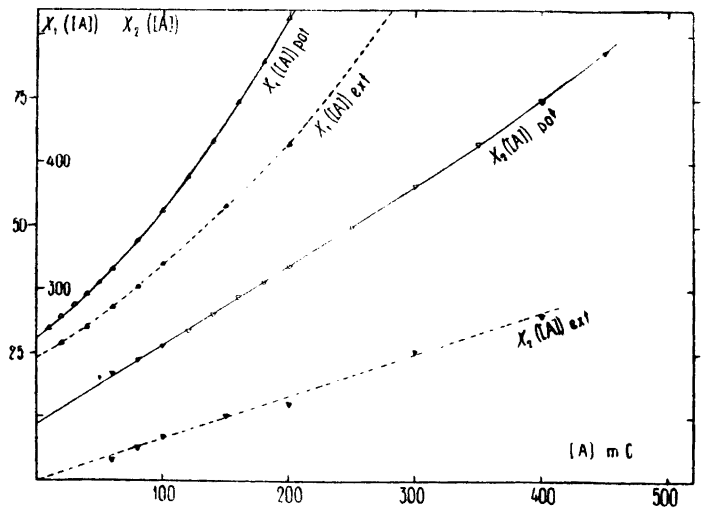

Fig. 3. The functions $X_1([A])$ and $X_2([A])$ which give the complexity constants.

The accidental error is determined by the reproducibility of the emfs which was 0.2 mV, corresponding to ≈ 2 % in [A]. At low $\bar{n}$, this error can be neglected. At high $\bar{n}$, on the other hand, where C_A — [A] is a comparatively small difference between two big numbers, it becomes important and causes a considerable spreading of the highest points on the complex formation curve (Fig. 1).

To secure a good test of polynuclear complex formation, measurements have been carried out with three uranyl solutions of highly different strengths. Their concentrations at the outset were $C'_M = 25$, 50 and 100 mC.

The E' and E given below are mean values from at least two titrations. At $C_M = 0$ three and at $C'_M = 25$ mC two different buffer solutions of different age have given identical results, as was mentioned above.

The results are found in Tables 1 and 2. The found values of $\bar{n}$ and log [A] are plotted in Fig. 1 and in Fig. 2 $\bar{n}/[A]$ is given as a function of [A]. Within the limits of error, the course of these functions are entirely the same for the different C'_M, so no polynuclear complexes seem to exist.

The ligand number $\bar{n}$ reaches a value ≈ 2.5 in our measurements. So we have to calculate with at least three complexes: MA, MA_2 and MA_3.

For the calculation of the complexity constants, we now compute corresponding values of $X([A])$ and [A] by integrating the $\bar{n}/[A]$-function of Fig. 2. As very low [A]-values have been determined, the function can be extrapolated to [A] = 0. Thus we may integrate according to (5a). From $X([A])$ hence obtained, $X_1([A])$, $X_2([A])$ and $X_3([A])$ are formed according

to (7). Corresponding values of these functions for the round [A], choosen as upper limits of integration, are found in Table 3. The functions $X_1([A])$ and $X_2([A])$ are plotted in Fig. 3. By extrapolation to $[A] = 0$, one gets the constants $\beta_1 = 27.5 \pm 0.5$ C^{-1}; $\beta_2 = 195 \pm 20$ C^{-2}. The function $X_3([A])$ is practically constant over the whole [A]-range and gives $\beta_3 = 625 \pm 150$ C^{-3}.

These values of the constants are rounded off according to the indicated errors which are the maximal accidental ones as they are estimated from the spreading of the $(\bar{n}/[A], [A])$-points of Fig. 2. — The systematic errors of $\bar{n}/[A]$, mentioned above, in all probability cause the calculated values of β_1 and β_2 to be 5—10 % too high.

With the constants obtained, $\bar{n}$ is calculated according to (2) for some round [A]. (Table 4.) The complex formation curve thus found (fulldrawn in Fig. 1) is seen to fit the experimental points very well. For the same [A], the composition of the system is calculated according to (8) and also given in Table 4.

THE EXTINCTIOMETRIC MEASUREMENT

The experiments are photo-electric determinations of the extinction E of the solutions. The apparatus used is built by Olerup [3] who mainly has followed the designs of Kortüm and v. Halban [8], Deck [9], and Kortüm [10, pp. 93 and 111]. As Olerup has given a close description, only a brief summary of the main points will be given here. On this occassion some improvements which extend the use in the ultra-violet, will also be mentioned.

Light from a mercury lamp passes a quartz double monochromator. The beam leaving the monochromator is made parallell by a quartz lens and then divided into two by a quartz plate. In the first beam, the absorption cells are placed, and the intensity of light of this beam may be weakened by means of a non-central rotating sector which can be adjusted during rotation. The light falls finally onto a sodium phototube. The second beam of light is reflected by a mirror of aluminium (which has good reflecting properties even at as short a wave-length as 3 130 Å) onto a second phototube which is coupled so as to compensate the current of the first one. An electrometer is coupled as a zero instrument between the tubes. The whole forms a compensated two phototubes apparatus, working according to the method of substitution. First, a cell with the solution to be measured is inserted in the first beam and the photo-current is compensated to zero by adjustment of the potential over one of the phototubes. Second, the cell of solution is changed for a similar cell containing solvent, and the sector is started. E of the solute is E of the sector, when compensation to zero is reached again. — By this arrangement, no errors are caused even if the light source fluctuates or the photo-current is not proportional to the intensity of light.

The E determined are not the true E of the wave-length used, however, on account of two sources of error, always involved in extinctiometric measurements: the light is not strictly monochromatic, and it partly passes the absorption cell more than once by re-

peated reflection at the end-plates. But as the measurements are treated as relative ones in the following calculations, it is not essential that the E:s measured are true. On the other hand, however, it is necessary that the error of E is a constant for all solutions which are compared in the calculations. We have to state how this condition may be fulfilled.

The error owing to lacking monochromaticity is examined by Kortüm [10, p. 10], Olerup [3, p. 47] and, especially, Fronaeus [5, p. 95]. It proves difficult to determine its magnitude, but one is able to establish that a constant error is obtained if a) E is kept constant (which is easy to realize), and b) the solutions measured have extinction curves of the same shape. For a system of only mononuclear complexes, this latter condition is fulfilled as soon as the solutions have the same [A] (Olerup [3, p. 48]). By the method of calculation used below, this is the case for all solutions which are compared. Thus both the methodical conditions mentioned are observed.

The error owing to reflection at the end-plates has been thoroughly examined by Olerup [3, p. 50]. He finds that also this error has a constant size when E is kept constant, and, moreover, that its relative size decreases when E increases.

The true E:s of the solutions were not essential, thus the absolute thicknesses of the absorption cells are not required, but only the ratios between them. The cell of 1 cm is set = 1,000. The determination of these ratios is made by picrate solutions containing an excess of NaOH. The cell of comparison only contains the NaOH-solution. This standard of calibration is recommended by v. Halban, Kortüm and Szigeti [11].

These measurements give the ratios between the 'effective' thicknesses, *i. e.* all existing differences between the cells are involved in this calibration and expressed as differences of thickness. It is evident that such an 'effective' thickness is valid only at a certain E. Therefore all calibrations are performed at one and the same E, and this E is then also used at all the following complex measurements, although the errors mentioned above only required the same E for solutions to be compared. The constant E is selected = 0.7, as the error of adjustment of the sector and the error from repeated reflection both become very small at this value.

The calibration has been performed before and after every series of measurements. As a rule the same results have been obtained within $2-3\ ^0/_{00}$. Thus the determined ratios of thickness are very well reproducible quantities, which reflects the precise construction of the apparatus used.

On account of the construction of the apparatus, the measurements must always be carried out at some strong mercury line within the range of sensitivity of the phototubes used. To select the most suitable one where the complex formation in question shows the greatest effect on the extinction of UO_2^{2+}, the whole extinction curve of a complex solution of the composition

$$C_M = 10 \text{ mC}; \ C'_A = C'_{HA} = 100 \text{ mC}; \ NaClO_4 \text{ to } I = 1$$

is photographed, as described in I p. 375. The extinction curve found is compared in Fig. 4 with the curve of UO_2^{2+}, determined in I. The strong mercury lines of the range in question are at 3 130, 3 660, 4 050 and 4 360 Å. One sees that the greatest differences between the curves are found for 3 130

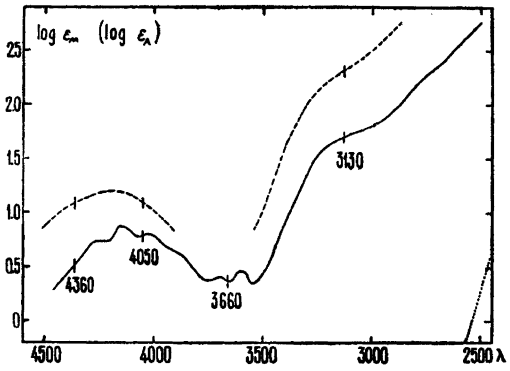

Fig. 4. Extinction curves of a) uranyl ion (fulldrawn), b) complex solution with $C_M = 10$ mC, $C_{HA} = C'_A = 100$ mC (dashed) and c) 1 : 1 chloroacetate buffer (dotted low to the right; the stoicheiometric molar extinction in this case, ε_A, is defined according to $\varepsilon_A = \frac{E}{d\,(C_A + C_{HA})}$.

and 4 360 Å. As ε_M is so low at 4 360 Å that inconveniantly high C_M should be necessary in the available cells, 3 130 Å has been selected. This selection also proved to be very good on account of the sensitiveness to light of the system (see below). The extinction curve of the buffer has been determined, too, and is given in Fig. 4. It is seen that this extinction is to be neglected within the whole range discussed.

From the directly measured E, the stoicheiometric molar extinction ε_M is immediately obtained, and according to Beer it is valid ((2) and (3) of I):

$$E/d = \varepsilon_M \cdot C_M = \varepsilon_0 \cdot C_0 + \varepsilon_1 \cdot C_1 + \varepsilon_2 \cdot C_2 + \ldots \tag{19}$$

The possibilities of calculating the composition of a complex system from the variation of the ε_M:s obtained, when C_M and C_A is varied, have been very thoroughly investigated by Olerup [3, p. 57], Bjerrum [4] and Fronaeus [5, p. 87]. They agree that no reliable complexity constants at all can be calculated if polynuclear complexes exist. If, on the other hand, mononuclear complexes are entirely formed, the composition is possible to calculate, and the result may become fairly accurate, if the ε:s of the system have suitable size in proportion to each other.

In the system in question, the potentiometric measurements indicate that the complex formation in all probability is mononuclear. Thus the calculation is possible, and is performed as follows, in close connection to Fronaeus and Olerup.

If (19) is applied on a mononuclear complex system, we obtain with (4) of I:

$$\frac{E}{C_M \cdot d} = \varepsilon_M = \frac{\varepsilon_0[\mathrm{M}] + \varepsilon_1[\mathrm{MA}] + \varepsilon_2[\mathrm{MA_2}] + \varepsilon_3[\mathrm{MA_3}] + \ldots}{[\mathrm{M}] + [\mathrm{MA}] + [\mathrm{MA_2}] + [\mathrm{MA_3}] + \ldots} \tag{20}$$

Introducing (6) of I p. 378, and (3) above, we get:

$$\varepsilon_M = \frac{\varepsilon_0 + \varepsilon_1 \beta_1[\mathrm{A}] + \varepsilon_2 \beta_2[\mathrm{A}]^2 + \varepsilon_3 \beta_3[\mathrm{A}]^3 + \ldots}{X([\mathrm{A}])} \qquad (21)$$

From (21), a new function $\varepsilon_M - \varepsilon_0$ is formed by subtracting ε_0 from both membra:

$$\varepsilon_M - \varepsilon_0 = \frac{(\varepsilon_1 - \varepsilon_0)\beta_1[\mathrm{A}] + (\varepsilon_2 - \varepsilon_0)\beta_2[\mathrm{A}]^2 + (\varepsilon_3 - \varepsilon_0)\beta_3[\mathrm{A}]^3 + \ldots}{X([\mathrm{A}])} \qquad (22)$$

As ε_0 may be separately determined, $\varepsilon_M - \varepsilon_0$ is experimentally determinable, as was ε_M.

As seen from (21) and (22), ε_M and $\varepsilon_M - \varepsilon_0$ are both functions of [A] only. A certain constant value of ε_M or $\varepsilon_M - \varepsilon_0$ thus corresponds to a certain constant value of [A]. The functions are thus equivalent; in the following calculation $\varepsilon_M - \varepsilon_0$ is preferred, however, as the course of $(\varepsilon_M - \varepsilon_0)/C_A = f(\varepsilon_M - \varepsilon_0)$, used there, is more advantageous than that of the corresponding $\varepsilon_M/C_A = f(\varepsilon_M)$.

The property of $\varepsilon_M - \varepsilon_0$ to be a function of [A] only is used to determine corresponding values of $\bar{n}$ and [A], *i. e.* the complex formation curve. For that purpose, $\varepsilon_M - \varepsilon_0$ is determined for increasing buffer concentrations C'_A. For every C'_A, measurements are performed at three different C_M. By this, the same three C_M cannot be choosen at different C'_A, on account of the condition that E has to be a constant. C_M must be varied as C'_A is varied. Therefore the cell-thickness d is choosen as a convenient parameter of the measurements: $\varepsilon_M - \varepsilon_0$ are determined for every C'_A with three different d, corresponding to three different C_M.

That C_M, which in a given case gives the proper $E \approx 0.7$ is found by trying. So the C_M:s of a certain d are not *a priori* connected in some known manner. As it is necessary for the following calculations to know C_M at any pair of $(\varepsilon_M - \varepsilon_0, C_A)$ along the curves of constant d, the first task is to establish such a connection.

An examination shows that the demand of a constant E causes that the function $\varepsilon_M - \varepsilon_0 = f(C_M)$ has a very curved course for a given d. If, however, the logarithmic function $\log(\varepsilon_M - \varepsilon_0) = f(\log C_M)$ is introduced, the points are found to fall along an almost straight line *. The best straight line through

* The corresponding connection between $\log \varepsilon_M$ and $\log C_M$ is an exactly straight line, provided E is kept quite constant. For $E/d = \varepsilon_M \cdot C_M$; hence $\log \varepsilon_M = \log E/d - \log C_M$, where $\log E/d$ is a constant.

Table 5. Determined values of $\varepsilon_M - \varepsilon_0$ at given C'_A and C_M.

$d \rightarrow$ cm	0.1		0.3		1		3	
C'_A mC	C_M mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$	C_M mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$	C_M mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$	C_M mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$
25			21.44	49.3	6.23	58.9	2.076	61.6
50			16.75	86.5	4.49	96.4	1.541	99.4
75			14.34	114.5	3.819	124.6	1.273	124.8
100	44.9	112.6	13.00	137.0	3.483	145.4		
150	31.49	155.6	10.05	170.7	3.014	176.4		
200	27.86	183.4	9.25	195.2	2.680	198.6		
300	24.65	220.6	8.04	228.2	2.412	231.1		
400	22.78	245.5	6.70	251.5	2.224	254.7		

Table 6. $\varepsilon_M - \varepsilon_0$ at given C'_A, corrected so as to fit those connections between $\varepsilon_M - \varepsilon_0$ and C_M which are established by the straight lines of Fig. 5. — The transformation from C'_A to C_A.

$d \rightarrow$ cm	0.1		0.3		1		3	
C'_A mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$	C_A mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$	C_A mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$	C_A mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$	C_A mC
25			47.9	27.7	58.6	27.1	61.5	27.0
50			86.7	52.5	96.2	52.2	99.4	52.1
75			115.3	77.4	124.5	77.2	124.7	77.1
100	113.8	103.5	138.0	102.4	145.5	102.2		
150	155.2	153.0	170.6	152.3	176.4	152.2		
200	183.2	202.7	195.4	202.3	198.6	202.2		
300	220.6	302	228.2	302	231.1	302		
400	245.5	402	251.2	402	254.7	402		

the experimental points of every series is now introduced as the connection searched for. These lines namely fit the points so well that one may with great certainty determine graphically those $\varepsilon_M - \varepsilon_0$, which exactly correspond to the established C_M-function at a given C'_A and d. This will be clear from a survey of Fig. 5.

These so modified $\varepsilon_M - \varepsilon_0 = f\ (C'_A)$ functions, with C_M known for every $\varepsilon_M - \varepsilon_0$, are now transformed into $\varepsilon_M - \varepsilon_0 = f\ (C_A)$. The transition from C'_A

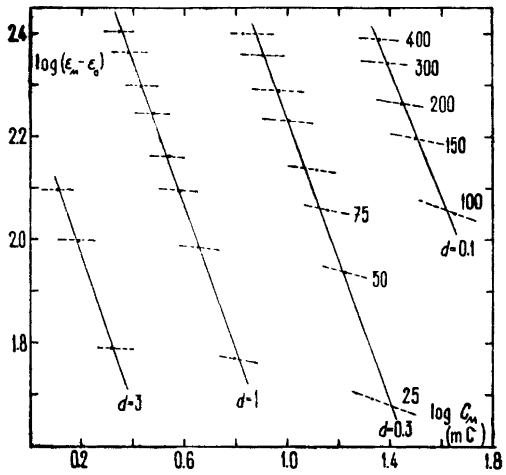

Fig. 5. log $\varepsilon_M - \varepsilon_0$ as a function of log C_M at different d. — ●: experimentally determined points. — Fulldrawn curves: the established connections between $\varepsilon_M - \varepsilon_0$ and C_M for given d. — Dashed curves: position of points with constant C'_A, given to the right.

to C_A is easily performed according to (14), by the aid of the potentiometrically determined $[H^+]$. Thus the preceding measurements are used to a certain extent. It must be emphasised, however, that this does not mean that the extinctiometric measurements hereby become dependent on the potentiometric ones. $[H^+]$ is namely only a correction term of C_A. Thus even a very large error in its determination has a very small influence. Moreover, most C_M used are so low that the $[H^+]$ of their solutions does not differ very much from that of pure buffer. The correction introduced thus depends very little on C_M, *i. e.* on the potentiometric complex titration.

Now the function $\varepsilon_M - \varepsilon_0 = f\ (C_A)$ should be cut at constant $\varepsilon_M - \varepsilon_0$. However, the curves for the different d are so close to each other that such a graphical determination would require an inconveniently large scale. By transforming the functions according to

$$\frac{\varepsilon_M - \varepsilon_0}{C_A} = f\ (\varepsilon_M - \varepsilon_0) \tag{23}$$

new functions are obtained which permit the same accuracy of the graphical determination at a much more convenient size (*cf.* Olerup [3, p. 66]).

The functions (23) now obtained are cut at a number of constant $\varepsilon_M - \varepsilon_0$, each of them corresponding to a certain C'_A (C_A) originally used (see Fig. 6). To diminish the errors of interpolation, the sections are choosen in a neighbourhood as close as possible to the experimental points. The solutions of a certain $\varepsilon_M - \varepsilon_0$ have, according to (22), the same [A], and, according to (2), also the same $\bar{n}$, but varying C_A and C_M. The values of C_A are immediately

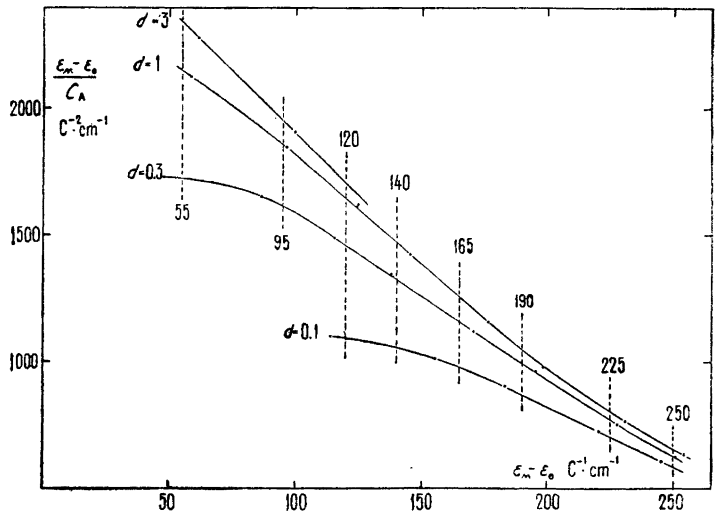

Fig. 6. $(\varepsilon_M-\varepsilon_0)/C_A$ *as a function of* $\varepsilon_M-\varepsilon_0$ *at different d. — The curves are cut at eight* $\varepsilon_M-\varepsilon_0$*, each of them representing a certain constant pair* $(\bar{n}, [A])$*.*

obtained from the values of $(\varepsilon_M-\varepsilon_0)/C_A$ of the intersections. The corresponding values of C_M are found by seeking up in the (log C_M, log $(\varepsilon_M-\varepsilon_0)$)-diagram (Fig. 5) the values of C_M which correspond to the given $\varepsilon_M-\varepsilon_0$ at the d in question.

Thus, for a constant $\varepsilon_M-\varepsilon_0$, C_M and C_A may be considered as the variables of the equation of the ligand number (1), whereas [A] and $\bar{n}$ are constants. The equation is of the first grade; in a (C_M, C_A)-diagram it means a straight line with the intercept on the C_A-axis = [A] and the slope = $\bar{n}$. By plotting the (C_A, C_M)-pairs found for each $\varepsilon_M-\varepsilon_0$ in such a diagram, the unknown constants [A] and $\bar{n}$ of every $\varepsilon_M-\varepsilon_0$ are obtained, and thereby the complex formation curve searched for.

If the pairs of (C_M, C_A) obtained at constant $\varepsilon_M-\varepsilon_0$ do not fall along straight lines it means polynuclear complex formation. For if polynuclear complexes exist neither [A] nor $\bar{n}$ are constants at constant $\varepsilon_M-\varepsilon_0$ (Fronaeus[5, p. 88]) and (1) may thus become curved. But it is by no means certain that this really occurs in all such cases. The lines may also be approximately straight but have a slope differing from $\bar{n}$ (see Fronaeus[5, p. 109]). The straight-lined course of (1) at constant $\varepsilon_M-\varepsilon_0$ therefore is a necessary but not sufficient condition for the mononuclear complex formation postulated in our calculations.

Table 7. C_A as a function of C_M at the eight selected $\varepsilon_M - \varepsilon_0$ and the corresponding values of [A], $\bar{n}$ and $\bar{n}/[A]$ hence determined.

$d \rightarrow$ cm	0.1		0.3		1		3				
$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$	C_A mC	C_M mC	C_A mC	C_M mC	C_A mC	C_M mC	C_A mC	C_M mC	$C_M=0$ $C_A=$ [A] mC	n	$\bar{n}/[A]$ C^{-1}
55			31.9	22.8	25.6	6.8	23.5	2.3	22.6	0.42	18.6
95			59.0	15.4	51.3	4.6	48.7	1.6	47.7	0.77	16.2
120	110.1	40.1	82.2	12.9	73.2	3.9	70.6	1.3	69.2	1.02	14.8
140	133.2	35.3	105.7	11.5	95.2	3.5			91.4	1.20	13.2
165	168.8	30.9	142.8	10.2	131.4	3.1			128.2	1.35	10.5
190	217.9	27.5	190.9	9.2	181.0	2.8			176.9	1.51	8.6
225	318	24.0	289.5	8.1	279.5	2.5			275	1.8	6.5
250	426	22.0	395	7.5	381	2.3			377	2.3	6.1

In the present case this condition is very well fulfilled, and as also the criterion applied in the potentiometric measurements gave the same result, there is no doubt, that the complex formation in question is entirely mononuclear.

However it is necessary to remember that our fundamental equations (21) and (22) may be invalid even if the chloroacetate complex formation is strictly mononuclear. Of course, this is the case if a reaction occurs in the solutions. In uranyl solutions one has moreover to take into consideration that reactions often are induced by the action of light. To investigate this, E was determined for a solution:

$$C_M = 10.98 \text{ mC}; \; C'_A = C'_{HA} = 100 \text{ mC}; \; NaClO_4 \text{ to } I = 1$$

when it was lighted in different ways. The observations then made, which have been fully confirmed in the later measurements, may be summed up as follows:

a. The solution is not changed during twentyfour hours in the dark, or in ordinary lamp-light.

b. The solution is not changed during several hours if lighted with UV-light of the wave-length of measuring, 3 130 Å.

c. The solution is rapidly changed in diffuse day-light, even behind glass. An exposure of one hour altered E of 3 130 Å by $\approx$ 10 %, whereas another

sample of the same solution, kept in the dark during the same time, gave the original E.

Thus it seems to be the absorption range in the violet which is sensitive to light. In the measurements, the solutions must therefore be prepared and stored only in lamp-light. Then reproducible E are obtained indicating that the system is in its original state and so (21) and (22) are valid.

Another fact which may cause (21) and (22) to be invalid is the hydrolysis of the solutions. It would be of no importance, if the solutions which are compared in the measurements had the same degree of hydrolysis. This would mean a constant error of ε_M which has no influence by the method of calculation used, as was mentioned above. The degree of hydrolysis depends on both p[H^+] and C_M, as was shown in I. As the solutions to be compared have different C_M and p[H^+], their errors of ε_M are different. They may, however, be roughly estimated from I where the effect of the hydrolysis on ε_M is determined. The error is important only at low C'_A where [UO_2^{2+}] is large. At the lowest C'_A it may cause an error of ≈ 1 % in [A] which means that the finally computed quantity $\bar{n}$/[A] becomes ≈ 5 % too high.

Now this error must be largely compensated by the error of C_M caused by the hydrolysis. As at the potentiometric measurements above, the C_M:s really taking part in the complex formation at low C'_A are 3—5 % lower them the stoicheiometric ones. Hence the corresponding $\bar{n}$/[A] come out 3—5 % too low. The total effect of the hydrolysis therefore seems to be rather small.

The uncertainty involved in these estimations of the effect of hydrolysis, together with the desire to keep [H^+] as a minor part of C_A, has lead to that C'_A lower than 25 mC have not been used.

The ε_M directly determined are found as the mean of at least three adjustments of the sector. Before every adjustment, the compensation of the apparatus is checked. As a control, about one third of the solutions is prepared and measured twice, sometimes with use of different preparations of buffer. In all cases the reproducibility is $\approx \pm 2$ ‰, or almost as good as was found by the calibration. At low C'_A this may cause an accidental error of $\bar{n}$/[A] which may amount ≈ 3 %, thus rather insignificant. At the higher C'_A where the variation of ε_M by C'_A is less, the error of $\bar{n}$/[A] becomes larger and may amount ≈ 10 %.

ε_0 is separately determined with two C_M : 47.11 mC ($d = 0.3$ cm) and 14.13 mC ($d = 1$ cm). One finds $\varepsilon_0 = 47.93$ and 48.09; mean 48.0 $C^{-1} \cdot cm^{-1}$. It is evident that Beers law is strictly valid for the uranyl ion within the errors of measurement.

Table 5 gives the determined values of $\varepsilon_M - \varepsilon_0$ for given C'_A and C_M. In Fig. 5 the corresponding values of log C_M and log $(\varepsilon_M - \varepsilon_0)$ are plotted. In the

Table 8. $X([A])$, $X_1([A])$, $X_2([A])$ and $X_3([A])$ for given $[A]$ as obtained by numerical integration of the extinctiometrically determined function of Fig. 2. — The complexity constants and the ligand number obtained from these functions.

	$\beta_1 = 24 \pm 3$ C^{-1}		$\beta_2 = 150 \pm 40$ C^{-2}	$\beta_3 = 350 \pm 150$ C^{-3}			
[A] mC	(5b) $\ln \frac{X([A])}{X(0.02)}$	$\frac{X([A])}{X(0.02)}$	$X([A])$	(7a) $X_1([A])$ C^{-1}	(7b) $X_2([A])$ C^{-2}	$X_3([A])$ C^{-3}	(2) $\bar{n}$
0				*24.0*	*147*	*350*	
20	0	1.00	1.54	27.0			0.39
40	0.3585	1.43	2.20	30.0			0.675
60	0.6781	1.97	3.03	33.9	165		
80	0.9653	2.63	4.04	38.0	175		
100	1.2258	3.41	5.24	42.4	184	370	1.21
150	1.7768	5.91	9.10	54.0	200	355	
200	2.2217	9.23	14.2	66.0	210	315	1.69
300	2.9984	20.06	30.9	99.7	252	350	
400	3.5870	36.13	55.4	136	280	335	2.14

best agreement with the points, straight lines are drawn which constitute the known connection between $\varepsilon_M - \varepsilon_0$ and C_M. The course of the curves for constant C'_A are sufficiently well known to enable us to state the value of $\varepsilon_M - \varepsilon_0$ at a certain C'_A, which corresponds to the stated ($\varepsilon_M - \varepsilon_0$, C_M)-connection. The ($\varepsilon_M - \varepsilon_0$, C'_A) thus found are in Table 6. There are also $C_A = C'_A + [H^+]$, $[H^+]$ being known from the potentiometric measurements. Hence $(\varepsilon_M - \varepsilon_0)/C_A$ is calculated. The function $(\varepsilon_M - \varepsilon_0)/C_A = f(\varepsilon_M - \varepsilon_0)$ (23) is then plotted in Fig. 6. The curves are cut at eight constant $\varepsilon_M - \varepsilon_0$. The $(\varepsilon_M - \varepsilon_0)/C_A$ found give the corresponding C_A:s, while the C_M:s are obtained from Fig. 5. The (C_A, C_M)-values at constant $\varepsilon_M - \varepsilon_0$ are found to be along straight lines. Their

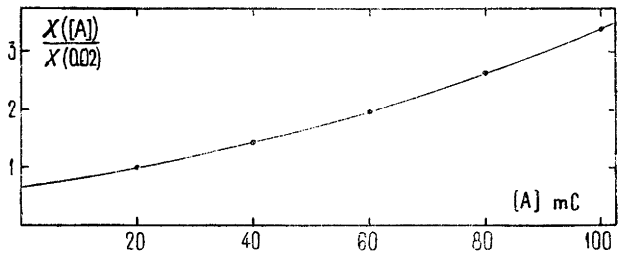

Fig. 7. Determination of $\lim_{[A]\to 0} \frac{X([A])}{X(0.02)} = \frac{1}{X(0.02)}$ *(Table 8).*

extrapolation to $C_M = 0$ gives [A], and their slope gives $\bar{n}$, hence $\bar{n}$/[A] is known. The values found are collected in Table 7. The complex formation function is plotted on Fig. 1 and $\bar{n}$/[A] as a function of [A] on Fig. 2 (filled points).

For the calculation of the complexity constants, the X([A]) function is computed by graphical integration of the $\bar{n}$/[A] function of Fig. 2. As this function in the present case is not determined at sufficiently low [A], it is not possible to extrapolate to [A] = 0, as was done in the potentiometric measurements. Another lower limit must be used at the integration and we chose $[A]_0 = 20$ mC. The integration gives $X([A])/X(0.02)$. This function, Fig. 7, is easily extrapolated to [A] = 0, and gives $1/X(0.02) = 0.65 \pm 0.05$. Hence the function $X([A])$ is known, and from this $X_1([A])$, $X_2([A])$ and $X_3([A])$ are formed and the constants are calculated as before. The values obtained are found in Table 8.

As it can be seen from Table 8, the accidental errors of the constants are fairly large here as two extrapolations must be performed, both of them over a rather great [A]-range. In return the systematic errors seem to be rather small, as was proved above.

For some round [A], $\bar{n}$ is calculated with the constants obtained, and given in Table 8 and on Fig. 1 (dashed curve). The calculated values fit the corresponding experimental ones very well.

COMPARISON BETWEEN THE RESULTS OF THE POTENTIOMETRIC AND THE EXTINCTIOMETRIC MEASUREMENTS

According to both the methods used, three mononuclear complexes exist in solutions of uranyl chloroacetate, *viz.* MA_1 MA_2 and MA_3. It has not been possible to observe any signs of polynuclear complex formation according to any method.

The following complexity constants have been calculated:

potentiometric: $\beta_1 = 27.5 \pm 0.5\ C^{-1}$; $\beta_2 = 195 \pm 20\ C^{-2}$; $\beta_3 = 625 \pm 150\ C^{-3}$

extinctiometric: $\beta_1 = 24 \pm 3\ C^{-1}$; $\beta_2 = 150 \pm 40\ C^{-2}$; $\beta_3 = 350 \pm 150\ C^{-3}$

Within the limits of the accidental errors indicated, the agreement may be considered as almost complete. The potentiometric values are throughout somewhat higher which may be due to the effect of their systematic errors as was discussed above.

The final result is that potentiometric and extinctiometric methods, properly used in determinations of complexity, give entirely the same results.

The constants thus obtained may therefore be considered as true constants of equilibrium.

Similar comparisons on systems involving several complexes are rather uncommon. The most important are those found in the work by Fronaeus [5] and Bjerrum [4]. — Fronaeus investigated some cupric salts. He found good agreement between potentiometric and extinctiometric results as long as the complex formation was mononuclear. Bjerrum found the same complex formation curve for the cupric ammine system according to both methods.

SUMMARY

The complexity of uranyl monochloroacetate has been investigated according to two methods: potentiometrically (by $p[H^+]$-measurements with the quinhydrone electrode) and extinctiometrically (at the Hg-line of 3 130 Å).

Both methods indicate that the complex formation is entirely mononuclear; there is no sign of polynuclear complexes.

The complexity constants of the three mononuclear complexes existing in the range of concentration used are calculated according to both methods. The results agree quantitatively.

The conclusion is that potentiometric and extinctiometric methods of measurements of complexity give consistent results if properly used. The constants obtained may therefore with great certainty be considered as the true constants according to the law of mass action.

My thanks are due to *Försvarets Forskningsanstalt (FOA)*, Stockholm, which has financially supported the work.

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Received May 9, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 809—834

Die Kristallstruktur von $Mg_2Cu_6Al_5$

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In dem ternären System Mg-Cu-Al war seit einiger Zeit eine Phase bekannt, der man die ungefähre Zusammensetzung $Mg_3Cu_7Al_{10}$ zuschrieb (Laves und Werner, 1936). In der zitierten Arbeit wird an Hand von Pulveraufnahmen festgestellt, dass die Phase wahrscheinlich analog aufgebaut ist wie die zinkreiche Magnesiumverbindung von der ungefähren Zusammensetzung Mg_2Zn_{11}. Verdoppelt man nämlich die erste Formel und verdreifacht man die letzte, ergeben sich die Formeln $Mg_6Cu_{14}Al_{20}$ und Mg_6Zn_{33}. Das Verhältnis der Magnesiumatome zu den Nichtmagnesiumatomen ist also in beiden Fällen von derselben Grössenordnung. Beide Phasen sind kubisch. Bei der ternären Phase wurde die Kantenlänge $a = 8{,}29$ kX, bei der binären $a = 8{,}54$ kX festgestellt. Die Interferenzmuster der Pulverphotogramme beider Phasen sind einander sehr ähnlich (Laves und Werner, 1936).

In der zuletzt zitierten Arbeit von Laves ist ein Ausschnitt aus dem Konzentrationsdreieck des Mg-Cu-Al-Systemes gegeben, der die ternäre Phase umfasst. Genaue Angaben über die Zusammensetzung der letzten konnten jedoch nicht gemacht werden. Ferner führten Laves und Werner u. a. auch sehr gründliche analytische und röntgenographische Untersuchungen an zahlreichen Mg-Zn-Legierungen in dem Gebiete zwischen 82,4 Atom% und 86,7 Atom% Zink aus. Pulverphotogramme, die von getemperten Legierungen der Zusammensetzung $MgZn_{5,5}$ oder sehr nahe derselben aufgenommen wurden, zeigten keine Fremdlinien. Da das Gewichtsverhältnis zwischen den Bestandteilen (Mg : Zn = 1 : 15) sehr ungünstig ist, konnte bezüglich der Zusammensetzung der reinen Phase kein absolut sicheres Ergebnis erzielt werden. Wenn auch die Formel Mg_6Zn_{33} (drei Formeleinheiten Mg_2Zn_{11} in der Elementarzelle) am wahrscheinlichsten wirkte, konnten die Verfasser die Möglichkeiten Mg_6Zn_{32} und Mg_6Zn_{34} nicht ausschliessen.

Ein weiterer Versuch, eventuelle Analogien zwischen der binären und ternären Phase zu entdecken, wurde in einer Arbeit von Schütz (1937) behan-

delt. Mit Hilfe von thermischen Untersuchungen und Schliffen wurde die Formel $Mg_2Cu_{5,5}Al_{5,5}$ als wahrscheinlich befunden. Eine eindeutige Formel konnte sich aber aus den Analysen nicht ergeben. So wurde einerseits die »Bedingung der gleichen Raumerfüllung isomorpher Verbindungen» (Beziehung zwischen Zellvolumen und mittlerem Verbindungsradius), andererseits die Valenzelektronenregel (Regel von Hume-Rothery) * herangezogen, um zu prüfen, in welchem zahlenmässigen Verhältnis die Kupfer- und Aluminiumatome in der ternären Phase zueinander stehen müssen, wenn man annimmt, dass die binäre und ternäre Phase dem gleichen Typus Mg_2X_{11} (X = Cu, Al; Zn) angehören. Auf Grund der ersten Regel liess sich keine Entscheidung treffen, während die Regel von Hume-Rothery unter Annahme der Formeln $Mg_2Cu_{5,5}Al_{5,5}$ und Mg_2Zn_{11} erfüllt wurde.

Da gemäss Laves und Werner drei Formeleinheiten Mg_2Zn_{11} in der Elementarzelle enthalten sein sollen, müsste im Falle einer Isomorphie eine Formeleinheit $Mg_6Cu_{16,5}Al_{16,5}$ in der Zelle vorliegen, wenn die Regel von Hume-Rothery erfüllt werden soll.

Die entgültige Beantwortung der Frage, welche exakten Zusammensetzungen den genannten Verbindungen zuzuschreiben sind und welche Strukturanalogien zwischen ihnen bestehen, ist also von grösserem Interesse und soll im folgenden durch die Ausführung von Strukturanalysen erfolgen.

In der vorliegenden Arbeit wird zunächst die Kristallstruktur der ternären Phase abgeleitet. Die Strukturbestimmung der binären Phase ist bereits in Angriff genommen und wird demnächst abgeschlossen und veröffentlicht werden.

DIE BESTIMMUNG DES ELEMENTARKÖRPERS

Um die Strukturanalyse so weit wie möglich zu erleichtern, schien es am Platze zu sein, noch einmal zu versuchen, die wirkliche Zusammensetzung der ternären Phase so genau wie möglich zu ermitteln. Dabei sollte aber ein anderer Weg als bisher beschritten werden. Da die Verbindung durch eine langsame peritektische Reaktion entsteht und erfahrungsgemäss solche Reaktionen oft am besten ausserhalb des Homogenitätsgebietes der reinen Phase verlaufen und da ferner die reine Phase dort am besten Kristalle bildet, wurde nicht angestrebt, wie bisher eine homogene Schmelze mit der exakten Zusammensetzung der reinen Phase herzustellen, sondern in verschiedenen Schmelzen eine grosse Anzahl von Einkristallen oder sehr wohl ausgebildeten Kristalliten zu züchten, von denen man annehmen könnte, dass sie die reinen Phasen

* Die Regel von Hume-Rothery verlangt für Verbindungen gleichen Strukturtyps die gleiche Anzahl Valenzelektronen pro Atom.

darstellten. Sämtliche Bestimmungen sollten also an Einkristallen oder reinen Kristalliten vorgenommen werden.

Es wurden zwei Schmelzen hergestellt und zwar mit den ungefähren Zusammensetzungen $Mg_6Cu_{13}Al_{20}$ und $Mg_6Cu_{18}Al_{18}$. Als Ausgangsmaterial dienten die reinsten Metalle (für analytische Zwecke) von Kahlbaum. Die Komponenten wurden in kleinen Graphittiegeln eingewogen, die ihrerseits in Quarzrohren unter Vakuum eingeschmolzen wurden. Die Legierungen wurden auf etwa 1200° C eine halbe Stunde lang erhitzt, mehrmals geschüttelt und schliesslich abgeschreckt. Danach wurden beide Proben etwa drei Wochen lang bei 600° C getempert. Die erhaltenen Reguli wurden vorsichtig zerstossen. Dabei sprangen sehr wohl ausgebildete Kristallite ab. Unter dem Mikroskop wurde eine grosse Anzahl von Kristalliten herausgesucht, von denen man annehmen konnte, dass sie entweder Einkristalle waren oder aus mehreren solchen bestanden. Die am besten ausgebildeten Einkristalle dienten zur Herstellung von Dreh- und Weissenbergaufnahmen. Ein Teil der Kristallite wurde fein zerrieben und davon wurden mit CuK-Strahlung Pulverphotogramme hergestellt. Sämtliche Photogramme zeigten einfach kubische Symmetrie und keine systematischen Auslöschungen. Die an den Pulverphotogrammen berechnete Länge der Zellkante $a = 8{,}294 \pm 0{,}003$ kX $= 8{,}311 \pm 0{,}003$ Å stimmte mit der von Laves gefundenen überein (siehe in der Einleitung). Die Pulverphotogramme waren frei von Fremdlinien, was durch eine einwandfreie Indizierung festgestellt wurde (siehe Tabelle 2).

Nachdem nun Kriteria dafür vorlagen, dass die ausgewählten Kristallite die reine Phase repräsentierten, wurde an ihnen mit grösstmöglicher Genauigkeit die Dichte durch Auftriebsmessungen in Benzol zu $\delta = 4{,}94 \pm 0{,}04$ bestimmt. (Die Kristallite wurden zwecks Austreibung von Luft in dem Messgefäss unter Benzol längere Zeit unter Vakuum gehalten.) Die angegebene Fehlergrenze bedeutet in diesem Falle die festgestellte Reproduzierbarkeit bei drei Bestimmungen.

Legt man die Kantenlänge $a = 8{,}29$ kX zugrunde, berechnet sich die theoretische Dichte unter Annahme der von Laves (Laves und Werner, 1936) angegebenen Formel $Mg_3Cu_7Al_{10}$ (zwei Formeleinheiten pro Elementarzelle) zu $\delta = 4{,}56$, was darauf hindeutet, dass die Verbindung mehr Kupfer enthalten muss als die Formel angibt. Die von Schütz gefundene Formel $Mg_2Cu_{5,5}Al_{5,5}$ ergibt eine Dichte von $\delta = 4{,}74$ (drei Formeleinheiten pro Elementarzelle). Setzt man in diese Formel ganze Zahlen ein, so dass sich $Mg_2Cu_6Al_5$ ergibt, wird die theoretische Dichte $\delta = 4{,}90$. Die Übereinstimmung wird dann also überraschend gut.

An reinen Kristalliten wurden nun zwei Analysen ausgeführt, in denen nur Kupfer und Aluminium bestimmt wurden. Kupfer wurde auf übliche Weise

elektrolytisch bestimmt, während Aluminium mit Ammoniak gefällt und als Al_2O_3 gewogen wurde (Kolthoff und Sandell, 1943). Magnesium wurde als Rest berechnet. Die gefundenen Verhältniszahlen Mg : Cu : Al waren 1) 2 : 6,0 : 5,0, 2) 2 : 6,1 : 5.0. Die oben genannte Formel $Mg_2Cu_6Al_5$ wirkt also sehr wahrscheinlich.

Um einen sicheren Weg zu gehen, soll bei der folgenden Strukturbestimmung diese Formel dennoch als nur beinahe richtig angesehen werden. Da drei Formeleinheiten $Mg_2Cu_6Al_5$ in der Elementarzelle vorkommen, soll zunächst die Formel $Mg_6Cu_{18\pm3}Al_{15\pm3}$ angenommen werden.

RAUMGRUPPE

Der zur Herstellung der Dreh- und Weissenbergaufnahmen angewandte Einkristall war sehr gut ausgebildet. Er bestand aus einer ziemlich dicken quaderförmigen Platte und gab keine sichtbaren Absorptionseffekte auf den Photogrammen der *hk*0- und *hk*1-Reflexe. Es wurde CuK-Strahlung verwendet.

An den Weissenbergphotogrammen sowie an den Pulverphotogrammen liessen sich keine systematischen Auslöschungen erkennen (siehe die Tabellen 2 und 3). Dies ist charakteristisch für die Raumgruppen mit den niedersten Symmetrien der verschiedenen Kristallklassen des kubischen Systems (T^1-$P23$, T_h^1-$Pm3$, T_d^1-$P\bar{4}3m$, O^1-$P43$, O_h^1-$Pm3m$). Laves will die Lauesymmetrie O_h festgestellt haben (Laves und Werner, 1936), die aus Schwenkaufnahmen (Schwenkbereich jeweils 15°) um die Richtungen [100], [110] und [111] hervorgegangen sein soll, indem Aufnahmen um [100], geschwenkt von $x°$ bis $(x + 15)°$ und von $(x + 90)°$ bis $(x + 105)°$ die gleichen Interferenzmuster zeigten. Dasselbe galt von Aufnahmen um [111], geschwenkt von $x°$ bis $(x + 15)°$ und von $(x + 120)°$ bis $(x + 135)°$ gemäss der oben zitierten Arbeit.

Leider liegt keine Indizierung der Schwenkaufnahmen von Laves vor. Es muss mit der Möglichkeit gerechnet werden, dass bei den Photogrammen von Laves in jeweils zwei Schwenkbereichen Reflexe aufgenommen worden sind, die zufälliger Weise ziemlich ähnliche Interferenzmuster lieferten.

Bei den von mir aufgenommenen Weissenbergphotogrammen *hk*0 und *hk*1 zeigte sich bei einer Anzahl von nahe beieinander liegenden Reflexen, dass die Intensitäten von *hk*0 und *kh*0 verschieden waren. Der Unterschied war teilweise sehr gross (siehe Tabelle 3). Analoges konnte bei entsprechenden Weissenberg-Aufnahmen von Mg_2Zn_{33} (siehe Samson, 1949) festgestellt werden. Der Unterschied zwischen den genannten Reflexen konnte nicht durch Absorption im Kristall erklärt werden. Ferner zeigten sowohl bei der ternären als auch bei der binären Phase Laueaufnahmen parallel [100] und [010]

deutlich die Abwesenheit tetragonaler Achsen. Im übrigen lieferten sie für die kubische Tetartoedrie und Paramorphie charakteristische Interferenzmuster.

Somit schien es hinreichend begründet zu sein, die Raumgruppen O^1-$P43$, O_h^1-$Pm3m$ und T_d^1-$P\bar{4}3m$ als unwahrscheinlich anzusehen. Es lag also am nächsten, die Raumgruppen T_h^1-$Pm3$ und T^1-$P23$ einer Untersuchung zu unterwerfen.

DIE VORLÄUFIGE BESTIMMUNG DER ATOMLAGEN MIT HILFE EINER PATTERSON-PROJEKTION

Die zu untersuchende Verbindung stellt einen neuen Strukturtyp dar. Ähnliche Verbindungen, aus denen man in struktureller Hinsicht irgendwelche Schlüsse für die zu untersuchende Phase ziehen könnte, wurden in der Literatur nicht gefunden.

Bei der Durchführung der Strukturanalyse schien es am praktischten zu sein, mit der Raumgruppe höherer Symmetrie (T_h^1-$Pm3$) zu beginnen. Die Punktlagen in dieser Raumgruppe sind:

1:	(*a*)	000	(*b*)	$\frac{1}{2}\frac{1}{2}\frac{1}{2}$					
3:	(*c*)	$0\frac{1}{2}\frac{1}{2}$,	$\frac{1}{2}0\frac{1}{2}$,	$\frac{1}{2}\frac{1}{2}0$	(*d*)	$\frac{1}{2}00$	$0\frac{1}{2}0$,	$00\frac{1}{2}$	
6:	(*e*)	$x00$,	$0x0$,	$00x$,	$\bar{x}00$,	$0\bar{x}0$,	$00\bar{x}$		
	(*f*)	$x0\frac{1}{2}$,	$\frac{1}{2}x0$,	$0\frac{1}{2}x$,	$\bar{x}0\frac{1}{2}$,	$\frac{1}{2}\bar{x}0$,	$0\frac{1}{2}\bar{x}$		
	(*g*)	$x\frac{1}{2}0$,	$0x\frac{1}{2}$,	$\frac{1}{2}0x$,	$\bar{x}\frac{1}{2}0$,	$0\bar{x}\frac{1}{2}$,	$\frac{1}{2}0\bar{x}$		
	(*h*)	$x\frac{1}{2}\frac{1}{2}$,	$\frac{1}{2}x\frac{1}{2}$,	$\frac{1}{2}\frac{1}{2}x$,	$\bar{x}\frac{1}{2}\frac{1}{2}$,	$\frac{1}{2}\bar{x}\frac{1}{2}$,	$\frac{1}{2}\frac{1}{2}\bar{x}$		
8:	(*i*)	xxx	$\bar{x}\bar{x}x$,	$\bar{x}x\bar{x}$,	$x\bar{x}\bar{x}$,	$\bar{x}\bar{x}\bar{x}$,	$\bar{x}x\bar{x}$,	$x\bar{x}x$,	$\bar{x}xx$,
12: *	(*j*)	$0yz$,	$z0y$,	$yz0$,	$0\bar{y}z$,	$z0\bar{y}$,	$\bar{y}z0$,		
		$0\bar{y}z$,	$\bar{z}0y$,	$y\bar{z}0$,	$0y\bar{z}$,	$z0y$,	$y\bar{z}0$		
	(*k*)	$\frac{1}{2}yz$,	$z\frac{1}{2}y$,	$yz\frac{1}{2}$,	$\frac{1}{2}\bar{y}z$,	$z\frac{1}{2}\bar{y}$,	$\bar{y}z\frac{1}{2}$		
		$\frac{1}{2}\bar{y}z$,	$\bar{z}\frac{1}{2}y$,	$y\bar{z}\frac{1}{2}$,	$\frac{1}{2}y\bar{z}$,	$z\frac{1}{2}y$,	$y\bar{z}\frac{1}{2}$		

Auf diese Punktlagen sind sechs Magnesiumatome, fünfzehn bis einundzwanzig Kupferatome und zwölf bis achtzehn Aluminiumatome so zu verteilen, dass sich die Formel $Mg_6X_{33\pm3}$ (X = Cu oder Al) ergibt. Die Anzahl der

* Der Verfasser hat auf analoge Weise wie im folgenden auch bewiesen, dass die Cu- und Al-Atome nicht auf eine 24-zählige Punktlage verteilt sind. Wegen Platzmangel wird diese sehr umfangreiche Beweisführung hier nicht veröffentlicht.

möglichen Kombinationen ist also recht beträchtlich. Es soll versucht werden, diese mit Hilfe einer Patterson-Projektion so weit wie möglich zu begrenzen.

Die Fig. 1 zeigt eine zweidimensionale Patterson-Projektion auf die xy-Ebene. Sie wurde mit Beevers-Lipson Streifen ausgeführt. Die hierbei angewandten S^2-Werte wurden an den Intensitäten des Weissenbergphotogrammes der $hk0$-Reflexe mit grösster Sorgfalt abgeschätzt, wobei die Abhängigkeit der Intensitäten vom Streuwinkel berücksichtigt wurde.

Die Aluminiumatome müssen in mindestens zwei verschiedenen Punktlagen vorkommen, sofern sie nicht eine 12-zählige Punktlage besetzen, da aus räumlichen Gründen jede 6-zählige Punktlage nur einmal auftreten kann. Die Kupferatome müssen auf jeden Fall in mindestens zwei verschiedenen Punktlagen vorkommen. Somit müssen die Aluminiumatome mindestens eine, die Kupferatome mindestens zwei der im folgenden aufgeführten Punktlagen oder Punktlagenkombinationen besetzen, wenn die vorerst angenommene Raumgruppe T_h^1-$Pm3$ die richtige ist:

1. $12(j)$ od. $12(k)$ od. $8(i)$
2. $8(i) + 6(e, f, g, h)$ od. $8(i) + 3(c) + 3(d)$
3. $8(i) + 3(c, d)$
4. $2 \times 8(i)$
5. $6(e, f, g, h) + 6(e, f, g, h)$
6. $6(e, f, g, h) + 3(c) + 3(d)$
7. $6(e, f, g, h) + 3(c, d)$

Jetzt soll untersucht werden, ob es in der Patterson-Projektion ein starkes Maximum gibt, dass nur von einigen wenigen oder einer einzigen dieser Möglichkeiten erklärt werden kann. Da die Abstandsvektoren auf den Geraden $x = 0$, $x = \frac{1}{2}$, $y = 0$ und $y = \frac{1}{2}$ sehr mannigfaltig sind und ferner die Maxima der Patterson-Projektion auf diesen Geraden durchweg sehr breit sind, scheint es praktisch zu sein, diese vorerst auszuschalten und zunächst nur das starke Maximum A in $X \sim 0{,}16$, $Y \sim 0{,}27$ der Projektion (siehe Fig. 1) zu betrachten. Alle projizierten Vektoren, die auf Diagonalen liegen, werden somit auch ausgeschaltet. Die Abstandsvektoren von 3. in der obigen Aufstellung sind in 2., und diejenigen von 7. sind in 6. mit enthalten. Einzählige Punktlagen, kombiniert mit 3-, 6- und 8-zähligen Punktlagen ergeben nur Vektoren auf den oben genannten Geraden. Die Fälle 2, 4, 5 und 6 umfassen also sämtliche Kombinationsmöglichkeiten der minder als zwölfzähligen Punktlagen, die für die Deutung des Maximum A in Frage kommen. Sollten die Vektoren dieser nicht das Maximum A erklären können, muss also eine 12-zählige Punktlage oder eine Kombination einer solchen mit den anderen dies tun. Die Komponenten der Abstandsvektoren für die obige Aufstellung sind in der Tabelle 1 übersichtlich geordnet.

Die auf den genannten Geraden liegenden Vektoren sind ausgelassen. Einer eventuellen Vertauschung der X- und Y-Komponenten der Fig. 1 kann durch Vertauschen der Komponenten in der Tabelle 1 Rechnung getragen werden.

In dieser Arbeit sollen Vektoren mit grossen und Parameter mit kleinen Buchstaben bezeichnet werden.

Tabelle 1 (ν = Frequenzzahl).

1 a. $12(j)$:

		ν
Z_j	Y_j	8
Y_j	Y_j-Z_j	4 *
Y_j	Y_j+Z_j	4 *
Y_j-Z_j	Z_j	4 *
Y_j+Z_j	Z_j	4 *
$2Y_j$	$2Z_j$	1

1 b. $12(k)$:

$\frac{1}{2}-Z_k$	$\frac{1}{2}-Y_k$	8
$\frac{1}{2}-Y_k$	Y_k-Z_k	4 **
$\frac{1}{2}-Y_k$	Y_k+Z_k	4 **
Y_k-Z_k	$\frac{1}{2}-Z_k$	4 **
Y_k+Z_k	$\frac{1}{2}-Z_k$	4 **
$2Y_k$	$2Z_k$	1

2 a. $8(i)$ + $6(e)$:

X_e-X_i	X_i	4 *
X_e+X_i	X_i	4 *
X_i	X_e-X_i	4 *
X_i	X_e+X_i	4 *

2 b. $8(i)$ + $6(f)$:

$\frac{1}{2}-X_f$	X_f	2 ***
X_f-X_i	X_i	4 *
X_f+X_i	X_i	4 *
$\frac{1}{2}-X_i$	X_f-X_i	4 **
$\frac{1}{2}-X_i$	X_f+X_i	4 **
X_i	$\frac{1}{2}-X_i$	8 ***

2 c. $8(i)$ + $6(g)$:

X_g	$\frac{1}{2}-X_g$	2 ***
X_g-X_i	$\frac{1}{2}-X_i$	4 **
X_g+X_i	$\frac{1}{2}-X_i$	4 **
X_i	X_i-X_g	4 *
X_i	X_i+X_g	4 *
$\frac{1}{2}-X_i$	X_i	8 ***

2 d. $8(i)$ + $6(h)$:

X_h-X_i	$\frac{1}{2}-X_i$	4 **
X_h+X_i	$\frac{1}{2}-X_i$	4 **
$\frac{1}{2}-X_i$	X_h-X_i	4 **
$\frac{1}{2}-X_i$	X_h+X_i	4 **

2 e. $8(i)$ + $3(c)$ + $3(d)$:

		ν
X_i	$\frac{1}{2}-X_i$	8 ***
$\frac{1}{2}-X_i$	X_i	8 ***

4. $8(i)$ + $8(i)$:

$X_{i_1}-X_{i_2}$	$X_{i_1}+X_{i_2}$	8 ****
$X_{i_1}+X_{i_2}$	$X_{i_1}-X_{i_2}$	8 ****

5 a. $6(e)$ + $6(f)$:

X_f	X_e	2
$\frac{1}{2}-X_f$	X_f	2 ***
$\frac{1}{2}-X_e$	X_f	2

5 b. $6(e)$ + $6(g)$:

X_g	$\frac{1}{2}-X_g$	2 ***
X_e	X_g	2
X_g	$\frac{1}{2}-X_e$	2

5 c. $6(e)$ + $6(h)$:

$\frac{1}{2}-X_e$	X_h	2
X_h	$\frac{1}{2}-X_e$	2

5 d. $6(f)$ + $6(g)$:

$\frac{1}{2}-X_f$	X_f	2 ***
X_g	$\frac{1}{2}-X_g$	2 ***
X_f	X_g	2

5 e. $6(f)$ + $6(h)$:

$\frac{1}{2}-X_f$	X_f	2 ***
$\frac{1}{2}-X_f$	X_h	2
$\frac{1}{2}-X_h$	$\frac{1}{2}-X_f$	2

5 f. $6(g)$ + $6(h)$:

X_g	$\frac{1}{2}-X_g$	2 ***
$\frac{1}{2}-X_g$	$\frac{1}{2}-X_h$	2
X_h	$\frac{1}{2}-X_g$	2

6 a. $6(e)$ + $3(c)$ + $3(d)$:

—	—	—

6 b. $6(f)$ + $3(c)$ + $3(d)$:

$\frac{1}{2}-X_f$	X_f	2 ***

6 c. $6(g)$ + $3(c)$ + $3(d)$:

X_g	$\frac{1}{2}-X_g$	2 ***

6 d. $6(h)$ + $3(c)$ + $3(d)$:

—	—	—

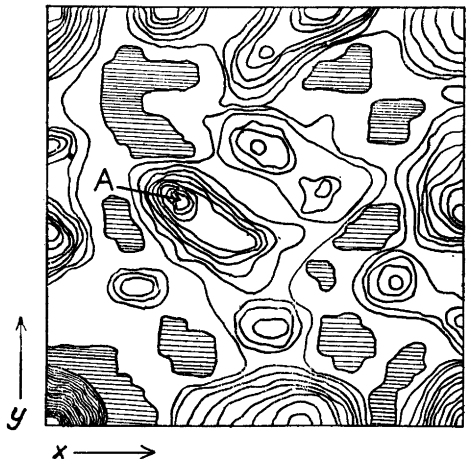


Fig. 1. Patterson-Projektion P(xyp) von $Mg_2Cu_6Al_5$ über das Gebiet $0 \leqq x \leqq \frac{1}{2}$; $0 \leqq y \leqq \frac{1}{2}$. Die schraffiert gezeichneten Gebiete bedeuten Minima.

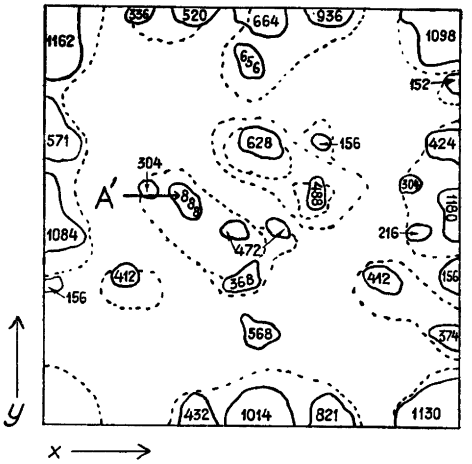


Fig. 2. Die auf die xy-Ebene projizierten Abstandsvektoren der gefundenen Struktur von $Mg_2Cu_6Al_5$. Die innerhalb der ausgezogenen Linien stehenden Zahlen geben die aus dem Streuvermögen der Atome und den Frequenzen der Vektoren berechneten Höhen der Maxima an. Die gestrichelten Linien stellen die ungefähren Umrisse der Maxima der Fig. 1 dar.

Bei den folgenden Betrachtungen wird, um einen sicheren Weg zu gehen, der kleinstmögliche Abstand Cu—Cu = 2,30 Å für die Begrenzung der Parameter gemäss den Raum- und Symmetriebedingungen angenommen. Somit ergibt sich:

$$0{,}14 \leqq (y_{12j},\ z_{12j}) \leqq 0{,}36,\quad 0{,}14 \leqq (y_{12k},\ z_{12k}) \leqq 0{,}36,\quad 0{,}14 \leqq (x_{6g},\ x_{6f},\ x_{8i}) \leqq 0{,}36$$
$$0{,}19 \leqq x_{6e} \leqq 0{,}36,\quad 0{,}14 \leqq x_{6h} \leqq 0{,}31.$$

Für die Vektorkomponenten $(X_f \pm X_i)$, X_i (siehe 2b in Tabelle 1) können sich folgende Parameterwerte ergeben:

a)	$X_f - X_i \sim 0{,}16,\ X_i \sim 0{,}27$:	$x_f \sim 0{,}43,\ x_i \sim 0{,}27$
a_1)	$X_i - X_f \sim 0{,}16$, —»—	$x_f \sim 0{,}11,\ x_i \sim 0{,}27$
b)	$X_i \sim 0{,}16,\ X_f - X_i \sim 0{,}27$:	$x_f \sim 0{,}43,\ x_i \sim 0{,}16$
b_1)	—»— $X_i - X_f \sim 0{,}27$:	$x_f \sim 0{,}11,\ x_i \sim 0{,}16$
c)	$X_f + X_i \sim 0{,}16,\ X_i \sim 0{,}27$:	$x_f \sim 0{,}11,\ x_i \sim 0{,}27$
d)	$1-(X_f + X_i) \sim 0{,}16,\ X_i \sim 0{,}27$:	$x_f \sim 0{,}57,\ x_i \sim 0{,}27$
e)	$X_i \sim 0{,}16,\ X_f + X_i \sim 0{,}27$:	$x_f \sim 0{,}11,\ x_i \sim 0{,}16$
f)	$X_i \sim 0{,}16,\ 1-(X_f + X_i) \sim 0{,}27$:	$x_f \sim 0{,}57,\ x_i \sim 0{,}16$

Die Fälle a), b), d) und f) können ausgeschaltet werden, da bei ihnen die Parameterwerte sehr weit ausserhalb der oben angegebenen Grenzen liegen. Nimmt man einen Fehler in der Patterson-Projektion an, könnte in den übrigen Fällen der Parameter x_f

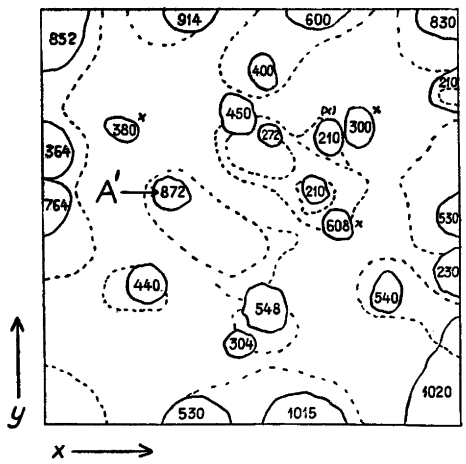


Fig. 3. Die projizierten Abstandsvektoren für den Fall, dass beide 12-zähligen Punktlagen besetzt sind. Die mit einem Kreuz versehenen Maxima kommen in der Fig. 1 und 2 nicht vor.

vielleicht den Grenzwert noch erreichen. Der Wert von x_i dürfte dann aber nicht oder nur unwesentlich grösser werden. Setzt man die gemäss dieser Überlegung eventuell möglichen Parameterwerte bei den anderen Fällen ein, erhält man Vektoren, die an denjenigen Stellen Maxima geben, wo in der Patterson-Projektion keine solchen vorliegen. Ferner würden bei einer solchen Parameterwahl die Komponenten X_i, $\frac{1}{2}-X_i$ bezw. $\frac{1}{2}-X_i$, X_i in 2b der Tabelle 1 ein doppelt so starkes Maximum ($\nu = 8$) dort ergeben, wo in der Patterson-Projektion ausgesprochene Minima liegen. Alle Vektoren von der hier behandelten Art sind in der Tabelle 1 mit einem Stern (*) versehen. Sie können überall auf analoge Weise ausgeschaltet werden.

Die Vektoren von der Art $(\frac{1}{2}-X_i)$, $(X_f \pm X_i)$ (siehe 2b) sind in der Tabelle 1 mit zwei Sternen (**) versehen. Für diese ergeben sich auf analoge Weise wie oben die Parameter: a) $x_i \sim 0{,}34$, $x_f \sim 0{,}61$ b) $x_i \sim 0{,}34$, $x_f \sim 0{,}07$ c) $x_i \sim 0{,}23$, $x_f \sim 0{,}39$ d) $x_i \sim 0{,}23$, $x_f \sim 0{,}07$ e) $x_i \sim 0{,}34$, $x_f \sim 0{,}39$.

Sollte analog dem voraufgegangenem Falle der Parameter von x_f in c) und e) den möglichen Grenzwert von $x_f = 0{,}36$ noch erreichen, dürfte x_i nicht wesentlich grösser werden. Legt man die dann eventuell möglichen Parameterwerte bei der Berechnung der übrigen Vektoren zugrunde, treten ebenfalls starke »falsche Maxima» auf. Die mit zwei Sternen versehenen Vektoren können also ebenfalls nicht mit dem Maximum A identisch sein.

Die Abstandsvektoren für die Möglichkeit 4 in der Tabelle 1, in der die achtzählige Punktlage zweimal vorkommt, ergeben Parameterwerte, die sehr weit ausserhalb der möglichen Grenzen liegen, wie die folgende Aufstellung zeigt: a) $x_{i_1} \sim 0{,}22$, $x_{i_2} \sim 0{,}06$ b) $x_{i_1} \sim 0{,}44$, $x_{i_2} \sim 0{,}28$ c) $x_{i_1} \sim 0{,}56$, $x_{i_2} \sim 0{,}28$ d) $x_{i_1} \sim 0{,}78$, $x_{i_2} \sim 0{,}06$.

Es soll noch einmal darauf hingewiesen werden, dass bei den drei bisher untersuchten Fällen die zugrundegelegten Grenzwerte der Parameter die äussersten sind, die sich ergeben, wenn man mit den kleinsten in der Struktur vorkommenden Atomen (Cu) und ebenfalls mit einer Kontraktion rechnet. Die genannten »falschen Maxima» werden wegen des starken Streuvermögens der Kupferatome auch noch besonders stark.

Alle in der Tabelle 1 mit drei Sternen versehenen Vektoren sind von der Art $(\frac{1}{2}-X_f)$, X_f (siehe 2b in der Tabelle). Sie können mit dem Maximum A nicht identisch sein, da der geometrische Ort dieser von dem genannten Maximum zu weit entfernt liegt.

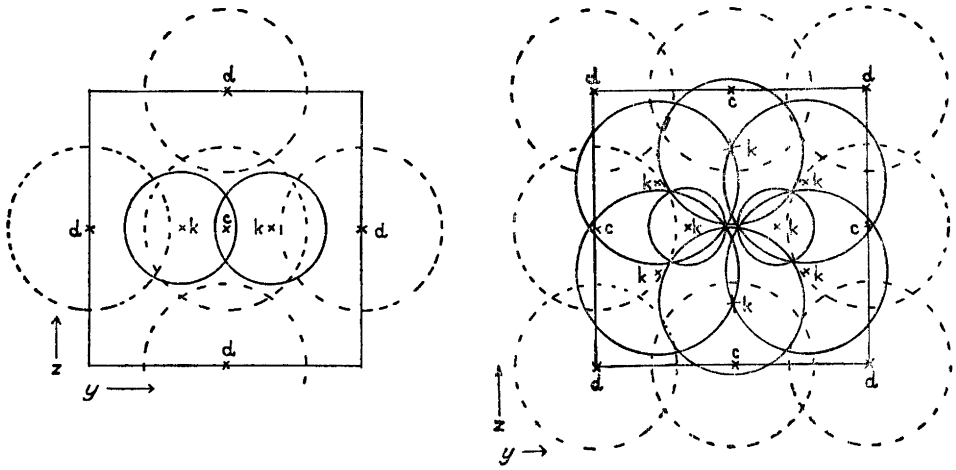


Fig. 4. Schnitte durch die Elementarzelle in den Höhen a) $a \cdot x = 0$ Å und b) $a \cdot x = 4{,}15$ Å. Die Kreise stellen Schnitte durch die um die Atome gedachten Kugelschalen dar. Die eingeschriebenen Buchstaben geben die Art der Punktlagen an. Für die dreizähligen Punktlagen sind die Kreise gestrichelt gezeichnet.

Werden die Parameter der 6-zähligen Punktlagen so gewählt, dass die bisher noch nicht behandelten Abstandsvektoren der Möglichkeiten 5a bis 5f der Tabelle 1 mit dem Vektor des Maximum A der Patterson-Projektion identisch werden, ergeben sich gleichzeitig Vektoren, die in der genannten Projektion nicht vorkommen. In sämtlichen Fällen haben die letzten eine doppelt so hohe Frequenz wie die ersten. Ferner könnten die zuerst genannten Vektoren wegen ihrer kleinen Frequenzzahl ($\nu = 2$) nur dann die erforderliche Stärke annähernd erreichen, wenn mehr als zwei 6-zählige Punktlagen kombiniert werden und wenn man annimmt, dass dann in diesen die stark streuenden Kupferatome liegen. Die genannten falschen Maxima werden aber dann besonders stark. Die Kombinationen 5a bis 5f können also abgesehen von den sich ergebenden falschen Maxima schon wegen der genannten kleinen Frequenzzahl nicht allein dass Maximum A in der Projektion erklären.

Da nun alle möglichen Vektoren der minder als 12-zähligen Punktlagen, sofern sie für das Maximum A in Frage kommen, behandelt worden sind, wirkt es wahrscheinlich, dass eine 12-zählige Punktlage in der Struktur vorliegt.

In 1a und 1b der Tabelle 1 können die Vektoren von der Art 2Y, 2Z wegen der kleinen Frequenz ($\nu = 1$) nicht das Maximum A erklären. Bei der Punktlage 12(k) würde das Maximum A durch die Komponenten $(\frac{1}{2} - Z) \sim 0{,}16$ und $(\frac{1}{2} - Y) \sim 0{,}27$ sehr gut erklärt werden, zumal der sich ergebende Vektor eine hohe Frequenzzahl ($\nu = 8$) hat. Daraus ergeben sich die ungefähren Parameterwerte $z_{12k} \sim 0{,}34$, $y_{12k} \sim 0{,}23$. Alle übrigen Vektoren dieser Punktlage passen sehr gut in die Patterson-Projektion hinein. Die Punktlage 12(j) bildet die gleichen Vektoren und Frequenzzahlen wie 12(k), wenn $z_{12j} \sim 0{,}16$ und $y_{12j} \sim 0{,}27$ gewählt werden. In beiden 12-zähligen Punktlagen können keine anderen als die genannten ungefähren Parameterwerte gewählt werden, ohne dass sich »falsche Maxima» ergeben.

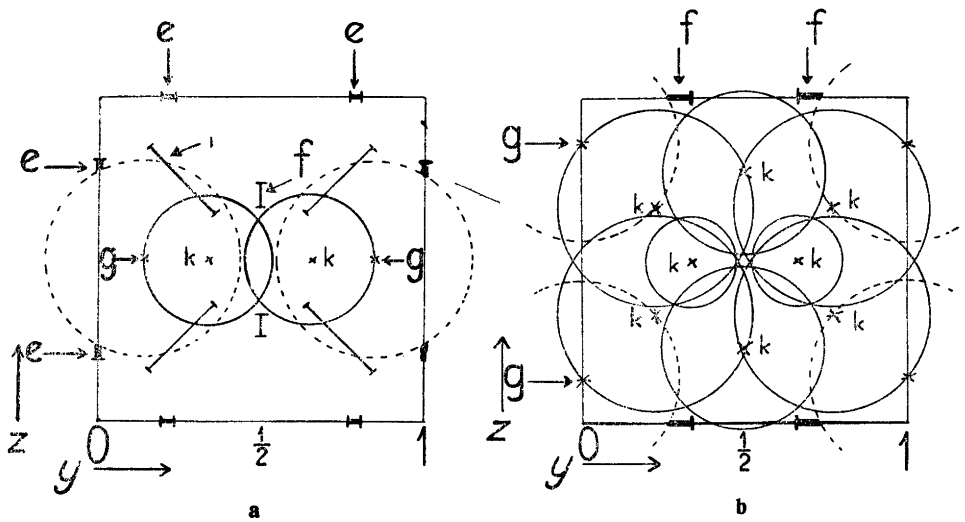


Fig. 5. Schnitte durch die Elementarzelle in den Höhen a) $a \cdot x = 0$ Å und b) $a \cdot x = 4{,}15$ Å. Gestrichelte Kreise = Schnittkreise der Kugelschalen der Punktlage 6 (g).

Zusammenfassend soll das bisherige, für die Struktur grundlegende Ergebnis wie folgt ausgedrückt werden: *Das Maximum A in $X \sim 0{,}16$, $Y \sim 0{,}27$ der Patterson-Projektion der Fig. 1 kann nur dann erklärt werden, wenn man annimmt, dass in der Struktur mindestens eine 12-zählige Punktlage vorliegt. Die ungefähren Parameterwerte müssen sein:*

$$y_{12j} \sim 0{,}27,\ z_{12j} \sim 0{,}16;\ y_{12k} \sim 0{,}23,\ z_{12k} \sim 0{,}34.$$

DIE VORLÄUFIGE BESTIMMUNG DER MÖGLICHEN ATOMLAGEN GEMÄSS DEN RAUM- UND SYMMETRIEBEDINGUNGEN

Da es nun gemäss dem vorigen Kapitel als sehr wahrscheinlich angesehen werden kann, dass mindestens eine 12-zählige Punktlage in der Struktur vorkommt, soll untersucht werden, wie sich die übrigen Atome unter Annahme der oben angegebenen ungefähren Parameterwerte der 12-zähligen Punktlagen gemäss den Raum- und Symmetriebedingungen in der kubischen Zelle von der Kantenlänge $a = 8{,}29$ kX einordnen lassen.

Für die folgenden Betrachtungen wurden die in anderen Strukturen gefundenen Abstände der hier vorkommenden Atomarten untersucht. Es ergab sich, dass die Abstände nicht kleiner oder nur unwesentlich kleiner angenommen werden konnten als: Al—Al = 2,60 Å, Al—Cu = 2,50 Å, Al—Mg = 2,70 Å, Mg—Cu = 2,60 Å, Mg—Mg = 2,80 Å, Cu—Cu = 2,30 Å, selbst wenn mit einer Kontraktion gerechnet wird.

Nun denken wir uns den Mittelpunkt eines jeden Cu-Atomes mit einer Kugelschale vom Radius $r = 2{,}50$ Å oder $r = 2{,}60$ Å umgeben. Es ist unwahrscheinlich, dass z. B. die Mittelpunkte von Al-Atomen innerhalb der Kugelschale von 2,50 Å oder die der Mg-Atome innerhalb der Schale von 2,60 Å liegen. Andererseits dürfen die Mittelpunkte nicht weiter als höchstens 0,3 Å ausserhalb der Schalen gelegen sein, wenn die Atome einander wirklich berühren sollen. Analoges gilt für die übrigen Atome.

Wir wollen zunächst sehen, wie sich das Strukturbild gestaltet, wenn die parameterfreien Punktlagen 3(*c*) und 3(*d*) mit 12(*k*) kombiniert werden. Hierzu genügt es, Schnitte durch die Elementarzelle in den Höhen $ax = 0$ Å und $ax = 4{,}15$ Å ($x = \frac{1}{2}$) zu betrachten. Die Fig. 4a und b geben diese wieder. Die eingeschriebenen Buchstaben bedeuten die Mittelpunkte der Schnittkreise der um die betreffenden Punktlagen gedachten Kugelschalen. Für die letzten wurde der Radius Cu—Al = 2,50 Å zugrunde gelegt.

Der Abstand $3(c)-12(k) \sim 2{,}3$ Å erscheint ziemlich kurz (der Mittelpunkt von 3(*c*) fällt in die Schnittkreise von 12(*k*) hinein). 3(*c*) soll dennoch nicht ausgeschaltet werden, da die Parameterwerte von 12(*k*) nur ungefähre sind. Die vorliegende Kombination schliesst aber die Punktlagen 6(*f*), 6(*g*) und 6(*h*) mit Sicherheit aus, da die Abstände $6(f)-3(c,\ d) \leqq 2{,}0$ Å, $6(g)-3(c,\ d) \leqq 2{,}0$ Å und $6(h)-12(k) \sim 1{,}4$ Å. Sieht man zunächst von der Punktlage 12(*j*) ab (die Kombination 12(*j*) + 12(*k*) soll später für sich behandelt werden), kann also weder 3(*c*) noch 3(*d*) mit 12(*k*) kombiniert werden, wenn für alle übrigen Atome noch Platz vorhanden sein soll.

In der Fig. 5 a und b sind wieder die Schnittkreise von 12(*k*) wiedergegeben. Die Mittelpunkte für die Atome in $(0x\frac{1}{2})$ und $(0\bar{x}\frac{1}{2})$ der Punktlage 6(*g*) können wegen der Spiegelebene (*x*0*z*) nur an den in der Fig. 5 a mit Pfeilen angedeuteten Punkten liegen. Der Parameter dieser Punktlage wird also sehr gut festgelegt ($x_{6g} \sim 0{,}14$). Die Kreise vom Radius $r = 2{,}5$ um die genannten Mittelpunkte von 6(*g*) und die beiden Spiegelebenen (*xy*0) und (*x*0*z*) begrenzen den Parameter der Punktlage 6(*e*): $0{,}19 \leqq x_{6e} \leqq 0{,}23$ (siehe die abgegrenzten Strecken auf den Kanten der Fig. 5 a). Der Parameter der Punktlage 6(*f*) wird einerseits vom Kreise mit $r = 2{,}5$ um den Mittelpunkt von 6(*g*) in Fig. 5 b, andererseits infolge der Spiegelebene $(x\frac{1}{2}z)$ begrenzt auf $0{,}26 \leqq x_{6f} \leqq 0{,}36$ (siehe die horizontalen Strecken auf der Kante der Fig. 5 b).

Die Mittelpunkte der Atome in 8(*i*) können sich wegen der Spiegelebenen (*x*0*z*)und (*xy*0) einerseits und $(x\frac{1}{2}z)$ und $(xy\frac{1}{2})$ andererseits nur auf den Teilen der Raumdiagonalen befinden, die in der Fig. 5 a eingezeichnet sind. Setzt man den Radius des Schnittkreises der Kugelschale um 8(*i*) auf den Ebenen $ax = 0$ Å und $ax = 4{,}15$ Å als Funktion von x_{8i} ab, kann man leicht feststellen, dass der Parameter $x_{8i} \sim 0{,}22$ sein muss, wenn diese Schnittkreise nicht die Punktlagen 6(*e*), 6(*g*) und 12(*k*) ausschliessen sollen. Der einzige noch nicht besetzte Punkt ist das Zentrum der Zelle $(\frac{1}{2}\frac{1}{2}\frac{1}{2})$, Punktlage 1(*b*).

Verfährt man von der Punktlage 12(*j*) ausgehend auf analoge Weise, ergibt sich ebenfalls nur eine Möglichkeit und zwar die Kombination 12(*j*) + 8(*i*) + 6(*g*) + 6(*f*) + 6(*h*) + 1(*a*) mit den ungefähren Parametern: $y_{12j} \sim 0{,}27$, $z_{12j} \sim 0{,}16$, $x_{6f} \sim 0{,}36$, $0{,}14 \leqq x_{6g} \leqq 0{,}24$, $0{,}27 \leqq x_{6h} \leqq 0{,}31$, $x_{8i} \sim 0{,}28$. Diese Kombination ist gleichwertig der oben gefundenen, da sie durch Parallelverschiebung um $\frac{1}{2}\frac{1}{2}\frac{1}{2}$ aus dieser hervorgeht.

In den Fig. 6 a und b sind die Schnittkreise von sowohl 12(*j*) als auch 12(*k*) eingezeichnet. 3(*c*) und 3(*d*) können wie im ersten Beispiel ausgeschaltet werden, wenn alle Atome Platz haben sollen. Die Punktlagen 6(*e*) und 6(*h*) können in der Kombination nicht vorkommen, da die Abstände $6(h)-12(k) \leqq 1{,}4$ Å und $6(e)-12(j) \leqq 1{,}4$ Å. 8(*i*) kann nicht vorkommen, da $12(j)-8(i) = 12(k)-8(i) \leqq 2{,}1$ Å. Das für die Punktlagen 6(*f*) und 6(*g*) noch verfügbare Gebiet ist durch Pfeile in der Fig. 6 ausgemerkt. Die

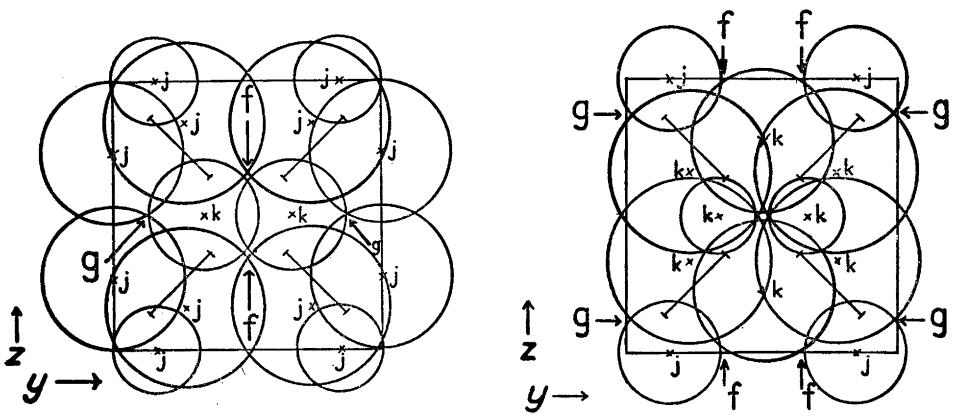


Fig. 6. Schnitte durch die Elementarzelle in den Höhen a) $a \cdot x = 0$ Å und b) $a \cdot x = 4{,}15$ Å. Der für die Mittelpunkte von 6 (f) und 6 (g) noch freie Platz wird durch Pfeile ausgemerkt.

ungefähren Parameterwerte sind: $x_{6g} \sim 0{,}14$, $x_{6f} \sim 0{,}36$ und für die 12-zähligen Punkt lagen wie oben angegeben. An den Ecken der Zelle ist für die Punktlage 1(*a*) und im Zentrum für 1(*b*) noch Platz vorhanden.

Zusammenfassend kann gesagt werden, dass es gemäss den Raum- und Symmetriebedingungen nur zwei Möglichkeiten gibt, die 12-zähligen Punktlagen unter Anwendung der mit Hilfe der Patterson-Projektion berechneten ungefähren Parameterwerte mit anderen Punktlagen zu kombinieren. Diese sind:

a) $12(k) + 8(i) + 6(e) + 6(f) + 6(g) + 1(b)$
$y_{12k} \sim 0{,}23$, $z_{12k} \sim 0{,}34$, $x_{8i} \sim 0{,}22$, $0{,}19 \leqq x_{6e} \leqq 23$, $0{,}26 \leqq x_{6f} \leqq 0{,}36$, $x_{6g} \sim 0{,}14$

b) $12(j) + 12(k) + 6(f) + 6(g) + 1(a) + 1(b)$
$y_{12j} \sim 0{,}27$, $z_{12j} \sim 0{,}16$, $y_{12k} \sim 0{,}23$, $z_{12k} \sim 0{,}34$, $x_{6f} \sim 0{,}36$, $x_{6g} \sim 0{,}14$.

Nun stellt sich die Frage, welche Atomarten die verschiedenen Punktlagen besetzen. Es wird zunächst die Möglichkeit a) betrachtet (siehe Fig. 5 a und 5 b). Wären in 6(*g*) und 6(*f*) gleichartige Atome, z. B. 6Cu + 6Cu oder 6Al + 6Al, müssen die sechs Magnesiumatome in 6(*e*) liegen. Die Parameter für 6(*g*) und 6(*f*) müssten dann annähernd dieselben Werte annehmen und bei der Punktlage 12(*k*) würde $y \cong z$ werden, d. h. die Struktur würde in eine höhere Symmetrie übergehen, was gemäss dem auf Seite 813 gesagten sowie auch auf Grund der Patterson-Projektion unwahrscheinlich ist. Da für die Punktlage

6(*g*) sehr wenig, für 6(*f*) dagegen sehr viel Platz vorhanden ist, wirkt es wahrscheinlich, dass in der letzten die grossen Mg-Atome liegen.

Nur so kann überhaupt der unterschiedliche Wert zwischen dem *y*- und *z*-Parameter in 12(*k*), d. h. die auf Seite 813 festgestellte niedere Symmetrie der Struktur der vorliegenden Verbindung räumlich begründet werden.

Rein Zahlenmässig ergeben sich somit noch folgende Möglichkeiten der Atomverteilung:

	12(*k*)	+ 8(*i*)	+ 6(*e*)	+ 6(*f*)	+ 6(*g*)	+ 1(*b*)	Formel
1.	12Al	8Cu	6Cu	6Mg	6Al	1Cu od. 1Al	$Mg_6Cu_{15(-1)}Al_{18(+1)}$
2.	12Al	8Cu	6Al	6Mg	6Cu	—»—	—»—
3.	12Al	8Cu	6Cu	6Mg	6Cu	—»—	$Mg_6Cu_{20(+1)}Al_{13(-1)}$
4.	12Cu	8Cu	6Al	6Mg	6Al	—»—	—»—
5.	12Cu	8Al	6Al	6Mg	6Cu	—»—	$Mg_6Cu_{18(+1)}Al_{15(-1)}$
6.	12Cu	8Al	6Cu	6Mg	6Al	—»—	—»—

Es wurden sämtliche, in der genannten Punktlagenkombination vorkommenden Abstandsvektoren berechnet. Die Höhen der Maxima, zu welchen die Vektoren Anlass geben, wurden aus dem Produkt von Atomnummern und Frequenzzahl berechnet. In der Fig. 2 bedeuten die umrandeten Zahlen diese Produkte oder die Summen aller derjenigen, die jeweils innerhalb der umrandeten Gebiete liegen. Die Fig. 2 gilt für den Fall, dass in 12(*k*) und 6(*e*) Kupferatome liegen (Fall 6 in der obigen Aufstellung). Für die Berechnung der Lagen der Maxima in Fig. 2 wurden die oben abgeleiteten ungefähren Parameterwerte angewendet. Man sieht, dass die Übereinstimmung dieser »konstruierten» Patterson-Projektion mit der aus den Intensitäten des Weissenbergphotogrammes berechneten überraschend gut ist (vergleiche die Fig. 1 mit der Fig. 2). Es werden in der Fig. 2 sogar sämtliche schwachen Maxima der Fig. 1 wiedergegeben. In der ersten wurden der Übersicht wegen für sich allein liegende Vektoren von sehr schwachen Maxima (kleiner als 100) ausgelassen, da sie ohnehin in der letzten nicht sichtbar sein können.

Die sich für die Fälle 1 bis 5 ergebenden Vektorenbilder sind wegen Raummangel hier nicht wiedergegeben. Die berechneten Höhen der Maxima A' für die Möglichkeiten 1 bis 3 verhalten sich zu denjenigen der Möglichkeiten 4 bis 6 wie 1 : 3. Bei den ersten drei Fällen sind ausserdem die Maxima in der Umgebung von A' wesentlich stärker als A' selbst (in der Fig. 2). Sie stehen so stark im Widerspruch zu der Pattersonanalyse, dass sie als unwahrscheinlich betrachtet werden können.

Bei den Möglichkeiten 4 bis 6 lässt sich auf Grund der Patterson-Projektion keine sichere Entscheidung treffen, wenn auch der Fall 6 günstiger wirkt als die anderen. Die Fälle 4 und 5 ergeben aber recht eigentümliche Struktur-

bilder, insofern als im Falle 4 nicht weniger als 20 Kupferatome, und im Falle 5 14 Aluminiumatome für sich alleinstehende Gruppen bilden. In dem Fall 6 wirkt dagegen die Verteilung der Atome normaler, wie unten aus S. 830 hervorgeht.

Die Fig. 3 zeigt die projizierten Vektoren der Kombination $12(j) + 12(k) + 6(f) + 6(g) + 1(a) + 1(b)$ (Möglichkeit b auf Seite 821). Es wurden 12 Al in $12(j)$, 6 Al in $6(g)$ und 12Cu in $12(k)$ angenommen. Die mit einem Stern versehenen »falschen Maxima» sind teils recht stark und liegen teils dort, wo gemäss der Patterson-Projektion Minima sein sollen (vergleiche die Fig. 3 mit der Fig. 1). Die genannten »falschen Maxima» ergeben sich lediglich aus denjenigen Vektoren, die bei dem Kombinieren der beiden 12-zähligen Punktlagen für sich allein entstehen. Sie bleiben also unverändert, wenn man die Atomverteilung ändert. In Anbetracht der im oben behandelten Fall erhaltenen sehr guten Übereinstimmung kann diese Kombination ausgeschlossen werden, zumal es unwahrscheinlich wirkt, dass zwölf der Aluminiumatome genau dieselbe Anordnung haben sollen wie die zwölf Kupferatome.

DIE GENAUE BESTIMMUNG DER ATOMLAGEN

Gemäss dem auf Seite 822 gesagten hebt sich der Fall 6 von den Fällen 4 und 5 ab. Die letzten beiden sollen aber vorläufig nur als minder wahrscheinlich angesehen werden, also nicht ausgeschlossen werden.

Für den Fall 6 wurden die Intensitäten der $hk0$-Reflexe berechnet, wobei die Parameter weit ausserhalb der auf Seite 821 angegebenen Werte variiert wurden. Es wurde immer mit der Möglichkeit gerechnet, dass entweder ein Aluminiumatom oder ein Kupferatom die Punktlage $1(b)$ besetzen kann und auch, dass $1(b)$ unbesetzt sein kann. Es wurde immer eine ungefähre Übereinstimmung zwischen den berechneten und beobachteten Intensitäten festgestellt, wenn die Parameter so gewählt wurden, dass für die Punktlage $1(b)$ genügend Platz vorhanden war. Für eine feinere Variierung der Parameter der Punktlagen $6(g)$ und $6(f)$ erwiesen sich die Intensitäten wegen des kleinen Streuvermögens der Aluminium- und Magnesiumatome sehr unempfindlich. Deshalb wurden zunächst beim Variieren der Parameter von $12(k)$, $8(i)$ und $6(e)$ in kleineren Interwallen die wahrscheinlichen Parameter der beiden oben genannten 6-zähligen Punktlagen konstant gehalten. Die Parameter der letzten konnten dann infolge der Beschränkung des in der Zelle noch freien Raumes gemäss den Symmetriebedingungen ziemlich gut bestimmt werden, wobei die anderen, genauer berechneten Parameter für $12(k)$, $8(i)$ und $6(e)$ zugrundegelegt wurden. Danach wurden die Intensitäten erneut berechnet. Diese Operation wurde mehrmals wiederholt.

Eine sehr gute Übereinstimmung zwischen den berechneten und beobachteten Intensitäten wurde erzielt, wenn in der Punktlage 1(*b*) ein Aluminiumatom angenommen wurde. Die Übereinstimmung wird wesentlich verschlechtert, wenn in dieser Punktlage ein Kupferatom angenommen wird oder wenn 1(*b*) unbesetzt gehalten wird. *Der Unterschied ist so stark merkbar, dass 1 Al in 1(b) als gesichert angesehen werden kann.*

Im folgenden wird das Resultat kurz zusammengefasst:

Raumgruppe: T_h^1—$Pm3$

12	Cu	in	12 : (*k*),	$y = 0{,}243 \pm 0{,}005$, $z = 0{,}336 \pm 0{,}005$
8	Al	in	8 : (*i*),	$x = 0{,}215 \pm 0{,}005$
6	Al	in	6 : (*g*),	$x = 0{,}16 \pm 0{,}01$
6	Cu	in	6 : (*e*),	$x = 0{,}225 \pm 0{,}005$
6	Mg	in	6 : (*f*),	$x = 0{,}32 \pm 0{,}01$
1	Al	in	1 : (*b*)	

Mit diesen Parameterwerten sind die Intensitäten, aufgeführt in den Tabellen 2 und 3, berechnet worden unter Verwendung der Formeln:

$$\text{Weissenbergphotogramm: } I \sim \frac{1 + \cos^2 2\Theta}{\sin 2\Theta} \cdot S^2$$

$$\text{Pulverphotogramm: } I \sim \frac{1 + \cos^2 2\Theta}{\sin^2\Theta \cdot \cos\Theta} \cdot p \cdot S^2 \cdot A$$

(p = Flächenhäufigkeitsfaktor, A = Absorptionsfaktor.)

Für die Fälle 4. und 5. auf Seite 822 wurden nun die Strukturfaktoren berechnet, wobei die auf Seite 821 angegebenen ungefähren Parameterwerte zugrundegelegt wurden. In beiden Fällen ergaben sich für alle Strukturfaktoren derjenigen *hk*0-Reflexe, die gemäss dem Weissenbergphotogramme stark oder sehr stark sein sollen, die gleichen Vorzeichen wie für den Fall 6. — Auf eine Variation der Parameter in kleineren Interwallen wurde verzichtet. — Nur bei einer geringen Anzahl Strukturfaktoren, deren *hk*0 schwachen und sehr schwachen Intensitäten (gemäss dem Photogramme) zukamen, waren die Vorzeichen zweifelhaft oder abweichend. Somit müsste eine Fourier-Projektion endgültig entscheiden können, ob die Möglichkeiten 4 und 5 ausgeschlossen werden können oder nicht.

Legt man die Vorzeichen der S-Werte für den Fall 6 zugrunde, die ja nur unwesentlich von den Fällen 4 und 5 abweichen, und schätzt man die S-

Tabelle 2. Pulverphotogramm von $Mg_2Cu_6Al_5$. CuK-Strahlung.

hkl	$10^4 \sin^2\Theta$ ber.	$10^4 \sin^2\Theta$ beob.	Intens. ber.	Intens. beob.
110	0172	0170	34	st—
111	0258	—	0,3	—
200	0344	—	1,6	—
210	0430	0426	16,9	m
211	0516	0513	8,2	s
220	0687	0679	4,9	ss
300, 221	0773	0769	10,0	s (+)
310	0859	0844	2,4	sss—
311	0945	—	0,4	—
222	1033	1030	5,1	ss
320	1119	1117	42	st
321	1205	1206	37	st (+)
400	1377	1376	40	st
410, 322	1463	1457	89	sst
411, 330	1549	1543	18,4	m
331	1633	—	0,7	—
420	1721	1716	17,4	m (—)
421	1807	1797	11,4	s +
332	1890	—	0,5	—
422	2062	—	0,0	—
500, 430	2151	2152	9,3	s
510, 431	2237	2235	6,8	s —
511, 333	2323	2328	2,3	sss —
520, 432	2495	2494	3,8	sss
521	2582	2580	1,3	sss —
440	2754	2753	5,3	s —
522, 441	2840	2856	3,8	ss
530, 433	2925	2932	2,5	sss
531	3011	—	0,9	—
600, 442	3098	3097	10,6	s
610	3184	—	0,9	—
532, 611	3270	3267	18,5	s +
620	3342	3433	7,0	ss
621, 540, 443	3528	3525	9,7	s
541	3614	3612	16,1	s
533	3700	3697	3,7	sss —
622	3786	3784	6,1	sss
630, 542	3872	3869	15,3	s
631	3958	3961	9,2	ss
444	4130	—	0,7	—
700, 632	4216	4212	26,5	m —
710, 550, 543	4303	4287	5,1	sss
711, 551	4389	4375	5,0	sss —
640	4475	4469	18,8	s

Tabelle 2 (Fortsetzung).

hkl	$10^4 \sin^2\Theta$ ber.	$10^4 \sin^2\Theta$ beob.	Intens. ber.	Intens. beob.
720, 641	4561	4553	5,9	sss
721, 633, 552	4647	4631	4,4	sss —
642	4819	4808	10,0	ss
722, 544	4905	4900	10,0	ss (+)
730	4991	—	0,0	—
731, 553	5077	(5062)	3,0	(sss —)
650, 643	5249	5245	11,7	ss (+)
732, 651	5335	5330	19,7	s (—)
800	5507	5497	5,1	sss —
810, 740, 652	5593	5590	4,8	sss —
811, 441, 554	5679	5678	17,1	s —
733	5765	(5751)	2,3	(sss —)
820, 644	5851	5850	8,4	ss —
821, 742	5937	5940	6,6	ss —
653	6024	6025	6,5	sss
822, 660	6196	—	2,3	—
830, 661	6282	(6286)	4,8	(sss —)
831, 750, 743	6368	—	0,3	—
751, 555	6454	(6442)	3,7	(sss —)
662	6540	6538	8,1	sss —
832, 654	6626	(6617)	4,3	(sss —)
752	6712	—	0,7	—
840	6884	(6862)	4,6	(sss —)
900, 841, 744, 663	6970	6952	6,4	sss
910, 833	7056	(7056)	3,4	(sss —)
911, 753	7142	—	4,3	—
842	7228	(7208)	3,0	(sss —)
920	7314	(7293)	3,6	(sss —)
921, 761, 655	7400	7401	14,5	s —
664	7572	(7567)	3,4	(sss —)
922, 850, 843, 762	7658	7655	35,1	m (—)
930, 851, 754	7745	7735	15,9	s —
931	7831	—	0,9	—
852	8003	(7981)	5,9	sss —
932, 762	8089	8086	23,5	m —
844	8261	—	3,0	—
940	8347	8343	26,0	s +
941, 853, 770	8433	8430	13,6	ss —
933, 771, 755	8519	(8496)	7,2	sss —
10 00, 860	8605	8599	23,0	s +
10 10, 942, 861, 764	8691	8687	25,7	s
10 11, 772	8777	8777	28,0	s
10 20, 862	8949	8948	24,6	s
10 21, 854	9035	9039	8,0	sss —

Tabelle 2 (Fortsetzung).

hkl	$10^4 \sin^2\Theta$ ber.	$10^4 \sin^2\Theta$ beob.	Intens. ber.	Intens. beob.
950, 945	9121	9114	7,5	sss —
951, 773	9207	9208	28,3	s (+)
10 22, 666	9293	9290	16,3	sss
10 30	9379	9380	38,0	m —
10 31, 952, 765	9466	9464	47,8	m —
10 32, 944, 870	9724	9725	140,0	st
871, 855, 774	9810	9814	33,5	m —

$$\text{Mittelwert } \frac{\sin^2\Theta}{\Sigma h^2} = 0{,}008605$$

$$a = 8{,}294 \pm 0{,}003 \text{ kX} = 8{,}311 \pm 0{,}003 \text{ Å}$$

Bei der Berechnung der Intensitäten sind die β-Reflexe in allen denjenigen Fällen mit einbezogen worden, wo sie mit den α-Reflexen zusammenfallen. Die in Klammern gesetzten Intensitäten (sss —) sind gerade noch erkennbar. Ihre $\sin^2\Theta$-Werte wurden bei der Berechnung der Gitterkonstante nicht mit einbezogen, da bei dem Vermessen dieser schwachen Reflexe eine Unsicherheit besteht.

Werte an den für Polarisations- und Lorenzfaktor korrigierten Intensitäten des Weissenbergphotogrammes der *hk*0 genauestens ab, ergibt die Fourier-Projektion auf die *xy*-Ebene das Bild der Fig. 7.

Gemäss dem auf Seite 18 zusammengefassten Resultat (siehe auch die Projektionen in den Fig. 8 und 9) sollen in der Fourier-Projektion folgende Maxima die Projektionen folgender Atome wiedergeben:

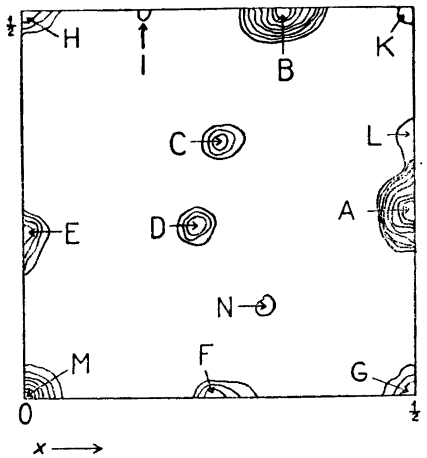


Fig. 7. Fourier-Projektion von $Mg_2Cu_6Al_5$ auf die xy-Ebene.

Tabelle 3. Berechnete und beobachtete Intensitäten der hk0 in einer Weissenberg-Aufnahme von $Mg_2Cu_6Al_5$. CuK-Strahlung.

h	*h*00	*h*10	*h*20	*h*30	*h*40	*h*50	*h*60	*h*70	*h*80	*h*90	*h*100
0	—	st—	—	—	sst+	s	st+	m+	st(—)	m	m(—)
		10	0,00	0,00	206	3,7	36	14	27	17	11
1	st—	st+	s	(sss—)	s+	m	s(—)	(sss—)	ss	sss	ss
	10	43	4,4	0,00	2,9	8,5	2,0	0,5	1,7	1,1	2,8
2	—	st	m+	s	m(+)	ss	m	s	s	s—	s
	0,00	28	12	5,2	13	2,4	14	4,0	4,0	0,6	4,4
3	—	s(—)	sst	m+	sss—	s(—)	st—	(sss—)	sss	m(—)	m+
	0,00	3,5	91	11	0,4	2,6	12,1	0,01	1,1	6,6	16
4	sst+	m+	st	st(—)	st(—)	st(—)	s(—)	sss—	s	sst	
	206	18	36	23	15	17	2,3	0,00	4,2	55	
5	s	s	—	sss—	—	m+	s(—)	sss—	s—	m	
	3,7	7,7	0,00	0,01	0,00	9,0	2,6	0,01	1,2	10	
6	st+	sss	m—	st—	st(+)	ss—	ss	s—	st+		
	36	0,6	5,3	17	48	0,2	0,3	2,0	48		
7	m+	sss—	s	(sss—)	m	—	ss—	m			
	14	0,5	2,9	0,01	6,8	0,01	0,00	5,2			
8	st(—)	sss—	m—	m—	m—	s	s—				
	27	0,5	5,8	6,4	4,7	2,1	1,3				
9	m	sss—	s	s—	m	m—					
	17	0,1	6,7	0,8	8,4	2,8					
10	m(—)	m	m(—)	sss							
	11	17	10	0,6							

Um einerseits die Unterschiede bei den starken und sehr starken Intensitäten erkennen zu können, andererseits aber auch die schwächsten Intensitäten zu erfassen, wurde von ein- und demselben Kristall ein schwach und ein sehr stark exponiertes Photogramm aufgenommen. Die Tabelle wurde somit mit Hilfe zweier Photogramme aufgestellt.

A = 2Cu(k), B = 2Cu(k), C = 1Cu(k), D = 2Al(i), E = 1Al(g) + 1Cu(e), F = 1Mg(f) + 1Cu(e), G = 2Al(g), H = 2Mg(f), I = 1Al(g), K = 1Al(b), L = 1Mg(f), M = 2Cu(e).

Die Magnesium- und Aluminiumatome haben ungefähr das gleiche Streuvermögen. Die Kupferatome streuen etwa doppelt so stark wie die ersten. Demnach sollen die Maxima ungefähr folgende Höhen haben:

$$A = B = M = 4;\ C = D = E = F = G = H = 2;\ I = K = L = 1.$$

Die Fourier-Projektion bestätigt also eindeutig das oben erhaltene Resultat (siehe Seite 824) und gibt auch die dort aufgeführten Parameterwerte mit grosser Genauigkeit wieder. Das kleine falsche Maximum N könnte nur durch Schätzungs- und Abbruchfehler entstanden sein. Die Möglichkeiten 4 und 5 können also als unwahrscheinlich, das erhaltene Resultat kann dagegen als gesichert angesehen werden.

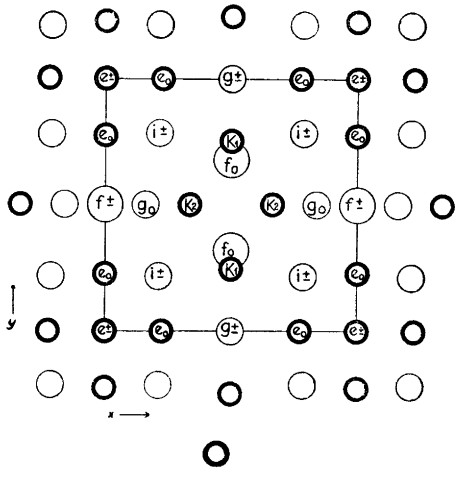
Was die auf Seite 813 genannte Raumgruppe T^1—$P23$ betrifft, soll noch folgendes gesagt werden. Die Raumgruppe T^1 unterscheidet sich von T_h^1 insofern, als bei ihr die 12-zählige Punktlage verallgemeinert ist. Ferner geht die 8-zählige Punktlage von T_h^1 durch Fortfall des Symmetriezentrums in eine 4-zählige der Raumgruppe T^1 über. Die 1-, 3- und 6-zähligen Punktlagen sind in beiden Raumgruppen gleich.

Die Raumgruppe niederer Symmetrie könnte die vorliegende Struktur nur dann beschreiben, wenn die 8-zählige Punktlage der Raumgruppe T_h^1—$Pm3$ in zwei 4-zählige Punktlagen der Raumgruppe T^1—$P23$ aufgeteilt wird und gleichzeitig eine Verallgemeinerung der Punktlage $12(k)$ erfolgt. Dies hätte aber nur dann einen Sinn, wenn man in den beiden 4-zähligen Punktlagen verschiedenartige Atome annähme, da sonst gleichartige Atome in verschiedenen Teilen der Zelle unbegründet erscheinender Weise verschiedene Abstände hätten. Die Struktur würde somit ihr Bauprinzip grundsätzlich ändern, welches in Anbetracht der guten Übereinstimmung zwischen den beobachteten und berechneten Intensitäten einerseits und der Patterson- und Fourier-Projektion andererseits unwahrscheinlich wirkt.

DISKUSSION DER STRUKTUR

Eine Projektion der oben abgeleiteten Struktur auf die xy-Ebene in der Höhe $x = 0$ ist in Fig. 8 wiedergegeben. Fig. 9 stellt eine solche in der Höhe $x = \frac{1}{2}$ dar. Alle Atome, die auf der Höhe $x = \frac{1}{2}$ liegen, sind der Übersicht wegen in Fig. 8 nicht mit angegeben. Entsprechend fehlen in der Fig. 9 die Atome mit der Höhe $x = 0$. Die in den Kreisen angegebenen Ziffern geben die Höhen der Atome, die Buchstaben die Art der Punktlagen an.

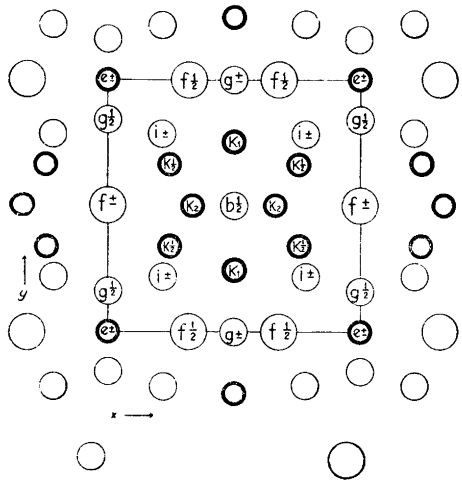
Die räumliche Darstellung des Gesamtbildes der Struktur schien am einfachsten durch die Beschreibung zweier Schichten zu erfolgen.



Cu

$e_0 : x = 0$
$e \pm : x = \pm\ 0.225$
$K_1 : x = \pm\ 0.336$
$K_2 : x = \pm\ 0.243$
$K\,\frac{1}{2} : x = \frac{1}{2}$

Fig. 8. Projektion der Struktur von $Mg_2Cu_6Al_5$ auf die xy-Ebene in der Höhe $x = 0$. Die Atome mit der Höhe $x = \frac{1}{2}$ sind hier nicht mit aufgeführt.



Al

$g_0 : x = 0$
$g\,\frac{1}{2} : x = \frac{1}{2}$
$g \pm : x = \pm\ 0.16$
$i \pm : x = \pm\ 0.215$
$b\,\frac{1}{2} : x = \pm\,\frac{1}{2}$

Mg

$f_0 : x = \pm\ 0$
$f\,\frac{1}{2} : x = \pm\frac{1}{2}$
$f \pm : x = \pm\ 0.32$

Fig. 9. Projektion der Struktur von $Mg_2Cu_6Al_5$ auf die xy-Ebene in der Höhe $x = \frac{1}{2}$. Die Atome mit der Höhe $x = 0$ sind hier nicht mit aufgeführt.

An jeder Ecke der Zelle befindet sich eine Gruppe von sechs Kupferatomen (*e* in Fig. 8). Die *e*-Atome bilden regelmässige Oktaeder und sind 2,64 Å voneinander entfernt. Über jeder Oktaederfläche liegt ein Aluminiumatom (*i*) in der Weise, dass es von jedem der drei Eckpunkte dieser Fläche den gleichen Abstand (2,54 Å) hat. Die Cu-Cu-Abstände innerhalb der Oktaeder sind also wesentlich grösser als die Cu-Al-Abstände. Die Aluminiumatome sind also in die Oktaederflächen hineingedrückt und drücken die Kupferatome auseinander. Dieser Befund wirkt insofern ziemlich sicher, als eine Anzahl berechneter Intensitäten für eine Variation der Parameter der Punktlagen 8(*i*) und 6(*e*) in kleinen Interwallen besonders empfindlich ist. Werden die Parameter nur wenig geändert, treten bei diesen Intensitäten Unstimmigkeiten auf, die durch Änderung der anderen Parameter nicht beseitigt werden können.

In der Fig. 10 ist eine Schicht durch die Elementarzelle parallel der xy-Ebene durch die Höhe $-0{,}320 \leqq z \leqq +0{,}320$ räumlich dargestellt. Die Oktaederflächen sind schraffiert gezeichnet.

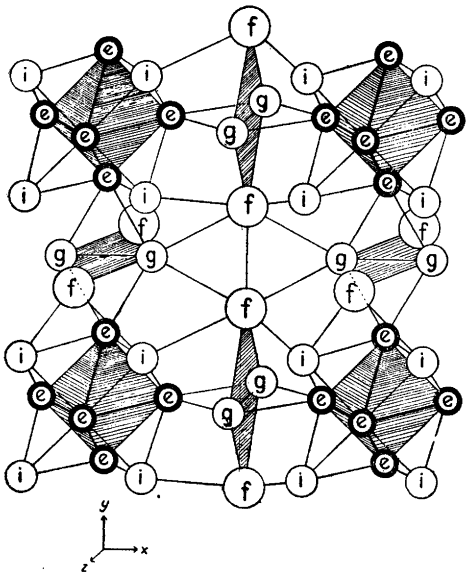


Fig. 10. Schicht durch die Elementarzelle parallel der xy-Ebene in der Höhe $-0{,}320 \leqq z \leqq +0{,}320$. *Die Anordnung der Atome ist räumlich dargestellt. Der Übersicht wegen sind die Kupferatome (k) hier fortgelassen.*

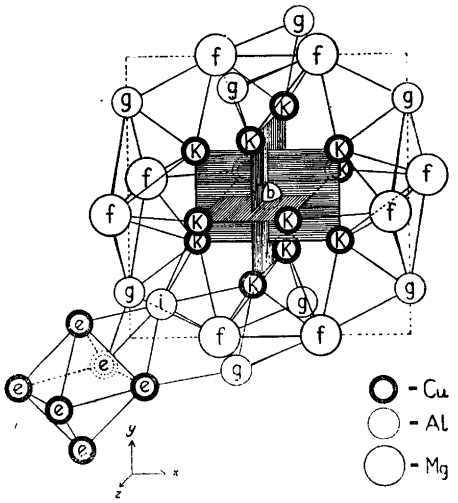


Fig. 11. Schicht durch die Elementarzelle parallel der xy-Ebene in der Höhe $\frac{1}{2} - 0{,}340 \leqq z \leqq \frac{1}{2} + 0{,}340$. *Die Anordnung der Atome ist räumlich dargestellt. Der Übersicht wegen ist nur ein Aluminiumatom (i) links unten eingezeichnet. Ein Oktaeder, gebildet von den Kupferatomen (e) ist ebenfalls links unten eingezeichnet. Im übrigen sind die Kupferatome (e) fortgelassen.*

Wie die Aluminiumatome (*g*) und die Magnesiumatome (*f*) die »Eckengruppen» in den Richtungen [100] und [010] verbinden, geht aus der Fig. 10 hervor. Die Verkettung in der [001]-Richtung erfolgt auf analoge Weise, indem die schraffiert gezeichneten Rhomboide in der Fig. 10 (der kubischen Paramorphie entsprechend) zweimal um 90° gedreht werden. Die Abstände sind: Al(*g*)—Cu(*e*) = 2,65 Å, Al(*g*)—Mg(*f*) = 2,97 Å, Al(*i*)—Mg(*f*) = 3,09 Å.

Ein räumliches Bild von der Anordnung der Kupferatome in 12(*k*) wird durch die Fig. 11 wiedergegeben, welche eine Schicht durch die Elementarzelle parallel der *xy*-Ebene durch die Höhe $\frac{1}{2} - 0{,}340 \leqq z \leqq \frac{1}{2} + 0{,}340$ räumlich darstellt (siehe auch die Projektion Fig. 9). Die zwölf Kupferatome liegen auf drei senkrecht zueinander stehenden Spiegelebenen in der Weise, dass sie die Eckpunkte dreier senkrecht aufeinanderstehender Rechtecke besetzen, welche in der Fig. 11 schraffiert gezeichnet sind. Der Schnittpunkt dieser

drei Ebenen bildet den Mittelpunkt des Aluminiumatoms in einzähliger Lage im Zentrum der Zelle. In der Fig. 11 ist dieser mit (*b*) bezeichnet. Alle Kupferatome (*k*) haben vom Zentrum (*b*) den Abstand 2,54 Å. Die Gesamtheit der Atome (*k*) und das Atom (*b*) soll »Zentrum-Gruppe» genannt werden. Die Zentrumgruppe steht teils in direkter teils in indirekter Verbindung mit den acht oben beschriebenen »Eckengruppen». Die direkte Verbindung erfolgt durch die Aluminiumatome (*i*). Jedes *i*-Atom ist von drei Kupferatomen (*k*) gleich weit entfernt (2,57 Å).

Die Kupferatome (*k*) werden analog dem oben behandelten Fall einerseits durch das Aluminiumatom (*b*), andererseits durch das Al-Atom (*i*) auseinandergedrückt. (Grösster Abstand Cu(*k*)—Cu(*k*) = 2,72 Å.) In der Fig. 11 ist der Übersicht wegen nur ein i-Atom (links unten) eingezeichnet.

Eine indirekte Verbindung zwischen den »Eckengruppen» und der »Zentrumgruppe» wird durch die Al-Atome (*g*) und Mg-Atome (*f*) hergestellt, die ihrerseits die »Eckengruppen» miteinander verketten (Abstände: Cu(*k*)—Al(*g*) = 2,49 Å, Cu(*k*)—2Mg(*f*) = 2,86 Å.)

Die Raumdiagonale der Zelle kann aufgefasst werden als eine Kette, die aus Aluminiumatomen besteht, die so angeordnet sind, dass zwischen jeweils zwei Al-Atomen ein Ring von drei Kupferatomen liegt. Ähnliche Ketten, in denen die Al-Atome ganz oder teilweise durch Mg ersetzt sind, verlaufen in verschiedenen anderen Richtungen.

Tabelle 4. Die Atomabstände in $Mg_2Cu_6Al_5$.

Art des Atoms	Anzahl Nachbarn	Art der Nachbarn	Abstände Å	Art des Atoms	Anzahl Nachbarn	Art der Nachbarn	Abstände Å
Cu (*e*)	4	Cu (*e*)	2,64	Al (*g*)	2	Cu (*e*)	2,65
	2	Al (*g*)	2,65		2	Cu (*k*)	2,49
	4	Al (*i*)	2,54		1	Al (*g*)	2,66
	2	Mg (*f*)	3,50		4	Al (*i*)	3,00
					2	Mg (*f*)	2,97
Cu (*k*)	1	Cu (*k*)	2,72		2	Mg (*f*)	3,19
	4	Cu (*k*)	2,66				
	1	Al (*b*)	2,54	Al (*i*)	3	Cu (*e*)	2,54
	1	Al (*g*)	2,49		3	Cu (*k*)	2,57
	2	Al (*i*)	2,57		3	Al (*g*)	3,00
	1	Mg (*f*)	2,86		3	Mg (*f*)	3,09
	2	Mg (*f*)	2,86				
				Mg (*f*)	2	Cu (*e*)	3,50
Al (*b*)	12	Cu (*k*)	2,54		2	Cu (*k*)	2,86
					4	Cu (*k*)	2,86
					2	Al (*g*)	3,19
					2	Al (*g*)	2,97
					4	Al (*i*)	3,09
					1	Mg (*f*)	2,99

Eine Übersicht über die Atomabstände und Koordination gibt die Tabelle 4.

Die gefundenen Al—Al-Abstände entsprechen annähernd denjenigen, die u. a. in Co_2Al_5 festgestellt worden sind (Bradley and Cheng, 1938). Die Cu—Al-Abstände liegen u. a. sehr nahe den Werten wie in Cu_9Al_4 (Bradley and Jones, 1933). Die Abstände Mg—Mg und Mg—Cu entsprechen sehr gut den von Laves (Laves, F., 1936) tabellierten Werten.

In Anbetracht der guten Übereinstimmung der berechneten und beobachteten Intensitäten, infolge der die Parameterwerte ziemlich gut bestimmt werden konnten, dürften eventuelle Fehler bei den Atomabständen ziemlich klein sein. Eine kleine Unsicherheit liegt bei den Aluminiumatomen (*g*) und den Magnesiumatomen (*f*) vor, da die Parameter dieser Atome wegen der kleinen Beiträge, die sie zu den Intensitäten liefern, auf Grund von Raumbedingungen festgelegt werden mussten.

EINE ÜBERSICHT ÜBER DIE IM Mg-Cu-Al-SYSTEM BISHER BESTIMMTEN TERNÄREN PHASEN

Die in der vorliegenden Arbeit behandelte Verbindung ist die dritte, bisher vollständig bestimmte Phase in dem Mg—Cu—Al-System. An weiteren Phasen sind vorher MgCuAl und $MgCuAl_2$ vollständig bestimmt worden. Die erste ist hexagonal (Laves und Löhberg, 1934), die letzte rhombisch (Perlitz und Westgren, 1943). Irgendwelche Strukturanalogien zwischen den zuletzt genannten und der von mir bestimmten Phase lassen sich nicht feststellen.

Es scheint noch eine weitere Phase vorzuliegen, welche die ungefähre Zusammensetzung Mg_4CuAl_6 haben soll. Sie ist kubisch ($a \sim 14{,}25$ k*X*) (Vogel, 1919; Laves, Löhberg und Witte, 1935).

Da man in der Literatur weitere ungefähre Formeln vorfindet, soll hier, um eine Übersicht zu geben, kurz gesagt werden, dass die Phasen »$Mg_2Cu_2Al_5$« und »$Mg_8Cu_7Al_{13}$« identisch sind mit $MgCuAl_2$ (Perlitz und Westgren, 1943).

ZUSAMMENFASSUNG

Durch vollständige Strukturanalyse wird festgestellt, dass die ternäre Verbindung, der man zuerst die ungefähre Formel $Mg_3Cu_7Al_{10}$ und später die ungefähre Formel $Mg_2Cu_{5,5}Al_{5,5}$ zuschrieb, die Zusammensetzung $Mg_2Cu_6Al_5$ hat. Die Verbindung stellt einen neuen Strukturtyp dar. Sie kristallisiert im kubischen System in der Raumgruppe T_h^1-$Pm3$. Die Elementarzelle mit der Kantenlänge $a = 8{,}294 \pm 0{,}003$ kX $= 8{,}311 \pm 0{,}003$ Å enthält drei Formeleinheiten $Mg_2Cu_6Al_5$.

Über Atomlagen und Atomabstände siehe Seite 824 und 832. Die Parameter sind auf Seite 824 zusammengestellt. Das Gesamtbild der Struktur wird an Hand von Schnitten und Projektionen auf Seite 829 erläutert.

Welche Strukturanalogien zwischen der hier behandelten Verbindung und der binären Phase Mg_2Zn_{11} bestehen, soll in einer weiteren, bald zu erscheinenden Arbeit behandelt werden[3].

Herrn Professor Arne Ölander möchte ich an dieser Stelle für das grosse Interesse, dass er dieser Arbeit gewidmet hat, herzlichst danken. Herrn Professor L. G. Sillén und Herrn Fil. lic. Anders Byström sei für ihre wertvollen Ratschläge ebenfalls herzlichsten Dank ausgesprochen. Zum Schluss möchte ich nicht versäumen, *Statens Naturvetenskapliga Forskningsråd* für die finanzielle Unterstützung dieser Arbeit zu danken.

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Eingegangen am 10. Mai 1949.

CTA CHEMICA SCANDINAVICA 3 (1949) 835–843

Die Kristallstruktur von Mg_2Zn_{11}

Isomorphie zwischen Mg_2Zn_{11} und $Mg_2Cu_6Al_5$

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In einer kürzlich von mir in dieser Zeitschrift veröffentlichten Arbeit [1] wurde die Kristallstruktur der ternären Phase $Mg_2Cu_6Al_5$ vollständig bestimmt. Ferner wurde dort auf die Wahrscheinlichkeit hingewiesen, dass diese Phase isomorph ist mit der Verbindung der noch nicht sicher bestimmten Zusammensetzung Mg_2Zn_{11}.

Die vorliegende Arbeit, in der die binäre Phase vollständig bestimmt werden soll, schliesst sich direkt an die zitierte [1] an. Es wird die Kenntnis der letztgenannten Arbeit angenommen und deshalb vieles darin erwähnte hier nicht mehr wiederholt.

BESTIMMUNG DES ELEMENTARKÖRPERS

Laves und Werner [2] führten analytische und röntgenographische Untersuchungen an zahlreichen Mg-Zn-Legierungen in dem Gebiete zwischen 82,4 Atom% und 86,7 Atom% Zink aus und fanden, dass dort eine Phase existiert, dessen Pulverphotogramm dem von $Mg_2Cu_6Al_5$ (die Verfasser nahmen in ihrer Arbeit die ungefähre Formel $Mg_3Cu_7Al_{10}$ an) sehr ähnlich war (kubische Zelle $a = 8{,}54$ kX).

Sichere Angaben über die genaue Zusammensetzung dieser Phase konnten wegen des sehr ungünstigen Gewichtsverhältnisses zwischen den Bestandteilen (Mg : Zn $\sim$ 1 : 15) nicht gemacht werden. Die Formel Mg_6Zn_{33} (drei Formeleinheiten Mg_2Zn_{11} in der Elementarzelle) wirkte am wahrscheinlichsten. Jedoch konnten die Möglichkeiten Mg_6Zn_{32} und Mg_6Zn_{34} nicht ausgeschlossen werden.

Es soll daher versucht werden, auf analoge Weise wie beim $Mg_2Cu_6Al_5$ die Zusammensetzung der binären Phase durch Analysen und Dichtebestimmun-

gen an wohl ausgebildeten Kristalliten zu ermitteln, von denen man annehmen könnte, dass sie die reine Phase darstellten.

Zunächst wurde eine Schmelze von der ungefähren Zusammensetzung Mg_2Zn_{11} hergestellt. Als Ausgangsmaterial dienten die reinsten Metalle (für analytische Zwecke) von Kahlbaum. Die Komponenten wurden in kleinen Graphittiegeln eingewogen, die ihrerseits in Quarzrohren unter Vakuum eingeschmolzen wurden. Die Legierung wurde auf etwa 700 ° C erhitzt, mehrmals geschüttelt und schliesslich abgeschreckt. Danach wurde sie etwa 60 Stunden lang bei 350° C getempert.

Das Pulverphotogramm, welches von Feilspänen dieses Regulus aufgenommen wurde, zeigte Interferenzlinien von sowohl $MgZn_2$, Zn als auch von der neuen zu bestimmenden Phase, was sich mit früheren Beobachtungen deckt, dass die letzte durch eine ziemlich langsame peritektische Reaktion zwischen $MgZn_2$ und Zn entsteht.

Der Regulus wurde daher zusätzlich noch etwa drei Wochen lang bei 350° C getempert und zeigte danach ein grobkristallines Gefüge. Beim vorsichtigen Zerstossen sprangen sehr viele, wohl ausgebildete Kristallite ab. Unter dem Mikroskop wurde eine grosse Anzahl hiervon herausgesucht. Die am besten ausgebildeten Kristallite dienten zur Herstellung von Dreh- und Weissenberg-Aufnahmen und erwiesen sich dabei als Einkristalle. Zur Herstellung des Pulverphotogrammes wurde ein Teil der Kristallite zerrieben und bei etwa 300° C rekristallisiert. Das jetzt erhaltene Pulverphotogramm (mit CuK-Strahlung) liess sich einwandfrei indizieren und wies keine Fremdlinien auf (siehe Tabelle 1). Die an ihm bestimmte Länge der Zellkante $a = 8{,}535 \pm 0{,}005$ kX $= 8{,}552 \pm 0{,}005$ Å deckt sich mit der von Laves bestimmten [2] ($a = 8{,}54$ kX).

Die bisher angewandten Kristallite stellten also die reine Phase dar. Es wurde angenommen, dass die übrigen dies auch taten.

Da das ungünstige Mengenverhältnis zwischen Magnesium und Zink eine ausserordentlich grosse Genauigkeit beim Analysieren erfordert, wurden zunächst verschiedene Analysenmethoden an Mischungen gestellter Mg- und Zn-Lösungen geprüft. Die beste Reproduzierbarkeit zeigte sich, wenn die Komponenten mit Schwefelwasserstoff in 0,01 *N* schwefelsaurer Lösung getrennt wurden [3] und Zink mit Kaliumferrocyanid massanalytisch bestimmt wurde [4]. Magnesium wurde im Filtrat der Schwefelwasserstoffällung (nach Austreiben von H_2S) mit $(NH_4)_2HPO_4$ gefällt und als Pyrophosphat gewogen [4].

Beim Analysieren der oben genannten Kristallite von Mg_2Zn_{11} (etwa 100 mg) wurden diese Methoden angewandt. Sie ergaben 6,39 % Mg und 93,35 % Zn. Dies entspricht der Formel $Mg_6Zn_{32,6}$.

Es ist also wahrscheinlich, dass die exakte Zusammensetzung der Formel Mg_6Zn_{33} entspricht.

Die Dichte wurde an den oben genannten Kristalliten vor dem Analysieren durch Auftriebsmessungen in Benzol mit grösster Genauigkeit bestimmt. Aus sechs Bestimmungen ergab sich der Mittelwert von $\delta = 6{,}16 \pm 0{,}02$ $g \cdot cm^{-3}$, wobei der angegebene Fehler die maximale Abweichung von dem Mittelwert bedeutet. Laves erhielt den Wert $\delta = 6{,}06\ g \cdot cm^{-3}$, nimmt aber einen Fehler von 1 bis 3 % an, da in seinem Fall die Dichte an einem ganzen Regulus bestimmt wurde und mit eingeschlossenen Luftblasen gerechnet werden musste.

Legt man die Kantenlänge $a = 8{,}552 \pm 0{,}005$ Å zugrunde, berechnet sich die theoretische Dichte unter Annahme der Formel Mg_6Zn_{33} (eine Formeleinheit in der Elementarzelle) zu $\delta = 6{,}11$, während die Formel Mg_6Zn_{32} $\delta = 5{,}94$ und Mg_6Zn_{34} $\delta = 6{,}29$ ergibt. Die erstgenannte Formel hebt sich also von den anderen ab.

RAUMGRUPPE

Zur Herstellung von Dreh- und Weissenbergaufnahmen wurden mehrere Einkristalle geprüft. Es liessen sich aber keine völlig fehlerfreien ausfindig machen. Das für die folgenden Untersuchungen angewandte Weissenbergphotogramm zeigte an einer in der Tabelle 2 ausgemerkten Stelle Absorptionseffekte. Die Absorptionseffekte sind aber von der Art, dass sie eine Bestimmung der Parameter mit üblicher Genauigkeit nicht oder nur unwesentlich beeinträchtigen dürften.

Analog wie beim $Mg_2Cu_6Al_5$ zeigten die Photogramme keine systematischen Auslöschungen. Ferner waren die Intensitäten der $hk0$ und $kh0$ bezw. der $hk1$ und $kh1$ verschieden. Die Photogramme von Mg_2Zn_{11} sind also im allgemeinen gesehen analog denen von $Mg_2Cu_6Al_5$. Die zu vermutende Raumgruppe ist also T_h^1—$Pm3$ oder T^1—$P23$.

DIE GENAUE BESTIMMUNG DER ATOMLAGEN

Wenn auch die Weissenbergphotogramme von $Mg_2Cu_6Al_5$ und Mg_2Zn_{11} einander ähnlich wirken, zeigt sich beim Photogramm von Mg_2Zn_{11}, dass die Unterschiede zwischen den Intensitäten der $hk0$ und $kh0$ bezw. der $hk1$ und $kh1$ teilweise stärker sind als bei dem Photogramm von $Mg_2Cu_6Al_5$ (vergleiche die Tabelle 2 mit der Tabelle 3 der zitierten Arbeit[1]). Die Erscheinung könnte entweder auf einen grösseren Unterschied zwischen dem y- und z-Parameter der Punktlage $12(k)$ in Mg_2Zn_{11} und einer damit verbundenen Verschiebung der anderen Parameter beruhen oder darauf, dass die fünfzehn

Tabelle 1. Pulverphotogramm von Mg_2Zn_{11} · CuK-Strahlung.

hkl	$10^4\sin^2\Theta$ ber.	$10^4\sin^2\Theta$ beob.	Intens. beob.	*hkl*	$10^4\sin^2\Theta$ ber.	$10^4\sin^2\Theta$ beob.	Intens. beob.
110	0163	0162	m+	640	4225	4231	s+ +β)
111	0244	0242	s+	720, 641	4306	4308	s +β)
200	0325	0327	s +β)	721, 633, 552	4387	4381	sss
210	0406	0403	m	642	4550	4558	ss−
211	0488	0485	s +β)	722, 544	4631	4631	s−
220	0569	—	—	730	4713	—	—
300, 221	0731	0730	s+ +β)	731, 553	4793	4798	sss +β)
310	0813	0813	s	650, 643	4956	4957	sss
311	0894	0890	s	732, 651	5037	5045	s+
222	0975	0972	st− +β)	800	5200	5193	sss
320	1056	1055	st− +β)	810, 740, 652	5281	5282	sss
321	1138	1137	st+ +β)	811, 441, 554	5362	5369	s+ +β)
400	1300	1297	st	733	5443	—	—
410, 322	1381	1380	sst	820, 644	5525	5522	sss
411, 330	1463	1464	m	821, 742	5606	5606	ss
331	1544	—	—	653	5687	(5699)	(sss−)
420	1625	1624	m+	822, 660	5850	5854	sss +β)
421	1706	1707	m	830, 661	5931	—	—
332	1788	—	—	831, 756, 743	6013	—	—
422	1950	(1958)	(sss−)	751, 555	6094	6097	sss−
500, 430	2031	2029	s+	662	6175	(6184)	(sss−)
510, 431	2113	2118	ss(+)	832, 654	6256	6255	ss− +β)
511, 333	2194	—	—	752	6338	—	—
520, 432	2356	2359	ss	840	6500	—	—
521	2437	2440	ss− +β)	900, 841, 744, 663	6581	6586	sss
440	2600	2599	s+	910, 833	6663	—	—
522, 441	2681	2677	ss	911, 753	6744	—	—
530, 433	2762	2771	sss	842	6825	—	—
531	2844	—	—	920	6906	—	—
600, 442	2925	2927	m− +β)	921, 762, 655	6987	6993	ss
610	3006	2990	sss +β)	664	7150	(7160)	(sss−)
532, 611	3087	3092	m+	922, 850, 843, 762	7231	7227	ss
620	3250	3252	ss +β)	930, 854, 754	7312	7307	ss− +β)
621, 540, 443	3331	3331	m−	931	7393	—	—
541	3412	3413	s+	852	7556	—	—
533	3494	—	—	932, 762	7637	7644	s+
622	3575	3580	s−	844	7800	—	—
630, 542	3656	3661	s+	940	7881	7871	ss
631	3737	3742	s−	941, 853, 770	7962	7956	ss−
444	3900	—	—	933, 771, 755	8044	—	—
700, 632	3981	3987	m+	10 00, 860	8125	8123	ss−
710, 550, 543	4062	4060	ss−	1010, 942, 861, 764	8206	8208	sss
711, 551	4143	—	—	10 11, 772	8287	—	—

hkl	$10^4 \sin^2\Theta$ ber.	$10^4 \sin^2\Theta$ beob.	Intens. beob.	*hkl*	$10^4 \sin^2\Theta$ ber.	$10^4 \sin^2\Theta$ beob.	Intens. beob.
10 20, 862	8449	8447	ss	10 31, 952, 765	8937	—	(sss—)
10 21, 854,	8530	—	—	10 32, 944, 870	9181	(9195)	(sss—)
950, 945	8613	—	—	871, 855, 774	9262	9266	sss
951, 773	8693	8689	sss	10 40, 864	9424	(9433)	(sss—)
10 22, 666	8775	—	—	10 41, 960, 872	9506	(9511)	(sss—)
10 30	8856	8858	sss				

$$\text{Mittelwert: } \frac{\sin^2\Theta}{\Sigma_h^2} = 0{,}008125$$

$$a = 8{,}535 \pm 0{,}005 \text{ kX} = 8{,}552 \pm 0{,}005 \text{ Å}$$

Die in Klammern gesetzten Intensitäten sind gerade noch erkennbar. Ihre $\sin^2\Theta$-Werte sind wegen der unsicheren Vermessbarkeit bei der Berechnung der Gitterkonstanten nicht mit einbezogen.

schwach streuenden Aluminiumatome (drei Formeleinheiten $Mg_2Cu_6Al_5$) durch stark streuende Zinkatome ersetzt werden. Wäre die letzte Ursache die wesentliche, müsste man (abgesehen von den Höhen der Maxima) eine annähernd gleiche Fourierprojektion erhalten wie beim $Mg_2Cu_6Al_5$, wenn man die Vorzeichen der Strukturfaktoren für Mg_2Zn_{11} unter Zugrundelegung der in $Mg_2Cu_6Al_5$ erhaltenen Parameterwerte berechnet und die *S*-Werte an den für Polarisation und Lorenzfaktor korrigierten Intensitäten des Weissenbergphotogrammes der *hk*0 genauestens abschätzt.

Die Fig. 1 gibt eine auf oben genannte Weise erhaltene Fourierprojektion auf die *xy*-Ebene wieder. Den Absorptionseffekten bei den 0*k*0-Reflexen wurde dadurch Rechnung getragen, dass die Intensitäten der 0*k*0-Reflexe mit denen der *h*00-Reflexe gleich gesetzt wurden.

In der Fig. 1 sollen folgende Maxima die Projektionen folgender Atome wiedergeben: A = 2 Zn(*k*), B = 2 Zn(*k*), C = 1 Zn(*k*), D = 2 Zn(*i*), E = 1 Zn(*g*) + 1 Zn(*e*), F = 1 Mg(*f*) + 1 Zn(*e*), G = 2 Zn(*g*), H = 2 Mg(*f*), I = 1 Zn(*g*), K = 1 Zn(*b*), L = 1 Mg(*f*), M = 2 Zn(*e*).

Da die Zinkatome etwa doppelt so stark streuen wie die Mg-Atome, sollen die Maxima ungefähr folgende Höhen haben: A = B = D = E = G = M = 2, F = 1,5, C = H = K = I = 1, L = $\frac{1}{2}$.

Die Lage des Maximum F deutet darauf hin, dass beim Mg_2Zn_{11} der Parameter der Punktlage 6(*e*) etwas grösser ist als beim $Mg_2Cu_6Al_5$. Dies gibt sich ebenfalls in dem Maximum E insofern zu erkennen, als es ziemlich breit ist und eine kleinere Höhe hat als zweien Zn-Atomen entspricht (grössere Differenz zwischen den Parametern x_{6e} und x_{6g}).

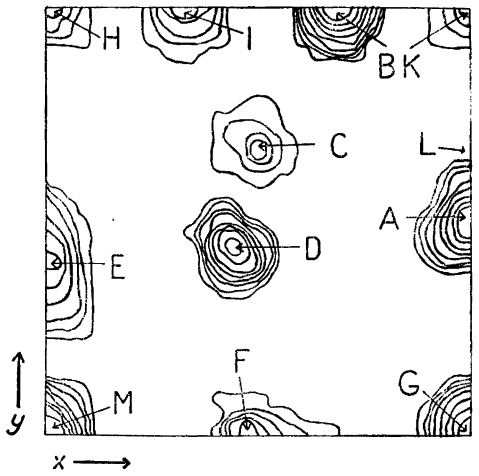

Fig. 1. Fourier-Projektion von Mg_2Zn_{11} auf die xy-Ebene über das Gebiet $0 \leqq x \leqq \frac{1}{2}$, $0 \leqq y \leqq \frac{1}{2}$.

Vergleicht man die Fourierprojektion der Fig. 1 mit der von $Mg_2Cu_6Al_5$ (Fig. 7 in der zitierten Arbeit [1]), sieht man deutlich, wie die schwächer streuenden Aluminiumatome der Punktlagen 8(*i*), 6(*g*) und 1(*b*) durch die stärker streuenden Zinkatome ersetzt worden sind. Die Maxima A, B, C, E, F, H und M sind in beiden Projektionen ungefähr gleich, während die Maxima D, G, I und K in der Fig. 1 etwa doppelt so stark sind wie in der genannten Fig. 7. Das schwache Maximum L, welches in der zuletzt genannten Figur schon einen Ansatz zum Zusammenfliessen mit dem Maximum A zeigt, ist in der Fig. 1 nicht mehr zu erkennen.

Das kleine falsche Maximum N der Fourierprojektion von $Mg_2Cu_6Al_5$ wurde in der voraufgegangenen Arbeit [1] durch Schätzungs- und/oder Abbruchfehler erklärt. Da in der Fig. 1 ein entsprechendes solches nicht vorkommt, und die Anzahl der Reflexe in Mg_2Zn_{11} grösser ist, scheint die Annahme von Abbruchfehlern in der zitierten Arbeit berechtigt gewesen zu sein.

Nun wurden die Intensitäten der *hk*0-Reflexe berechnet, wobei die Parameter um die aus der Fourierprojektion abgelesenen Werte herum variiert wurden. Eine gute Übereinstimmung zwischen den berechneten und beobachteten Intensitäten wurde erzielt, wenn die Parameterwerte des im folgenden kurz zusammengefassten Endergebnisses angenommen wurden.

Raumgruppe: $T_h^1 - Pm3$

12 Zn in	12:(*k*),	$y = 0{,}243 \pm 0{,}005$, $z = 0{,}343 \pm 0{,}005$
8 Zn in	8:(*i*),	$x = 0{,}222 \pm 0{,}005$
6 Zn in	6:(*e*),	$x = 0{,}235 \pm 0{,}005$
6 Zn in	6:(*g*),	$x = 0{,}160 \pm 0{,}005$
6 Mg in	6:(*f*),	$x = 0{,}32 \pm 0{,}1$
1 Zn in	1:(*b*)	

Tabelle 2. Berechnete und beobachtete Intensitäten der hk0 in einer Weissenberg-Aufnahme von Mg_2Zn_{11} · CuK-Strahlung.

h	*h*00	*h*10	*h*20	*h*30	*h*40	*h*50	*h*60	*h*70	*h*80	*h*90	*h*10 0
0	—	—	m—	m	sst+	m	m+	m	m(+)	s	ss
	—	4,0	11	18	440	19	45	38	74	29	9,5
1	—	m—	ss	s	s+	s	sss	—	ss—	—	—
	4,0	40	4,7	7,0	12	6,1	0,64	0,40	2,3	0,05	0,02
2	m—	st	ss	—	s	—	ss	m—	ss—	ss	sss—
	11	49	7,9	0,03	5,3	0,24	1,5	28	2,9	9,5	2,5
3	m	ss—	sst	s—	s+	s—	s+	sss	—	s+	sss—
	18	0,50	70	6,8	9,6	4,0	12	3,2	0,18	15	1,3
4	sst+	sst	sst+	m+	st—	st+	sss+	—	s	st	ss—
	440	55	87	22	36	54	0,64	0,49	13	120	11
5	m	ss(+)	m—	s+	—	m—	ss—	s	sss—	s	
	19	3,8	9,4	6,8	0,11	11	0,25	3,2	0,33	8,4	
6	st+	ss—	m+	st—	sst	ss(+)	ss	sss—	st+	m+	
	45	1,1	20	19	49	4,4	5,8	0,43	46	24	
7	st	ss—	m—	—	m+	ss—	sss—	st	m+		
	38	0,53	7,8	0,16	24	2,2	1,5	26	16		
8	sst	ss	ss(+)	ss(+)	m—	s+	s+	sss—			
	74	1,4	2,9	3,6	14	6,9	8,2	0,45			
9	m+	ss(+)	ss(+)	ss(+)	ss	m	m				
	29	1,4	2,6	4,0	5,8	13	6,7				
10	m	st	m	—	st						
	9,5	26	15	0,3	20						

Von der in der Tabelle eingezeichneten vertikalen Linie ausgehend, macht sich eine nach rechts hin kontinuierlich zunehmende Absorption bemerkbar, die auf Fehler im angewandten Einkristall beruht. Die Übereinstimmung zwischen den berechneten und beobachteten Intensitäten ist in dem ausgemerkten Teil der Tabelle in vertikaler Richtung am besten (nahe beieinander liegende Reflexe). Unstimmigkeiten in eigentlichen Sinne treten in dem genannten Teil der Tabelle wie auch im übrigen nicht auf. Die Reflexe 100 und 010 sind wegen ihrer kleinen Θ-Werte nicht auf dem Weissenbergphotogramm vorhanden.

Mit diesen Parameterwerten sind die Intensitäten, aufgeführt in der Tabelle 2, berechnet worden unter Verwendung der Formel:

$$I \sim \frac{1 + \cos^2 2\Theta}{\sin 2\Theta} \cdot S^2$$

DISKUSSION DER STRUKTUR

Das gesamte Bild der Struktur von Mg_2Zn_{11} ist dem von $Mg_2Cu_6Al_5$ [1] sehr ähnlich. Es wird somit auf die Fig. 8, 9, 10 und 11 der zitierten Arbeit [1] und die dort geführte Diskussion verwiesen.

An dieser Stelle soll nur noch betont werden, dass sogar die Atomabstände in den verschiedenen Richtungen bei beiden Strukturen völlig analog sind. So fällt insbesondere auf, dass auch bei der hier bestimmten Struktur die *i*-Atome einerseits in die Flächen der aus den *e*-Atomen gebildeten Oktaeder, andererseits in die Dreieckflächen der aus den *k*-Atomen gebildeten Zentrumgruppe hineingedrückt sind. Die *i*-Atome drücken somit die *e*- und *k*-Atome auseinander, wie aus den Abständen *e-i*, *e-e*, *i-k*, *k-k* in der folgenden Tabelle hervorgeht.

Tabelle 3. Die Atomabstände in Mg_2Zn_{11}.

Art des Atoms	Anzahl Nachbarn	Art der Nachbarn	Abstände Å	Art des Atoms	Anzahl Nachbarn	Art der Nachbarn	Abstände Å
Zn(*e*)	4	Zn(*e*)	2,84	Zn(*g*)	2	Zn(*e*)	2,65
	2	Zn(*g*)	2,65		2	Zn(*k*)	2,61
	4	Zn(*i*)	2,69		1	Zn(*g*)	2,74
	2	Mg(*f*)	3,56		4	Zn(*i*)	3,09
					2	Mg(*f*)	3,29
Zn(*k*)	1	Zn(*k*)	2,68		2	Mg(*f*)	3,06
	4	Zn(*k*)	2,72				
	1	Zn(*b*)	2,58	Zn(*i*)	3	Zn(*e*)	2,69
	1	Zn(*g*)	2,61		3	Zn(*k*)	2,60
	2	Zn(*i*)	2,60		3	Zn(*g*)	3,09
	1	Mg(*f*)	3,00		3	Mg(*f*)	3,16
	2	Mg(*f*)	2,92				
				Mg(*f*)	2	Zn(*e*)	3,56
Zn(*b*)	12	Zn(*k*)	2,58		2	Zn(*k*)	3,00
					4	Zn(*k*)	2,92
					2	Zn(*g*)	3,06
					2	Zn(*g*)	3,29
					4	Zn(*i*)	3,16
					1	Mg(*f*)	3,08

Die gefundenen Zn-Zn, Mg-Zn und Mg-Mg-Abstände entsprechen annähernd den von Laves in $MgZn_2$ bestimmten [5], welche 2,59 kX, 3,02 kX resp. 3,17 kX waren.

DIE VALENZELEKTRONENKONZENTRATION IN DER BINÄREN UND TERNÄREN PHASE

In Mg_2Zn_{11} ist die Zahl der Valenzelektronen pro Atom 2,0. Nimmt man in $Mg_2Cu_6Al_5$ alle sechs Kupferatome als einwertig an, ergibt sich hier die Zahl 25/13 = 1,92.

Wollte man Hume-Rothery's Regel auf diese Phasen beziehen, erhielte man für beide Phasen die Zahl 2,0, wenn man in der ternären Phase fünf einwertige und ein zweiwertiges Kupferatom annimmt. In der Elementarzelle müssten dann drei zweiwertige Kupferatome vorliegen. Wie aus der Tabelle 4 der zitierten Arbeit [1] hervorgeht, zeichnen sich die Abstände Cu(k)—Al(g) = 2,49 Å als etwas kürzer als die übrigen aus. Da in der Struktur zwölf solche Abstände vorliegen, könnte man vielleicht jedem dieser Kupferatome eine höhere mittlere Valenz zuschreiben, d. h. neun von ihnen sind als einwertig und drei als zweiwertig zu betrachten, wobei diese regellos gemischt sind. Die Abstände dieser Kupferatome von den anderen Nachbarn sind normal.

ZUSAMMENFASSUNG

Durch vollständige Strukturanalyse wird festgestellt, dass die binäre Phase der Zusammensetzung Mg_2Zn_{11} isomorph ist mit $Mg_2Cu_6Al_5$.

Die Verbindung Mg_2Zn_{11} kristallisiert in der Raumgruppe T_h^1—$Pm3$. Die Elementarzelle mit der Kantenlänge $a = 8{,}535 \pm 0{,}005$ kX = $8{,}552 \pm 0{,}005$ Å enthält drei Formeleinheiten Mg_2Zn_{11}.

Die Atomlagen sind auf Seite 840, die Atomabstände auf Seite 842 zusammengestellt.

Was die bildliche Darstellung der Struktur betrifft, wird auf die zitierte Arbeit [1] verwiesen.

Statens Naturvetenskapliga Forskningsråd soll an dieser Stelle für die finanzielle Unterstützung dieser Arbeit gedankt werden.

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Eingegangen am 29. Juni 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 844–849

Oxidation of Ascorbic Acid in the Presence of Copper

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Numerous investigations of the effect of copper on the oxidation of ascorbic acid[1–12] have revealed *inter alia* that hydrogen peroxide is formed as an oxidation product, simultaneously as the copper reduced to univalent cuprous ion is reoxidized, through the action of air, to bivalent cupric. The amount of oxygen used has also been studied and was shown to vary in copper-catalyzed oxidation from 0.5 to 1 mol O_2 for each ascorbic acid molecule[5,7,8]. This variation in amount of oxygen used is explained by an intermediate reaction product, hydrogen peroxide, being changed by further reactions. Should the hydrogen peroxide decompose completely, as for instance in the presence of catalase, the oxygen amount used is only one O-atom; on the other hand, should the hydrogen peroxide remain entirely as such in the solution the oxygen amount used is 2 atoms. Schümmer[4] and Mystkowski[5] reported, that hydrogen peroxide markedly accelerates the oxidation of ascorbic acid. Also Dekker and Dickinson[3] stressed this fact and assumed that in the initial phase of the reaction mechanism the ascorbate oxidizes slowly, through the agency of cupric ions, to an ion akin to semiquinone, which in the subsequent phase immediately and rapidly oxidizes, through the action of air, to dehydro-ascorbic acid.

Some observations made while investigating the oxidation of ascorbic acid near neutral reaction and in comparatively high copper concentrations promted the study reported here. An interesting fact was then observed, namely, that the consumption of oxygen was exceedingly small during complete oxidation of ascorbic acid, while red cuprous oxide was simultaneously precipitated. As the precipitation of cuprous oxide, obviously, played a decisive role in the reaction mechanism the present work was initiated with the view to clarify this phenomenon. The fact that precipitation of copper in conjunction with oxidation of ascorbic acid has hitherto passed unnoticed may chiefly be due to the fact that most of the kinetic investigations concerning

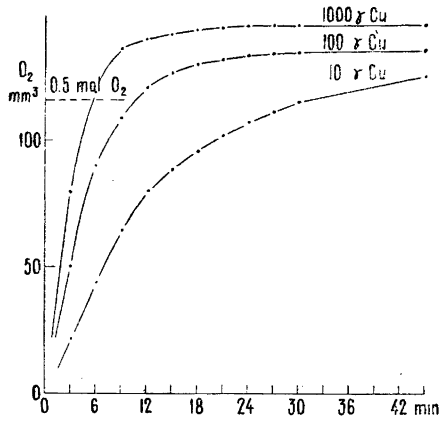

Fig. 1. Effect of copper concentration on the amount of oxygen used in the oxidation of ascorbic acid in acetate buffer of pH 4.20.

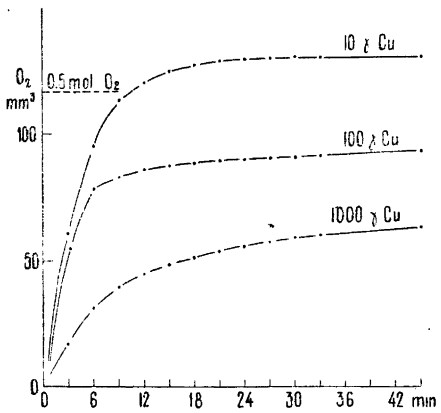

Fig. 2. Effect of copper concentration on the amount of oxygen used in the oxidation of ascorbic acid in acetate buffer pH 5.95.

ascorbic acid oxidation were carried out in relatively acid solutions, and moreover in the presence of comparatively low concentrations of copper. Mystkowski [6] is probably the only one who mentions precipitation of oxidulated copper. He, however, found no strict parallel between the amount of ascorbic acid oxidized and Cu_2O formed.

EXPERIMENTAL

Figs. 1 and 2 show the amounts of oxygen used as measured with the Warburg manometer in experiments wherein 1.84 mg *l*-ascorbic acid were oxidized in the presence of varying concentrations of $CuSO_4$ in a 0.1 *N* acetate buffer at 30° C and at two different pH-levels, at 4.20 and 5.95 respectively. Shaking rate 100 per minute, fluid volume 5 ml, ascorbic acid put in side arm and added after temperature equilibration and zero readings taken.

It is obvious that in the two experiments the effects of copper on the amount of oxygen used are in opposition to each other. On employing 10 γ of copper, the oxygen amount used in both experiments is approximately the same, *i. e.* about 0.55 moles. In copper concentrations higher than this the oxygen amount used increased at pH-level 4.20 but decreased perceptibly at pH-level 5.95, sinking to about 0.28 moles on employing 1000 γ copper. Despite this fact the ascorbic acid oxidized completely as indicated by titration at the end of the experiment.

To compare the oxidation rates a test was made: employing 1000 γ of copper under the above given conditions the ascorbic acid oxidized was determined by titrating samples taken at 3 minute intervals. Figure 3 shows that ascorbic acid oxidized very rapidly at pH-level 5.95. At the same time the red precipitate formed during the very first minutes of the reaction. At pH 4.20 the oxidation rate was markedly slower.

The decrease in the amount of oxygen used is coupled with the cuprous oxide precipitated during the reaction. Besides the case in which maximum copper concentration (1000 γ Cu) was employed a red turbidity was also observed at pH 5.95 when 100 γ of copper were used. In examining the effect of pH on the precipitation of cuprous oxide under the above conditions (0.01 mmol ascorbic acid, 0.016 mmol $CuSO_4$ in 5 ml 0.1 N acetate buffer) it was found that precipitation began at a pH-level above 4.5. In phosphate buffer having acid reaction no red precipitate could be detected, but the copper phosphate precipitated in neutral or alkaline reactions turned yellow after the addition of ascorbic acid.

The addition of protein prevented cuprous oxide from precipitating and increased the total amount of oxygen used (Fig. 4). The effect of ovalbumin is similar to that of casein, and cooked and uncooked vegetable proteins. Aspartic acid and glutamine also prevent the precipitation of cuprous oxide.

DISCUSSION

The kinetic investigations concerning the oxidation of ascorbic acid indicate that in neutral and acid reactions the monovalent ion of ascorbic acid is coupled with the absorption of oxygen [3,9,10]. The fact that the oxidation rate is independent of ascorbic acid concentration indicates that decomposition of the copper ascorbate complex (CuA) to an intermediate product of semiquinon-like structure seems to be the rate-determining step. Dekker and Dickinson[3] assumed the following reaction series to take place:

$$Cu^{++} + H_2A \rightleftharpoons CuA + 2H^+ \quad (1)$$

$$CuA \rightarrow Cu^+ + \text{Intermediate products} \quad (2)$$

$$\text{Intermediate products} \rightarrow \text{Dehydroascorbic acid} \quad (3)$$

B^- denoting the intermediate its aerobic decomposition is as follows:

$$B^- + O_2 + H^+ \rightarrow D + HO_2 \quad (4)$$

$$2\ HO_2 \rightarrow H_2O_2 + O_2 \quad (5)$$

or the anaerobic decomposition:

$$B^- + Cu^{++} \rightarrow D + Cu^+ \quad (6)$$

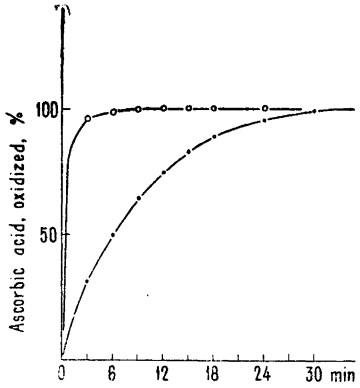

Fig. 3. The oxidation of ascorbic acid in acetate buffer. Systems contained in 50 ml: 36 ml acetate buffer; 18.4 mg ascorbic acid; 39.3 mg Cu $SO_4 \cdot 5\ H_2O$.

●——● *pH 4.20*
○——○ *pH 5.95*

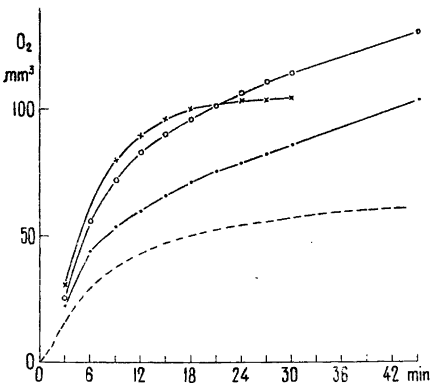

Fig. 4. Effect of different casein concentrations on the oxygen uptake of ascorbic acid in acetate buffer of pH 5.95 and with 1000 γ of copper.

– – – – *without protein*
●——● *3 mg casein*
○——○ *10 mg casein*
×——× *50 mg casein*

The cuprous ion formed re-oxidizes to cupric ion:

$$Cu^{+} + O_2 + H^{+} \rightarrow Cu^{++} + HO_2 \quad (7)$$
$$2\ HO_2 \rightarrow H_2O_2 + O_2 \quad (8)$$

In the reaction series given above the oxygen amount used is 1 mol per molecule of ascorbic acid oxidized. If, however, the oxidation of cuprous ions is prevented, for instance by its precipitating from the solution as cuprous oxide, the oxygen amount used decreases in aerobic oxidation to 0.5 mol and in anaerobic oxidation to zero.

The decomposition of hydrogen peroxide formed in the reaction has not been considered in the reaction series above. This would complicate the reaction considerably as copper is capable of decomposing hydrogen peroxide both catalatically and peroxidatively. Dekker and Dickinson assumed cupric ion together with hydrogen peroxide to catalyze the oxidation of the ascorbate ion so that the hydrogen peroxide itself does not decompose but accumalates in the solution:

$$Cu^{++} + H_2O_2 \rightarrow CuH_2O_2^{++} \quad (9)$$
$$CuH_2O_2^{++} + A^{--} \rightarrow Cu^{+} + H_2O_2 + B^{-} \quad (10)$$

Should hydrogen peroxide react quantitatively according to reactions (9) and (10) it would not change the oxygen amount used although the reaction rate would be accelerated. Obviously the catalatic or peroxidative decomposition of hydrogen peroxide would, on the other hand, decrease the oxygen amount used. It is probable that both in the catalatic and in the peroxidative reactions the copper peroxide functions as an active oxygen donator (*cf.* Krause [13]). This compound, however, forms only during the precipitation of copper hydroxide. The cupric ion does not decompose hydrogen peroxide.

The solubility product of copper hydroxide is 3.9×10^{-19} (Näsänen [14]) wherefore in the concentration employed it should precipitate at pH-levels below 6. The acetate buffer, however, prevents it from precipitating. Consequently it is probable that under the conditions employed the hydrogen peroxide does not notably partake in the reactions. The small oxygen amount used on employing 1000 γ of copper may, consequently, be due primarily to the precipitation of cuprous oxide.

In comparing the curves depicting oxygen amounts used with those for ascorbic acid oxidation (Figs. 2 and 3) it is seen that oxygen is consumed even after all ascorbic acid has been oxidized in the presence of 1000 γ copper. This fact indicates that dehydroascorbic acid is formed chiefly anaerobically, and that oxygen is used solely for the oxidation of cuprous ions. According to Dekker and Dickinson the aerobic reaction (4) is, however, markedly faster than the anaerobic reaction (6) probably because of the instability of perhydroxyl, HO_2, in acid solutions. Obviously the precipitation of cuprous oxide greatly accelerates the anaerobic reaction by shifting the equilibrium thereof to the side of dehydroascorbic acid.

Should the reaction mixture contain compounds such as amino acids or proteins, which form complex linkages with copper, the precipitation of cuprous oxide is prevented. A natural result of this is an increase in the oxygen amount used, as shown in Figure 4. The intermediate B^- decomposes aerobically and also the cuprous ion re-oxidizes to cupric ion. The oxidation of cuprous ion may, according to Mapson [11], also take place with hydrogen peroxide. Mapson is of the opinion that the reaction

$$2\ Cu^+ + 2H^+ + H_2O_2 \rightarrow 2Cu^{++} + 2H_2O$$

is the most natural explanation for the decomposition of hydrogen peroxide.

SUMMARY

The effect of copper on the oxidation of ascorbic acid is studied. It is shown that in acetate buffer the ascorbic acid causes precipitation of cuprous

oxide in the presence of copper salt concentrations of approximately 0.001 *M* or more. As a result of this phenomenon the oxygen amount used in ascorbic acid oxidation is found to decrease so that considerably less than one atom is required for each molecule of ascorbic acid. This evidence promted some kinetic investigations.

It is probable that the oxidation of ascorbic acid in copper catalysis is an anaerobic process, and the accelerating effect which oxygen has on the reaction is based mainly on the reoxidation of the cuprous ion.

The proteins and some amino acids prevent cuprous oxide from precipitating and the oxygen amount used from decreasing.

The writer is indebted to students N. Petterson and B. Rehell for their valuable assistance with the experiments.

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Received May 21, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 850–857

Effect of Copper on the Iron Uptake of Plants

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There are some reports on copper-caused-chlorosis in green plants. Willis and Piland [1] observed the unfavorable effect of copper on the iron content of corn and stressed the action of copper on the oxidation-reduction potential in soil. Chapman and co-workers [2] found in *Citrus* that copper in nutrient solutions sometimes causes chlorosis. They also suggested that the action of copper may be due to reactions affecting the oxidation of iron and hence the absorbability of iron by plant roots. Ødelien [3] made similar observations of copper-caused-chlorosis on oats.

On the other hand, according to Maquenne and Demoussy [4], the poisonous effect of ferrous ion can be prevented in nutrient solution with copper sulfate.

The purpose of this investigation was to examine the effect of copper on the iron uptake of peas in nutrient solutions containing ionizable or complex-bound iron and different nitrogen sources. In order to explain the facts observed on basis of oxidation-reduction conditions, potential measurements were carried out in nutrient solutions during growth.

EXPERIMENTAL

The peas were grown in sterilized nutrient solutions under daylight conditions in the spring and summer of 1948. Erlenmeyer flasks (1000 ml) were used for culture flasks except in the experiment with inoculated peas, where Woulffe flasks (3000 ml) were used. Two sterile seedlings were transferred to each of the culture flasks. In the experiment without nitrogen nutrition the nutrient solution was inoculated with 1 ml of aqueous suspension of H 7 *Rhizobium* strain on transfer of the seedlings. Experiments with nitrate and ammonium nitrogen comprised two parallel series and that with inoculated plants four parallel series.

The nutrient solutions contained per one litre of glass-distilled water the following salts in grams:

	I (with NO_3—N)	II (with NH_4—N)	III (inoculated)
$Ca(NO_3) \cdot 4H_2O$	1.0	—	—
$(NH_4)_2\ SO_4$	—	1.0	—
KH_2PO_4	0.85	0.85	0.85
K_2SO_4	0.4	—	0.4
$MgSO_4 \cdot 7H_2O$	0.3	0.3	0.3
$CaSO_4$	—	0.5	0.5

Copper was removed from concentrated stock solutions with an acid dithitzone treatment. Calcium sulphate was prepared from a copper-free calcium chloride solution by precipitating with a copper-free potassium sulphate solution. Micronutrients were used as a combination A-Z-a according to Hoagland and Broyer but without copper. 10 mg iron were supplied as ferric chloride or ferric citrate to a litre of nutrient solution. Also experiments without iron were carried out in series with nitrate and ammonium nitrogen. Copper was added as copper sulphate. The solutions were adjusted to pH 6.0 with 5 % NaOH.

Iron was estimated colorimetrically with the thiocyanate method according to Scott [5].

Two platinum electrodes were immersed in culture flasks, and daily E_h measurements were made with the Beckman potentiometer. All the E_h values reported are mean values of four electrodes in two culture flasks of parallel experiments. All the E_h values were calculated for pH 6.

RESULTS

The effect of copper addition on the dry matter of sprouts and their ash content in different series appears from the figures in Table 1.

It is seen, that increasing copper content promotes the growth of peas most in ammonium nitrogen solution with ferric citrate as the source of iron. This is in agreement with Arnon [6] who used ferric tartrate as iron source in experiments with barley. Contrarily, with ferric chloride as iron source, copper in ammonium nitrogen solution has a deleterious effect on growth. In all the solutions containing nitrate nitrogen, copper had a beneficial effect on growth. In inoculated nutrient solutions without nitrogen low copper concentrations

Table 1. Dependence of dry weight and ash content of sprouts on copper concentration in nutrient solutions of different composition.

N-source	Iron source	Addition of copper γ/l	Duration of series	Dry weight of sprouts g	Ash content %
NO_3—N	Ferric chloride	0	May 5—June 1	0.398	17.9
»	»	5	»	0.425	19.4
»	»	500	»	0.431	18.0
»	Ferric citrate	0	»	0.842	23.8
»	»	500	»	0.886	24.8
NH_4—N	Ferric chloride	0	June 29—July 20	0.213	33.0
»	»	500	»	0.126	14.5
»	Ferric citrate	0	»	0.195	24.4
»	»	500	»	0.400	22.8
Inoculated	»	0	March 24—May 10	0.640	17.0
»	»	5	»	0.700	17.0
»	»	20	»	0.668	15.9
»	»	200	»	0.608	16.4
»	»	1000	»	0.554	17.7

stimulated the growth of plants, but concentrations above approximately 20 p. p. m. seem to be harmful to plants. Copper seems to increase the ash content of plants in nitrate nutrient solutions, but strongly decreases it in solutions with ammonium nitrogen. With the exception of plants having ferric citrate and copper in nutrient solution, all the plants with ammonium sulphate as nitrogen source grew very poorly. An appreciable acidification of nutrient solution was reached, and in many cases the plants died before flowering.

The iron content of sprouts is graphically presented in Fig. 1.

In nitrate plants copper did not markedly affect iron content when iron was supplied as ferric citrate; with ferric chloride in nutrient solution copper had, however, a very marked antagonistic effect on the iron content of the sprout. In ammonium plants the iron content in ash of sprouts increased markedly on adding copper to cultures with ferric chloride iron as well as with ferric citrate iron. In inoculated plants, small amounts of copper in nutrient solution increased the iron content of sprouts, but the higher copper concentrations had the opposite effect.

The results of the measurements of the oxidation-reduction potentials in nutrient solutions are given in Figs. 2 to 4.

Examination of the curves in Fig. 2 clearly shows that in nitrate nitrogen solutions the addition of copper distinctly prevents the fall in electrode

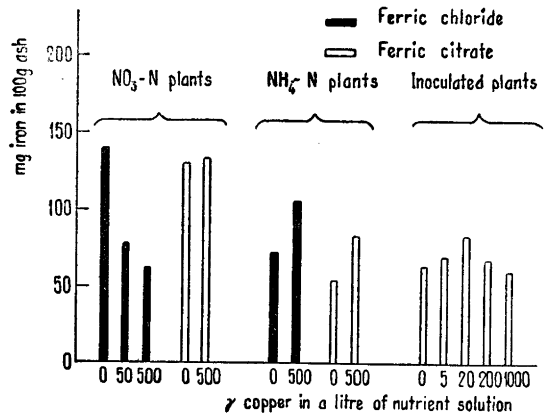

Fig. 1. Effect of copper on iron content of sprouts.

potentials during growth when ferric chlorid is used as the iron source. With ferric citrate as iron source the addition of copper has, however, no marked effect on the potential. In nutrient solutions with ammonium nitrogen (Fig. 3) the addition of copper promotes the fall of the potential in cultures with ferric chloride, later on also in cultures with ferric citrate. The potential curves in inoculated solutions (Fig. 4) are very peculiar. No definite electrode potentials are established in the culture solutions. The wide oscillations of the potential during the initial stage of the growth period dampen later on and become confused. However, a very striking conformity exsists between the potential curves of all the different series, also during the last period of the growth. Therefore, the oscillations can not be accidental only. Higher concentrations of copper have a poising effect on the oscillations of the potential. After a growth period of about three weeks iron in nutrient solution begins to precipitate as ferric hydroxide. This phenomenon is evidently caused by the utilization of citric acid. From this time on the smaller copper concentrations have a reducing effect on the potential, but, on the contrary, the higher concentrations of copper prevent the falling of potential to a low level.

A more detailed study of the reasons for the oscillations of the potentials is beyond the scope of this work. The phenomenon probably is not due to the actual variation of the average potential in the culture solution, but to the variable oxygen concentration in the immediate neighbourhood of the platinum electrode, caused by the varying activity of bacteria.

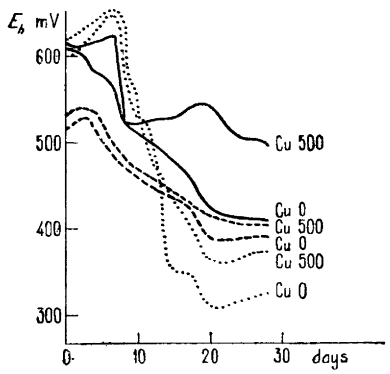

Fig. 2. Effect of copper on electrode potential in nitrate nutrient solution.

..... *Without iron.*
_____ *With ferric chloride.*
– – – *With ferric citrate.*

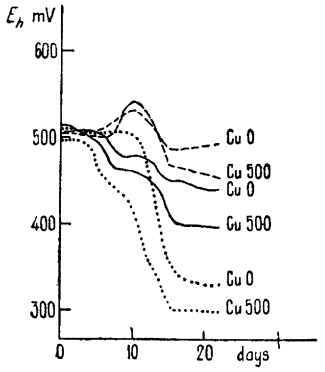

Fig. 3. Effect of copper on electrode potential in ammonium nutrient solution.

..... *Without iron.*
_____ *With ferric chloride.*
– – – *With ferric citrate.*

DISCUSSION

It has often been demonstrated that plants take up iron better in the presence of an organic non-ionized iron complex, such as citrate, tartrate, oxalate, glycerophosphate, or humic acid iron complex [7–11]. On the other hand, plants seem to absorb the ionized iron for the most part as ferrous cation. Ferric iron must be reduced before absorption, *e. g.*, by the reducing substances in soil or by the reducing action of microorganisms or the epidermis of the plant root [9, 12, 13].

In the experiments described above the iron supplied as ferric chloride always precipitated through hydrolysis as ferric hydroxide during the autoclaving of the culture solution. Considering the very little solubility product of $Fe(OH)_3$ (only 10^{-37} at pH 6.0) the actual concentration of ionized ferric iron in culture solution is negligible, and, under these conditions, cannot have any poising effect on the oxidation-reduction potential in the solution. On the contrary, the oxidation-reduction conditions determine the equilibrium $Fe^{+++} + e^- \rightleftharpoons Fe^{++}$ in the solution, and, therefore, decisively influence the ferrous iron uptake of plant.

When iron is supplied as ferric citrate, *i. e.* as a non-ionized organic complex, the oxidation-reduction conditions in nutrient solution cannot exert a decisive effect on the form of occurrence of iron, nor, on the iron uptake as long as there are enough citrate ions in the solution. According to Swenson

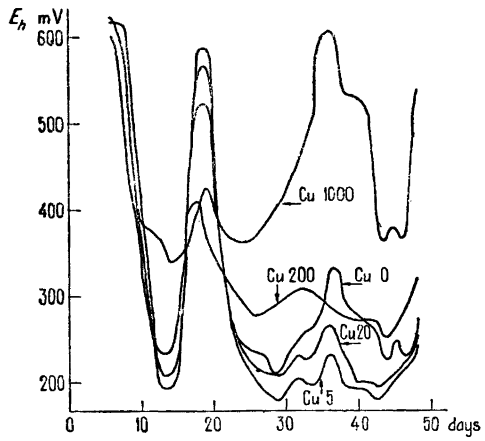

Fig. 4. Effect of copper on electrode potential in inoculated nutrient solution with ferric citrate as the iron source.

et al. [14] of several anions (citrate, mucate, fluoride, gallate, tartrate, gluconate, and salicylate) citrate is the most effective in its ability to coordinate with iron. An increase of the pH value from 3.4 to 6.3 increases the effectiveness of citrate to replace iron from phosphate more than can be attributed to the additional hydroxyl alone.

The results obtained indicate that the correlation between the effect of copper on the iron uptake and on the potential in nutrient solution is very distinct in all the experiments. Thus, the effect of copper on the iron uptake seems to be due to the altering of the oxidation-reduction conditions in the culture solution. In nitrate cultures with ferric chloride where the fall in potential is prevented by the addition of copper, and where copper thus increases the ratio Fe^{+++}/Fe^{++}, copper decreases the iron uptake. In ammonium cultures with ferric chloride as the iron source where copper, on the contrary, promotes the fall in potential, and thus decreases the ratio Fe^{+++}/Fe^{++}, it also promotes the iron uptake. In nitrate cultures with ferric citrate iron, copper has no effect on potential nor on the iron uptake. Even in ammonium cultures with ferric citrate as well as in inoculated cultures the effect of copper on the iron uptake is in correlation with the effect on the potentials. The effect of copper on potential, noticed in older cultures, is possibly caused by the uptake of citric acid from the culture solution.

Lin [15] studied the appearance of iron chlorosis in rice cultures supplying nitrogen as ammonium or nitrate, and never obtained chlorosis in ammonium cultures, but did in nitrate cultures. The experiments described above show that copper may cause iron deficiency in nitrate cultures when organic non-ionizable iron complexes capable of being absorbed are not present. The

fact that Lin was unable to prevent chlorosis by the addition of iron as tartrate can be accounted for by the more rapid decomposition of the iron tartrate complex. In preceding experiments with ferric citrate, iron did not begin to precipitate until about three weeks had elapsed.

Hence we may draw the conclusion that copper has a marked effect on the availability of iron for plants. This effect is very important considering the plant nutrition from the practical point of view.

SUMMARY

The iron uptake of peas was studied in sterile water cultures containing 10 mg Fe in a litre by using different nitrogen sources, and varying the copper concentrations. Moreover, the oxidation-reduction potentials of the culture solutions were observed.

The effect of copper on the iron uptake always was in correlation with the effect on the electrode potential in the nutrient solution.

When iron was supplied as ferric chloride and precipitated by autoclaving, copper (50 and 500 γ Cu in a litre) decreased the iron uptake and also prevented the fall in electrode potential in nitrate culture solution, but in ammonium culture solutions copper increased the iron uptake (calculated on the ash basis) and promoted the fall in potential.

When iron was supplied as ferric citrate, 500 γ of copper had no marked effect on the iron uptake of plant in nitrate culture solution, nor on the potential of the culture solution. In ammonium culture solution with ferric citrate iron, copper increased the iron uptake, and also promoted the fall in potential in nutrient solution after a growth period of about two weeks.

The experiments with inoculated peas in the presence of ferric citrate as the iron source gave wide oscillations of electrode potentials, which in different series were very well synchronized. Also now a negative corralation was observed between the final level of electrode potential and the iron content of plants.

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Received May 21, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 858–861

Studies on the Metabolism of *Aspergillus niger*

I. The Effect of Aeration on the Formation of Citric and Oxalic Acids in Surface Mould Cultures

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As early as 1927 Rippel and Bortels[1] advanced the assumption that carbon dioxide is essential for the metabolism of moulds and accordingly, not only an unnecessary end product of metabolism. This assumption was later confirmed by the experiments of Foster *et al.*[2] who, using C^{11} as a tracer in carbon dioxide, found *Aspergillus niger* to yield citric acid from sugar containing the tracer carbon in the carboxyl groups. They used a submerged culture according to Kluyver and Perquin[3].

The fact that carbon dioxide participates in the formation of citric acid in *Aspergillus* prompted the present investigation which deals with the effect of aeration on the citric and oxalic acid formation in surface mould cultures. It is not improbable that a decrease in the percentage of carbon dioxide developed in respiration affects the amounts of fermentation products formed.

EXPERIMENTAL

Aspergillus niger (strain HC I of the laboratory) was grown as a surface culture in 1000 ml Erlenmeyer flasks containing 100 ml nutrient solution. The composition of the nutrient solution was the following: 2.25 g ammonium nitrate, 0.6 g potassium dihydrogenphosphate, 0.4 g potassium monohydrogenphosphate, 0.25 g magnesium sulphate, 0.02 g $ZnSO_4 \cdot 7H_2O$, 0.003 g $MnSO_4 \cdot 4H_2O$, 0.50 g $FeCl_3 \cdot 6H_2O$, 0.1 mg $CuSO_4 \cdot 5H_2O$, 130 g sucrose, and glassdistilled water to one litre. Prior to autoclaving the media were adjusted with HCl to pH 2.0.

The flasks were plugged with cotton, and into some of them sterile air was admitted near the mould pellicle. The fermentations were allowed to continue for 13 to 16 days. The nutrient solution gave a 2 cm layer of medium when the flasks were placed in a horizontal position.

Acid formation was followed by taking samples from the flasks with a sterile pipette the lower end of which was passed through the mould pellicle into the nutrient solution. Care was taken not to mix the nutrient solution while moving the flasks. Citric acid was determined according to Pucher *et al.*[4] by oxidizing at room temperature with $KMnO_4$ in a KBr-Br solution to pentabromacetone, by de-halogenizing, and by titrating the bromine ion argentometrically. Oxalic acid was estimated by precipitation as the calcium salt at pH 5 and by subsequent permanganate oxidation. The results recorded here are mean values of two parallel experiments.

In aerated cultures a uniform pellicle of mycleium developed about 2 to 3 days later than in unaerated cultures. Correspondingly, in the latter ones fermentation started earlier. In the aerated cultures the total amount of sugar consumed was greater but the titratable acidity did not markedly differ from that of unaerated cultures (Table 1).

Table 1. Consumption of sugar and increase in titratable acidity of aerated and unaerated cultures.

Duration of fermentation days	Sugar left in medium %		0.125 *N* NaOH per ml fermented medium ml	
	Aerated	Unaerated	Aerated	Unaerated
4	98.0	68.5	0.3	0.4
6	90.0	52.5	0.3	2.0
8	50.0	45.0	1.5	2.5
10	33.0	36.0	2.6	2.9
12	26.5	33.5	3.4	3.1
14	24.9	32.5	3.8	3.2
16	23.5	—	3.8	—

In comparing the aerated cultures and unaerated cultures with each other quite noteworthy differences were observed in the relationships between the citric and oxalic acids produced (Fig. 1).

The relation between the maximum yields of citric and oxalic acids is given below:

	Citric acid m*M*	Oxalic acid m*M*
Aerated	0.0145	0.170
Unaerated	0.0645	0.060

Thus the aeration nearly trebles oxalic acid formation at the same time decreasing citric acid production to one-fourth.

DISCUSSION

Some of the hypotheses which have been proposed for the explanation of the mechanism of citric acid fermentation involve *inter alia* the breakdown of the sugar molecule to simpler compounds, such as acetaldehyde or acetic acid,

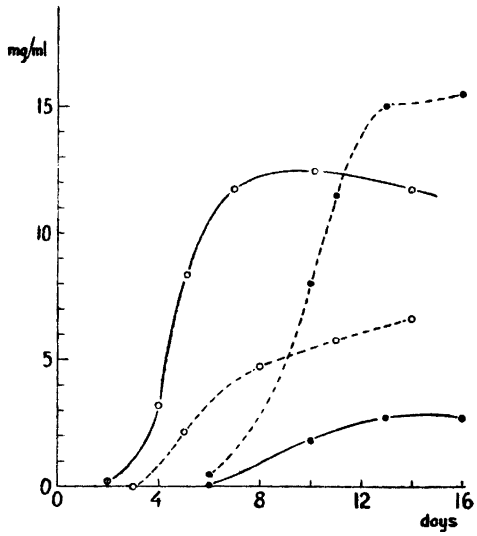
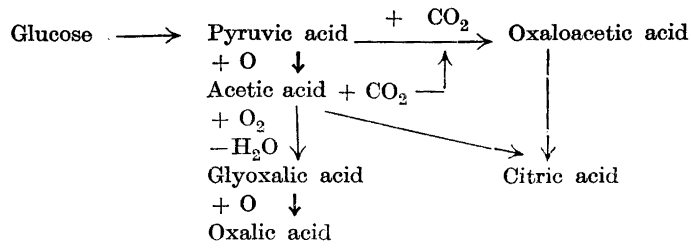


Fig. 1. *The effect of aeration on the production of citric and oxalic acids.*

—— *Citric acid*
····· *Oxalic acid*
● *Aerated*
○ *Nonaerated*

which are then used for the synthesis of citric acid (*cf. e. g.* [5,6,11]). This assumption has been critisized because carbon dioxide is not regularly formed in the citric acid fermentation to the extent presumed on decarboxylation of pyruvic acid. Wells *et al.*[7] showed that such a hypothesis is incompatible with the high yields of citric acid from sugar under some conditions. High yields of citric acid were observed also by Raistrick and Clutterbuck [8] and by Butkewitsch and Gajewskaja [9]. On the contrary, it has been noted that *Aspergillus niger* forms from sugar small amounts of acetaldehyde which proves at least a partial decomposition of sugar through the C_3-compounds [10,11]. In fact, as early as 1928 Virtanen [11] advanced the idea that citric acid is formed as a condensation product of oxaloacetic acid and acetic acid.

The observation made by Foster *et al.*[2] that carbon dioxide takes part in the formation of citric acid *via* the Wood-Werkman reaction (pyruvic acid + CO_2 → oxaloacetic acid) throws new light on the mechanism of citric and oxalic acid formation. The mechanism may now be interpreted as follows:



According to the above mechanism it is possible to understand the great quantities of citric acid present in such reaction series where an incomplete alcohol fermentation forms the initial stage of the reaction chain. The carbon dioxide liberated in incomplete alcohol fermentation is re-fixed by the Wood-Werkman reaction wherefore the quantitative conversion of sugar to citric acid is theoretically possible. The formation of citric acid, on the other hand, is restricted in the above scheme by the removal of carbon dioxide from the reaction system. When the pH of the nutrient solution is 2 it is incapable of fixing CO_2 as a bicarbonate. In unaerated cultures the CO_2 arising as a respiration product accumulates as gas in the very near vicinity of the mycelium, whereas in aerated cultures it follows the outflowing current of air. Foster and Davis [12] could not achieve a CO_2-deficiency in the mycelium of vigorously fermenting *Rhizopus nigricans* in a high vacuum. In the aerobic experiments described above the fermentation is, however, so much slower that it is possible to remove by aeration significant amounts of CO_2 from the interior of the fungus cells. Now, the CO_2 fixation is suppressed and with that also the citric acid formation. The acetic acid, consequently, is converted into oxalic acid in greater quantities than in unaerated cultures. It may be possible that the abundant formation of oxalic acid in neutral reaction can also be explained by the fixation of carbon dioxide as a carbonate.

SUMMARY

Aeration of the surface culture of *Aspergillus niger* nearly trebles oxalic acid formation while decreasing citric acid production to one-fourth.

On the basis of this result the significance of the Wood-Werkman reaction in citric acid fermentation has been discussed.

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Received May 21, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 862–866

Studies on the Metabolism of *Aspergillus niger*

II. Effect of Iron on the Production of Citric and Oxalic Acids

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The effect of iron on the production of citric acid by *Aspergillus niger* continues to be a subject of controversy. Bernhauer[1] observed no notable effect on the addition of 0.005 % ferric chloride. Porgas[2] as also Chrzaszcz and Peyros[3] ascertained the accelerative effect of iron on the citric acid formation by certain mould strains. On the contrary, Knobloch and Sellmann[4] as well as Giordani[5] and Perquin[6] are of the opinion that iron in most cases has an unfavourable effect on the production of citric acid. This unfavourable effect is contrary to the effect of iron on mould development which, according to various investigators, is promoted by iron, (*cf. e. g.* Bernhauer and Knobloch[7]). Perlman *et al.*[8] have studied the effect of iron on the production of citric acid by several mould strains and found that different mould strains vary greatly in their behaviour.

Characteristic of all the above mentioned investigations is the fact that the nutrient solutions of the control experiments were not free of iron. Thus, for instance, Knobloch and Sellmann used in their investigations tap water and Perlman *et al.* employed nutrient solutions having an iron content of 162 to 102 γ per liter, as determined spectrographically. In most instances no detailed information was given as regards the culture conditions employed; nor was the continuous observation of acid production mentioned.

The present experiments were made with nutrient solutions having an accurately determined iron content, and the nutrient solutions of the control experiments were freed of iron. During the experiments the acid production was under continuous observation.

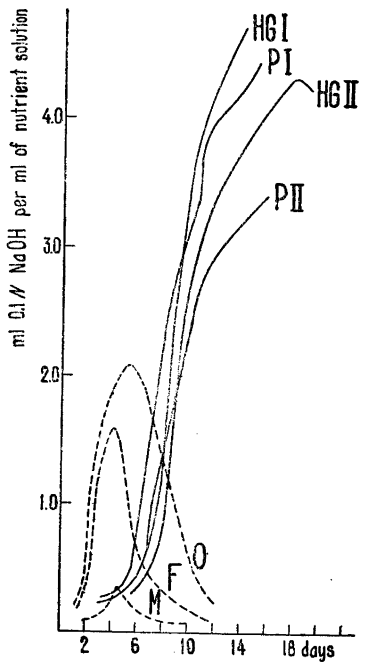

Fig. 1. Acid forming potency of various Aspergillus strains.

——— *fair acid production*
– – – – *poor acid production*

EXPERIMENTAL

The nutrient solution used in the experiments was similar to that previously employed by Erkama *et al.*[9]. Prior to the addition of magnesium sulphate and the heavy metals the concentrated nutrient solution was, according to Waring and Werkman [10], purified of iron by treating it with a chloroform solution of 8-hydroxyquinoline at pH 6.2. The magnesium sulphate was purified of the heavy metals according to Steinberg by treating with calcium carbonate in an autoclave [11]. In all the experiments the same iron content, *i. e.* 10 mg Fe per liter, was employed. The fermentations were carried out in 1 liter Erlenmeyer flasks. 100 ml of medium were added to each flask, and the flasks were plugged with cotton wool. All the results given below are mean values of two test flasks.

Determination of citric acid was made according to Pucher *et al.*[12]. The previously used method of Kometiani [13] gave unsatisfactory results. The oxalic acid was precipitated at pH 5 as calcium salt, then dissolved in sulphuric acid and titrated with potassium permanganate. The sugar was determined according to Bertrand.

Of the various mould strains isolated, generally the poor acid producers developed more rapidly than the fair ones. As a result of rapid growth the poor acid producers consumed more sugar and consequently, the fermentation products disappeared quickly from the solutions in the absence of sugar. Fig. 1 illustrates some typical examples of acid production by fair and poor acid producer strains.

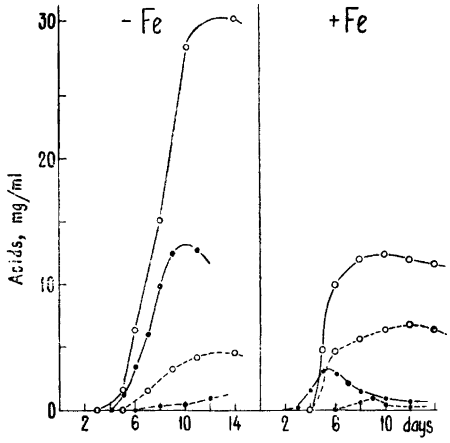

Fig. 2. Effect of iron on the citric and oxalic acid formation by different A s p a r g i l l u s strains.

——— *citric acid*
– – – – *oxalic acid*
○ *strain HGI*
● *strain O*

The effect of iron on the production of acids is evident from fig. 2. In these experiments the strains HG I and O were used, the former producing about 50 % acid from the fermented sugar and the latter about 10 %.

Greater yields of citric acid are obtained in iron-free solution than in iron-containing solution. On the other hand addition of iron promotes oxalic acid production to some extent provided there is enough sugar in the solution. In comparing acid production and sugar consumption (Fig. 3) with each other it is seen that the iron addition does not appreciably affect the sugar consumption of fair acid producers but greatly increases the sugar consumption of poor acid producers. In poor acid-producer strains this fact leads to cessation of acid production and relatively rapid disappearance of acids from iron-containing solution.

DISCUSSION

In the present experiments the strains of *Aspergillus* were observed to differ in the acid production ability in that the oxalic acid formation was induced by the poor acid-producing strains 2 to 4 days later than the citric acid formation, whereas the fair acid producers started the oxalic acid production almost simultaneously with the formation of citric acid, especially in the presence of iron, or only 1 to 2 days later. Moreover the formation of oxalic acid by poor acid producers was very slight, but in some instances increased, whereas the percentage of citric acid decreased in the absence of sugar.

It is generally known that mould thrives on acids it has produced in the nutrient solution after complete consumption of the carbohydrate nutrition

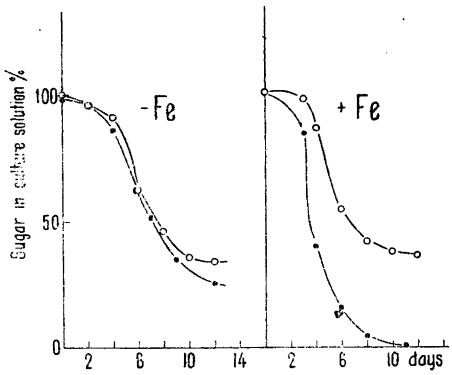

Fig. 3. Effect of iron on the sugar consumption by different Aspargillus strains.

○ *strain HGI*
● *strain O*

proper. Consequently, citric acid and oxalic acid may be regarded as constituting a reserve set aside by the organism when carbohydrate nutrient is promptly on hand. During carbohydrate starvation conditions the reserve acids are oxidized.

In the different experiments comparison of sugar concentrations corresponding to the maximum yields of citric acid reveals that said concentrations are then approximately on the same level: with fair acid producers the level is about 35 % and with poor acid producers about 25 to 30 % of the initial sugar concentration irrespective of the iron content of the nutrient solution. In many instances on employing poor acid producers the formation of oxalic acid begins only in sugar concentrations below 25 per cent. This observation is confirmed by the investigations of Kusnetzow [14] which shows that sugar concentration determines in which direction fermentation will proceed, *i. e.*, whether citric or oxalic acid is formed. On the basis of the investigations of Kusnetzow it seems that oxalic acid is formed directly from the sugar. Butkewisch [15], again, has shown that oxalic acid can be formed from citric acid. According to the present experiments oxalic acid formation by poor acid-producer strains appears chiefly to be effected by oxidation of citric acid but fair acid-producer strains form oxalic acid directly from sugar simultaneously with citric acid formation.

Iron greatly accelerates growth of poor acid producers whose mycelium dry weight in iron-containing solution is almost regularly greater than in iron-free solution. The effect of iron on the development of fair acid producers is not as distinct although the mycelium dry weight in iron-containing solution is generally greater than in ironfree solution. In the light of our experiments it seems that the effect of iron on the acid formation of poor acid-producer strains is indirect in that it accelerates the growth greatly and thus leads to the rapid disappearance of sugar from the nutrient solution. Possibly there

may be a question of great acceleration of iron respiration, the mechanism for regulating the relationship between respiration and fermentation being more poorly developed than in strains forming acid abundantly.

The sugar content in nutrient solutions of fair acid-producers never dropped during the experiment as low as to cause starvation conditions. Nor did the iron have an appreciable effect on the consumption of sugar. Possibly the effect of iron on acid formation by strains producing acid abundantly is of a more primary nature than on that by poor acid producers.

SUMMARY

The effect of iron on the production of citric acid and oxalic acid was studied by comparing the fair and poor acid-forming strains of *Aspergillus niger*. 10 mg of iron added to a liter of medium containing sucrose and the usual salts decreased citric acid production by about 60 per cent when strains forming acid abundantly were employed and by about 75 per cent when strains forming acid weakly were employed. The oxalic acid production was increased by about 45 per cent on addition of iron when strains forming acids abundantly were employed.

The iron appreciably increased the sugar consumption of strains forming acids weakly.

We are indebted to Mrs. Inkeri Heikkinen for assistance in some of the experiments.

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Received May 21, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 867–871

Studies on the Metabolism of *Aspergillus niger*

III. The Effect of Oxygen Tension on the Formation of Citric and Oxalic Acids in Surface Mould Cultures

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Little information is available concerning the effect of aeration and oxygen concentration on the oxydative fermentation by *Aspergillus niger*. Most of the studies reported so far on the utilization of oxygen by moulds have been conducted with the view of obtaining data on the effect of respiration on metabolism in general rather than on oxidative fermentations (*cf. e. g.*, Tamiya [1]).

This paper presents the results obtained from a study of citric and oxalic acid formations in surface cultures of moulds on employing different oxygen pressures. Since the previous experiments (Erkama *et al.*[2]) showed iron to have an appreciable effect on acid formation and since the effect of iron in some instances at least could be explained by accelerated respiration, the formation of acids in iron-free and iron-containing solutions was also studied.

EXPERIMENTAL

Culture conditions were similar to those used in the previous experiments except that the growth flasks now were plugged with rubber stoppers provided with an inlet tube for admittance of air near the mycelium surface and an outlet tube for taking samples from near the bottom of the flask. Moreover, the flasks were provided with two platinum electrodes and an agar bridge for making potential measurements as well as with a capillary tube for taking gas samples.

A mixture of oxygen and nitrogen was admitted at the rate of about 1.25 litres per hour. In the experiments three different gas mixtures containing 1 %, 9 %, and 71 % oxygen by volume were used. The mould strain employed was HG I. The results given are mean values of two experimental flasks.

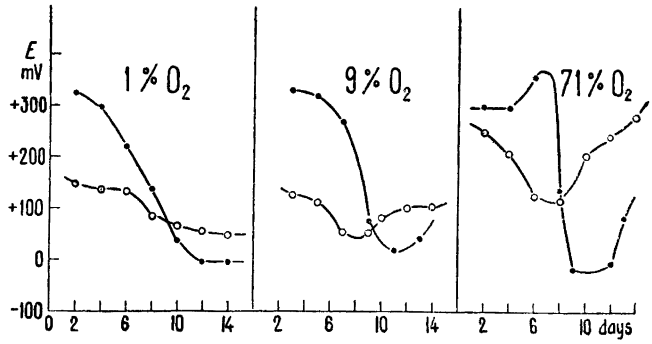

Fig. 1. Potential time curves.

● *Without Fe*
○ *With Fe*

Effect of oxygen content on electrode potential

It was found that when a uniform mould pellicle had developed the potential of the nutrient solution dropped quite abruptly. This drop in potential invariably occurred earlier in iron-free nutrient solutions than in those containing iron, the former developing mould more rapidly in the beginning than the latter. The higher the oxygen content, the more abrupt was the drop in potential (Fig. 1). In iron-containing nutrient solutions the potential always dropped more abruptly than in iron-free solutions.

According to Kusnetzow [3] citric acid cannot be formed in a solution if the potential underneath the mycelium does not drop below rH 17. In the present experiments the rH values corresponding to the potentials measured and referred to the saturated calomel electrode were generally smaller than 17 during intense acid formation; on employing the oxygen content of 71 % a rH-value of 11 was attained in iron-containing solution. On the other hand, on employing the 71 % oxygen concentration the potential did not drop below rH-value 18 in iron-free nutrient solution. Despite this fact the maximum yield of citric acid from the mould was obtained in this experiment.

Effect of oxygen content on acid formation

Examination of the curves in Fig. 2 reveals that when higher oxygen concentrations are employed also the yields of acid obtained are greater. Moreover, the fact that iron has an opposite effect in lower and higher oxygen concentrations deserves attention. In low oxygen concentrations the iron seems

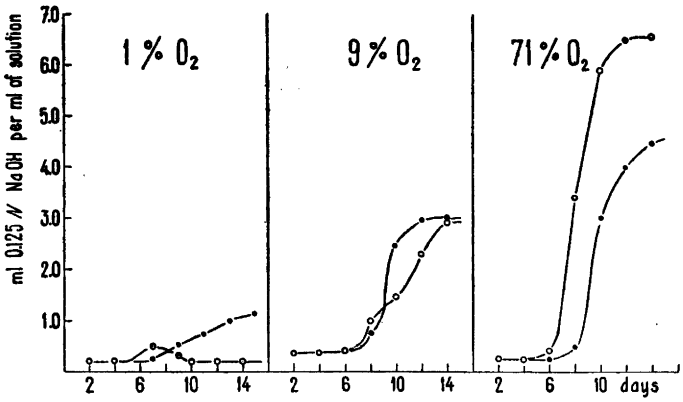

Fig. 2. Titratable acidity time curves.

○ *Without Fe*
● *With Fe*

to speed up acid formation, but in high oxygen concentrations the maximum yields of acid are obtained in iron-free nutrient solutions.

It was demonstrated previously [4] that aeration of the mycelium surface decisively affects the ratio of citric and oxalic acids formed in the nutrient solution, lower yields of citric acid and higher yields of oxalic acid being obtained in aerated cultures than in non-aerated cultures. Although in the investigations referred to above aeration was more intense than in the present experiments the gas in the growth flasks even here was renewed at the rate of about once an hour. Aeration as poor as this even sufficed to decrease markedly the yield of citric acid which in all the oxygen concentrations employed was lower than in non-aerated culture.

Tables 1 and 2 show the ratios between the yields of the two acids as compared with the results of the above-mentioned experiments [2, 4].

Examination of the tables shows that the unfavourable effect of iron on the formation of citric acid becomes evident either in non-aerated cultures or

Table 1. Formation of citric acid in different oxygen concentrations.

	Time of incubation, days	Citric acid formed in nutrient solution, mg/ml			
		unaerated	1 % O_2	9 % O_2	71 % O_2
Without iron	14	31.0	1.5	4.0	18.4
With iron	14	11.8	1.4	5.8	10.6

Table 2. Formation of oxalic acid in different oxygen concentrations.

	Time of incubation, days	Oxalic acid formed in nutrient solution, mg/ml			
		unaerated	1 % O_2	9 % O_2	71 % O_2
Without iron	14	4.6	0	5.0	15.0
With iron	14	6.2	4.0	4.5	9.9

in very high oxygen concentrations only. This observation provides a possible explanation to the disagreement in the reports on the effect of iron on citric acid formation. Aeration affects citric acid formation considerably more than oxalic acid formation. It is very likely that the direct effect of oxygen concentration is much smaller than that caused by the outflow of carbon dioxide through aeration which in turn results in the arrest of the Wood-Werkman reaction (*cf.* Erkama *et al.*[4]). This also partly explains why submerged cultures (*cf. e. g.*, Kluyver and Perquin [5]) never give as great yields of citric acid as surface cultures.

The above explanation is also in agreement with the fact that only in the highest oxygen concentration employed did the yield of oxalic acid equal that obtained on admitting atmospheric air at a rate of approximately 50 times greater than the rate used with oxygen. By using air the yield of oxalic acid increased during 14 days to 15.2 mg per ml while the yield of citric acid was only 2.8 mg per ml (Erkama *et al.*[4]). Consequently, it seems as if the effect of aeration on oxalic acid formation were chiefly due to the effectiveness with which carbon dioxide is blown out and not to the increase in oxygen concentration. This observation is of significance on studying the formation of fruit acids in higher plants.

The effect of iron on formation of oxalic acid in different oxygen concentrations is interesting. As seen from Table 2 iron seems to prevent formation of oxalic acid in high oxygen concentrations, but speeds it up in low oxygen concentrations.

Effect of oxygen content on catalase activity of moulds

It is likely that the respiration system of a surface mould culture differs from that of a submerged mould culture. Tamiya [1] remarks that a surface mould culture is more sensitive to variations in external oxygen pressure than a submerged hyphae which phenomenon he attributed to the fact that the respiration system of a surface mould culture which probably is a flavine

system, has less affinity for oxygen than the relatively more active iron system of a submerged hyphae. According to Tamiya iron respiration even in young surface cultures seems to be most intense underneath the mycelium.

Catalase determinations of moulds cultivated in different oxygen concentrations (Table 3) show that the catalase activity of a mould is greatest in low oxygen concentrations. Likewise the »pseudocatalatic» effect noted in moulds cultivated in iron-free nutrient solution was greatest in the moulds developed in low oxygen concentration.

Table 3. Catalase activity of Aspergillus niger cultivated under different oxygen pressures.

	Kat f/g dry matter		
	1 % O_2	9 % O_2	71 % O_2
Without iron	0.0047	0.0022	0.0015
With iron	0.0960	0.0039	0.0026

The iron porphyrine content of moulds seems to adapt itself as regards catalase at least, smoothly according to external oxygen pressure.

SUMMARY

The formation of citric and oxalic acids was examined in the surface cultures of *Aspergillus niger* under varying oxygen tension and iron concentration. The redox-potential of the nutrient solution as well as the catalase activity of the mould were also considered in these experiments. The effect of these various factors on acid formation is discussed on the basis of the results obtained.

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Received May 21, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 872–885

The Iodine-azide Reaction

I. The Catalytic Effect of the Tetrathionate Ion

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No reaction will take place in a solution containing triiodide and azide ions without the presence of a catalyst. Raschig[1], who was the first to describe this phenomenon, was of the opinion that the iodine-azide reaction was a typical catalytic reaction. Raschig ascertained that sodium thiosulphate and sodium sulphide act as catalysts.

The overall equation of the iodine-azide reaction is:

$$2\ N_3^- + I_2 = 3\ N_2 + 2\ I^-$$

Raschig [2] has proposed the following reaction mechanism for the iodine-azide reaction catalyzed by thiosulphate:

$$Na_2S_2O_3 + I_2 = NaIS_2O_3 + NaI$$
$$NaIS_2O_3 + 2\ NaN_3 = NaI + Na_2S_2O_3 + 3\ N_2$$

while tetrathionate is formed at the same time according to the equation:

$$NaIS_2O_3 + Na_2S_2O_3 = Na_2S_4O_6 + NaI$$

In his proposal, Raschig assumes that the tetrathionate does not catalyze the iodine-azide reaction. This has now been proved incorrect, and for this reason alone his mechanism of reaction must be held as rather unlikely.

Feigl [3] and others have shown that many compounds, which contain sulphide sulphur, catalyze the iodine-azide reaction. Examples are all inorganic sulphides [3], thiocyanates [3], carbon disulphide [4], tri-, tetra- and pentathionate [5] and many organic sulphur compounds [6] containing sulphidic sulphur.

Neither the authors mentioned above, nor the rather many others who have made use of the iodine-azide reaction, have tried to elucidate the reaction mechanism by kinetic measurements. Alkali metal salts of tetrathionic acid should be suitable for that purpose, since according to the literature, tetrathionate ions do not react with iodine or azide ions. According to Kurtenacker *et al.* [7], tetrathionate decomposes in aqueous solution to tri- and pentathionate. The rate of decomposition is fairly small at 25° C and is, within a pH-range of 9—5, independent of the pH.

REAGENTS

Sodium azide. Sodium azide, *p. a.* — after drying — was analyzed by oxidizing hydrazoic acid to free nitrogen by means of ceric salt [8] in excess.

NaN_3 (65.02) Calc. N 64.63 Found N 64.64, 64.59

Potassium tetrathionate: This salt is prepared according to the method of Raschig [9] which, in principle, consists of oxidation of thiosulphate by means of cupric ions:

$$3\ K_2S_2O_3 + 2\ CuSO_4 = Cu_2S_2O_3 + 2\ K_2SO_4 + K_2S_4O_6$$

The raw product of potassium tetrathionate was recrystallized four times from water. A polythionate analysis, as outlined by Kurtenacker [10] was carried out. The improved method of Foss [11] was used for analysis of the reaction between tetrathionate and potassium cyanide:

$$S_4O_6^{--} + 3\ CN^- + H_2O = S_2O_3^{--} + SO_4^{--} + SCN^- + 2\ HCN$$

This consists in principle of the titration of the thiosulphate formed with iodine in a solution acidified with acetic acid instead of sulphuric acid.

The potassium tetrathionate prepared was found to be 99.6 % pure and to contain no sulphate or potassium pentathionate. Furthermore, it was ascertained that the use of further recrystallized sodium azide and potassium tetrathionate did not alter the experimental results.

The other reagents used were all of analytical grade.

FIRST EXPERIMENTAL METHOD

According to Raschig [1, 2], the iodine-azide reaction takes place without interfering side reactions, and the amount of nitrogen evolved is proportional to the amounts of iodine and azide consumed. This result has been confirmed by Sommer and Pincas [12] and by Browne [13]. The present author has analyzed the gas evolved using tetrathionate as the catalyst and found that it consists only of nitrogen.

The original experiments were carried out by measuring the pressure, at constant volume, of the nitrogen evolved in a shaking apparatus rather similar to that described in detail by Brønsted[14] in 'Nitramidkatalytische Studien. II'. The volume of the flask, in which the reaction takes place, was about 200 ml. The flask — through an elastic glass spiral — was connected to a manometer, which was filled with mercury. The other side of the manometer was connected, also through an elastic glass spiral, to a compensation flask matching the reaction flask. During an experiment, the compensation flask contained sodium azide and iodine solution of the same concentration as in the reaction flask. Thus it was possible to compensate for vapour pressure. The necks of the two flasks were provided with a ground male connection and could be closed with a cap with a corresponding female connection. At the bottom of the cap a glass rod was fastened, the lower end of which was hook shaped. On this hook a small glass container — a 'vessel' could be placed. The catalyst solution was placed in this 'vessel'. By shaking, the 'vessel' can be made to fall into the solution of sodium azide and iodine in potassium iodide. The entire shaking apparatus, including the manometer, was, during an experiment, lowered into a water thermostat, which was kept at 25.00° ± 0.02° C. Stopcocks and ground joints were greased with a wax-containing stopcock grease which was not attacked by water at 25° C.

Performance of an experiment: A known amount of sodium azide solution and iodine, in potassium iodide solution, was pipetted with a Krogh syringe pipette into the reaction flask as well as into the compensation flask. Into the 'vessel', which was placed on the hook in the reaction flask, a known amount of potassium tetrathionate solution was introduced. When all the joints had been greased and connected, the entire shaking apparatus was lowered into the thermostat and the system evacuated until the solutions of azide and iodined bubble briskly. At this time the apparatus was allowed to stand for 30 minutes for the sake of complete temperature adjustment. The experiment was started by switching on the motor which works the shaking mechanism. The 'vessel' containing the tetrathionate thereby fell into the azide-iodine solution, and the evolution of nitrogen began. The nitrogen pressure, which is directly proportional to the amounts of iodine and azide consumed, was read at definite intervals. The shaking is sufficient when a still more rapid shaking does not alter the pressure-time curve.

Solutions used in the experiments: The concentrations of the solutions used were adjusted so that the ionic strength in the reaction mixture was as close to 0.37 as possible. The sodium azide solutions were made by weighing dried sodium azide and dissolving in water in a volumetric flask. For each experiment, 11.344 ml sodium azide solution was pipetted off. The iodine, in potassium iodide solution, was made by weighing out dried potassium iodide, *p. a.*, and resublimated iodine, and dissolving in water in a volumetric flask. Furthermore, the iodine concentration was determined by titration with standard sodium thiosulphate. 11.344 ml of the iodine in potassium iodide solution was used in each experiment. Potassium tetrathionate was weighed off in the 'vessel', after which 1.038 ml of water was added. The volumes mentioned above are the volumes of the syringe-pipettes measured at 20° C. In the following calculations the expansion of the reacting mixture from 20 to 25° C is not compensated for. This correction is, for pure water, about 0.12 %. Neither, is the alteration of volume due to the addition of potassium tetrathionate nor to the mixing taken into consideration. Since the largest amount of tetrathionate added is 20 mg, this correction is also without importance.

EXPERIMENTAL RESULTS

From the experimental results it is evident that the rate of reaction is proportional to the concentration of potassium tetrathionate and approximately proportional to the concentration of sodium azide. Consequently we have:

$$\frac{da}{dt} = k \cdot a \cdot c$$

where c is the concentration of potassium tetrathionate, and a is the concentration of sodium azide. The integrated form of this rate equation is:

$$k = \frac{2.303}{c \cdot t} \log \frac{a}{a-x} \qquad (1)$$

where t is the time of reaction, a is the initial concentration of sodium azide, and $a-x$ is the concentration of sodium azide at the time t.

To illustrate the treatment of the experimental results, an experiment (expt. no. 1) with the appurtenant calculations (Table 1) is shown; for each reading of the nitrogen pressure p (mm Hg) at the time t, the rate constant k according to (1) is calculated.

The rate constants of two other experiments, calculated in an analogous manner, are given in Table 2.

Many other experiments have been carried out, but as it turned out to be possible to use a much simpler method, the results of these experiments are not tabled. All the experiments carried out with a shaking apparatus show that the rate of reaction is: 1) directly proportional to the concentration of the catalyst, 2) approximately proportional to the concentration of sodium azide, and 3) independent of the concentration of iodine. Furthermore, the experiments seem to indicate that the iodide ions have a specific effect on the absolute rate of reaction; but there does not seem to be a simple dependence between rate constant and iodide ion concentration. The large rate constant in expt. no. 3 is partly due to the large concentration of iodide ions and partly to the very small concentration of sodium azide. A small concentration of azide ions seems to give a higher rate constant than a large concentration does (see later).

As the rate of reaction is independent of the concentration of iodine, it should be possible to carry out the rate experiments in such a manner that a relatively small amount of iodine solution is added to a solution which contains potassium tetrathionate, a relatively large amount of sodium azide, and

Table 1. Reaction between sodium azide and iodine at 25° C. Catalyst: Potassium tetra thionate. The initial concentrations were: $a = 0.2719\ M$; $c = 0.00279\ M$; $c_{I^-} = 0.0957\ M$; $c_{I_2} = 0.0476\ N$. *x, which is the amount of azide consumed, is calculated from the equation:*

$$x = \frac{p}{13.41} \cdot 0.0476.$$

The ionic strength was 0.376

Experiment 1

p mm Hg	t Time in min	x	$a-x$	$\log \frac{a}{a-x}$	$k = \frac{2.303}{c \cdot t} \cdot \log \frac{a}{a-x}$
0	0	0	0.2719		
0.71	1	0.0025	0.2694	0.0040	3.30
1.52	2	0.0054	0.2665	0.0087	3.59
2.21	3	0.0079	0.2640	0.0128	3.52
2.99	4	0.0106	0.2613	0.0173	3.57
3.78	5	0.0134	0.2585	0.0219	3.62
4.47	6	0.0159	0.2560	0.0262	3.60
5.14	7	0.0182	0.2537	0.0301	3.55
5.80	8	0.0206	0.2513	0.0342	3.53
6.47	9	0.0230	0.2489	0.0384	3.52
7.16	10	0.0254	0.2465	0.0426	3.52
7.76	11	0.0276	0.2443	0.0465	3.49
8.32	12	0.0295	0.2424	0.0499	3.43
8.92	13	0.0317	0.2402	0.0538	3.42
9.50	14	0.0337	0.2382	0.0575	3.39
9.96	15	0.0354	0.2365	0.0605	3.33
10.53	16	0.0374	0.2345	0.0642	3.31
11.10	17	0.0394	0.2325	0.0679	3.30
11.61	18	0.0412	0.2307	0.0714	3.27
12.13	19	0.0431	0.2288	0.0749	3.26
12.64	20	0.0449	0.2270	0.0784	3.24
13.17	21	0.0467	0.2252	0.0819	3.22
13.41	22	0.0476			
13.41	23	0.0476			

some starch solution. When all iodine is used up, the blue colour of the solution should disappear, and the time of reaction should be inversely proportional to the rate of reaction. This assumption has proved to be correct.

NEW EXPERIMENTAL METHOD

Into a 300 ml Erlenmeyer flask was pipetted 30 ml of a solution, which was 4.013 M with respect to sodium nitrate and 0.1 M to potassium iodide, 10 ml potassium tetrathionate solution, 0.2 ml 0.5 % starch solution, and 10 ml of a solution, which was 0.01 N

Table 2. Reaction between sodium azide and iodine at 25° C. Catalyst: Potassium tetrathionate. The initial concentrations in experiment No. 2 were: $a = 0.1767\ M$; $c = 0.00279\ M$; $c_{I^-} = 0.1903\ M$; $c_{I_2} = 0.0473\ N$; and the ionic strength was 0.375. The initial concentrations in experiment No. 3 were: $a = 0.02389\ M$; $c = 0.00279\ M$; $c_{I^-} = 0.2721\ M$; $c_{I_2} = 0.0480\ N$; and the ionic strength was 0.304.

Experiment 2			Experiment 3		
p mm Hg	t Time in min	$k = \frac{2.303}{c \cdot t} \log \frac{a}{a-x}$	p mm Hg	t Time in min	$k = \frac{2.303}{c \cdot t} \log \frac{a}{a-x}$
0.55	1	3.96	0.33	1	17.4
1.20	2	4.33	0.62	2	16.6
1.83	3	4.43	0.90	3	16.4
2.43	4	4.44	1.16	4	16.3
3.04	5	4.46	1.42	5	16.3
3.62	6	4.43	1.68	6	16.4
4.20	7	4.42	1.91	7	16.3
4.75	8	4.40	2.12	8	16.2
5.30	9	4.38	2.36	9	16.4
5.81	10	4.36	2.60	10	16.7
6.83	12	4.33	3.00	12	16.7
7.81	14	4.29	3.39	14	17.0
8.82	16	4.28	3.78	16	17.4
9.78	18	4.26	4.11	18	17.6
10.70	20	4.24	4.42	20	17.9
11.58	22	4.22	4.99	24	18.6
12.41	24	4.19	5.43	28	19.1
13.22	26	4.16	5.80	32	19.7
13.61	28		6.10	36	20.4
13.61	30		6.29	40	20.5
			6.42	44	20.3
			6.53	50	19.3
			6.64	60	17.6
			6.70	70	16.1
			6.78	90	
			6.83	110	
			7.00	310	
			7.00	390	

with respect to iodine and 0.02 M to potassium iodide. The flask was placed in a water thermostat, so that only the neck was above the water. Behind the flask a plate of white glass was inserted, and in front of the thermostat, the front of which was clear glass, an electric bulb was mounted and screened so that a ray of light was directed through the Erlenmeyer flask against the white glass plate. It was now very easy to see any colour change. Before continuing the experiment the Erlenmeyer flask was allowed to stand in

Table 3. Reaction between sodium azide and iodine at 25° C. Catalyst: Potassium tetrathionate. In all four experiments — besides the solutions mentioned in the table — were added 0.2 ml starch solution and 39 ml solution, which was 4.013 M with respect to sodium nitrate and 0.1 M to potassium iodide. In experiments 2 and 4 (in which 5 ml 0.5 M sodium azide is added instead of 10 ml) for the sake of constant ionic strength, 5 ml 0.5 M sodium nitrate was added. The concentrations of the stock solutions used were: Potassium tetrathionate: 0.01 M; iodine: 0.00982 N with respect to iodine and 0.02 M to potassium iodide; and sodium azide: 0.5 M.

Expt. no.		c	a	x	t Time in min	$k = \frac{2.303}{c \cdot t} \log \frac{a}{a-x}$
1	5 ml tetrathionate 5 ml water 10 ml iodine 10 ml sodium azide	0.000831	0.0831	0.001631	5.70	4.13
2	5 ml tetrathionate 5 ml water 5 ml iodine 5 ml water 5 ml sodium azide 5 ml sodium nitrate	0.000831	0.04153	0.000816	5.57	4.31
3	5 ml tetrathionate 5 ml water 5 ml iodine 5 ml water 10 ml sodium azide	0.000831	0.0831	0.000816	2.78	4.19
4	10 ml tetrathionate 10 ml iodine 5 ml sodium azide 5 ml sodium nitrate	0.001661	0.04153	0.001631	5.42	4.45

the thermostat for 15 minutes (this has proved to be sufficient for temperature adjustment). In addition a bottle containing 0.5 *M* sodium azide solution was placed in the thermostat. After the elapse of 15 minutes, 10 ml 0.5 *M* sodium azide was sucked into a pipette. This was immediately allowed to run down into the Erlenmeyer flask, which

Table 4. Effect of addition of various salts on the rate of reaction of the tetrathionate-catalyzed iodine-azide reaction. In each experiment 10 ml 0.00982 N iodine in 0.02 M potassium iodide, 10 ml 0.01 M potassium tetrathionate, 0.2 ml starch solution, and 5 ml 0.5 M sodium azide were used. Furthermore, the various solutions mentioned in the table were added. The concentrations in the reacting solutions were: $c = 0.003105$; $a = 0.07762$ and $x = 0.00305$.

Expt. no.		t Time in min	$k = \frac{2.303}{c \cdot t} \log \frac{a}{a-x}$
1	5 ml water	10.13	1.28
2	5 ml 0.5 *M* sodium chloride	9.17	1.42
3	5 ml 0.5 *M* potassium iodide	5.57	2.33
4	5 ml 0.5 *M* sodium nitrate	9.37	1.39
5	5 ml 0.5 *M* sodium acetate	9.32	1.39
6	5 ml 6 *M* sodium nitrate	5.57	2.33
1 a	5 ml water	10.15	1.28

at the same time — partly immersed in the thermostat water — was kept in a rotary motion with the left hand. When half the sodium azide had run out of the pipette, a stopwatch was started. The last drop in the pipette was blown out, and the flask was kept rotating for an additional 10 sec. The instant the colour in the experimental solution disappeared, the watch was stopped.

The volume in each experiment was 60.2 ml. The reason the solution was made approx. 0.05 *M* with respect to potassium iodide, is that the reduction of iodine to iodide ions would otherwise cause a considerable increase in the concentration of iodide ions. All the pipettes used were controlled by weighing out with water.

The results (see Table 3) of this experimental method are quite in accordance with those previously obtained, since the rate of reaction is found to be independent of the concentration of iodine, proportional to the concentration of the catalyst, and approximately proportional to the azide ion concentration, although decreasing azide ion concentration seems to give a slightly too large rate of reaction.

Table 4 represents an investigation of the effect of addition of various salts. It will be noticed that increasing concentration of a salt causes an increase in the rate of reaction. This is to be expected since the rate determining reaction step is a reaction between an azide ion and a tetrathionate ion (see later),

which are both negatively charged. Of course, the ionic strength of the solutions in question is too large to allow for quantitative considerations. From the results it is seen that sodium chloride, sodium nitrate and sodium acetate have practically the same effect. Potassium iodide, however, has a specific rate increasing effect, which is in accordance with what the original measurements seemed to indicate. This effect cannot be explained by a shift in the equilibrium $I_2 + I^- \rightleftharpoons I_3^-$. There does not seem to be a simple dependency between iodide ion concentration and rate of reaction.

THE EFFECT OF pH ON THE RATE OF REACTION

Hydrazoic acid is approximately of the same strength as acetic acid. According to Hughes [15] the ionization constant of hydrazoic acid is about 2×10^{-5} at 25° C, *i. e.* the pH of a 0.1 *M* sodium azide solution is roughly 9, and very close to 100 % of the sodium azide dissolved will be on hand as free azide ions.

Two experiments with tetrathionate as the catalyst were carried out in boric acid — borate buffered solutions with identical concentrations of sodium nitrate, sodium azide, potassium tetrathionate, and starch. As usual the time of decoloration of identical amounts of iodine were measured at 25° C. In two solutions of pH 9.19 and 8.69 respectively, the times of decoloration were the same within 1 %. From this fact it follows that it is the azide ions which take part in the reaction, because the concentration of free azide ions is practically constant, whereas the concentration of undissociated hydrazoic acid varies considerably in the two experiments.

A series of experiments was carried out (without addition of sodium nitrate), in such a way that the concentrations of sodium azide, potassium tetrathionate and iodine were the same in all experiments. In addition, varying amounts of hydriodic acid were added, so that the azide ions were partly converted into un-ionized hydrazoic acid. Accordingly varying amounts of potassium iodide were added, so that the total concentration of iodide ions was the same in all experiments. The solution of hydriodic acid was made from iodine and water by reduction with hydrogen sulphide. The excess of hydrogen sulphide was removed by boiling out in a current of carbon dioxide. The concentration of hydriodic acid was determined by titration with standard sodium hydroxide. The result showed an approx.- but not exact-proportionality between the rate of reaction and the concentration of free azide ions. When an excess of hydriodic acid was added, so that all azide ions were converted into hydrazoic acid, the rate of reaction was extremely small. Consequently we have good reason to assume that only free azide ions — and not hydrazoic acid — take part in the iodine-azide reaction.

EXPERIMENTS WHICH SHOW THAT TETRATHIONATE IONS DO NOT DECOMPOSE WHEN CATALYZING THE IODINE-AZIDE REACTION

A few experiments were carried out as follows: A small amount of tetrathionate solution and some starch were added to a solution with a large concentration of sodium azide and small concentration of iodine. The time of decoloration was determined. At the instant the colour changed from light blue to colourless, a small amount of additional iodine solution was added, and the time of decoloration determined. From these two reaction times two rate constants can be calculated according to equation (1). The two constants were the same within the range of experimental error.

In this connection it is worth mentioning that a water solution of potassium tetrathionate shows an increased activity after standing some days, and the older the solution becomes the more the activity increases. This phenomenon is undoubtedly due to the fact that tetrathionate gradually decomposes to tri- and pentathionate [7].

Although tetrathionate does not seem to decompose essentially when acting as catalyst, it has been nevertheless ascertained that when rather concentrated solutions of sodium azide and potassium tetrathionate are mixed, a visible reaction takes place evolving a gas. An experiment was carried out by dissolving 1 g potassium tetrathionate in 10 ml 0.5 *M* sodium azide. After two hours approx. 10 ml of a gas had been evolved. The gas was analyzed * according to Christiansen and Wulff [16]. The result was 98.9 % nitrogen and 1.2 % oxygen. This small percentage of oxygen was probably due to contamination by atmospheric air. Consequently, it is believed that tetrathionate ions oxidize azide ions essentially to free nitrogen.

ENERGY OF ACTIVATION

The energy of activation of the tetrathionate catalyzed iodine-azide reaction was determined by experiments analogous to those previously described. The rate of reaction was determined in the temperature range 20° to 40° C. The experiments were carried out partly without addition of sodium nitrate and partly in a solution, which was 1 *M* with respect to sodium nitrate. In each experiment of the first series 10 ml 0.0200 *N* iodine in 0.500 *M* potassium iodide, 1 ml 0.05 *M* potassium tetrathionate, 9 ml water, 20 ml water, 0.2 ml starch solution and 10 ml 0.1 *M* sodium azide were used. In the second series the 20 ml water was substituted by 20 ml 2.5 *M* sodium nitrate solution.

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* I am greatly indebted to Miss I. Wulff, who carried out the analysis.

Table 5. Energy of activation of the tetrathionate catalyzed iodine-azide reaction. Concentrations in all experiments: $c = 0.000996$ M; $a = 0.01992$ M; $x = 0.00398$. $k_{exp.}$ is the rate constant calculated in each case from equation (1). $k_{calc.}$ is calculated from the equation $\log k = H - \frac{A}{T}$, which is a straight line fitted to the experimental k and T values.

	No sodium nitrate added			The solution is 1 *M* as to sodium nitrate		
Temp. °C	Time in min	$k_{exp.}$	$k_{calc.}$	Time in min	$k_{exp.}$	$k_{calc.}$
20°	102.03 103.25	2.194 2.168	2.168	48.57 48.33	4.608 4.631	4.603
25°	70.40 70.42	3.179 3.179	3.126	33.18 33.38	6.745 6.708	6.730
30°	50.67 50.55	4.418 4.428	4.467	23.60 23.58	9.484 9.493	9.728
35°	36.72 36.80	6.095 6.083	6.295	16.55 16.52	13.52 13.55	13.86
40°	24.63 24.82	9.089 9.018	8.790	11.32 11.33	19.77 19.75	19.54

Using the method of least squares, the numerical values of H and A were calculated according to the equation

$$\log k = H - \frac{A}{T}$$

In the first series of experiments, in which no sodium nitrate was added, the result is

$$\log k = 9.842 - \frac{2786}{T}$$

Values of k calculated from this equation are, together with the experimental values, shown in Table 5. The energy of activation was:

$$A \times 4.571 = 2786 \times 4.571 = 12740 \text{ kcal/mole}$$

From the second series of experiments, in which the solution was 1 M with respect to sodium nitrate, was analogously calculated

$$\log k = 10.47 - \frac{2874}{T}$$

The experimental as well as the calculated values of k are shown in Table 5. The energy of activation was calculated to be 13140 kcal/mole. The frequency exponents H, which had values of 9.842 and 10.47, were — with 1 sec. as unit — respectively 9.84 — log 60 = 8.06 and 10.47 — log 60 = 8.69. These values agree well with those to be expected, when an ion of two negative charges (the tetrathionate ion) reacts with an ion of one negative charge (the azide ion) (*cf.* Moelwyn-Hughes: *Kinetics of reactions in solution* [17]). As was expected the addition of a neutral salt increased the rate of reaction.

DISCUSSION

It has been shown that the rate of the tetrathionate catalyzed iodine-azide reaction is proportional to both the concentration of potassium tetrathionate, and to the concentration of sodium azide, but is independent of the concentration of iodine. From the investigations of the effect of pH on the rate of reaction it is evident that only azide ions participate in the process. The integrated rate equation is consequently:

$$k = \frac{2.303}{c \cdot t} \log \frac{a}{a-x} \qquad (1)$$

where k is the rate constant, c is the concentration of potassium tetrathionate, t is the time, a is the initial concentration of azide ions, and $a-x$ is the concentration of azide ions at the time t.

Tetrathionic acid is a rather strong acid and therefore potassium tetrathionate, in a slightly basic solution as is the case in a sodium azide solution, will be on hand as potassium ions and tetrathionate ions. The rate determining reaction step must therefore be:

$$N_3^- + S_4O_6^{--} \rightarrow N_3S_4O_6^{---} \qquad (2)$$

The reversal of reaction (2) must be without importance in this case. This reaction (2) can be shown to take place (*cf.* a following publication) even if iodine is not present. Therefore it is reasonable to assume that it is also the

first step in a sequence of reactions. As the reaction between the complex and the iodine is instantaneous, it is impossible by kinetic investigations to elucidate the mechanism of this reaction.

Feigl [4] assumes, on the basis of diverse qualitative experiments and the fact that carbon disulphide and azide ions in water solution react with the formation of azido-dithiocarbonate ions, that the mechanism of the carbon disulphide catalyzed iodine-azide reaction is as follows:

$$2\ NaN_3 + 2\ CS_2 \longrightarrow 2\ N_3\ S\ C\ S\ Na \qquad (I)$$

$$2\ N_3\ S\ C\ S\ Na + I_2 \longrightarrow N_3{-}C(=S){-}S{-}S{-}C(=S){-}N_3 + 2\ NaI \qquad (II)$$

$$N_3{-}C(=S){-}S{-}S{-}C(=S){-}N_3 \longrightarrow 2\ CS_2 + 3\ N_2 \qquad (III)$$

$$2\ NaN_3 + I_2 \longrightarrow 3\ N_2 + 2\ NaI \qquad (IV)$$

Browne [13] has also investigated the iodine-azide reaction with carbon disulphide as the catalyst — although without carrying out kinetic measurements. Browne has shown the existence of the azido-carbondisulphide, which Feigl had postulated, and shown that it reacts very rapidly with sodium azide solution with the liberation of nitrogen. Browne proposes a reaction mechanism rather similar to that of Feigl, but with the difference that the azido-dithiocarbonate ion is assumed to be the actual catalyst.

As long as no other mechanism is proved, it is natural to formulate the mechanism of the tetrathionate catalyzed iodine-azide reaction analogous with the mechanism of the carbon disulphide catalyzed reaction:

$$N_3^- + S_4O_6^{--} \longrightarrow N_3S_4O_6^{---} \qquad (2)$$

$$2\ N_3S_4O_6^{---} + I_2 \longrightarrow (N_3S_4O_6)_2^{----} + 2\ I^- \qquad (3)$$

$$(N_3S_4O_6)_2^{----} + 2\ N_3^- \longrightarrow 3\ N_2 + 2\ \overset{*}{N_3}S_4O_6^{---} \qquad (4)$$

$$\overset{*}{N_3}S_4O_6^{---} \longrightarrow N_3^- + S_4O_6^{--} \qquad (5)$$

where (2) is the rate determining reaction step, and (3), (4) and (5) are instantaneous. $\overset{*}{N_3}S_4O_6^{---}$ is assumed to be an activated ion which decomposes readily. As the reaction step, in which iodine participates, is instantaneous, it is impossible to decide whether it is triiodide ions or iodine molecules — or both — which react. It is worth mentioning that a solution of iodine in alcohol reacts in the same way as a solution of iodine in potassium iodide.

SUMMARY

Kinetic investigations were carried out on the iodine-azide reaction catalyzed by potassium tetrathionate. The reaction was a second order reaction with respect to azide ions and tetrathionate ions. The rate of reaction was found to be independent of the concentration of iodine. The rate determining reaction step was a reaction between azide ions and tetrathionate ions. The old theory of Raschig [2] and Feigl [3], assuming formation of complex compounds between iodine and sulphur compounds, must be abandoned, and in its place a reaction mechanism is proposed, which shows a considerable analogy with that proposed by Feigl [4] and Browne [13] for the carbon disulphide catalyzed iodine-azide reaction. The energy of activation of the tetrathionate catalyzed iodine-azide reaction was determined.

The author wishes to thank Professor J. A. Christiansen for his interest in this investigation and for many helpful discussions.

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Received June 2, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 886–894

Investigations on Malt Amylase

III. On the Colorimetric Determination of α-Amylase

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Several investigations have been performed on the colour reaction between starch and iodine, and several methods have been proposed making use of this reaction for the determination of amylase activity. This paper is intended to discuss some aspects of this subject and to give an account of a rapid method for the colorimetric determination of α-amylase.

A quantitative colorimetric assay of amylase was first described by Roberts [1], who measured the time necessary for the break down of a starch solution — prepared in a certain way — to the achroic stage and calculated amylase activities therefrom.

Hanes and Cattle [2] investigated the alteration in iodine coloration during the action of various amylases on starch. The extinction was measured at wave-lengths between 4300 and 7500 Å. The wave-length for maximum absorption was found to change towards shorter wave-lengths as the break down proceeds from about 6000 Å for soluble starch. Hanes and Cattle found a filter corresponding to the wave-length 5700 Å to be suitable for the colorimetric assay of amylase. This is due to the fact that they followed mainly the early part of the break down.

By plotting extinction values at 5700 Å against the corresponding values for the reducing power of the starch solution submitted to hydrolysis by various amylases, these authors found pronounced differences between the starchsplitting enzymes and even between α-amylases of varying origin. They explained this by the different capacities of the various enzymes to attack the large starch molecules and the smaller dextrin molecules.

Bernfeld and Fuld [3] have recently compaired various α-amylases by calculating the quotient saccharogenic activity divided by colorimetrically deter-

minated dextrinogenic activity, but did not find any differences in the early part of the break down.

A difference in the capacity of the various α-amylases to break down large and small polysaccharide molecules has been demonstrated quantitatively by Myrbäck and co-workers [4–7]. Their results seem to indicate that differences in the capacities of the reactiou products to give coloured compounds with iodine may be expected first at a late stage in the depolymerization.

It has been reported in several papers, *e. g.* those by Blom, Bak and Braae [8] and especially by Hanes and Cattle [2], that in the colorimetric determination of malt amylase the enzymic action depends on both the α- and the β-amylase.

The influence of various quantities of β-amylase on the results of colorimetric assays of malt α-amylase was investigated by Sandstedt, Kneen and Blish [9] *. They found that on the addition of increased amounts of β-amylase, the time necessary for the break down to a certain stage first diminished rapidly, but soon reached a value where addition of more β-amylase had no appreciable influence.

Thus, by keeping the amount of β-amylase sufficiently high in the reaction mixture, it is possible to get an assay of the amount of α-amylase in a mixture of α-and β-amylase.

Moreover, it might be possible (but probably not practical) to assay the β-amylase colorimetrically in solutions free from α-amylase by preparing a reaction mixture from a given amount of α-amylase, the β-amylase solution and starch solution.

In the last few years several authors [12–19] have proposed various methods for determining a suitable stage in the break down up to which the reaction time is counted. In order to render colorimetric amylase determinations comparable, a conversion factor should always be included, giving the capability of the enzyme to form reducing sugars, *e. g.* milliequivalents reducing sugars per minute and gram, or maltose equivalents. It must be observed [15, 20] that the time necessary for the break down of solutions of starch of different initial degree of polymerization differs somewhat.

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* They characterize their method as a standardized Wohlgemuth procedure. This seems to the present author to be erroneous. Wohlgemuth's method [10] is characterized by the simultaneous running of several experiments with various amounts of amylase for a constant time. Roberts [1] on the other hand, withdraws samples at suitable times from one reaction mixture. Roberts' method is furthermore the earlier. It is evident from this that the standardized method should be regarded as a modification of Roberts' method. This is true also of Ehrnst and coworkers' procedure [11, 12].

EXPERIMENTAL

On the shape of the extinction curves

The works of the present author on the colorimetric determination of α-amylase aimed first at finding out how a high degree of accuracy could be attained in a simple way. It is thereby desirable to get the same relative accuracy irrespective of the amount of α-amylase. This can be effected in the way suggested by Wohlgemuth [10] by preparing several reaction mixtures with the amylase activities forming a geometric series [10, 21]. Another way is to take the samples at times, forming a geometric series. A time table was thus prepared, in which the logarithm of the quotient of one time divided by the preceding was always 0.1. A table for more accurate determinations was also prepared, the logarithm of the quotients being 1/30.

The selection of a suitable colour standard for the determination of the end point is rendered difficult by the fact that the wave-length of the adsorbtion maximum is displaced during the break down towards or perhaps even outside the violet part of the visible spectrum [2]. In order to avoid this difficulty and to treat the problem more accurately, the extinction coefficient of the solutions were determined with a step-photometer, Leitz' Leifometer. The time necessary for the break down until the extinction at 5300 Å was 1, *i. e.* the transmission of light through a 1 cm layer was 10 %, was chosen as the reaction time.

The reaction mixture was made up of 3 ml of enzyme solution, 5 ml of an acetate buffer solution, an appropriate amount of a stock starch solution to render the reaction mixture 1 %, and water to 50 ml. In certain experiments part of the water was exchanged for appropriate amounts of β-amylase prepared as in Ohlsson [21].

The stock starch solution was prepared from Lintner [22] starch suspended in cold water and poured into boiling water. It was preserved in a flat-bottomed flask fitted with a stopper with an adapter and a glass tube leading from the bottom of the flask to the side tube of a burette. The system was sterilized by steam and the adapter and the burette plugged with cotton. Thus the starch solution could easily be preserved for some time and an appropriate amount easily withdrawn. The starch concentration of the solution — about 2 % — was determined from the dry weight of an aliquot part of the solution. When solutions were prepared from this stock solution, the solution withdrawn was first heated until it became quite clear.

The iodine reagent was prepared as suggested by Sandstedt, Kneen and Blish [9], and 1 ml of the reaction mixture added to 5 ml of the iodine solution. The extinction was read at about 20° C.

In three series, the apparent activities of α-amylase solutions were determined in the presence of various amounts of β-amylase. The results are given in Table 1.

Table 1. Determination of α-amylase activity in the presence of various amounts of β-amylase.

α-Amylase solution %	β-Amylase activity of the reaction mixture mg maltos/min · ml	Apparent α-Amylase activity mg starch/min · ml
0.08	0	11
	0.2	14
	1.0	30
	2.4	41
	4.9	48
	9.7	62
0.008	0	0.09
	0.2	4.2
	2.4	5.8
	4.9	6.2
0.0008	0	0.06
	0.2	0.65
	2.4	0.66

The concentration of the α-amylase solution is given in % of dry preparation, manufactured as described earlier [23]. The β-amylase activity of the reaction mixture was calculated from the activity and the amount of the β-amylase solution added.

By plotting the extinction coefficients E against the logarithms of time in these experiments, Figure 1 was obtained. In order to faciliate a close comparison of the three series with different amounts of α-amylase, the time notations in the series with the 0.008 % solution was divided by 10 and in the series with the 0.0008 % solution by 100.

When the extinction coefficients are plotted against log time and not against time, curves from assays of amylase solutions of different activities can be compared. In Fig. 1 the extinction coefficient curves from experiments with various amounts of α-amylase and β-amylase have the same shape if they correspond to apparent activity values that are the same fraction of the highest possible activity (in the presence of enough β-amylase).

From Fig. 1 we also see that to achieve considerable accuracy in the determinations it is desirable to take samples and colorimeter readings from the reaction mixture at times forming a geometric series in which the logarithm of the quotient is 1/10 to 1/30.

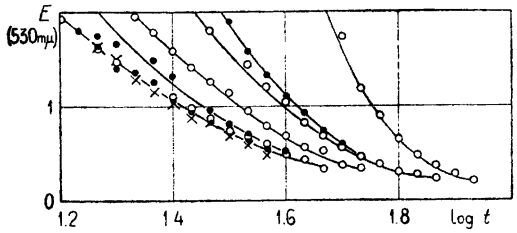

Figure 1. The extinction at 530 mμ as a function of log time with various amounts of α- and β-amylase.
The series of rings correspond to the activities 62, 48, 41 and 30. The series of dots correspond to the activities 6.2, 5.8, and 4.2. The series of crosses corresponds to the activity 0.66.

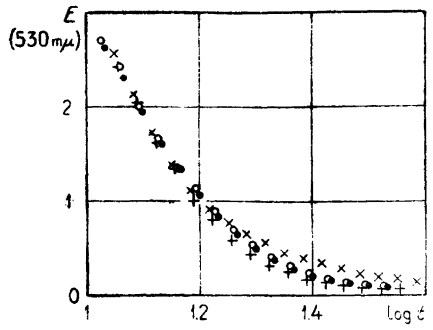

Figure 2. Extinction against log time curves from the break down of starch with malt α-amylase (dots), pancreatic amylase (rings), mould amylase (crosses ·) and salivary amylase (crosses –).

Comparison of extinction curves using various α-amylases

Some experiments were performed in the manner described with malt α-amylase, pancreatic amylase, mould amylase (Takadiastase) and salivary amylase in order to compare the shape of the extinction curves in the final part of the break down. The extinction values were plotted against the time, and to faciliate a close comparison, a suitable constant was added to the logarithm of the time notations in order to render the initial parts of the extinction curves coincident. The results given in Fig. 2 indicate that the differences in the action on starch by the various amylases give raise to slight differences in the shape of the extinction curves in the part near the achroic stage.

The influence of the initial molecular weight of the starch

The rapid method described later in this paper was used in these experiments. Two starch solutions were prepared, from common potato starch and from a lot of potato starch treated with 1.5 *N* hydrochloric acid, washed and dried. The molecular weight of the two lots were determined from viscosi-

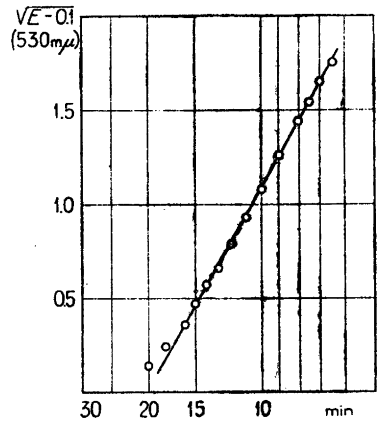

Figure 3. $\sqrt{E-0.1}$ *plotted against the logarithm of the time. It should be observed that the inclination of the line is always the same.*

metric measurements after extrapolation to zero concentration, using Staudinger and Husemann's value for the viscosity-molecular weight constant $K_m = 0.63 \cdot 10^{-4}$.

The mol. wt. and the time necessary for the break down until the extinction $E = 1$ was reached are given in Table 3.

Table 3. Comparison of the times required for the enzymatic breakdown of starch of various molecular weights.

M	t min
470 000	25.3
27 000	21.3

It is evident that the reaction time is influenced both by the initial and the final polymerization degree of the starch, and that it is rather vague to speak about the amount of starch broken down in colorimetric amylase assays.

A rapid method

For a rapid assay of the activity of amylase solutions containing no β-amylase, the present author has developed the following procedure, which renders the simultaneous performance of 5 assays possible.

2 ml of enzyme solution were pipetted into each of five 100 ml Erlenmeyer flasks and placed in a thermostat at 30° C. A discussion on the necessary accuracy of the temperature assay is given in a previous paper [24] by the present author. A 1 % buffered starch solution, made up from a suitable amount of a stock starch solution, buffer and water, was also preserved in the termostat. A stop watch was started, and at the time 1 minute 20 ml of the buffered starch solution was pipetted into the first Erlenmeyer flask and the flask shaken. When the stop watch showed 2 minutes, 20 ml was pipetted into the second flask and so on. 1 ml of the reaction mixture was withdrawn from each flask 10, 15, 25, 40 and 60 minutes after the starch had been added (if necessary) and poured into 5 ml of the iodine solution. The extinction coefficient was measured at 5300 Å for the solutions suitably coloured.

The moment corresponding to the extinction coefficient $E = 1$ could easily be obtained with tolerable accuracy in the following way. The square root — calculated on a slide rule — of the extinction coefficient was plotted on logarithm paper against the time (*i. e.* the square root plotted against the logarithm of the time). Then an almost straight line is obtained, and the time corresponding to the square root = 1 was read off. A still better result was obtained if the square root of the extinction coefficient minus 0.1 was taken, and the time read off as corresponding to the value 0.95 of this root. The values obtained from a control series are shown in Fig. 3.

Hanes and Cattle [2] found that the extinction decreases linearly with prolonged breakdown at the early part. That the square root of the extinction decreases linearly during some later part of the breakdown is only due to the fact that the reaction is retarded towards the end. If this happens at a stage where the colour may easily be assayed, one can make use of it to faciliate the calculation.

A conversion factor was determined by running assays of a malt α-amylase solution colorimetrically according to the method given here and by iodimetric determinations of reducing sugars according to Blom and Rosted's [25] modification of Linderstrøm-Lang and Holter's [26] method. The amylase amount 1 mg starch/min corresponded to 0.14 mg maltose/min if no β-amylase was present.

The principles of this procedure may of course also be applied to the Sandstedt, Kneen and Blish [9] method. The buffered β-amylase limit dextrin solution is then substituted for the buffered starch solution.

SUMMARY

1. A short review is given of the colorimetric assay of amylase. Some methods said to be modifications of Wohlgemuth's method should instead be characterized as modifications of Roberts' method.

2. If the extinction values are plotted against the time in colorimetric assays with various amounts of α- and β-amylase, only curves corresponding to activity values that are the same fraction of the highest possible activity (in the presence of enough β-amylase) have the same shape.

3. Extinction/log time — curves for α-amylases of different origin differ somewhat at the latest stage of the break down.

4. A rapid procedure for the simultaneous running of 5 colorimetric assays is described. 20 ml of a 1 % buffered starch solution is added at intervals of 1 minute to Erlenmeyer flasks containing 2 ml of enzyme solution. Samples are withdrawn after 10, 15, 25, 40, and 60 minutes if necessary and pipetted into iodine solution. Extinction values E are taken at 5300 Å. The values of $\sqrt{E-0.1}$ are plotted against the logarithm of the time and lie on a straight line at a suitably late stage of the break down. If the reaction time is counted until $E = 1$ (corresponding to $\sqrt{E-0.1} = 0.95$), the amylase amount 1 mg starch/min corresponds to 0.14 mg maltose/min.

This investigation was financially supported by *Statens Tekniska Forskningsråd* and *Statens Naturvetenskapliga Forskningsråd*. Prof. Karl Myrbäck kindly granted me the use of his laboratories. Miss Margareta Kjellén and Miss Irma Sjögårdh assisted in performing the experiments. The English text was revised by Mrs William Cameron. For all this help I wish to express my cordial thanks.

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Received June 20, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 895–897

Investigations on Malt Amylase

IV. An Enzymic Method for the Determination of Viscosity-Molecular Weight Constants

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Viscosimetrical methods have frequently been used for the assay of the molecular weight of polymeric homologous substances giving solutions of high viscosity. An expression for the connection between the viscosity and the molecular weight is given by the modified Arrhenius-Staudinger formula [1, 2]

$$\ln \eta_r = K_m c_{gm} M \qquad (1)$$

where

η_r = the relative viscosity,
K_m = the viscosity-molecular weight constant,
c_{gm} = the concentration in primary moles per litre, and
M = the molecular weight.

This formula, however, is not valid for all polymeric homologous substances [3, 4]. Reviews on this subject have recently been published by Ewart [5] and by Kinell [6].

The substances for which the modified Arrhenius-Staudinger formula is valid, are characterized by one constant, K_m. If such a substance can be submitted to enzymatic depolymerization under such conditions that all linkages are broken with equal ease, and if the break down can be followed both viscosimetrically and by end group determinations, the viscosity-molecular weight constant can easily be determined, if some approximations are made. This will be shown in the case of starch and malt α-amylase in this article. Starch is certainly not one substance but two, amylose and amylo-

pectin, occuring in given proportions. Thus the values obtained from this mixture will depend on the K_m values for each of them and the proportions. This has, however, no influence on the principles of the method, and as starch has also been used in earlier measurements, the results from these measurements can be compared with the present results.

The number of moles of reducing sugars liberated per litre of a starch solution in unit time when submitted to hydrolysis by malt α-amylase can be determined both viscosimetrically and iodometrically. The number determined viscosimetrically is [7, 8]

$$2K_m M_o c_{gm}^2 \cdot \frac{\mathrm{d}\,\frac{1}{\ln \eta_r}}{\mathrm{d}t} \tag{2}$$

where

M_o = the primary molecular weight, and
t = the time

This expression includes two approximations, both founded on the presumption that the number of linkages broken is small in comparison with the number of linkages at complete polymerization (see equations (5) and (7) in the article quoted[7]).

The enzyme activity determined viscosimetrically, expressed in μA unites, is [7, 9, 10]

$$\mu A = c_s^2 \cdot \frac{\mathrm{d}\,\frac{1}{\ln \eta_r}}{\mathrm{d}t} \cdot 10^6 \tag{3}$$

A conversion factor for the amylase activities expressed in μA units and in mg maltose/min · ml is given in a recent paper by the present author [11]: the amylase unite 1 μA liberates reducing sugars corresponding to 0.018 mg maltose/min. If we want to calculate the number of moles reducing sugar liberated in unit time in one liter of starch solution on addition of the enzyme amount 1 μA, we must bear in mind that the activity of the enzyme in the reaction mixture is 0.001 μA and that the time is here counted in seconds. Hence, as the molecular weight of maltose is 342 and $c_s = c_{gm} \cdot 162 \cdot 10^{-3}$,

$$\frac{0.018 \cdot 10^{-3}}{342 \cdot 60} = 2K_m \cdot 162 \cdot \frac{10^{-3}}{162^2}$$

and $K_m = 0.71 \cdot 10^{-4}$.

This constant was calculated previously by Staudinger and Eilers [12] from measurements made by Biltz [13] and they found values between $0.9 \cdot 10^{-4}$ and $2.7 \cdot 10^{-4}$. Staudinger and Husemann [14] have also calculated this constant from experiments where starch was dissolved in formamide. They found the value to be $0.63 \cdot 10^{-4}$ with an uncertainty of 10 %.

SUMMARY

If the number of linkages broken pro unit time in enzymic depolymerization processes, where all linkages are broken with equal ease, can be calculated from end group determinations, and if the modified Arrhenius-Staudinger formula is valid for the substrate, the viscosity-molecular weight constant K_m of the substrate can be calculated from a conversion factor for the enzymatic activity. In this way the constant was calculated for starch, giving $K_m = 0.7 \cdot 10^{-4}$.

I wish to express my gratitude to *Statens Naturvetenskapliga Forskningsråd* for financial support in this investigation and to Mrs William Cameron, who revised the English text.

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Received June 20, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 898–900

Die Konstitution der Harzphenole und ihre biogenetischen Zusammenhänge

XII *. Die Konfiguration des Phillygenols

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Die in verschiedenen Arten von *Forsythia* und *Phillyrea* vorkommenden Glycoside Forsythin und Phillyrin geben bei der Hydrolyse die Aglycone Forsythigenol und Phillygenol, die nach der Annahme von Kunimine und Suzuki [1] wahrscheinlich identisch sind. Sosa [2] hat neuerdings gezeigt, dass das Glycosid von *Forsythia suspensa* Vahl. identisch ist mit dem Glycosid von *Phillyrea latifolia* L.[3]. Dadurch scheint die Annahme von Kunimine und Suzuki bestätigt zu sein und ich schlage vor die älteren Namen Phillyrin und Phillygenol für das Glycosid bzw. das Aglycon beizubehalten.

Kunimine und Suzuki nahmen weiter an, dass das Phillygenol in naher Beziehung zu Pinoresinol und Eudesmin stehe. Dies konnte von Kunimine und Wada [4] durch Überführung des Methyläthers des Phillygenols in Pinoresinoldimethyläther bewiesen werden. Dadurch ist Phillygenolmethyläther ein Stereoisomeres des Pinoresinoldimethyläthers.

Die Angabe von Kaku, Ri und Hara [5], dass Phillygenol bei der Methylierung ein Gemisch von Pinoresinoldimethyläther und Epipinoresinoldimethyläther gibt, ist wohl darauf zurückzuführen, dass sie die Spaltung des Glycosids mit verdünnter Säure vorgenommen haben, die eine teilweise Epimerisierung des Aglycons bewirkt hat.

Wie schon vor längerer Zeit von Erdtman [6] hervorgehoben worden ist, kann ein Molekül mit der Struktur des Pinoresinoldimethyläthers in drei verschiedenen stereoisomeren Formen vorkommen, wovon zwei symmetrisch und eine unsymmetrisch sind. Für Pinoresinoldimethyläther ist ein symmet-

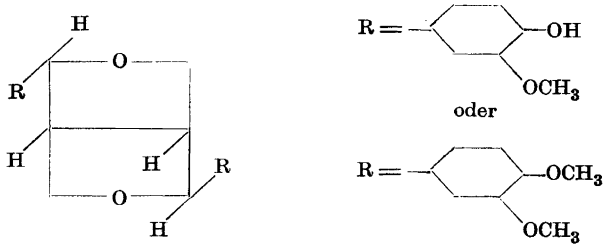
* XI Mitt. *Acta Chem. Scand.* 2 (1948) 82.

rischer Bau bewiesen [6, 7] und dem Phillygenolmethyläther käme danach entweder die andere symmetrische Form oder die unsymmetrische Form zu.

Durch freundliches Entgegenkommen von Herrn Dr. A. Sosa der mir eine kleine Menge Phillygenols zusandte, wofür auch an dieser Stelle bestens gedankt wird, bin ich nun in der Lage gewesen, einen endgültigen Beweis für die Konfiguration des Phillygenolmethyläthers zu führen.

Der Phillygenomethyläther erwies sich nämlich als identisch mit dem Epipinoresinoldimethyläther, für welchen die unsymmetrische Konfiguration bewiesen ist [8]. Diese Identität wurde durch Mischschmelzpunkt des Äthers sowie dessen Dibromderivats, welcher keine Depression gab, erwiesen. Leider konnten die Drehungen dieser beiden Substanzen nicht ermittelt werden.

Damit kommt dem Phillygenol die untenstehende Konfiguration zu,



wobei noch unentschieden bleibt, welcher der beiden unsymmetrisch gelagerten aromatischen Ringe die unmethylierte Hydroxylgruppe trägt.

VERSUCHSTEIL

Methylierung des Phillygenols

Entgegen der Angabe von Kaku, Ri und Hara [5] konnte die Methylierung des Phillygenols nicht mit Diazomethan bewirkt werden. Es wurde nur unverändertes Ausgangsmaterial zurückerhalten.

150 mg Phillygenol wurden in etwas Methanol gelöst und mit Dimethylsulfat und Alkali methyliert. Durch Eingiessen des Reaktionsgemisches in Wasser und Extrahieren mit Äther wurden 86 mg des Methyläthers erhalten. Aus der alkalischen Mutterlauge konnte unverändertes Phillygenol mit Kohlendioxyd ausgefällt werden.

Der Phillygenolmethyläther hatte nach Umkristallisieren aus Alkohol den Schmp. 129—129,5°. Der Mischschmelzpunkt mit Epipinoresinoldimethyläther (Schmp. 130—131°) war 129—130°.

Bromierung des Phillygenolmethyläthers

10 mg Phillygenolmethyläther wurden in Chloroform gelöst und mit einer Lösung von Brom in Chloroform versetzt, bis die Farbe deutlich rot war. Das Chloroform wurde verdampft und der ölige Rückstand mit Methanol verrieben. Er kristallisierte sofort

und wurde aus Alkohol umkristallisiert. Schmp. und Mischschmp. mit Dibromepipinoresinoldimethyläther 161—162°.

ZUSAMMENFASSUNG

Phillygenolmethyläther ist als identisch mit Epipinoresinoldimethyläther erkannt worden.

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Eingegangen am 29. Mai 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 901–903

Synthetic Plant Hormones

I. Sulphur Analogues of Some Phenoxy Acetic Acids (Preliminary note)

HOLGER ERDTMAN and GÖSTA NILSSON

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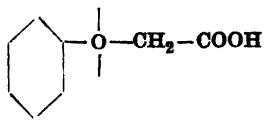
Derivatives of phenoxy acetic acid (I) *e. g.* 2-methyl-4-chlorophenoxy acetic acid and 2,4-dichlorophenoxy acetic acid possess great practical importance as 'selective weed killers'. It has been generally assumed that the herbicidic properties of this group of compounds is related to the physiological activity of β-indolyl acetic acid ('heteroauxin') (II).

A common feature of these compounds is the occurrence of an unsaturation (double bond or lone pairs of electrons) at the atom adjacent to the α-carbon atom of the acetic acid residue. Another requirement appears to be the presence of an aromatic nucleus. A somewhat similar relation appears to exist between N-dimethylamino acet-*o*-toluidide (III) and α-N-dimethylamino methyl indole (IV) the local anestetic properties of which were accidentally observed by the senior author [1, 2]. The similarity of IV (a C = C-double bond in the indole nucleus) and its precursor in the synthesis (III) which contains a C = O double bond led to the rediscovery of the local anestetic activity of anilides of type III [2] originally observed by Einhorn and Oppenheimer [3] and ultimately to the important anestetic 'xylocain' [4].

Experiments were initiated to prepare the sulphur analogues of 2-methyl-4-chlorophenoxy acetic acid and 2,4-dichlorophenoxy acetic acid. (Compounds V and VI.) If physiologically active, these compounds offer an opportunity to study the physiological effect of the transformation into the corresponding sulphoxides and sulphones, reactions in which the lone pairs of electrons of the sulphur atom are involved.

Compounds V and VI were prepared according to the general procedure outlined by Kalle and Co [5]. V melted at 127—128° and VI at 122—123°.



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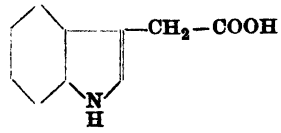

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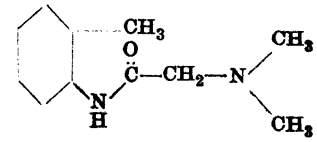

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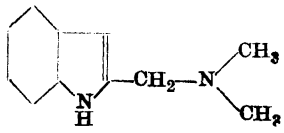

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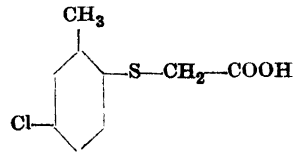

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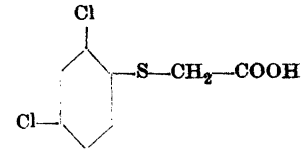

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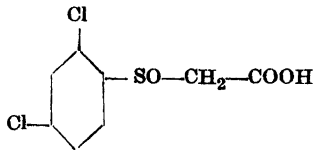

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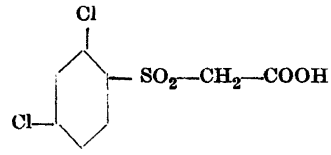

VIII

V was oxidised with potassium permanganate in the presence of sodium carbonate when the corresponding sulphone acid was formed (m. p. 147—148°). In the same manner VI was oxidised to the sulphone acid VIII m. p. 149.5—150.5°. The 2,4-dichloro phenyl sulphoxide acid VII was obtained from VI by oxidation with hydrogen peroxide in glacial acetic acid at room temperature. M. p. 145—146° (with decomposition).

Preliminary biological tests carried out in the laboratories of Professor H. Burström, Lund, by various methods indicate that the sulphur analogues of the phenoxy acetic acids show a diminished activity and that oxidation of the sulphur atom to give sulphoxides or sulphones results in complete disappearance of the activity.

SUMMARY

Some sulphur analogues of herbicidic phenoxy acetic acids have been prepared and found to possess herbicidic properties. Oxidation to the corresponding sulphoxides and sulhpones apparently destroys the activity.

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Received June 27, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 904–905

Synthetic Plant Hormones

II. β-Indolyl-methanesulphonic Acid *

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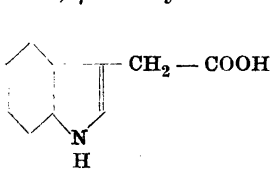
β-Indolyl acetic acid (I) possesses auxin activity and acts as a growth factor for certain microorganisms. Slight modification of the structure of biological factors frequently yields compounds exhibiting antagonistic properties and the study of such phenomena is important for the understanding of the physiological *rôle* of such substances. Hence it was of interest to have access to the hitherto unknown ** sulphonic acid analogue of β-indolyl acetic acid, β-indolyl methanesulphonic acid II.



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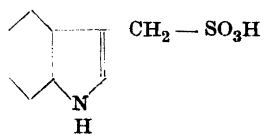

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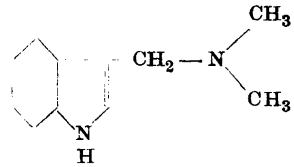

III

Recently it was found in these laboratories that certain substituted benzyl alcohols, *e. g.* vanillyl and veratryl alcohol are excellent model substances for lignin, being very easily transformed into sulphonic acids by heating with bisulphite cooking acid [1]. It was apparent, that the alkaloid gramine (III) possesses certain analogies with benzyl alcohols and that consequently sulphite

* Part I. *Acta Chem. Scand.* 3 (1949) 901.

** This work was completed in 1947 and a patent application was presented in July 29, 1948. Recently T. Wieland, E. Fischer and F. Moewus (*Ann.* 561 (1948) 47) described the synthesis of the sodium salt of β-indolyl-methanesulphonic acid and the important observation that this compound is an antagonist of heteroauxin as regards root growth. These authors work with sodium bisulphite solutions in the presence of methanol and the yield is inferior to that obtained according to our procedure. The publication of our experiences, therefore, appears not to be superfluous.

cooking of gramine might result in the formation of the desired sulphonic acid (II). This proved to be the case and it was found that almost quantitative yields were obtained when gramine was heated with a sodium sulphite solution.

Biological tests showed that β-indolyl methanesulphonic acid possesses no activity in the Avena test and that most microorganisms tested to date were not retarded in their growth by this acid. Only in the case of some yeasts, a slight inhibition could be observed, which was remedied by the addition of β-indolyl acetic acid.

EXPERIMENTAL

Preparation of β-indolyl-methanesulphonic acid

Gramine (20 g), sodium sulphite (19 g) and water (350 ml) were heated in a sealed tube to 110° for ten hours. After cooling the crystals (sodium salt of β-indolyl-methanesulphonic acid) were collected by filtration. The filtrate had a strong odour of dimethylamine. Yield of the sodium salt 94 %. It may be recrystallised from hot water.

$C_9H_8NO_3SNa$	Calc.	C 46.5	H 3.49	S 13.75	Na 9.9
	Found	» 46.7	» 3.57	» 13.8	» 9.9

β-Naphthylamine salt: This salt was prepared by neutralising a solution of the free acid with β-naphthylamine. The acid solution was obtained by filtration of a solution of the sodium salt through an organolith charged with hydrogen ions. The salt may also be prepared directly from the sodium salt and β-naphthylamine hydrochloride. The substance was recrystallised from butanol saturated with water. M. p. 228°.

$C_{19}H_{18}N_2O_3S$	Calc.	C 64.4	H 5.21
	Found	» 64.4	» 5.27

Several series of experiments were run in which the composition of the cooking acids and the heating time was varied. The results, however, were inferior. With only sulphur dioxide solutions a considerable amount of resin was formed together with small amounts of indole. With sodium bisulphite, oils were obtained from which unchanged gramine could be isolated in various amounts together with the sodium salt of the sulphonic acid.

SUMMARY

The sulphonic acid analogue of heteroauxin has been prepared.

We are grateful to Professor H. Burström, Lund, Docent N. Nielsen and Docent E. Rennerfelt, Stockholm, for their preliminary reports on the biological testing of the sulphonic acid and to *Statens Tekniska Forskningsråd* for financial support.

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Received June 27, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 906—911

Fungicidal Properties of some Constituents of the Heartwood of *Tetraclinis articulata* (Vahl) Masters

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The heart wood constituents of conifers belonging to different families show great and apparently characteristic chemical differences [1]. In the *Pinaceae* phenols are common. Examples are pinosylvin and compounds belonging to the flavone-flavanone group in the genus *Pinus*, conidendrin in the genera *Picea* and *Tsuga* and again a flavanone, taxifolin, in *Pseudotsuga*.

In the *Cupressaceae*, phenols appear to be less common although a few instances are known. The known heart wood constituents of this family generally belong to the terpene group. Some of these are open terpenoids related to geranic acid. Examples are dihydrogeranic acid (rhodinic or citronellic acid = 'callitrol') in several *Callitris* species, dehydrogeranic acid in *Neocallitropsis araucarioides* (Compton) Florin *(Callitropsis araucarioides* Compton). Cyclic terpenoids occur in *Libocedrus formosana* Florin (shonanic acid) and in *Thuja plicata* D. Don (the thujaplicins, which, however, do not obey the isoprene rule, but which, nevertheless, are undoubtedly related to the true terpenes). Another constituent of Thuja plicata is the 'dehydroperillic acid', the proposed structure of which has now been disproved [2].

In 1904 Grimal [3] investigated the volatile oil in a yield of two per cent obtained by steam distillation of the wood of *Callitris quadrivalvis* Ventenat *(Cupressaceae)*. He segregated the oil into a phenolic (about one per cent of the wood) and a neutral fraction. Acids appear to be absent. The phenolic fraction consisted of carvacrol (I) and hydrothymoquinone (II). The neutral fraction yielded thymoquinone (III). Hence, contrary to what is known about the chemistry of the genus *Callitris*, this species produces phenols. They differ, however, from those occurring in the *Pinaceae* by being elaborated on a terpenoid basis. *Callitris quadrivalvis* is also known in the older litterature under

the name *Thuja articulata* Vahl. (Theophrast's '*Thujon*'). Taxonomists now recognise that this conifer is the representative of a monotypic genus *Tetraclinis*. Its generic name is *Tetraclinis articulata* (Vahl) Masters. This species is peculiar in another chemical sense. Its lignin contains syringyl groupe just as the angiosperms [4]. Only a few conifers share this peculiarity of *Tetraclinis* namely *Podocarpus amarus* (Blume) and *Podocarpus pedunculata* (Bailey) (N. O. *Podocarpales*). The separation of the genera *Tetraclinis* and *Callitris* is entirely in harmony with existing chemical evidence. *Tetraclinis articulata* inhabits southern Spain and north western Africa. It is the source of the sandarac resin.

The occurence of thymoquinone in *Tetraclinis* is strange. Simple quinones are infrequent plant products. This quinone together with the corresponding hydroquinone has been isolated from the oil of *Monarda fistulosa (Labiatae)* [5]. This plant contains an oxydase which causes the dehydrogenetion of the phenol to the quinone. The latter, therefore, may be a secondary product.

The fungicidal properties of the substances isolated by Grimal from the wood of *Tetraclinis* have been investigated and in addition also those of thymol (IV), an isomeride of carvacrol. The two monophenols are fairly strong fungicides surpassing hydrothymoquinone in activity. Thymoquinone possesses increased toxicity. In view of the facile dehydrogeneration of the hydroquinone by oxidases or otherwise, it appears probable that in the wood of *Tetraclinis*, hydrothymoquinone functions as a potential antibiotic which is transformed into the more potent quinone when the wood due to damage is exposed to light and air. This will be investigated as soon as fresh samples of the wood are available.

MICROBIOLOGICAL SECTION

I. Activity of thymol and the constituents of the wood of *Tetraclinis articulata* against wood destroying fungi

Varying amounts of thymol, carvacrol, hydrothymoquinone or thymoquinone were added to test tubes charged with sterilized malt extract agar. The substrates vere inoculated with small pieces of the mycelium of different fungi. The growth of the mycelia was measured at suitable intervals. Four parallel runs were made with each fungus. Tables 1—4 show the results in per cent of the growth in control experiments containing no toxic substances.

Table 1. Relative growth in presence of thymol.

Fungus	Per cent thymol				
	0.001	0.002	0.005	0.01	0.02
Coniophora puteana	74	66	47	16	—
Lentinus lepideus	55	45	18	—	—
Merulius lacrymans	40	38	27	—	—
Poria vaporaria	80	55	—	—	—

Table 2. Relative growth in presence of carvacrol.

Fungus	Per cent carvacrol			
	0.002	0.005	0.01	0.02
Coniophora puteana	80	43	—	—
Fomes marginatus	86	59	16	—
Lentinus lepideus	80	56	18	—
Merulius lacrymans	41	14	—	—
Poria vaporaria	58	26	—	—

Table 3. Relative growth in presence of hydrothymoquinone.

Fungus	Per cent hydrothymoquinone			
	0.001	0.002	0.05	0.1
Coniophora puteana	73	34	—	—
Fomes marginatus	35	29	6	—
Lentinus lepideus	32	11	—	—
Merulius lacrymans	2	—	—	—
Poria vaporaria	65	46	14	—

Table 4. Relative growth in presence of thymoquinone.

Fungus	Per cent thymoquinone			
	0.002	0.005	0.01	0.02
Coniophora puteana	78	77	64	—
Fomes marginatus	99	90	37	14
Lentinus lepideus	109	74	49	—
Merulius lacrymans	52	47	14	—
Poria vaporaria	80	74	45	16

From Tables 1 and 2 it is seen that thymol and carvacrol are moderately strong fungicides preventing growth of the different fungi at a concentration of about 0.01 %.

Hydrothymoquinone (Table 3) is less toxic. Concentrations of about 0.05—0.1 % are needed to prevent growth. The activity is rather similar to that of phenol when tested on the same organisms [6]. *Merulius lacrymans* is checked by lower concentrations of hydrothymoquinone than the other fungi. This conforms with the known sensitivity of this fungus to pinosylvin [7] and to the thujaplicins [6].

Thymoquinone appears to be less toxic than thymol and carvacrol but possesses greater inhibitory activity than its hydrogenation product.

II. Activity against the germination of conidia of *Polyporus annosus*

Polyporus annosus, the common "root rot fungus", forms conidia which contribute to the spreading of this fungus. Unpublished observations show that certain natural fungicides, for example, pinosylvin inhibits the germination of these conidia at lower concentration (0.0025—0.005 % solution) than the growth of the mycelium (in 0.01—0.02 % solution). It was therefore of interest to investigate the influence of the *Tetraclinis* fungicides on the germination of the conidia of *Polyporus annosus*.

The experiments were carried out in 0.5 % malt extract solution and are summarised in Table 5.

Table 5. Inhibition of the germination of conidia of Polyporus annosus.

Substance	Concentration in per cent						
	0.1	0.05	0.025	0.01	0.005	0.0025	0.001
Thymol	−	−	(+)	+	+	++	++
Carvacrol	−	−	+	+	++	++	++
Hydrothymoquinone	−	+[1])	+++	+++	+++	+++	+++
Thymoquinone	−	−	(+)	+	++	++	+++

[1]) Saturated solution

− = 0 % germination
(+) = < 5 %
\+ = 5—20 %
++ = 20—50 %
+++ = 50—60 % germination
(= germination in the controls)

No remarkable difference between the inhibition of the growth of mycelia and the germination of the conidia is apparent from these figures. The low activity of hydrothymoquinone is, however, apparent.

III. Activity against blue stain fungi and organisms related to yeasts

Spore suspensions (1 ml containing 10^6 spores) were introduced into small paper boxes followed by sterilised malt agar solutions to which various amounts of the test substances were added. After five days incubation the growth was observed. The results are summarised in Tables 6—7. (— = no growth, (+) = isolated colonies, + = poor growth, ++ = good growth. All controles ++.)

Table 6. Growth of various lower fungi in the presence of thymol and carvacrol.

Fungus	Per cent thymol				Per cent carvacrol			
	0.05	0.02	0.01	0.005	0.05	0.02	0.01	0.005
Cladosporium herbarum	—	—	++	++	—	(+)	++	++
Ophiostoma piceae	—	—	++	++	—	(+)	++	++
» *pini*	—	—	++	++	—	(+)	+	++
Phialophora fastigiata	—	—	(+)	++	—	—	+	++
Phoma lignicola	—	—	+	++	—	(+)	+	++
Pullularia pullulans	—	—	+	++	—	—	++	++
Penicillium janthogenum	—	(+)	+	++	—	+	++	++
Rhodotorula glutinis	—	—	+	++	—	—	+	++
Torulopsis pulcherrima	—	—	+	++	—	(+)	+	++

Table 7. Growth of various lower fungi in the presence of hydrothymoquinone and thymoquinone.

Fungus	Per cent hydro-thymoquinone			Per cent thymo-quinone			
	0.05	0.02	0.01	0.02	0.01	0.005	0.002
Cladosporium herbarum	—	++	++	—	++	++	++
Ophiostoma piceae	—	++	++	—	—	++	++
» *pini*	—	+	++	—	—	+	++
Phialophora fastigiata	—	+	++	—	(+)	+	++
Phoma lignicola	—	+	++	—	+	++	++
Pullularia pullulans	—	+	++	—	—	—	++
Penicillium janthogenum	—	+	++	—	+	++	++
Rhodotorula glutinis	—	(+)	+	—	—	+	++
Torulopsis pulcherrima	—	(+)	++	—	—	+	++

As seen from these tables the limits of inhibition are approximately for thymol 0.01—0.02 %, for carvacrol 0.02—0.05 %, for hydrothymoquinone 0.02—0.05 % and for thymoquinone 0.01—0.02 %. All these fungi are inhibited by approximately the same amount of the various substances. Hydrothymoquinone possesses the lowest toxicity. *Penicillium janthogenum* appears to be somewhat more resistent than the other fungi tested. It is a common observation that moulds such as *Penicillium* and *Trichoderma* species are relatively resistant to natural fungicides. Mc Gowan found that fungi belonging to these genera are far more resistant to viridine and gliotoxine than other fungi. All fungi, however, were inhibited by approximately the same amount of mercuric cloride [8].

The toxicity of thymol to bacteria has already been investigated by several workers[9, 10].

SUMMARY

The toxicity against wood destroying fungi of thymol and three substances (carvacrol, hydrothymoquinone and thymoquinone) occurring in the wood of *Tetraclinis articulata* (Vahl) Masters, has been investigated. Hydrothymoquinone is approximately as active as phenol. Conversion into thymoquinone enhances the toxicity. The simple phenols, thymol and carvacrol, are more efficient fungicides than hydrothymoquinone.

The authors wish to acknowledge their indebtedness to *Statens Tekniska Forskningsråd* for financial support, and one of us, E. R., wishes to acknowledge the assistance of miss V. Edborg.

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Received July 4, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 912–913

Demethylation of Pinosylvin Monomethyl Ether

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The heart wood of Scots pine (*Pinus silvestris*) contains approximately 1 % of the mixed phenols pinosylvin and pinosylvin monomethyl ether. The relation of these two phenols appears to be rather constant (1 : 3—1 : 4)[1]. The isolation of the crude mixture is quite simple [2] but the separation of the mixture is laborious.

Since pinosylvin, generally, is more toxic and more important for physiological investigation, the conversion of the monomethyl ether (or the mixture) into pure pinosylvin is a matter of importance. Previous attempts to employ hydriodic acid or hydrobromic acid showed that these reagents were unsuitable for the demethylation of pinosylvin monomethyl ether.

The facile demethylation of the monomethyl ether has now been accomplished by means of pyridine hydrochloride, introduced as demethylating agent by Prey [3]. The yield of pure pinosylvin was about 90 %. Unfortunately the method is not suited for the demethylation of the crude, distilled phenol mixture. A lot of resinous, strongly coloured material is formed and the pinosylvin cannot be obtained in a pure state without considerable loss of substance. Thus the yield of pure pinosylvin never exceeded 50 % of the theoretical value, determined by oxidizing the phenol mixture with potassium permanganate and isolating the benzoic acid formed. (I am indebted to Mr. A. Frank for these determinations.) The isolation of pinosylvin monomethyl ether of sufficient purity, however, is facile.

EXPERIMENTAL

Pinosylvin monomethyl ether (13 g) and pyridine hydrochloride (26 g) were heated to 180° on a salt bath for two hours. The hot mixture was poured into 2 *N* hydrochloric acid (250 ml) and extracted with ether (3 · 150 ml). The brown ether solution was dried over anhydrous sodium sulfate and filtered through a column of aluminium oxide (10

cm · 5 cm^2). The faintly yellow filtrate was evaporated to dryness and the residue recrystallized twice from benzene. M. p. 154—155° (uncorr.). Concentration of the mother liquors yielded a second crop of pure pinosylvin. Total yield 11.0 g (90 %).

SUMMARY

The demethylation of pinosylvin monomethyl ether in a good yield by means of pyridinium hydrochloride is described.

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Received June 17, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 914–917

Some Observations on the Ability of Heme to Catalyze the Oxidation of Easily Oxidizable Substances by Peroxides of Fats and Fatty Acids *

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It is well known that hemin and related compounds show a peroxidase-like action by catalyzing the oxidation of easily oxidizable substances, for instance, benzidine, by hydrogen peroxide. This fact is the basis of the benzidine and similar reactions for the detection of blood. The peroxidase action of heme has been studied quantitatively by several workers, among them Kuhn and Brann [1] and Reuter *et al.* [2]

As far as the author has been able to determine an analogous reaction in which, instead of hydrogen peroxide, peroxides of fats and fatty acids took part, has been described only by Frehden [3], who used the conversion, by peroxides, of 2,7-diaminofluorene in the presence of hemine to blue meriquinoid oxidation products as a spot reaction for the detection of rancidity in fats. On the other hand, the catalytic influence of hemin on the oxidation of fatty acids by atmospheric oxygen to peroxides, has been studied by several workers, for instance, Robinson [4], Kuhn and Meyer [5] and Franke [6].

Since none of the investigators, who have studied other catalytic functions of hemin, have studied the reactions between peroxides of fats and fatty acids and easily oxidizable substances, this paper will deal with the study of such reactions.

EXPERIMENTAL

The heme used in most of the experiments was prepared by Anson and Mirsky's method [7]. In other experiments a commercial preparation of hemin was used ***.

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* A preliminary communication on this work was presented at the VI Scandinavian Physiological Congress, Glavind, J., and Hartmann, S. *Acta Physiol. Scand.* 16 (1948) suppl. 53, 26.

** This work was aided by a grant from Laurits Andersens Fond.

*** We are indebted to F. Hoffmann-La Roche & Co., Inc., Basle, for the kind supply of hemin.

Various peroxidized fats and fatty acids were tried, such as oleic acid, lard, cod liver oil, *etc.* The peroxide values were determined by the method of King *et al.*[8], and by the authors' method [9], and calculated as milliequivalents per 1000 g of fat.

Especially for quantitative studies, it was found necessary to use rigorously purified solvents. The acetone, pyridine, xylene, *etc.*, must not contain traces of either reducing or oxidizing substances. They can be tested by adding a few drops of a solution of leuco-dichlorophenolindophenol (containing a little dichlorophenolindophenol) dissolved in ethyl alcohol and acidified with acetic acid, to 20 ml of the solvent in a test tube. After heating this mixture in a water-bath to 70° C, or to the boiling-point of the solvent, the intensity of the red color of the indophenol should show neither a decrease nor an increase.

HEME-CATALYZED OXIDATION OF DIFFERENT SUBSTANCES BY PEROXIDES OF FATTY ACIDS

When benzidine is used as the oxidizable substance in the reaction, the following experiments can be made. In a test tube containing 1 ml of a 2 per cent solution of benzidine in 96 per cent ethyl alcohol, 0.1 ml of a 0.008 per cent solution of heme in distilled water and a few drops of an oil having a peroxide value of about 25 are added. After shaking, only a very faint bluish color can be observed. However, if instead of a peroxidized oil an equivalent amount of hydrogen peroxide is added, a strong blue color will appear.

The blue color produced by a peroxidized oil can be better observed when the benzidine and the heme solutions are mixed and placed on a porcelain dish, and the oil allowed to float on the dish. On the contact surface between the oil and the alcohol-water mixture, a faint but distinctive bluish-green color appears which reaches its maximum in the course ofa few minutes, and then fades out.

The color reaction is still more clearly seen on a filter paper. When the filter paper is impregnated with the benzidine-heme solution and a drop of the oil is brought onto it, a bluish-green ring appears around the oil in the course of a few minutes. It is possible by means of such a paper to make a rough estimation of peroxide values. Oils having a peroxide value of about 20 give a pronounced blue color. At a peroxide value of about 10, the color is fainter, and, due to the color of the oil and the heme, more greenish. A peroxide value of about 3 only makes the color of the paper darker brown, while still lower peroxide values will give no distinct color at all.

The results obtained with benzidine show that this substance reacts with fatty peroxides in the presence of heme, but that the reaction is much weaker than that with hydrogen peroxide. This is probably due to the fact that the reaction does not take place in one phase but in a non-homogenous system, and, consequently, is much slower. Therefore, the unstable colored products obtained by the oxidation of benzidine will not be formed in sufficiently great

amounts to be observed. In order to obtain a stronger color reaction an attempt was made to use substrates which on oxidation would produce more stable colors, and to carry out the reaction in one phase.

When the oxidation of benzidine is carried out in the presence of α-naphthol, a coupled reaction, with the formation of a stable compound — an indophenol — takes place. An intensive red color is developed when 1 drop of a peroxidized oil and 0.1 ml of the heme solution mentioned above are added to 1 ml of a solution containing 1 per cent of benzidine and 1 per cent of α-naphthol in 96 per cent ethyl alcohol.

Similar strong colors can be obtained by other coupled oxidations in the presence of peroxidized fat and heme. Dimethyl-*p*-phenylenediamine + α-naphthol, and *p*-phenylenediamine + α-naphthol will give blue and red colors, respectively.

The colors will appear faster when the mixtures are heated for a few minutes at 70° C on a water bath. Furthermore, faster reactions and more intensive colors are obtained when heme is used in the forms of imidazol-, pyridine-, or other hemochromogens. A solution of 20 mg hemin in a mixture of 120 ml pyridine and 40 ml distilled water, or in a mixture of 5 ml pyridine and 10 ml glacial acetic acid can be used. When 0.1 ml of such a solution is added to a solution of benzidine in alcohol and shaken with a drop of a peroxidized oil, a comparatively strong color is developed. Cyanide inhibits the reactions.

The colors are stronger if the peroxidized oil does not form a suspension in alcohol but is brought into a true solution, for instance, by the addition of a little ether or by carrying out the reaction in acetone. In xylene or other hydrocarbons in which the oil is easily soluble a strong reaction is also seen, but after a short time the hemin precipitates and the reaction stops. If a few drops of a peroxidized oil is shaken with an aqueous pyridine-hemochromogen solution, a weak reaction may sometimes be observed.

Certain other easily oxidizable substances give very strong colors in the reaction, especially when pyridine-hemochromogen is used. This is the case with leucomalachite green, and the colorless compound 3,5-dichloro-4,4'-hydroxyphenylenediamine which is obtained by reduction of the well known redox-indicator 2,6-dichlorophenolindophenol, for instance, with ascorbic acid. Guajac resin can also give a very strong color, whereas pyrogallol gives only a weak color, and tyrosin is not oxidized.

DISCUSSION

The experiments show that peroxides of fats and fatty acids react in the presence of heme, especially when it is in the form of a suitable hemochromogen, with a great variety of easily oxidizable substances. The reaction is

strong, especially if it is carried out in one phase, but the speed of the reaction decreases for more heterogenous mixtures.

The question arises as to whether or not the reaction has a biological significance or function, that is, if enzymes or other heme-containing compounds may function in the plant or animal organism in a manner analogous to peroxidase, but with fat peroxides as oxygen sources. Much evidence of the biological significance of peroxides of unsaturated fatty acids has accumulated in the past years [10], and an enzyme catalyzing the peroxidation has been isolated [11], but no enzyme catalyzing the transfer of peroxidic oxygen from such peroxides has been observed. It is well known that peroxides from horse-radish can react with certain organic peroxides, for instance, ethyl ether peroxide. We have been unable to observe any reaction between a potent extract of horse-radish, peroxidized fats, and easily oxidizable substances; not even when the fats were brought into a very fine suspension by means of 'Tween 80'.

A study of the reaction between peroxides and leucomalachite green, catalyzed by heme, in the presence of organic solvents will be reported in a following paper. A histochemical method for the demonstration of peroxidized fat, based on the reactions described here, has already been published [12].

SUMMARY

Heme, especially in the form of pyridine-hemochromogen, catalyzes the oxidation of benzidine, guajac resin, leucomalachite green and other easily oxidizable substances by peroxides of fats and fatty acids. The reaction is strong when carried out in acetone, ether-alcohol, or other suitable solvents.

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Received June 23, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 918–925

Electron Diffraction Investigation of α, β, γ, δ and ε Benzene Hexachloride

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According to the older conception 1,2,3,4,5,6-hexachlorocyclohexane may occur in eight different forms as far as optically active modifications are not taken into consideration. Only one of these forms, however, should be separable into optically active antipodes. The modern picture based on a non-planar carbon ring and the possibility of a conversion of the carbon ring does not alter this result although it deviates in essential features from the older one [1,2]. At present *five* of the eight benzene hexachlorides are known and we have thought it worth while to try to determine the molecular configurations of all these substances in the vapour state using the electron diffraction sector method.

The final results of our investigation has already been published in a condensed form [3]. We feel, however, that a more detailed report should be given, and the present paper therefore brings the experimentally obtained distribution curves and the discussion leading us to the conclusions already published.

We want first, however, to draw the attention to the circumstance that each molecular species may exist in two forms, generally different, which are transformed into each other by a conversion of the carbon ring. In those cases in which the energy of these two forms of a benzene hexachloride is different, the interaction of atoms not directly linked together will probably suffice to make the concentration of the less stable form in the vapour practically equal to zero.

The results obtained are summarized in Table 1 and Fig. 1.

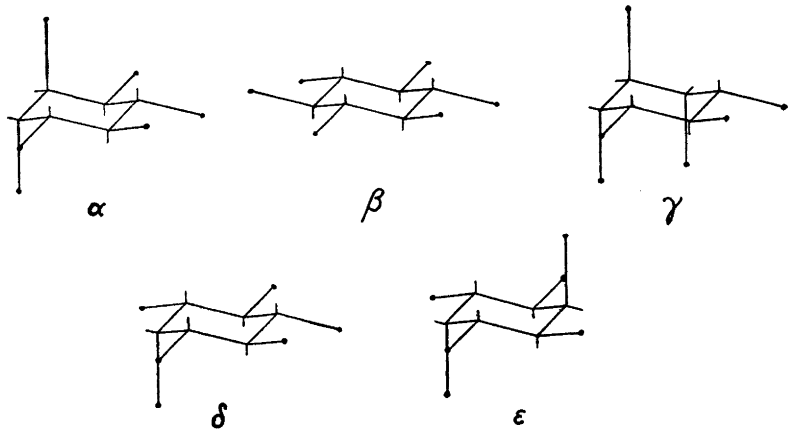

Fig. 1. Configurations of the five isomers.

Table 1. Configuration of isomers of benzene hexachloride.

Isomer	Configuration
α	$\varepsilon\ \varepsilon\ \varkappa\ \varkappa\ \varkappa\ \varkappa$
β	$\varkappa\ \varkappa\ \varkappa\ \varkappa\ \varkappa\ \varkappa$
γ	$\varepsilon\ \varepsilon\ \varepsilon\ \varkappa\ \varkappa\ \varkappa$
δ	$\varepsilon\ \varkappa\ \varkappa\ \varkappa\ \varkappa\ \varkappa$
ε	$\varepsilon\ \varkappa\ \varkappa\ \varepsilon\ \varkappa\ \varkappa$

In order to demonstrate the way leading us to these conclusions we start with an inspection of the $\frac{\sigma(r)}{r}$ curves of the five isomers (Fig. 2). It will be observed that these five experimental curves are indeed strikingly similar, all showing seven rather pronounced peaks (I to VII). The r-values of the first four peaks are nearly indentical, but in the case of number V (occuring approximately at $r = 4.7$ Å) a shift of the β peak towards a smaller r-value and the γ peak towards a greater r-value is observed. The distance responsible for this peak in the β compound is C_1—$Cl_{4\varkappa}$, whereas in the other compounds distances corresponding to greater r-values ($Cl_{1\varkappa}$—$Cl_{3\varepsilon}$ and $Cl_{1\varkappa}$—$Cl_{4\varepsilon}$) will also occur. The γ compound contains the greatest number of ε—Cl-atoms and consequently the r-value of peak V should be greatest for this substance.

The position of peak VI ($r \approx 5.45$ Å) is nearly the same in all the $\frac{\sigma(r)}{r}$-curves, but the height is markedly different for the five substances. The

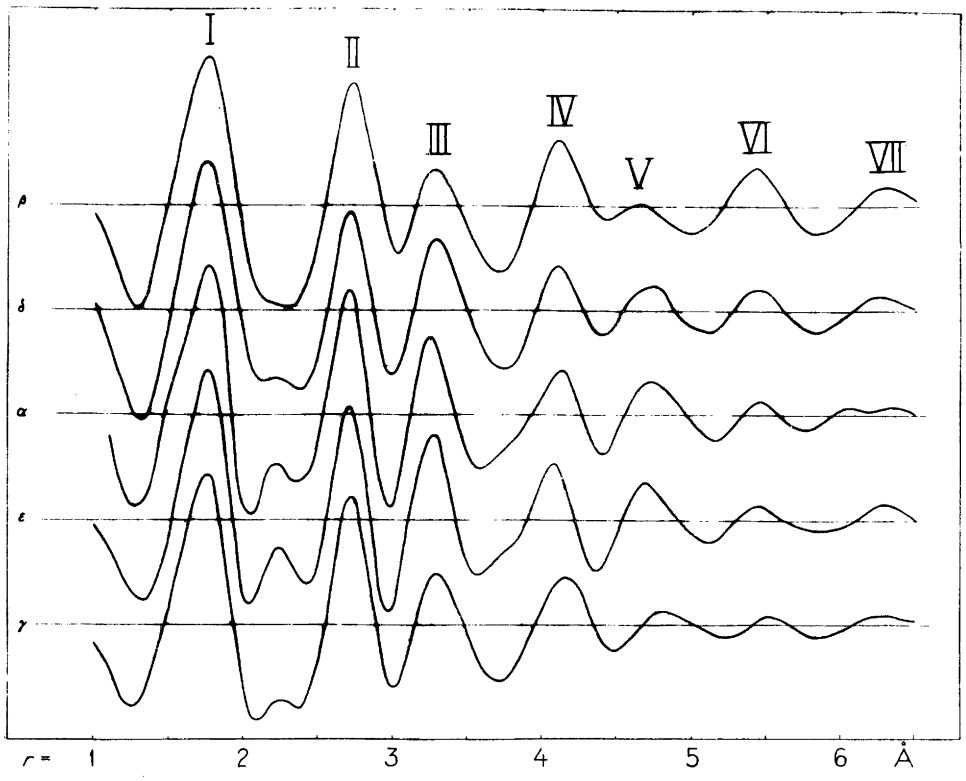

Fig. 2. Experimental $\frac{\sigma(r)}{r}$*-curves of the five isomers.*

internuclear distance responsible for this peak is $Cl_{1\alpha}$—$Cl_{3\alpha}$ and consequently the expected relative heights of this peak are:

$$6(\beta), \quad 4(\delta), \quad 2(\alpha), \quad 2(\varepsilon), \quad 1(\gamma)$$

From Fig. 1 it is seen that the heights are indeed decreasing from β to γ, those of α and ε being almost equal:

$$\beta > \delta > \alpha \approx \varepsilon > \gamma$$

The peak just discussed (VI) is especially well suited as a basis for comparison because it is no doubt caused by the $Cl_{1\alpha}$—$Cl_{3\alpha}$ distance with almost negligible disturbances caused by other distances.

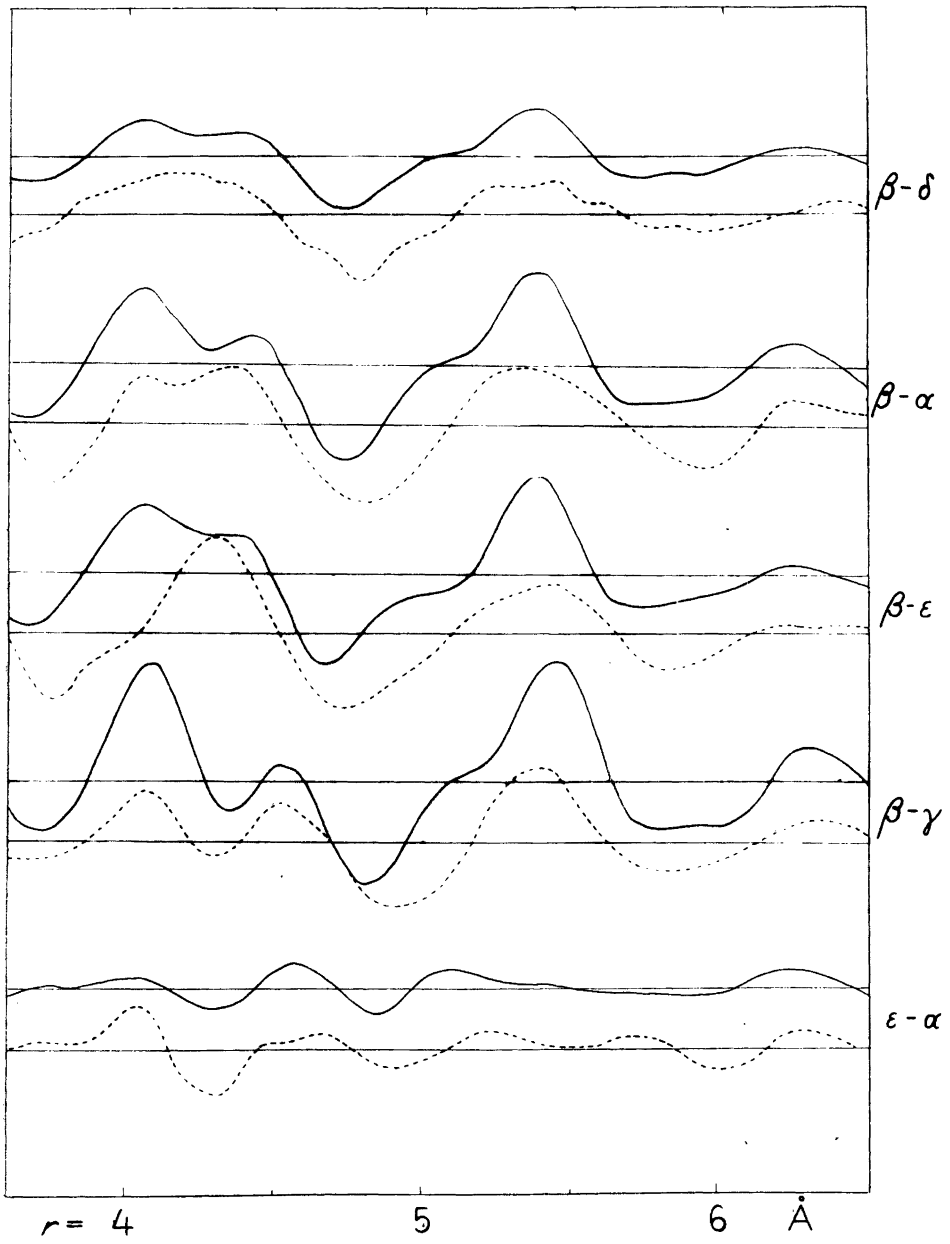

Fig. 3. Differential $\frac{\sigma(r)}{r}$*-curves. Fully drawn curves are theoretical curves, dotted curves experimental ones.*

Peak VII (due to the distance $Cl_{1\varkappa}$—$Cl_{4\varkappa}$) should be expected to be most pronounced in the case of the β isomer, the δ and ε isomers follow with the same weight factor, and the height should be still smaller for the α compound and the peak disappear for the γ compound. This is generally in good agreement with the heights observed in the experimental curves.

The most valuable informations, however, may be obtained when comparing differential $\frac{\sigma(r)}{r}$-curves — experimental and theoretical — each differential curve referring to a given pair of isomers. This procedure has the advantage that the contribution to the curve under discussion from a considerable number of internuclear distances occuring in both substances is automatically eliminated and the effects of distances which are different in the two substances are being accentuated. In the inner part of the differential curves ($r <$ about 3.6 Å) the amplitudes of the $\frac{\sigma(r)}{r}$-curves are comparatively great and here an error in one of the experimental curves will have more serious consequences for the resulting differential curve than a corresponding relative error will have in the outer part of the $\frac{\sigma(r)}{r}$-curve.

We have chosen the β compound as a standard substance of reference because the configuration of this substance may be regarded as definitively settled, and because it contains $\varkappa$—Cl atoms only. In Fig. 3 the experimental and theoretical differential curves are reproduced in the following order: (β—δ), (β—α), (β—ε), (β—γ) and as a supplement (ε—α). The theoretical curves have been calculated putting C—C = 1.54 Å, C—Cl = 1.76 Å, and assuming normal tetrahedral structures based on the symmetrical form of the carbon ring.

It is seen that the correspondance between theoretical and experimental curves is at least as good as might be expected. It must be admitted, however, that the (β—α)- and (β—ε)-curves are so similar that a final distinction between the α and ε isomers cannot be based on these curves only. This is the reason why we have included the (ε—α)-curves in our discussion. It is obvious from these curves that the ε and α isomers cannot be interchanged without totally spoiling the agreement between experimental and theoretical (ε—α)-curves.

Our investigation was of course not carried out without considering the possible configurations so far not mentioned in this paper:

$\varepsilon\varkappa\varepsilon\varkappa\varkappa\varkappa$
$\varepsilon\varepsilon\varkappa\varepsilon\varkappa\varkappa$
$\varepsilon\varkappa\varepsilon\varkappa\varepsilon\varkappa$

We have not been able, however, to obtain agreement between our experimental curves and the theoretical ones assuming any one of these configurations to be represented by one of the five substances under investigation.

It is of course of interest to examine the deviations from ideal structures actually occuring in the series of benzene hexachlorides. Most convenient for this purpose is of course the symmetrical β compound which contains only $\varkappa$-chlorine atoms and no ε bonded chlorine. In this case there is no reason to assume a deformation which would diminish the symmetry of the molecule. The $Cl_{1\varkappa}$—$Cl_{2\varkappa}$-distance of the ideal structure, however, would be somewhat smaller than those expected to be energetically favourable. We have therefore calculated the internuclear distances in a series of symmetrical models in which the C—C—Cl- and C—C—C-angles are different from 109.5°. The most satisfactory agreement between calculated distances and distances given by the positions of the maxima of the $\frac{\sigma(r)}{r}$ -curve was obtained with a C—C—C-angle equal to 114° and a C—C—Cl-angle of 112° (Table 2).

Table 2. Atomic distances in β isomer.

Type of distance	Exp.	Theoretical (ideal structure)	Theoretical (deformed)
C_1—Cl_1	1.76	1.76	1.76
C_1—Cl_2	2.73	2.70	2.74
Cl_1—Cl_2	3.28	3.18	3.25
C_1—Cl_3	4.12	4.08	4.14
C_1—Cl_4	4.65	4.56	4.64
Cl_1—Cl_3	5.44	5.39	5.46
Cl_1—Cl_4	6.33	6.26	6.35

The other benzene hexachlorides all contain ε-Cl-atoms and here the $Cl_{1\varepsilon}$—$H_{3\varepsilon}$-repulsion — the existence of which has been clearly demonstrated in earlier work on chlorinated cyclohexanes [4] — will no doubt cause the ε—Cl-bonds to be bent away from the principal axis of the carbon ring.

More serious deviations from tetrahedral valency angles must of course occur when two ε-chlorine atoms appear in 1,3 position. The γ compound is the only one of the known benzene hexachlorides having such an arrangement, and here the X-ray crystallographic work of Bijvoet and co-workers [5] clearly shows the effect of the $Cl_{1\varepsilon}$—$Cl_{3\varepsilon}$-repulsion. Our experimental differential (β—γ)-curve, however, does not demonstrate this effect because it begins with an r-value of 3.6 Å.

The dipole moments of the five known benzene hexachlorides have been measured by Hetland [6] in our laboratory and the results seem to be in accordance with our configuration determinations. The moments of the β and ε isomers are zero and the α and δ isomers both have a moment of 2.2 D. The γ isomer has the greatest moment (2.9 D). If we disregard the mutual interaction between the C—Cl bond moments and also the deviations from tetrahedral angles, rough values of the resultant moments may be computed which might be expected to give at least the right sequence of the dipole moment values. We find (Table 3):

Table 3. Dipole moments.

	Exp.	Calc.
α	2.2	3.4
β	0	0
γ	2.9	4.9
δ	2.2	3.4
ε	0	0

As already pointed out [2] the substance which has the $\varepsilon\varepsilon xxxx$-configuration should be the only benzene hexachloride capable of existing in d and l forms. It was therefore very interesting to learn that Cristol [7] has been able to prepare the l form of α benzene hexachloride.

Dr. L. K. Frevel of the Dow Chemical Company was kind enough to supply us with sufficient of the ε isomer both for the electron diffraction investigation and for the crystal structure determination. The latter is being carried out by cand.real. N. Norman and is now nearly completed. His results fully confirm the conclusions drawn from the electron diffraction analysis.

When comparing the $\frac{\sigma(r)}{r}$ -curves of closely related substances and studying differential curves it is important to eliminate — as far as possible — systematic differences in the r-scale due to inaccuracies in measurements of voltage and the effective distance from the point of diffraction to the photographic plate. In the curves reproduced in Fig. 1 a slight adjustment of the abscissa scales have been carried through with the aim of placing the first high peak corresponding to the C—Cl bond distance at exactly the same r-value, namely 1.76 Å. The r-values of this maximum in the original curves of the α, β, γ, δ and ε compounds were 1.79, 178, 177, 177 and 1.80 respectively, giving a mean value of 1.78 Å for the C—Cl-distance. If the true ratio of the C—C and C—Cl bond distances are 1.78 : 1.54 and not 1.76 : 1.54, the deviations from tetra-

hedral valency angles in the β compound would be a little larger than those indicated above.

The absolute heights of the peaks in the $\frac{\sigma(r)}{r}$-curves depend to some degree upon the time of exposure, the photographic material used and the drawing of the background in the intensity curves. In order to obtain ordinate values suited for a direct comparison of the $\frac{\sigma(r)}{r}$-curves of the different isomers and the evaluation of differential curves, the ordinate scales were so adjusted, that the height of the first pronounced peak at $r = 1.76$ Å was the same in all cases. The number of C—Cl bonds being the same for all the isomers we think that such a procedure should be the most satisfactory in the present case.

SUMMARY

The configurations of the five known isomers of benzene hexachloride have been studied in the vapour phase by the electron diffraction sector method. The distance distribution curves ($\frac{\sigma(r)}{r}$-curves) of these five isomers are very similar because the greater part of the internuclear distances are nearly identical in all the molecules in question. A careful examination of the curves, however, and the study of a selected set of differential curves has made it possible to decide which configuration corresponds to each of the individual isomers. The general types of deformations caused by repulsive forces acting between atoms not directly linked together and resulting in deviations from tetrahedral valency angles are discussed.

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Received July 2, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 926–929

Determination of Carbon Disulphide in Aqueous Solution

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In connection with an investigation of the carbon disulphide catalyzed iodine-azide reaction (to be published later), it became necessary to be able to — easily and rapidly — determine the concentration of carbon disulphide in an aqueous solution. A survey of the literature concerned showed that there was no suitable method for this purpose. Methods previously used are, for instance, conversion of carbon disulphide to sulphate or silver sulphide followed by a gravimetric determination. For the estimation of small amounts of carbon disulphide in air or in solution, colorimetric methods have been used, *e. g.* the formation of the yellow colour of cupric diethyldithiocarbamate. The best method for the estimation of considerable amounts of carbon disulphide in air or of pure carbon disulphide seems to be the xanthate method. Matuszak [1] has reviewed the literature and has shown that the method is quantitative. By this method, carbon disulphide is absorbed in or mixed with alcoholic potash, whereby a xanthate is formed:

$$CS_2 + KOH + C_2H_5OH = SC(OC_2H_5)SK + H_2O$$

The solution is then acidified with acetic acid until the reaction is acid to phenolphthalein, but still alkaline to litmus, and starch indicator is added. The solution can now be titrated with iodine solution according to the equation:

$$2\ SC(OC_2H_5)SK + I_2 = 2\ KI + (SCOC_2H_5S)_2$$

Matuszak mentions that the presence of water slows up the reaction between carbon disulphide and alcoholic potash. The reaction is instantaneous when absolute alcohol is used, but a solution made from 95 per cent alcohol reacts with sufficient rapidity. From this fact we concluded that the diffi-

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culty in solutions with higher concentrations of water might be overcome by allowing the mixture to stand for some time. Of course, carbon disulphide can be removed from an aqueous solution by aeration, and absorbed in alcoholic potash; but this is a tedious and complicated method. Harrower and Wiley [2] have, however, used this method when determining carbon disulphide in blood.

PROCEDURE

Into a 300 ml Erlenmeyer flask with glass stopper is introduced 20 ml of alcoholic potash (10 % potassium hydroxide in 96 % alcohol). Into the flask is now pipetted 10 ml of the aqueous solution of carbon disulphide, the stopper is replaced, the flask shaken for a few seconds, and left in darkness for 30 min. The stopper is then removed and washed with a small amount of water. One drop of phenolphthalein is added, and the solution is neutralized by dropwise addition of 60 % acetic acid. An excess of 3—4 drops is added. For the sake of imparting to the solution an adequate reaction, one gram of calcium carbonate, *p. a.*, is added, and the flask shaken for about 15 seconds. 1 ml starch indicator is added, and the solution is diluted with water to 150 ml. It is now ready for the titration with 0.01 *N* iodine. The iodine solution is added to an excess of about 0.5 ml (corresponding to a strong blue colour). The excess of iodine is determined by back-titration with 0.01 *N* thiosulphate.

According to Matuszak [1], it is important that titration of xanthate takes place in a nearly neutral solution. The addition of calcium carbonate to a xanthate solution, acidified with acetic acid, has previously been used by Lehmann [3]. If the alcoholic potash, used as reagent, is not freshly prepared, it is absolutely necessary to run a blank determination by substituting 10 ml water for the 10 ml carbon disulphide solution. Otherwise the blank determination is carried out exactly as the real determination. According to Harrower and Wiley [2], it is advisable to dissolve potassium hydroxide in alcohol at low temperature, and to store the reagent in an ice box. Prepared in this way, the reagent has a very small blind value when fresh.

Matuszak [1] titrates the xanthate formed with iodine until the formation of a blue colour. According to our experience, the addition of an excess of iodine and back-titration with thiosulphate seems to yield the most accurate results, especially when rather small amounts of carbon disulphide are to be determined.

EXPERIMENTAL RESULTS

After a few introductory experiments, it seemed most expedient to use 5 or 10 % alcoholic potash. The experiments were carried out in such a way that 10 ml of water were added (in expt. no. 11, 20 ml water were added) to a varying amount of alcoholic potash contained in a 300 ml Erlenmeyer flask. 15—20 mg carbon disulphide were now added (7.613 mg $CS_2 \sim$ 10 ml 0.01 *N* iodine). The carbon disulphide used was of analytical quality and was further purified by distillation. It was weighed out in a capillary tube on a micro balance. The tube was introduced into the alcoholic potash. After replacing

Table 1. Conversion of carbon disulphide to xanthate in 10 % alcoholic potash. The amounts of carbon disulphide added and found are given as the equivalent volumes of 0.01 N iodine. 'Reaction time' is the time allowed for the reaction between carbon disulphide and alcoholic potash. Temperature during the experiments: 16.5 – 17.0° C.

Expt. no.	Reaction time min	Alcoholic potash, ml	Water added. ml	CS_2 added	CS_2 found
1	30	10	10	18.10	17.92
2	30	15	10	21.05	21.03
3	30	20	10	20.21	20.21
4	30	20	10	25.12	25.11
5	30	20	10	14.03	14.04
6	30	20	10	19.71	19.67
7	30	20	10	17.31	17.32
8	30	20	10	23.51	23.49
9	30	40	10	22.12	22.13
10	30	80	10	27.26	27.28
11	30	20	20	23.24	23.07
12	60	20	10	19.01	19.00
13	120	20	10	27.31	27.30
14	20	20	10	22.13	22.10

the stopper, the carbon disulphide could be washed out of the tube by a slight shaking of the flask. The flask was then allowed to stand in darkness for a certain time (reaction time). The capillary tube was then crushed with a spatula, and neutralization and titration accomplished as described above.

From Table 1 it can be seen that, when using 20 ml of alcoholic potash, a reaction time of 30 min. is sufficient for the quantitative conversion of carbon disulphide to xanthate. In the column 'CS_2 added', the amounts of carbon disulphide used is given, — but expressed as equivalents, in ml, of 0.01 *N* iodine solution. The column 'CS_2 found' gives the corresponding experimental values — also in ml 0.01 *N* iodine solution. From Table 2 it can be seen that 5 % alcoholic potash may be used, but the reacting solution must be allowed to stand longer, or more alcoholic potash must be used.

SUMMARY

The well known xanthate method for the determination of carbon disulphide has been modified so that carbon disulphide, in an aqueous solution, can be directly titrated, without first separating the carbon disulphide from the water.

Table 2. Conversion of carbon disulphide to xanthate in 5 % alcoholic potash. The amounts of carbon disulphide added and found are given as the equivalent volumes of 0.01 N iodine. 'Reaction time' is the time allowed for the reaction between carbon disulphide and alcoholic potash. Temperature during the experiments: 15.6—16° C.

Expt. no.	Reaction time min	Alcoholic potash, ml	Water added, ml	CS_2 added	CS_2 found
15	30	20	10	21.65	21.10
16	40	20	10	27.25	27.20
17	60	20	10	25.12	25.11
18	30	30	10	30.40	30.38
19	30	40	10	20.73	20.73
20	40	30	10	19.01	19.00
21	40	40	10	20.05	20.06
22	30	80	10	24.14	24.16

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Received July 4, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 931–938

Microbiological Determinations of Amino Acids in Foodstuffs. I

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Many of the earlier methods for the determination of amino acids are applicable only to purified proteins. Interfering reactions, especially with carbohydrates, preclude their use for determining amino acids in foods. Great interest has recently developed in the amino acid content of foods, particularly nutritionally essential amino acids. In this field the microbiological amino acid determinations are chiefly being used to-day. In view of the use to which such data are put, it is rarely necessary, as Chibnall [1] emphasises, that the results should be more accurate than to within ± 10 per cent. Almost all present microbiological methods achieve or surpass this goal. The present paper describes the results obtained by the microbiological determination of eighteen amino acids in eight foodstuffs which are commonly used for animal nutrition in this country.

MATERIAL AND METHODS

Preparation of material. The materials were obtained from the National Animal Experimental Station, Uppsala. All samples were ground and thoroughly mixed to assure homogeneity. The lipids were extracted with ethyl ether and the residue air-dried and stored. It could be anticipted that more uniform analyses should be obtained when the materials were air-dried by equilibration with the moisture of the athmosphere and the samples were weighed for all analyses, including moisture and ash, during a short time interval. This procedure was followed in the present investigation.

Hydrolysates of tryptophan were prepared with $Ba(OH)_2$ according to Greene and Black [2]. Cystine was determined in hydrolysates prepared according to Miller and du Vigneaud [3]. Acid hydrolysates, used for the determinations of the other amino acids, were prepared by suspending 5 g of sample in 50 ml of 2 *N* HCl and autoclaving at 15 pounds pressure for 10 hours [4].

Methods of analysis. Microorganisms, basal media, ranges of standard curves, and incubation times are given in Table 1. The procedures followed for the cultures and

Table 1. Experimental conditions for the microbiological analyses.

Amino acid	Medium	Micro organisms	Standard curve γ per 2 ml	Incubation time, hours
Alanine	Sauberlich and Baumann [22]	*L. citrovorum* (8081)	0—80	24
Arginine	Henderson and Snell [23]	*L. casei* (7469)	0—20	72
Aspartic acid	» » »	*L. mesenteroides* P-60	0—40	72
Cystine	» » »	*L. arabinosus* 17—5	0—20	48
Glutamic acid	» » »	*L. arabinosus* 17—5	0—80	72
Glycine	Steele *et al.* [24]	*L. mesenteroides* P-60	0—20	30
Histidine	Henderson and Snell [23]	*L. mesenteroides* P-60	0—20	72
Isoleucine	» » »	*L. mesenteroides* P-60	0—30	72
Leucine	» » »	*L. arabinosus* 17—5	0—20	72
Lysine	» » »	*L. mesenteroides* P-60	0—20	72
Methionine	» » »	*L. arabinosus* 17—5	0—10	48
Phenylalanine	» » »	*L. arabinosus* 17—5	0—10	48
Proline	» » »	*L. mesenteroides* P-60	0—20	72
Serine	» » »	*L. casei* (7469)	0—20	72
Threonine	» » »	*L. fermenti* 36	0—20	72
Tryptophan	» » »	*L. arabinosus* 17—5	0—2	72
Tyrosine	» » »	*L. mesenteroides* P-60	0—20	72
Valine	» » »	*L. arabinosus* 17—5	0—20	72

inoculum have been described in previous papers [5, 6]. A Fisher automatic volustat was used for the serial pipettations. A casein hydrolysate was always included as an extra control in the determination of the amino acids. For each amino acid several separate assays were carried out. In each series, five assay levels were used.

RESULTS AND DISCUSSION

Several methods have been used for expressing the analytical data obtained by microbiological amino acid determinations of complex foodstuffs. The values may be calculated as the amino acid content of the foodstuff and, in such cases, it is necessary to provide other data on gross composition to facilitate a comparison of different samples. Usually this is done by calculating the data to a common nitrogen basis, 16 per cent [7, 8], even though it is recognized that the nitrogen content of most protein in foodstuffs is not 16 per cent. An alternative method of expressing the data, is to calculate the ratio of amino acid nitrogen to total nitrogen. This avoids any empirical assumption and permits comparison of different samples. This procedure is widely adopted in the analysis of foodstuff proteins [9]. A third method commonly used, is to calculate the amino acid content in per cent of the weight of foodstuff, either

Table 2. 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
Meat meal I, from S. G. S., Uppsala	12.9	80.5	18.3	6.5
Meat meal II, from Scan, Kävlinge	12.6	79.0	30.2	6.3
Meat meal III, from Tomelilla	10.4	65.0	36.6	5.8
Sweet yellow lupine, whole meal	7.70	48.4	3.9	10.1
Field bean, whole meal	5.17	32.4	2.5	11.1
Potato (Magnum bonum), unpeeled raw potato dried at room temperature	1.26	7.9	0.9	1.0
Golden Rain oats II, whole meal	2.26	14.1	2.4	13.6
Maja barley, whole meal	2.12	13.2	2.9	12.1

airdried by equilibration with moisture of the atmosphere or on a water and ash-free basis [10, 11]. To facilitate a comparison with previous data in the literature the microbiological data of the present investigation were calculated according to all three methods.

In Table 2 the nitrogen values and the content of crude protein of the materials are recorded.

Recent total nitrogen values [12] for meat scraps, ground barley, and ground oats are in agreement with the values of this investigation. As could be anticipated, only small differences exist between the total nitrogen values of the three commercial meat meals. These differences are more pronounced when the amino acid content of the three meat meals are compared (Table 3).

The most striking differences may be observed in the values of cystine, isoleucine, lysine, methionine, threonine and tyrosine. Recent data, from the literature, for the amino acid content of the foodstuffs investigated in this paper are given in Table 4.

Mainly, only values obtained by microbiological methods have been recorded. In general, there is comparatively good agreement with the values obtained in this investigation (Table 3). A discrepancy is observed in the trypthophan values calculated for ash- and moisture-free material, where lower values have been obtained in this work. One probable reason would appear to be the method of hydrolysis with barium hydroxide used by the author. In some preliminary analyses, on hydrolysates prepared according to Kuiken *et al.* [19] with sodium hydroxide and cysteine, somewhat higher values were obtained. These authors also compared their method with that of Greene and Black, and reported a higher tryptophan content in some other foodstuffs when the sodium hydroxide-cysteine hydrolysis was used.

With regard to the amino acid data for lupine meal in Table 4, it may be stated that they were obtained by chemical analyses quoted from Block and

Table 3. Amino acid content of the foodstuffs.

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	Meat meal I			Meat meal II			Meat meal III			Lupine meal		
	1	2	3	1	2	3	1	2	3	1	2	3
Alanine	7.2	8.4	8.2	7.1	8.5	8.3	5.3	7.2	7.1	1.7	3.9	3.9
Arginine	5.0	5.8	12.0	4.7	5.6	11.9	5.0	7.0	14.0	2.8	6.2	13.0
Aspartic acid	3.1	4.0	2.3	3.5	4.3	2.8	3.9	5.4	3.6	2.7	5.9	3.9
Cystine	0.25	0.26	0.21	0.31	0.42	0.30	0.34	0.47	0.40	0.35	0.70	0.50
Glutamic acid	9.8	11.5	6.8	9.1	11.0	6.5	9.4	12.0	7.8	10.0	23.0	17.0
Glycine	1.5	1.9	2.1	1.5	1.8	2.2	0.69	1.0	1.1	0.87	2.0	2.3
Histidine	1.7	2.0	3.5	2.3	2.8	4.8	1.9	2.4	4.6	0.90	1.9	3.5
Isoleucine	1.5	1.6	1.1	2.2	2.7	1.8	2.3	3.2	2.2	1.6	3.7	2.4
Leucine	10.1	11.0	7.5	6.3	8.5	5.5	7.0	9.6	6.4	3.4	7.5	5.1
Lysine	2.8	3.2	3.8	5.8	6.9	8.4	3.9	5.4	6.4	1.6	3.8	4.3
Methionine	1.1	1.2	0.7	1.5	1.9	1.1	2.1	2.8	1.7	0.44	0.98	0.57
Phenylalanine	2.8	3.3	1.8	3.2	3.9	2.1	2.9	4.0	2.2	1.7	3.9	2.1
Proline	5.5	6.3	4.8	3.8	4.5	3.5	4.6	6.4	4.9	1.7	3.9	3.1
Serine	4.4	5.1	4.3	4.9	5.6	4.8	4.6	6.4	5.3	1.2	2.7	2.3
Threonine	1.7	1.9	1.4	1.9	3.0	2.2	1.3	1.8	1.3	0.87	2.0	1.4
Tryptophan	0.18	1.0	0.8	0.14	1.0	0.8	0.2	1.0	0.8	0.1	1.0	0.8
Tyrosine	1.6	1.8	0.9	3.1	3.7	1.8	1.9	2.9	1.4	1.2	2.6	1.3
Valine	4.5	5.3	4.0	4.9	5.9	4.3	4.5	6.4	4.8	2.1	4.6	3.5
NH_3—N			7.0			5.2			5.3			13.0
Total	64.6	75.6	73.2	66.3	82.0	78.3	61.8	85.4	81.3	35.2	80.3	84.0

Table 3 (continued)

Amino Acid Content of the Foodstuffs.

Amino acid	Bean meal			Potato			Oat meal			Barley meal		
	1	2	3	1	2	3	1	2	3	1	2	3
Alanine	1.7	5.7	5.7	0.47	6.1	6.0	0.57	4.1	4.0	0.42	4.1	4.0
Arginine	1.7	5.7	12.0	0.54	7.1	14.0	0.79	6.31	3.0	0.59	5.7	11.0
Aspartic acid	2.0	6.7	4.5	0.89	11.5	7.6	0.56	4.5	3.1	0.29	2.5	1.7
Cystine	0.21	0.70	0.50	0.04	0.6	0.4	0.07	0.5	0.4	0.09	0.9	0.6
Glutamic acid	4.8	16.0	9.2	0.59	7.4	4.4	2.3	18.0	11.0	2.4	22.0	13.0
Glycine	0.52	1.7	2.0	0.28	1.9	2.2	0.86	3.1	3.6	0.59	2.9	3.3
Histidine	0.48	1.5	2.7	0.095	1.2	2.0	0.27	2.2	3.7	0.18	1.5	2.6
Isoleucine	1.3	4.3	2.8	0.45	5.9	4.0	0.67	5.4	3.6	0.57	4.8	3.3
Leucine	2.2	7.2	4.8	0.44	4.6	3.1	1.0	8.1	5.4	0.80	6.8	4.6
Lysine	1.6	5.3	6.2	0.38	3.7	4.3	0.46	4.3	5.2	0.38	3.3	4.0
Methionine	0.35	1.1	0.68	0.25	2.5	1.5	0.39	3.1	1.8	0.41	3.5	2.1
Phenylalanine	1.2	3.8	2.0	0.28	3.6	1.9	0.71	5.7	3.1	0.66	5.7	3.0
Proline	1.3	4.3	3.2	0.22	3.0	2.2	0.50	4.1	3.1	0.57	4.8	3.7
Serine	1.6	5.2	4.2	0.20	2.6	2.1	0.36	2.9	2.4	0.61	5.3	4.6
Threonine	0.8	2.6	1.9	0.19	2.5	1.8	0.27	2.1	1.6	0.35	3.2	2.2
Tryptophan	0.052	1.0	0.8	0.07	1.0	0.8	0.05	1.4	1.2	0.05	1.6	1.4
Tyrosine	0.81	2.6	1.3	0.25	2.5	1.2	0.45	3.6	1.7	0.37	2.8	1.4
Valine	1.5	4.8	3.5	0.44	4.3	3.5	0.48	3.8	2.8	0.45	3.8	2.8
NH_3—N			12.0			20.0			15.0			18.0
Total	24.1	80.2	79.8	6.08	72.0	82.7	10.8	83.2	85.7	9.78	85.2	87.1

Bolling [7]. The values do not seem to be calculated on the common nitrogen basis of 16 per cent, which may account for some of the discrepancies. The source of material is not well defined. The amino acid content of the field bean has not previously been investigated. For comparison, available data on soy bean meal are given. It is obvious that as a potential source of essential amino acids, the soy bean meal is far superior.

The protein of potatoes represents approximately 50 per cent of the total nitrogen content [20], and consists of the well known globulin 'tuberin' A comparatively large part of the remaining total nitrogen is composed of free amino acids (*cf.* Dent *et al.* [21]). Analysis of the amino acid content of unpeeled potatoes has not previously been carried out. For comparison the data obtained by Stokes *et al.* [18] on peeled potatoes may be of some interest. On the whole, the agreement is good. A real discrepancy may exist between the methionine values, where a rather low figure is recorded for the peeled potato. However, it may be mentioned that Stokes' methionine method has been reported to give low values [8].

Table 4. Amino acid content of some foodstuffs (literature values).

1 = Values expressed as percentage for ash- and moisture-free material
2 = Values expressed as percentage in crude protein (total nitrogen in hydrolysates × 6.25)

Amino acid	Meat scraps		Lupine meal	Soy bean meal		Peeled potato	Oat meal		Barley meal	
	1	2	2	1	2	1	1	2	1	2
	(12)*	(7.13)*	(7)*	(12)	(7.13)*	(18)*	(11, 12)* (14—17)*	(7)*	(12)* (14—17)*	(7)*
Alanine										
Arginine	3.4	7.0	12.6	3.0	7.1	0.37	0.7 1.26	5.8	0.5 0.53	4—6
Aspartic acid			5.6		3.7					
Cystine		1.0			1.9					
Glutamic acid	6.4		27.0	9.0	19.0		2.9	17.0	3.2	
Glycine		1—2								
Histidine	2.2	2.0	2.9	1.7	2.3	0.10	0.4 0.38	2.0	0.3 0.21	1—2
Isoleucine	2.1	6.3	9	3.5	4.7	0.45	0.7	4.0	0.7	
Leucine	5.0	8.0		4.5	6.6	0.56	1.1 1.36	6.5	1.0 0.89	
Lysine	3.8	7.0	3.1	3.0	5.8	0.33	0.6	3.3	0.4	1—3
Methionine	0.7	2.0		0.9	2.0	0.09	0.2	1.2	0.2	
Phenylalanine	3.2	4.5	4.5	2.8	5.7	0.43	0.8 0.79	4.8	0.7 0.68	
Proline		5—6	4.0		5.0					
Serine	2.3	4.5		2.4			0.4	2.4	0.5	
Threonine	2.2	4.0		2.1	4.0	0.37	0.4	2.4	0.4	
Tryptophan	0.4	0.7		0.6	1.2	0.13	0.1	0.6	0.2	
Tyrosine	1.4	3.2	5.6	1.2	4.1		0.2	1.2	0.2	
Valine	3.8	5.8	2	3.0	4.2	0.46	0.7	4.2	0.7	

* Literature references.

Finally, the data collected from microbiological amino acid analyses on whole meal from oat and barley grown in U. S. A. show a comparatively good agreement with the values obtained on Swedish oat and barley. In this connection it may be mentioned that Kuiken and Lyman [8] recently showed that soy bean meals, prepared from twenty strains of soy beans grown in different parts of U. S. A., showed a quite uniform amino acid distribution.

SUMMARY

Eight foodstuffs commonly used in animal nutrition have been analyzed by microbiological methods for the eighteen possible amino acids. Where a comparison is possible, the results agree closely with those obtained by other microbiological methods on similar materials.

The investigation was supported by a grant from the Swedish Agricultural Research Council. The technical assistance of Mrs. I. Kristenson is greatfully acknowledged.

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Received July 11, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 939–945

Studies Related to Pristane

III. The Identity of *Nor*phytane and Pristane

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In the two first communications in this series [1, 2] we have shown that the saturated hydrocarbon pristane, earlier considered a octadecane, is at least a nonadecane. As the eight possible mono-methyl-octadecanes all showed m. p. between — 16.5° and + 13° C whereas pristane does not solidify at — 80°, we concluded that pristane belongs to the more branched alkanes. The only branched alkanes that are found in living nature are the hydrogenated terpenes; therefore we have continued the comparison of pristane with saturated compounds related to known diterpenes.

Owing to its easy accessibility, we first [1] synthesized the previously known hydrocarbon crocetane = 2,6,11,15-tetramethyl-hexadecane. The physical constants of this compound we found to be in agreement with the data of Fischer and Löwenberg [3] who synthesized this hydrocarbon the first time. As will be seen crocetane comes rather close to the values given for pristane, but the crocetane values

	d_4^{20}	n_D^{20}
Crocetane		
(mean of Fischer, and our own data)	0.7880	1.4404
Pristane	0.7827–0.7843	1.4385–1.4398

fall a little higher. Terpenes with symmetrically transposed isoprene units such as in crocetane are only known in squalene and the carotenoids. The diterpenes of certainly known constitutional formula have either regularly connected isoprene units or the end unit distorted through a Wagner-rearrangement. [4] The regularly constructed diterpene alkane is phytane = 2,6,10,14-tetramethyl-hexadecane. Willstätter, Mayer and Hüni [5] produced phytane as early as 1911; unfortunately the only physical constant given was

$d_4^0 = 0.803$. We, therefore, had to prepare phytane once more and the synthesis followed the line:

Phytol → Dihydrophytol → Dihydrophytylbromide → Phytane

(for details see experimental part). Phytane thoroughly purified showed the following constants:

	d_4^{20}	n_D^{20}
Phytane:	0.7907	1.44225

The difference in density and refractive index between crocetane and phytane is in agreement with the difference expected from the regularities worked out by Calingaert [6] and Francis [7].

The two most probable terpenoid-alkanes thus definitely differ from pristane. The physical constants of this saturated diterpene strengthen the results of the molecular weight determinations of Berner [1], giving the molecular formula to $C_{19}H_{40}$. For comparison we therefore prepared *nor*phytane = 2,6,10,14-tetramethyl-pentadecane. According to Fischer and Löwenberg [8] phytol was degradated to 6,10,14-trimethyl-pentadecanone-2. CH_3MgI when added to this ketone gave 2,6,10,14-tetramethyl-pentadecanol-2. This carbinol was converted to the corresponding ethylene compound which was in turn hydrogenated to *nor*phytane = 2,6,10,14-tetramethyl-pentadecane, whose physical constants were as follows:

	d_4^{20}	n_D^{20}
*Nor*phytane	0.7833	1.4386
Pristane	0.7827—0.7843	1.4385—1.4398

The comparison with pristane shows the most excellent agreement. On the basis of the before mentioned regularities [6, 7] of the physical constants of the alkanes, it can be foreseen that no other nonadecane of reasonable structure will have density and refractive index as low as *nor*phytane; these regularities thus heavily augment the possibility that pristane is identical with *nor*phytane.

Of course, we have tried to demonstrate this identity through mixed melting point determinations, but these failed, as we did not succeed in crystallizing these hydrocarbons at all. Below — 85° C pristane and *nor*phytane as well as phytane and crocetane successively become thicker, between — 99 and — 100° C they all congeal. And this congelation appears at the same tem-

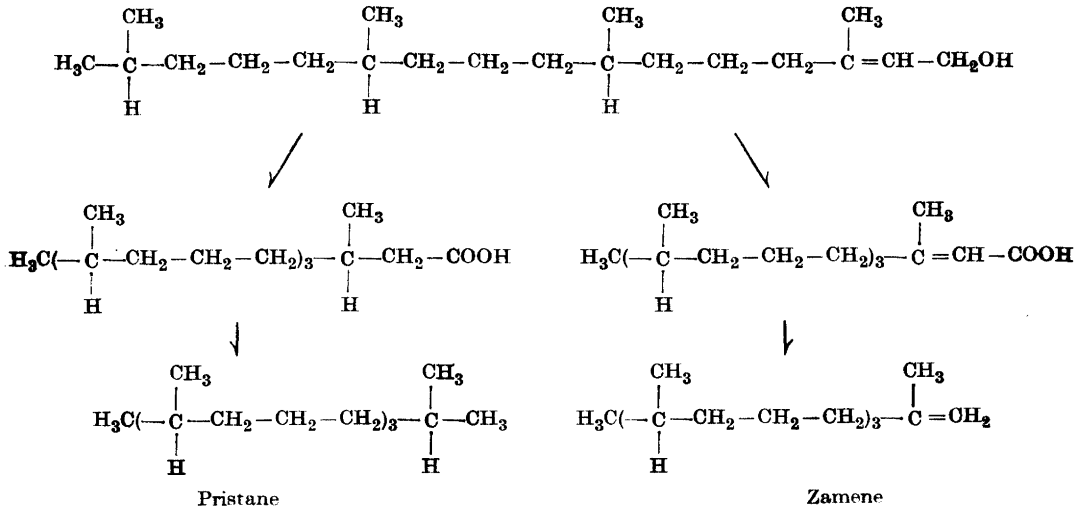
peratures with mixtures of pristane and *nor*phytane as with mixtures of pristane and for example phytane. Certainly the identical congelation circumstances of pristane and the said terpenoid alkanes significantly point to the correctness of our working hypothesis that pristane itself is of a terpenoid nature.

As mentioned in a lecture given by one of us [9] at the 6th Scandinavian chemical meeting in Lund the 28th August 1947, we have succeeded in degrading pristane to oxygen containing terpenoid compounds of lower molecular weight partially through chlorination with tert-butylchloride-$AlBr_3$ according to Bartlett, Condon and Schneider [10], splitting off HCl with alcoholic potassium hydroxide followed by ozonization, and partially through nitration with 100 % HNO_3 according to Grundmann [11] and ozonization of the alkali-soluble fraction of the nitro-compounds. Both methods, however, gave very complex mixture of compounds, and we have met great difficulties in the preparation of definite degradation products.

A confirmation of the identity of pristane and *nor*phytane was however achieved from quite another side. As is known, Tsujimoto [12] in 1935 demonstrated that the pristane fraction of Basking Shark liver oil contains quite small amounts of an ethylene hydrocarbon, called zamene, which on hydrogenation gave an alkane with constants identical with pristane. In cooperation with Chem. Eng. Per Koch Christensen we have tried to elucidate the structure of zamene. The details of this work will follow in another publication of this series. It may be mentioned, however, that zamene was transformed by peracids into its epoxide, the epoxide hydrolyzed to the corresponding α-glycol. This glycol which was separated from pristane through 'Entmischung' between petrol and 90 % methanol analyzed for $C_{19}H_{40}O_2 \pm CH_2$.

When this α-glycol was split up with lead tetraacetate according to Criegee, it gave two components; formaldehyde, and a liquid monooxygen-compound with physical constants and elementary composition very close to the ketone $C_{18}H_{36}O$ from phytol, *viz.* 6,10,14-trimethyl-pentadecanone-2. Hence the ethylene hydrocarbon zamene ought to have the constitution 2,6,10,14-tetramethyl-pentadecene-1.

The constitutional formulae deduced for pristane and zamene at once suggests a process for this unusual occurence of C_{19}-terpenes in nature. Both hydrocarbons bear close relations to the fundamental aliphatic diterpene phytol. Dehydrogenation of the primary alcohol group to carboxyl, accompanied or not by hydrogenation of the α,β-ethylene bond, gives rise to phytane- or to phytene-carboxylic acid respectively. On decarboxylation these acids generate the two hydrocarbons pristane and zamene.



It is impossible for the time being to predict at what stage these transformations of phytol take place in nature, the authors being inclined to guess at enzymatic processes of the bacterial flora of the intestine of either the zooplankton or the intermediate food animals, but the constitution of these hydrocarbons ought to exclude synthetic processes in the elasmobranch fishes themselves.

EXPERIMENTAL

Phytane

Phytol ('L. Light & Co. Ltd') was carefully fractionated at 0.001 mm, and the main fraction was hydrogenated at ordinary temperatures with PtO_2-catalyst. In accordance with the statements of Willstätter, the fractionation gave a small forerun of phytane, the main fraction boiling at 100° bath temperature at 0.001 mm was dihydrophytol, $n_D^{20} = 1.4541$, $d_4^{20} = 0.8431$. These constants are in agreement with older data: Willstätter [5] $d_4^{20} = 0.8398$, $n_D^{20} = 1.45213$, Kuhn and Suginomé [13] $n_D^{20} = 1.4538$.

Dihydrophytol was transformed into its bromide with anhydrous HBr-gas according to *Organic Synthesis* XV p. 35 a 24. Dihydrophytylbromide b. p. 105°, 0.001 mm $n_D^{20} = 1.4641$, $d_4^{20} = 0.9675$, $M_D = 103.1$, $M_{D,\,calc.} = 102.3$.

$C_{20}H_{41}Br(361.4)$	Calc.	C 66.45	H 11.43	Br 22.12
	Found	» 66.66	» 11.50	» 22.34

Kuhn and Suginomé [13] have emphasized the difficulties in getting Grignard-reactions of dihydrophytylbromide. Only with small amounts of completely dry, freshly destilled ether did the reaction start. When the reaction had ceased, dry NH_4Cl and then water

were added. Phytane b. p. 75° bath temperature 0.001 mm. The main fraction was purified with 100 % H_2SO_4, redestilled once over Na and then slowly fractionated at 0.001 mm, main fraction 69°—71° bath temperature. $d_4^{20} = 0.7907$ calc. by A.W. Francis $d_4^{20} = 0.7895$, the value given by Willstätter, $d_4^0 = 0.803$, corresponds exactly to $d_4^{20} = 0.7895$, using the mean temperature coefficient dD/dt = 0.000676 of $C_{18}-C_{21}$ alkanes given in Egloff: *Physical constants of hydrocarbons.* Vol. I (1939).

Table 1. Dispersion of phytane.

$$R_{\lambda,\,\text{calc.}} = \frac{92.381 \cdot \lambda^2}{\lambda^2 - 0.7883 \cdot 10^6} \qquad \text{o} : \lambda_0 = 887.8 \text{ ÅU}$$

λ	n_λ^{20}	$R_{\lambda,\,\text{obs.}}$	$R_{\lambda,\,\text{calc.}}$
6678.1	1.43961	94.03	94.04
5895.9	1.44225	94.52	94.52
5875.7	1.44234	94.54	94.54
5015.6	1.44688	95.38	95.37
4921.9	1.44752	95.50	95.49
4713.1	1.44911	95.79	95.78
4471.5	1.45113	96.17	96.17

2,6,10,14-Tetramethyl-pentadecanol-2

Phytol was freshly fractionated at 0.001 mm and treated with ozone in glacial acetic acid. The phytol-ozonide was decomposed with zink dust and water. Acidic reaction products were withdrawn from the ethereal solution of the ketone through repeated washings with sodium carbonate, and 2,6,10-trimethyl-pentadecanone fractionated cautiously at 0.0001 mm (b. p. bath temperature 76—80° C). The physical constants given in literature and found by us are as follows:

	n_D^{20}	d_4^{20}
Willstätter	1.4443	0.844
Fischer from phytol	1.4452	0.8357
» synthetic	1.4455	0.8351
Fischer & Löwenberg synthetic	1.4454	0.8371
Found this publ.	1.4445	0.8370

4.25 g 2,6,10-trimethyl-pentadecanone was slowly reacted at ordinary temperature with the calculated amount CH_3MgI and worked up in the usual way. 2,6,10,14-tetramethyl-pentadecanol-2 is an oil, b. p. 100° at 0.001 mm.

$C_{19}H_{40}O$ (284.4)	Calc.	C 80.21	H 14.17
	Found	» 79.71	» 14.14
	»	» 80.32	» 14.14

$d_4^{20} = 0.8367$.

Table 2. Dispersion of 2,6,10,14-tetramethyl-pentadecanol-2.

$$R_{\lambda,\,\text{calc.}} = \frac{89.131 \cdot \lambda^2}{\lambda^2 - 0.7832 \cdot 10^6} \qquad \text{o} : \lambda_0 = 885 \text{ ÅU}$$

λ	n_λ^{20}	$R_{\lambda,\,\text{obs.}}$	$R_{\lambda,\,\text{calc.}}$
6678.1	1.44653	90.71	90.72
5895.9	1.44927	91.19	91.19
5875.7	1.44938	91.21	91.20
5460.7	1.45118	91.53	91.53
5015.6	1.45394	92.01	92.00
4471.5	1.45823	92.76	92.76
4358.3	1.45935	92.96	92.96

Norphytane = 2,6,10,14-tetramethyl-pentadecane

By dehydration of 2,6,10,14-tetramethyl-pentadecanol-2 2 isomeric *nor*phytenes may arise, *viz.* one with *iso*propylidene (Δ2.3) and one with methylene-grouping (Δ1.2). We have made no efforts to determine the composition of the ethylenemixture formed by heating 2,6,10,14-tetramethyl-pentadecanol-2 with $KHSO_4-K_2SO_4$ at 150° C for 4 hours, as both of them will generate the same alkane on hydrogenation. The resulting liquid norphytene showed the following constants $n_D^{20} = 1.4482$, $d_4^{20} = 0.79899$, $M_{D,\,\text{obs.}} = 89.29$, $M_{D,\,\text{calc.}} = 89.47$.

*Nor*phytene was hydrogenated by bubbling hydrogen through the ethylene at 90° C in the presence of a $Pt-SiO_2$-catalyst (17 % Pt). *Nor*phytane was purified in the usual way with sulfuric acid and fractionation over Na. B. p. 0.001 mm 68° bath temperature. $d_4^{20} = 0.7833$.

Table 3. Dispersion of Norphytane = 2,6,10,14-tetramethylpentadecane.

$$R_{\lambda,\,\text{calc.}} = \frac{87.988 \cdot \lambda^2}{\lambda^2 - 0.7886 \cdot 10^6} \qquad \text{o} : \lambda_0 = 888 \text{ ÅU}$$

λ	n_λ^{20}	$R_{\lambda,\,\text{obs.}}$	$R_{\lambda,\,\text{calc.}}$
6678.1	1.43596	89.56	89.57
5895.9	1.43863	90.03	90.03
5875.7	1.43872	90.05	90.05
5460.7	1.44054	90.38	90.38
5015.6	1.44320	90.85	90.84
4921.9	1.44382	90.96	90.95
4713.1	1.44535	91.23	91.23
4471.5	1.44735	91.59	91.60
4358.3	1.44849	91.79	91.80

SUMMARY

For comparison with pristane, phytane was resynthesized, and *nor*-phytane = 2,6,10,14-tetramethyl-pentadecane synthesized the first time.

*Nor*phytane	$d_4^{20} = 0.7833$	$n_D^{20} = 1.4386$	Congelation point = — 100° C.
Our purest pristane	$d_4^{20} = 0.78267$	$n_D^{20} = 1.43848$	Congelation point = — 100° C.

As the regularities worked out by the petroleum chemists predict that no other nonadecane of reasonable structure will show density and refractive index as low as *nor*phytane, it is concluded that *nor*phytane and pristane are identical = 2,6,10,14-tetramethyl-pentadecane.

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Received July 5, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 946–953

Studies on the Reaction between Aromatic Nitro Compounds and Active Methylene Groups. XVI. The Mechanism of Neutralization of *Meta* Dinitrobenzene Derivatives by Aliphatic Amines at —72° C

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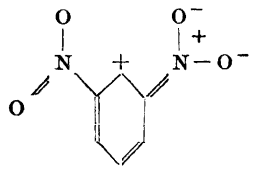
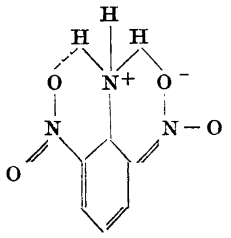
In a very interesting paper, Lewis and Seaborg[1] some years ago discussed the acidity of some aromatic nitro compounds towards amines at low temperatures. They investigated whether or not trinitrobenzene, in petroleum ether solution, was neutralized by ammonia, methylamine, dimethylamine and triethylamine, and found that trinitrobenzene only gave a slight colour with triethylamine but an intense crimson colour with the weaker base ammonia. The selective light absorption is due to a quinoid structure of the nitro compound stabilized by the addition of the amine. Even more remarkable, however, was that *meta* dinitrobenzene, the ionization constant of which in aqueous solution could not be greater than 10^{-17}, in petroleum ether solution was neutralized by ammonia and methylamine, while the stronger bases dimethylamine and triethylamine were without noticeable effect.

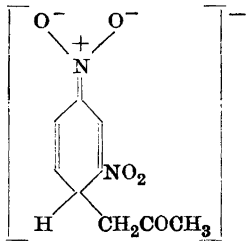
To explain this phenomenon Lewis and Seaborg assume the presence of a double chelation. The energy gained from the double chelation is then responsible for the attachment of ammonia and methylamine to the benzene ring, while dimethylamine, where only a single chelation is possible, and triethylamine, where no chelation is possible, are not attached. A condition precedent for this theory is that in the *meta* dinitrobenzene derivatives the carbon atom between the nitro groups is lacking in electrons, or, otherwise expressed, that an *ortho* quinoid resonance form:



dominates during the neutralization. They write the formula for the double chelation in the following way:



The investigations previously reported in this series [2, 3] have given evidence that the most important resonance form of the addition compound between *meta* dinitrobenzene and acetone in alkaline solution may be expressed by the formula:



This compound is an anion in which the benzene ring has a quinoid structure, and the acetone ion is assumed to be attached *ortho* respectively *para* to the two nitro groups.

The formula given by Lewis and Seaborg for the addition product between aromatic nitro compounds and amines thus does not agree with my formulation of the addition product between aromatic nitro compounds and acetone in alkaline solution. Although the experimental conditions are quite different — chelation is, for instance, impossible when the acetone anion is attached — and therefore *a priori* it is not necessary to assume the same mechanism for the addition of the different nucleophilic reagents (the weak nucleophilic amines and the strong nucleophilic acetone ion) to the ring, it was of interest to extend Lewis' and Seaborg's investigations to the *meta*

dinitrobenzene series in the hope of determining where the attachment of the amines actually takes place.

These experiments were performed at —71 to —73° C (ethanol + solid CO_2). Lewis and Seaborg do not report at what temperature they were working.

Skellysolve B was used as a solvent. The results are given in Table 1. Table 2 gives the results of Lewis' and Seaborg's measurements. In Table 1 are also included λ max. and ε max. in ultra violet (pentane), taken from Canbäck [3]. The measurements of 2,6-dinitrotoluene are new. See Fig. 1.

Table 1. Colour intensities produced on mixing different amines with m-dinitrobenzene derivatives in petroleum ether solution at — 72° C. Included are the UV absorption characteristics of the nitro bodies in pentane at + 20° C.

	1,3-Dinitro-benzene	2,4-Dinitro-toluene	2,6-Dinitro-toluene	4,6-Dinitro-1,3-dimethyl-benzene	Dinitro-mesitylene
Benzylamine	+ + +	+ +	—	—	—
Dimethylamine	+ +	+ +	—	—	—
Piperidine	+	+	—	—	—
Triethylamine	—	—	—	—	—
N-Ethylpiperidine	—				
Quinine	—				
Hexamethylene-tetramine	—				
ε max.	21000	15900	12000	15100	3900 *
λ max. mμ	227	232	227	243	238

* Inflexion point.

In Table 1 the approximative strengths of the colours are indicated by the number of +. *Meta* dinitrobenzene gave a clear crimson and 2,4-dinitrotoluene a blue-reddish colour.

As seen in Table 1, the same tendency is found in the *meta* dinitrobenzene series as Lewis and Seaborg observed. However, Lewis' and Seaborg's statement that *meta* dinitrobenzene does not give a colour with secondary amines must be corrected. At —71 to—73° C dimethylamine, as well as piperidine, gave bright colours, *e. g.* the amines did neutralize the nitro compound. Similarly 2,4-dinitrotoluene was neutralized by primary as well as by secondary amines. 2,6-Dinitrotoluene, dinitroxylene and dinitromesitylene were not noticeably influenced by any of the amines.

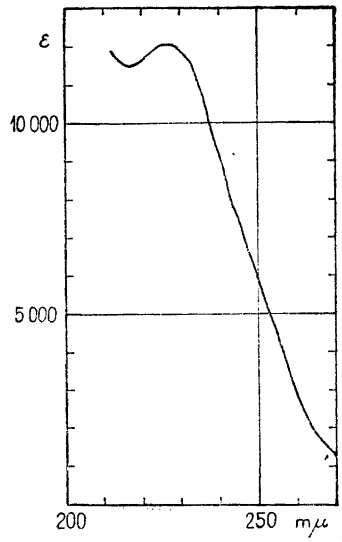


Fig. 1. Ultra-violet spectrum of 2,6 dinitrotoluene in petroleum ether.

Table 2. Colour production on mixing several bases with nitro compounds according to Lewis and Seaborg.

	1,3-Dinitrobenzene	1,3,5-Trinitrobenzene	2,4,6-Trinitrotoluene	2,4,6-Trinitroxylene	Trinitromesitylene
Ammonia	+	+	+	+	+
Methylamine	+	+	+	+	+
Dimethylamine	−	+	+	−	−
Triethylamine	−	+	+	−	−

Thus in principle Lewis' and Seaborg's statement, that *meta* dinitrobenzene is neutralized only when a double chelation is possible, is not true. The following description of the phenomenon might be more correct.

The amines in petroleum ether solution are such weak nucleophilic reagents that at the temperatures at which Lewis and Seaborg worked neutralization of *meta* dinitrobenzene only occurs when a double chelation is possible. At −72° C, however, the energy gained from a simple chelation is great enough to allow even secondary amines to be attached to the ring and thus to stabilize a quinoid form of the nitro compound.

The nitrogen atom in triethylamine is known [14] to be blocked by the bulky ethyl groups. To test if the phenomenon could possibly be explained by the

steric hindrance in the amine, quinine and hexamethylenetetramine were investigated. The nitrogen atom in the quinuclidine nucleus is not hindered. As seen in Table 1, neither quinine nor hexamethylenetetramine was neutralized by *meta* dinitrobenzene, indicating that the steric hindrance in triethylamine was not responsible for the failure of the reaction.

The attachment of the amines might occur at the carbon atom between the nitro groups since a positive reaction is obtained with 2,4-dinitrotoluene but not with 2,6-dinitrotoluene.

However, it is not absolutely certain that the attachment of the amines takes place in this position as 4,6-dinitro-1,3-dimethylbenzene, although sterically hindered but still capable of adding anions [3], gives a negative reaction even though the carbon atom between the nitro groups is unsubstituted. The fact that Lewis and Seaborg obtained a positive reaction with 2,4,6-trinitroxylene is explained by the presence of a nitro group *para* to the unsubstituted ring carbon atom. The latter compound is therefore much more acid than 4,6-dinitro-1,3-dimethylbenzene. It may also be that the small steric hindrance (see below) present in 2,6-dinitrotoluene (with both nitro groups hindered contrary to 2,4-dinitrotoluene in which only one nitro group is hindered) is sufficient to counteract the energy gained from a simple chelation even at —72°, *e. g.* if the attachment takes place in the position *ortho* — *para* to the nitro groups. Additional support of this theory is found in the investigations performed by Field, Garner and Smith [4] and Garner and Gillbe [5] on the ionization of aromatic nitro compounds in liquid ammonia. From their results it will only be mentioned here that conductiometric as well as photometric measurements showed that 1,3-dinitrobenzene, 2,4-, 3,5-, and 2,6-dinitrotoluene behaved in the same manner, while 1,4-, and 1,2-dinitrobenzene as well as 2,5-, 2,3-, and 3,4-dinitrotoluene behaved in a different manner. Of special interest in this connection is that 1,3-dinitrobenzene, 2,4-, 3,5-, and 2,6-dinitrotoluene gave blue or violet coloured solutions in liquid ammonia while 2,5-, 2,3-, and 3,4-dinitrotoluene and 1,2-, and 1,4-dinitrobenzene gave yellow solutions. It is thus highly improbable that the attachment of the ammonia takes place on the carbon atom between the two nitro groups, which are in a *meta* position to each other.

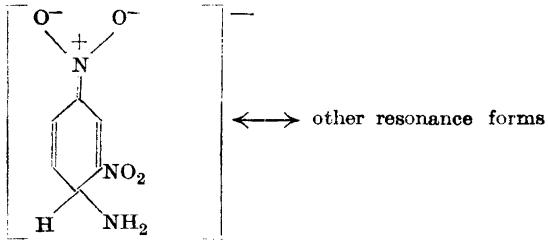
The molar extinction coefficient ε, as has been previously demonstrated [3], is a very sensitive measurement of the steric hindrance in *meta* dinitrobenzene derivatives. From Table 1 it is apparent that 2,4-dinitrotoluene and 4,6-dinitro-1,3-dimethylbenzene are hindered to about the same extent, while dinitromesitylene is very strongly hindered. *Cf.* Canbäck[3], Fig. 3.

2,6-Dinitrotoluene has λ max. = 227 mμ and ε = 12000 in petroleum ether. In agreement with the theory previously advocated [3], the methyl group in the

joint *ortho* position has no influence on λ max. in comparison with the parent compound *meta* dinitrobenzene. The latter compound has also λ max. = 227 mμ. ε for 2,6-dinitrotoluene may be considered to be twice that of *ortho* nitrotoluene[3], which according to Brown and Reagan[6] is 5950. The calculated value is then 11900 and the observed value 12000. The steric hindrance in 2,6-dinitrotoluene is thus very small.

In the reaction between acetone and *meta* dinitrobenzene derivatives in alkaline solution, the attachment of the acetone anion seems to occur at the ring carbon atom that is *para* to one of the nitro groups. Additional support for this point of view is obtained from the fact that 2,6-dinitrotoluene gives a positive reaction with λ max. 530 mμ and 'ε' = 1900 after 15 minutes. As in the case of the other sterically hindered alkyl *meta* dinitrobenzenes the colour developed slowly[3].

Till now in this paper only the place of attachment of the amines on the benzene ring has been discussed. No standpoint has been taken on the question of whether or not the addition product formed is a neutral one, as Lewis and Seaborg suggest, or if an anion is formed by a reaction analogous to the reaction between *m*-dinitrobenzene and acetone in alkaline solution, *e. g.* if an ion of the following type is formed by the addition of an *amide* ion. The latter



formulation of the addition product appears to be true when the nitro bodies are dissolved in liquid ammonia. The different behaviour of the tertiary amines contrary to the primary and secondary ones observed in this investigation, is then easily explained by the fact that the former amines cannot dissociate according to the scheme $2\ NH_3 \rightleftharpoons NH_4^+ + NH_2^-$ and hence the strongly nucleophilic amide ion is not formed. With this latter formulation of the neutralization reaction, it is not necessary to use conceptions such as double chelation to give a reasonable explanation of the observed phenomena.

EXPERIMENTAL

Melting points were determined on the Kofler block and corrected. UV and spectra in the visible were determined on a Beckman Quartz spectrophotometer model DU using 1 cm quartz cells.

2,6-Dinitrotoluene. The compound was isolated chromatographically * from a mixture of 2,4-dinitrotoluene and 2,6-dinitrotoluene obtained from Bofors Nobelkrut. M. p. 65.6—66.0°. Refractive index 1.5400 at 82—83° (Kofler [10] gives 80—82°). Transition point of the instable and the stable form, 40° (Schaum [7] gives 40.5°, and Kofler [8] 36°). The instable form melted by rapid heating at 58° (Kofler [10] gives 58°). The melting point, when mixed with 2,6-dinitrotoluene prepared from 2,4,6-trinitrotoluene by selective reduction and deamination according to Holleman and Böeseken [9], was 65°.

Petroleum ether for UV measurements was prepared from Skellysolve B which was freed from aromatic compounds by the method given by Weissberger [11]. B. p. 65—66°. Without noticeable absorption in 1 cm cells to 212 mμ.

Benzylamine, b. p. 185°, *dimethylamine*, b. p. 7°, *piperidine*, b. p. 106°, and *triethylamine*, b. p. 90°, were commercial reagents. They were distilled immediately before use. *N-Ethylpiperidine*, *quinine* and *hexamethylenetetramine* were commercial products which were used without further purification. Other compounds and reagents were the same as used previously [2, 3].

The neutralization at — 72° was tested in the following way. About 0.1 g of the nitro compound was dissolved in 25 ml Skellysolve B. To 5 ml of this solution was added 1 ml of the amine to be tested. The test tube was closed and placed for 30 minutes in an ethanol-solid CO_2 bath and the colour noted. The temperature varied between — 71 and — 73°. With some of the amines, parts of the solution sometimes solidified.

'ε' was determined as described previously [3].

Lepsius [12] has reported that 2,6-dinitrotoluene gives no colour with acetone and alkali. Rudolph [13], on the contrary, reports a red colour that develops slowly.

SUMMARY

Contrary to the statement of Lewis and Seaborg, 1,3-dinitrobenzene in petroleum ether solution gives a distinct crimson colour when secondary amines are added to the solution if the temperature is approx. —70° C. Tertiary amines give no positive reaction.

Assuming the mechanism of neutralization proposed by Lewis and Seaborg, the place of attachment of the amine has been discussed. It has been shown that the most probable place is ortho respectively para to the two nitro groups.

It is mentioned that the mechanism of the neutralization is better understood if an amide ion is assumed to be the nucleophilic reagent operating in the reaction, *e. g.* that a coloured, complex organic anion is formed. A reasonable, simple structure for the resonating anion is suggested.

* On Superfiltrol. Solvent Skellysolve B. Eluent 99 vol. Skellysolve B + 1 vol. acetone.

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Received July 8, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 954–958

A New Sensitive Method for the Determination of Peroxides of Fats and Fatty Acids *

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For the determination of peroxides in fatty products, several methods have been devised:

The oldest and best known is that of Lea [1], which is based on the liberation of iodine from potassium iodide by fatty peroxides in a glacial acetic acid-chloroform solution, followed by titration of the liberated iodine with thiosulfate after the addition of water. Various modifications of this method have found a widespread technical application. It has, however, several disadvantages. Apart from its failure to indicate very small amounts of peroxides, a more serious objection against it is that the iodine has a tendency to combine with the double bonds, especially those of the highly unsaturated fatty acids.

A method based on another principle has been developed by Chapman and McFarlane [2]. The principle is the oxidation, by peroxides in acetone or methyl alcohol, of ferrous sulfate to ferric sulfate, which is determined colorimetrically by means of ammonium thiocyanate. This method is more sensitive and gives higher values than Lea's method. It has, however, the disadvantage that atmospheric oxygen or oxygen dissolved in the solvents can also oxidize the reagent and cause considerable errors.

A new method has been proposed by Fondarai [3], who uses the principle of adding thiofluorescein by the iodometric method. The thiofluorescein, which in alkaline solution has an intensive blue colour, is oxidized into a colourless

* A preliminary communication of this work was presented at the VI Scandinavian Physiological Congress, Oslo, 1948 (S. Hartmann and J. Glavind, *Acta Physiol. Scand.* **16**, suppl. 53 (1948) 32.

** This work was aided by a grant from *Tuborgfondet*, Copenhagen.

compound, and the difference in colour intensity with, and without, peroxides can be tested colorimetrically. The method is highly sensitive, but rather troublesome and complicated.

In a previous paper, the authors reported that peroxides can react, in the presence of heme, with certain leuco-dyes to form coloured compounds. In the course of this work it was found that 3,5-dichloro-4,4'-dihydroxyphenylenediamine, the leuco-base of the well known redox-indicator 2,6-dichlorophenolindophenol, could react with considerable velocity with organic peroxides, even though heme was not present. The reaction did not show any tendency to have an autocatalytic character as did the heme-catalyzed reaction, and the dye formed has the advantage of being fat-soluble. Based on these observations, the authors have developed a new chemical method for the determination of peroxides, the details of which are given below.

METHOD

Preparation of dichlorodihydroxyphenylenediamine: 2 g of a commercial preparation of 2,6-dichlorophenolindophenol, as a rule of 65 per cent purity, and 1 g of ascorbic acid are dissolved in 100 ml of 50 per cent ethyl alcohol. After 10 minutes with occasional shakings, 200 ml of saturated aqueous sodium chloride solution, containing 20 ml glacial acetic acid, are added. The mixture is left standing for about 2 hours at 5° C., then decanted and filtered through a glass filter. The precipitate is rinsed with distilled water and further purified by repeated dissolutions in portions of about 25 ml absolute ethyl alcohol, from which it is precipitated by the addition of distilled water (300 to 500 ml portions). Eventually, the leuco-compound is dried *in vacuo* at room temperature. It is obtained as bluish-white metallic needles which should not contain traces of either sodium chloride or ascorbic acid.

The other reagents used in the reaction, such as xylene, butanol, and glacial acetic acid should be of a high degree of purity. By heating samples of them for 10 minutes in a boiling water-bath with dichlorodihydroxyphenylenediamine, or with a minute quantity of dichlorophenolindophenol, they should neither intensify nor bleach the pink colour. The test tubes used for the reaction should be rinsed with alcohol.

Preparation of the reagent: 0.1 g of dichlorodihydroxyphenylenediamine is dissolved in 10 ml of butanol or ethyl alcohol. The reagent should be kept in the refrigerator, and be discarded as soon as the red color becomes so intense as to give a considerable blank.

Procedure for the determination: From the oil, the peroxide value of which is to be determined, a suitable dilution is made by means of xylene, containing 5 per cent glacial acetic acid. 0.2 ml of the reagent is added to 5 ml of the oil dilution in a test tube, and the mixture heated in boiling water for 10 minutes. At the same time a blank determination is run. After cooling and adjusting the volumes, the intensities of the red colours are read in a Beckman spectrophotometer at 520 mμ.

Calculation of the results: A curve is constructed for the extinction of 2,6-dichlorophenolindophenol in relation to its concentration. The concentration of the indophenol can be determined by adding potassium iodide in acid solution, followed by titration with

thiosulfate. The peroxide content is calculated as milliequivalents per kg of oil. A content of 0.001 milliequivalent of peroxide in the reaction mixture will give an extinction, after adjustment of the volume to 5 ml, in a 1 cm layer of 1.17.

RESULTS

The method has been used for a considerable length of time in our laboratory with good results. It has very often been compared with the other methods mentioned in the introduction, especially the iodometric method in the modification of King *et al.*[4]. The method has been found to be by far much easier and less time consuming than any of the other methods. The results have been very reproducible, since the method is not much influenced by variations in external experimental conditions. All observations tend to indicate that the reaction is quantitative and specific. Therefore, especially in highly unsaturated compounds, considerably higher figures have often been found than when the same oil was tested by the method of King *et al.* As mentioned in the introduction, this discrepancy is probably explained by an addition of the iodine liberated by the peroxide to the double bonds of the oil. Table 1 presents typical examples of comparative results obtained by the two methods.

Table 1. Comparison of the methods of King et al., and the authors, for the determination of peroxides in a number of fats and fatty acids.

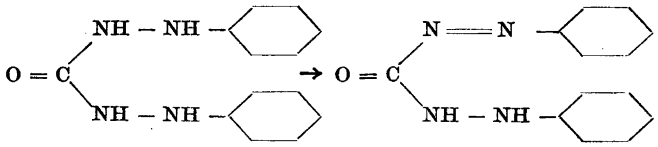
Type of fat	Milliequivalents of peroxide per kg fat by the method of	
	King *et al.*	The authors
Butterfat, oxidized	60	67
Margarine	4.8	5.0
Oleic acid, oxidized	1980	2500
Linseed oil	1800	4600
» »	500	1500
Arachid »	1.5	1.5

The method can be made still more sensitive by making the dichlorophenolindophenol solution alkaline, since the blue colour, of the alkaline dye, is much more intense than the red colour in acid solution. The red colour will change into blue by the addition of potassium palmitate. In alkaline solution, however, the leuco-compound has a great tendency to become oxidized by atmospheric oxygen. Therefore, for most purposes it will be most convenient to use the

light absorption of the red colour. As a rule, this will be strong enough, so that a conversion into the blue component is not necessary.

DISCUSSION

There can be very little doubt that the chemical group in rancid fats which reacts with leucodichlorophenolindophenol is a peroxide or hydroperoxide. In 1926 Stamm [5] proposed the use of diphenylcarbazide as an indicator for the decomposition of fats. In this reaction the colourless diphenylcarbazide is converted into diphenylcarbazone which is intensively red.



The reaction was explained by Stamm [5] as caused by free fatty acids, aldehydes, and ketones, and by other investigators [6-7] by oxyacids or catalytically acting unsaturated acids and aldehydes. It would appear to be a more simple explanation that this reaction is also caused by peroxide groups. In order to examine this question the authors synthesized diphenylcarbazid and studied its colour development with a variety of fats, the peroxide values of which had been determined by the indophenol method. A close parallelism was observed, which shows that the Stamm reaction is, in fact, also a peroxide reaction. The colour intensity of the two reactions is of the same order of magnitude. The diphenylcarbazide reaction can be carried out in exactly the same manner as the indophenol reaction, only a somewhat greater concentration of acetic acid must be present, *i. e.*, 10 per cent glacial acetic acid in xylene. However, the diphenylcarbazide reaction has the definite disadvantage that solutions of the diphenylcarbazide shows a much greater tendency to become oxidized by atmospheric oxygen than solutions of leucodichlorophenolindophenol.

SUMMARY

A new method for the determination of peroxides is described. The method consists of the oxidation of dichlorodihydroxyphenylenediamine by heating it with the peroxidized fat in a solution of an organic solvent. Dichlorophenolindophenol is thereby formed in a quantitative reaction, and can be determined colorimetrically.

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Received June 23, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 959–966

The Effect of Some Alkali Salts on the Light Absorption of the Aqueous Solutions of Cupric Sulphate and on the Dissociation of Cupric Sulphate

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In a previous paper[1] it has been shown that the light absorption of cupric sulphate solution can be interpreted by means of one complex, $CuSO_4$. In this paper a calculation method which permits the determination of the dissociation constant of this complex, within wide limits of ionic strength, was derived on the basis of Beer's law. With the aid of the dissociation constant values determined in this way and the corresponding extinction values, the molar extinction coefficients can be calculated at different wavelengths. The values of the molar extinction coefficients permit, on the one hand, the comparison of the results obtained at different wavelengths. On the other hand the dissociation constant can be calculated by means of the molar extinction coefficient values also in such cases where the original method cannot be used. This method has since been used by the present author for determination of the dissociation constant of cupric sulphate in the solutions of some alkali salts. The results of these measurements are reported below.

The equations used in the calculations are

$$e = E/d - \varepsilon_{Cu^{++}}[Cu^{++}] = \varepsilon_{CuSO_4}[CuSO_4] \tag{1}$$

and

$$K = \frac{(c_{Cu} - e/\varepsilon_{CuSO_4})\ (c_{SC_4} - e/\varepsilon_{CuSO_4})}{e/\varepsilon_{CuSO_4}} \tag{2}$$

where E is the extinction, d the thickness of the absorbing layer in cm, $\varepsilon_{Cu^{++}}$ and ε_{CuSO_4} molar extinction coefficients, c_{Cu} the total copper and c_{SO_4} the total sulphate concentration.

The measurements were carried out by means of a Beckman Quarz Spectrophotometer Model DU. The spectral band width was about 1 mμ. The temperature of the room, where the measurements were carried out, and that of the cell compartment was kept constant at 25°. The chemicals used when not pro analysis grade were prepared and purified as described earlier [1].

Most of the measurements were carried out at 272 mμ because the light absorption of the complex is relatively high at this wavelength but the light absorption of cupric ion is very low, even if not quite negligible, at the concentrations used. At longer wavelengths the absorption of cupric ion is still less but the absorption of the complex in question is also much smaller and therefore much higher copper concentrations must be used. Hence the measurements could not be done in as low ionic strength as required.

Table 1 shows some results of the measurements in lithium and sodium perchlorate solutions. These measurements were carried out at 272 mμ. At this wavelength the molar extinction coefficient of cupric sulphate has, according to earlier determinations [1], the value 120, which was used in the calculations. The molar extinction coefficient of cupric ion can be determined from the light absorption measurements in cupric perchlorate solutions as cupric perchlorate is obviously practically completely dissociated. The light absorption of cupric perchlorate solution seems, however, to be to some extent dependent on the pH of the solution, evidently because of the hydrolyse. Therefore the pH of the solutions was measured as well. For the molar extinction coefficient of cupric ion in the solutions investigated the value 0.8 was obtained at this wavelength. The correction of pK due to the light absorption of cupric ion amounts in these measurements at the most to 0.02 at low ionic

Table 1. Determination of the dissociation constant of cupric sulphate in lithium and sodium perchlorate solutions.

$\lambda = 272\ m\mu$, $\varepsilon_{CuSO_4} = 120$, $\varepsilon_{Cu^{++}} = 0.8$.

Salt	$c_{Cu} \cdot 10^3$	I	$e \cdot 10^3$	$[CuSO_4] \cdot 10^3$	$[Cu^{++}] \cdot 10^3$	pK
$LiClO_4$	15.32	0.705	141.0	1.175	14.14	0.770
»	»	1.35	122.6	1.022	14.30	0.699
»	»	2.00	132.0	1.100	14.21	0.735
»	»	2.65	145.0	1.208	14.11	0.783
$NaClO_4$	15.10	0.209	247.6	2.063	13.04	1.084
»	»	0.369	172.0	1.433	13.67	0.886
»	»	0.684	120.7	1.006	14.09	0.706
»	»	1.62	100.6	0.838	14.26	0.613
»	»	2.40	100.2	0.835	14.26	0.613
»	»	3.20	115.7	0.964	14.13	0.684

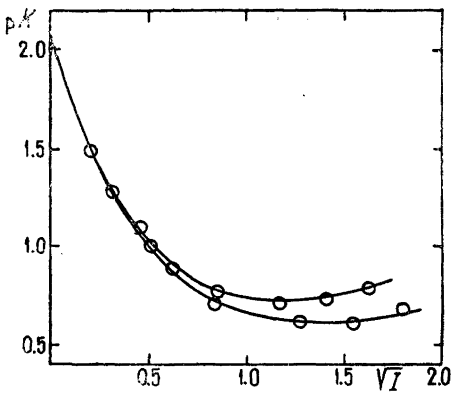

Fig. 1. Dissociation constant of cupric sulphate in lithium perchlorate (upper curve) and sodium perchlorate (lower curve) solutions.

strengths and to 0.05 at higher ionic strengths. The results can be represented by means of the Debye-Hückel equation

$$pK = pK_0 - \frac{4.05\sqrt{I}}{1 + \alpha\sqrt{I}} + BI \qquad (3)$$

For pK_0 the value obtained in the previous paper with a different method was adopted. The values of α and B were calculated using the method of least squares. The following values were obtained

	pK_0	α	B
$LiClO_4$	2.099	1.550	0.211
$NaClO_4$	2.099	1.527	0.161

In Fig. 1 the dissociation constant is represented in relation to ionic strength. As seen pK is higher in lithium than in sodium perchlorate solutions. The differences in pK values are, however, of a magnitude common in equilibrium constants of electrolytes. The values obtained for α and B are of reasonable magnitude.

In lithium sulphate solutions numerous measurements were performed. The results of some measurements are given in Table 2. In this case the correction due to the light absorption of cupric ion is smaller than in perchlorate solutions, amounting at most to 0.02 in pK at lower ionic strengths, and it is still smaller at higher ionic strength.

Table 2. Determination of the dissociation constant of cupric sulphate in lithium sulphate solutions. $\lambda = 272$ mμ. $\varepsilon_{CuSO_4} = 120$, $\varepsilon_{Cu} = 0.8$. $c_{Cu} = 0.01003$.

c_{SO_4}	I	$e \cdot 10^3$	$[CuSO_4] \cdot 10^3$	$[Cu^{++}] \cdot 10^3$	$[SO_4^{--}]$	pK
0.00412	0.0386	116	0.967	9.06	0.00315	1.529
0.00515	0.0409	138	1.150	8.88	0.00400	1.510
0.00618	0.0433	160	1.333	8.70	0.00485	1.500
0.00712	0.0455	178	1.483	8.55	0.00564	1.488
0.00823	0.0481	199	1.658	8.37	0.00657	1.479
0.00852	0.0491	198	1.650	8.38	0.00687	1.457
0.0103	0.0533	225	1.875	8.15	0.00843	1.437
0.0123	0.0587	250	2.08	7.95	0.01022	1.408
0.0128	0.0600	256	2.13	7.90	0.01067	1.403
0.0170	0.0711	299	2.49	7.54	0.01451	1.357
0.0213	0.0829	333	2.78	7.25	0.0182	1.316
0.0426	0.144	407	3.39	6.64	0.0392	1.115
0.0852	0.269	487	4.06	5.97	0.0811	0.924
0.128	0.397	534	4.45	5.58	0.124	0.808
0.213	0.649	621	5.18	4.85	0.208	0.711
0.426	1.284	705	5.86	4.15	0.420	0.528
0.852	2.559	794	6.62	3.41	0.845	0.361
1.28	3.841	883	7.36	2.67	1.273	0.335
1.70	5.099	933	7.78	2.25	1.692	0.310
2.13	6.387	1019	8.49	1.54	2.122	0.415

In Tables 3 and 4 some of the results in sodium and potassium sulphate solutions are recorded. The measurements in lithium sulphate solutions gave for the parameter pK_0 in the Debye-Hückel equation the value 2.122, calculated by method of least squares. The value 2.099, obtained in the previous paper, differs so little from this value that it was used in the calculations. For the parameters of the Debye-Hückel equation in sulphate solutions the following values were obtained:

Table 3. Determination of the dissociation constant of cupric sulphate in sodium sulphate solutions. $\lambda = 272$ mμ. $\varepsilon_{CuSO_4} = 120$. $\varepsilon_{Cu^{++}} = 0.8$. $c_{Cu} = 0.01150$.

c_{SO_4}	I	$e \cdot 10^3$	$[CuSO_4] \cdot 10^3$	$[Cu^{++}] \cdot 10^3$	pK
0.1316	0.384	667	5.55	5.95	0.869
0.232	0.702	764	6.36	5.14	0.737
0.493	1.464	887	7.39	4.11	0.568
0.733	2.190	955	7.96	3.54	0.491
0.976	2.924	1018	8.48	3.02	0.462

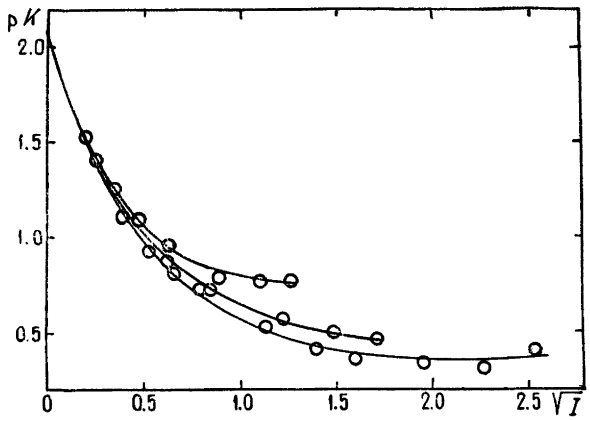

Fig. 2. Dissociation constant of cupric sulphate in lithium (lower curve), sodium (middle curve) and potassium (upper curve) sulphate solutions.

Table 4. Determination of the dissociation constant of cupric sulphate in potassium sulphate solutions. $\lambda = 272$ *mμ.* $\varepsilon_{CuSO_4} = 120.$ $\varepsilon_{Cu^{++}} = 0.8.$ $c_{Cu} = 0.01150.$

c_{SO_4}	I	$e \cdot 10^3$	$[CuSO_4] \cdot 10^3$	$[Cu^{++}] \cdot 10^3$	pK
0.0766	0.218	666	5.55	5.95	1.080
0.1415	0.393	792	6.60	4.90	0.970
0.271	0.795	857	7.14	4.36	0.793
0.402	1.202	960	8.00	3.50	0.763
0.531	1.580	1038	8.65	2.85	0.765

	pK_0	a	B
K_2SO_4	2.099	1.762	0.155
Na_2SO_4	2.099	1.676	0.0534
Li_2SO_4	2.099	1.536	0.0576

Fig. 2 shows the variation of the dissociation constant of cupric sulphate with ionic strength in alkali sulphate solutions. It is seen that pK is highest in potassium sulphate and lowest in lithium sulphate solutions. Thus at a given ionic strength

$$pK(LiClO_4) > pK(NaClO_4)$$

but

$$pK(Li_2SO_4) < pK(Na_2SO_4) < pK(K_2SO_4)$$

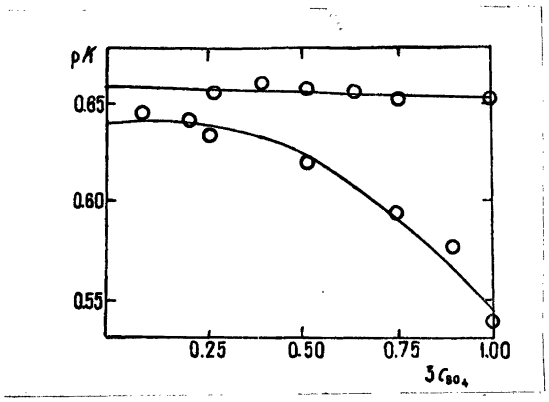

Fig. 3. Dissociation constant of cupric sulphate in mixed solutions. Upper curve: sodium perchlorate — sodium sulphate (I = 1.0). Lower curve: sodium perchlorate — lithium sulphate (I = 1.2.).

The order in perchlorate solutions is obviously the »normal» one. The reverse order in sulphate solutions may perhaps be attributed to a further complex formation or ionic association. The possibility of the existence of higher complexes between cupric and sulphate ions is discussed in the previous paper [1]. It seems, however, improbable that these complexes would exist to any appreciable extent. According to Onsager's [2] as well as to Righellato and Davies' [3] interpretation of conductance data such ions as $LiSO_4^-$, $NaSO_4^-$ and KSO_4^- are present in alkali sulphate solutions. The estimated dissociation constants were 0.23, 0.20 and 0.15 respectively. Accordingly the effect of these ions would be appreciable but the reverse order of the salt effect in sulphate solutions cannot be explained by means of them. But, by means of such ions as $LiCuSO_4^+$, $NaCuSO_4^+$ and $KCuSO_4^+$ the mentioned effect may be explained. The effect of these ions depends on their dissociation constant values as well as on the values of the molar extinction coefficients. The correction of pK is positive when the molar extinction coefficient of such an ion is small, and negative when the molar extinction coefficient is great. It may thus be possible that the calculated value of pK is too high in potassium sulphate solutions but too low in sodium and lithium sulphate solutions. This correction would change the results also in perchlorate solutions but in a still less degree.

Some measurements were carried out in mixed solutions of sodium perchlorate and sodium sulphate at constant ionic strength (I = 1.0). The results represented in Fig. 3 show that the pK values in these solutions vary linearly with the quantity 3 c_{SO_4}/I, as expected. The fact that the activity coefficients in sodium perchlorate and sodium sulphate solutions are nearly equal, permits determination of the dissociation constant and of the molar extinction coefficient of cupric sulphate at a constant ionic strength, by the use of equations (1) and (2), where K and ε_{CuSO_4} are the only unknowns.

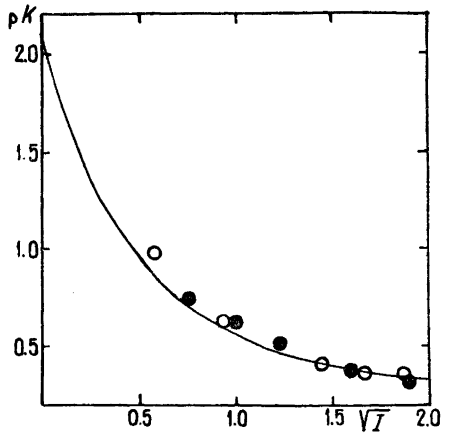

Fig. 4. Effect of wavelength and total copper concentration on the results. Solid line: $c_{Cu} = 0.01003$, $\lambda = 272$ *mμ. The empty circles:* $c_{Cu} = 0.1511$, $\lambda = 310$ *mμ. The filled circles:* $c_{Cu} = 0.259$, $\lambda = 310$ *mμ. At 310 mμ* $\varepsilon_{CuSO_4} = 3.80$, $\varepsilon_{Cu++} = 0.08$.

Similar series was carried out in mixed solutions of sodium perchlorate and lithium sulphate at constant ionic strength ($I = 1.2$). The results of these measurements are also given in Fig. 3. The relation between pK and $3\,c_{SO_4}/I$ is not linear in this case as expected on the basis of the above results, because, by adding lithium sulphate to sodium perchlorate solution the ion combination $Li^+ - SO_4^=$ obviously cause a lowering in pK values as pK is smaller in lithium sulphate solutions than in sodium perchlorate solutions, but simultaneously the combination $Li^+ - ClO_4^-$ causes a change in the opposite direction as pK is higher in lithium perchlorate than in sodium perchlorate solutions. This relation can be represented by means of the equation

$$pK = a + bx + dx(1 - x) \tag{4}$$

where a, b, d are parameters and $x = 3c_{SO_4}/I$. The calculation by the method of least squares gave

$$pK = 0.640 - 0.095\ x + 0.125\ x(1 - x)$$

Some measurements were carried out also at 310 mμ. The results of these measurements which were made in lithium sulphate solutions are given in Fig. 4. The solid line refers to measurements at 272 mμ at which the cupric sulphate concentration was 0.01003. The empty circles refer to measurements at 310 mμ, $c_{Cu} = 0.1511$ and the filled circles to measurements at the same wavelength, $c_{Cu} = 0.259$. The results at 310 mμ agree thus relatively well with results at 272 mμ. The two first points at low ionic strength refer to pure cupric sulphate. The dissociation constant of cupric sulphate in cupric

sulphate solutions is thus nearly equal to that in lithium or still better to that in sodium sulphate solutions. These measurements support the earlier results which seemed to prove that the first complex is the only between cupric and sulphate ions.

The accuracy of the results of this study depends chiefly on the reliability of the molar extinction coefficient values. This question was discussed in the previous paper [1]. It may be mentioned here that the results in perchlorate and diluted sulphate solutions are relatively insensitive to the error in the molar extinction coefficient. The concentrated sulphate solutions are in this respect more unfavourable.

SUMMARY

The dissociation constant of cupric sulphate has been determined in lithium and sodium perchlorate as well as in lithium, sodium and potassium sulphate solutions over a wide range of ionic strength from the light absorption measurements. The order of the salt effect is in alkali perchlorate solutions the opposite to that in alkali sulphate solutions.

The measurements in mixed solutions of sodium perchlorate and sulphate gave a linear relation between pK and the composition. Similar measurements in mixed solutions of lithium sulphate and sodium perchlorate proved that the relation between pK and the composition is not linear.

The possibility of existence of other complexes than $CuSO_4$ in the solutions investigated is discussed. The measurements at different wavelengths and total copper concentrations support the result of the previous paper [1] that the first complex $CuSO_4$ is practically the only between cupric and sulphate ions.

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Received June 8, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 967–970

An Apparatus for the Determination of Small Quantities of Carbonate

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Jensen [1] in 1931 described a new method for the determination of carbonate. The original apparatus was, however, owing to its large volume, rubber tubings and rubber stoppers, not suitable for smaller quantities than that corresponding to 30 mg of carbon dioxide. Since a reliable method for the determination of smaller amounts of carbonate was lacking, the apparatus described in the present paper has been constructed. By replacing rubber by glass, and by diminishing the volume from approx. 600 to approx. 50 ml, the lower limit has been reduced to about 0.2 mg carbon dioxide.

PROCEDURE

The sample is placed in vessel A (Fig. 1), the pipette P filled with hydrochloric or sulfuric acid, and a measured amount of 0.1 *N* barium hydroxide (containing 50 g $BaCl_2,2H_2O$ per litre in order to depress the solubility of barium carbonate) is introduced into the vessel B. A drop of 0.01 % phenolphthalein is added to vessel B. Before the system is closed the mercury level must stand in the upper position in order to avoid the formation of a pressure greater than 1 atm. After the vessels have been connected, the hydrochloric acid is led into vessel A by opening the stopcock S. By means of an up-and-down movement of the mercury level, the air in the apparatus is circulated in the direction shown by the arrows. The two vessels containing solution act as valves. 40—50 beats per minute are suitable. When all the carbon dioxide has been expelled from the solution in vessel A and absorbed in the barium hydroxide in vessel B, the latter solution is back titrated with 0.1 *N* hydrochloric acid from a Rehberg burette [2] connected to the apparatus.

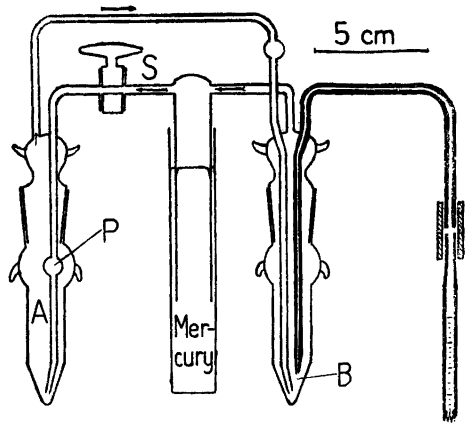

Fig. 1. The apparatus.

The necessary stirring during the titration is obtained by the air circulation. The same procedure is repeated without sample. The difference in the amount of 0.1 *N* hydrochloric acid used is equivalent to the carbonate in the sample.

EXPERIMENTAL

The amount of 0.1 *N* hydrochloric acid used in blank experiments is shown in Table 1. By extrapolation, one finds that the amount of 0.1 *N* hydrochloric acid corresponding

Table 1.

Approx. 0,1 *N* barium hydroxide ml	0.1 *N* hydrochloric acid used ml					Mean ml
0.250	0.255	0.255	0.257	0.258	0.255	0.256
0.500	0.538	0.541	0.542	0.537	0.538	0.539
0.750	0.822	0.819	0.818	0.823	0.820	0.820

to 0 ml of barium hydroxide is −0.023 ml. The content of carbon dioxide in the air in the apparatus is therefore equivalent to 0.023 ml of 0.1 *N* hydrochloric acid.

The influence of the time of circulation on the expulsion and absorption of carbon dioxide is shown in Table 2. It can be seen that a circulation time of 15−20 minutes is sufficient in most cases.

Table 2.

Time of circulation minutes	Potassium carbonate added mg	found mg	found / added
1	1.039	0.200	0.193
2	1.021	0.740	0.724
4	1.113	1.000	0.898
8	1.270	1.256	0.989
16	1.211	1.217	1.005
32	1.191	1.200	1.007
2	5.58	1.89	0.338
4	5.47	4.84	0.885
8	5.59	5.59	1.000
16	5.34	5.33	0.998

STANDARDIZATION OF 0.1 *N* HYDROCHLORIC ACID

The titration acid can be standardized in many ways, but it would appear to be most convenient to standardize against potassium, sodium, or calcium carbonate, exactly as used in the actual analysis. In the above experiments potassium carbonate was used. For control, the acid was standardized also against borax with methyl orange as indicator. The difference was negligible.

SOURCES OF ERROR

A change in the carbon dioxide content of the air in the laboratory will, of course, influence the results, and should, therefore, be avoided by means of ventilation of the room. If hydrochloric acid is used to expel the carbon dioxide care must be taken that the concentration in vessel A is not stronger than 3 *N*. Already at 4 *N* the amount of hydrogen chloride passing over into the barium hydroxide will be measurable.

The content of salts of volatile acids other than carbonates in the sample is another possible source of error. Fluosilicic acid, which may be formed from fluorides in the sample and silicon from the glass, is, for instance, able to pass over into the barium hydroxide. This error can be overcome by making vessel A of plexi-glass. It is not possible, however, to give a general method of overcoming all possible errors, but rather each case has to be considered separately.

EXAMPLES

Results of some analyses are shown in Table 3.

Table 3.

Substance	Sample wt. mg	0.1 *N* hydrochloric acid used		Carbon dioxide in the sample	
		in blank ml	with sample ml	mg	%
Hydroxylapatite containing carbonate I	76.3	0.540	0.112	0.942	1.234
	74.0	0.541	0.125	0.915	1.237
Do II	51.8	0.290	0.101	0.416	0.803
	49.9	0.286	0.104	0.400	0.802
Rock phosphate	43.8	0.987	0.037	2.090	4.77
	38.1	0.994	0.168	1.822	4.78

After the described apparatus was constructed, the attention of the author was drawn to the fact that Blom and Lund [3], in a modification of the original apparatus, have also used the two vessels as valves in connection with an injection syringe.

SUMMARY

An apparatus for the determination of carbonate in amounts corresponding to 0.2—2.0 mg of carbon dioxide has been described. Experiments showing the use of the apparatus have been given.

The author is indebted to professors Niels Bjerrum, S. Tovborg Jensen, and A. Tovborg Jensen, and to K. J. Pedersen, D. Sc., for valuable advice and kind help.

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Received July 9, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 971–974

An Easy Route to Methylene-labelled Glycine

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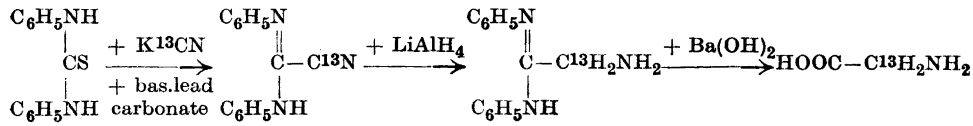
The preparation of glycine, labelled with C^{13} or C^{14} in the methylene group, is at present a rather tedious process, involving preparation of methyl-labelled acetic acid, subsequent halogenation, and exchange of the halogen atom against NH_2 [1, 2]. Using $KC^{13}N$ as the starting material we got an overall yield of round 12 % by the above route, included the preparation of methyl-labelled acetate by the reaction sequence: $KC^{13}N \rightarrow C^{13}O_2 \rightarrow C^{13}H_3OH \rightarrow C^{13}H_3J \rightarrow C^{13}H_3COOH$ [3].

In view of the usefulness of methylene labelled glycine for studies of intermediary metabolism, an easy route to this compound from the sources of C^{13} and C^{14}, at present available, would be of some interest. The following method involves only three isolation stages from $KC^{13}N$ to $HOOC\text{-}C^{13}H_2NH_2$, gives an overall yield of 50 %, or more, and could be carried out in about 10 hours. An outline of the procedure is shown by the following scheme:



(I) (II) (III) (IV)

N · N′-diphenyl-thio-urea (I) is condensed with $KC^{13}N$ in the presence of basic lead carbonate in water-alcohol mixture to N · N′-diphenyl-cyano-formamidine (II). Since the reduction of cyano-groups to $—CH_2NH_2$ proceeds with extreme ease in ether solution with lithiumaluminum hydride [4] it was to be expected that the cyanoformamidine should be reduced in a similar way to the corresponding glycine-N · N′-diphenyl-amidine, provided that the amidine structure was not to be touched by the hydride. We found that this, actually, was the case, and that the glycine-N · N′-diphenyl-amidine was formed in a yield of 65—70 %. In view of the easy reducibility of amides [5]

and anilides [6] by lithium-aluminum hydride it is somewhat surprising that the amidine structure should be stable against this reducing agent. It is, however, to our knowledge not yet known, whether amidine groups in general are stable against hydrides, but it seems worth investigating if the N′ · N′-diphenyl-amidine structure, in its stability against reduction, as observed by us, could be used for protection of carboxyl groups when working with lithium-aluminum hydride and other agents of the same kind. The hydrolysis of glycine-N · N′-diphenyl-amidine is very slow with concentrated HCl at 100°, but proceeds with great ease and almost quantitative yield with barium hydroxide in water solution at boiling during one hour, yielding glycine and aniline.

For preparation of C^{13}-labelled glycine the commercial $KC^{13}N$ (Eastman Kodak) could be used directly; for C^{14}-labelled glycine high yield syntheses from $BaC^{14}O_3$ have been worked out for preparation of $KC^{14}N$ [7], the latter being at now commercially available.

EXPERIMENTAL

N · N′-Diphenyl-cyano-formamidine (II)

The following preparation is a small-scale procedure, worked out from the data for large-scale preparation given by Sandmeyer [8]. 7 g 95 % Potassium cyanide with about 5 % C^{13}-excess was dissolved in 20 ml of water. To this solution 30 g basic lead carbonate, 20 g N · N′-diphenyl-thio-urea (I) and 50 ml 95 % ethanol were added, and the mixture heated to 50—55° during one hour under efficient stirring. Gradually the mixture turns black (PbS), the reaction being ready when the filtrate of a small sample does not discolorize a small amount of lead carbonate at short boiling. The reaction being complete, 200 ml of cold water was added to the reaction mixture. After cooling the precipitate was filtered off, washed with cold water and treated with 200 ml of boiling ethanol, whereby the N · N′-diphenyl-cyano-formamidine was extracted from the lead sulphide and excess lead carbonate. The alcoholic extract was evaporated to about 50 ml and kept at 0°, whereby the main part of the amidine crystallized in light yellow plates, m. p. 139° (litt. 137°). Yield 14 g. An additional amount of 6 g of product could be isolated from the mother liquor. (This latter crude material does, however, contain at least 80 % of the amidine, so it was found to be quite satisfactory to evaporate, in later runs, the alcoholic extract to dryness and directly use this material (19 g, 90 % yield) for the subsequent reduction.)

Glycine-N · N′-diphenyl-amidine (III)

In a 2 litre three-necked flask, equipped with reflux condenser, separating funnel, high-speed mercury-seal stirrer and nitrogen inlet, 16 g of lithium-aluminum hydride was dissolved in 500 ml anhydrous ether. A solution of 19 g crude, but alcohol-free, N · N′-diphenyl-cyano-formamidine in 300 ml anhydrous ether was added at room temperature under a period of about 2 hours. The rate of addition was regulated to keep the ether in gentle reflux. A yellow precipitate was formed at the beginning of the reaction, gradually changing to white. After addition of the cyano-formamidine the

mixture was stirred for another 20 minutes, cooled to 0° and water (500 ml) added, drop by drop. During the reduction procedure dry nitrogen was slowly passed through the mixture. The yellow ether layer, after addition of the water, was separated and the cloudy water-phase 3 times extracted with 100 ml of ether. The combined extracts, dried for a short time over anhydrous sodium sulphate, were evaporated to dryness leaving an yellow oil, the main part of which solidifies on scratching. This reaction product (17.5 g) consists of glycine-N · N′-diphenylamidine and a small amount of aniline. For isolation of the pure product a small sample (1 g) was treated with 2 ml of methanol, sucked dry (m. p. 106°) and recrystallized from petrol ether, m. p. 112°.

Calculated for $C_{14}H_{15}N_3$:	18.65 % N
Estimated:	18.7, 18.8 % N

Glycine (IV)

16.5 g crude glycine-N · N′-diphenyl-amidine was heated at reflux with 80 ml 10 % solution of $Ba(OH)_2$ for one hour (oilbath at 130°). Aniline was shown to separate from the waterphase after 15 minutes. After the hydrolysis the alcaline mixture was extracted 3 times with 50 ml ether each, and barium ions removed by adding an exact amount of 10 % sulphuric acid. $BaSO_4$ was centrifuged off, boiled with 150 ml water, centrifuged and the combined Ba- and sulphate-free solutions evaporated to 10 ml. A small amount of Norite was added, the solution filtered and the glycine precipitated by addition of 5 volumes of ethanol and cooling to 0°. The colourless crystals were washed with some absolute ethanol and dried. Yield 3.8 g. Overall yield from KCN 48 % (Higher yields being obtained).

Calculated for glycine, $C_2H_5NO_2$:	18.7 % N
Estimated:	18.6 » »

M. P. of the ethyl ester hydrochloride 143° (litt. 144°).

A paper partition chromatogram in phenol-water of our glycine preparation showed the right R_F-value. As for control of the actual position of the labelled carbon atom in the glycine prepared a small sample was totally combusted in oxygen to CO_2, trapped as $BaCO_3$. Another sample was treated with ninhydrine liberating its carboxyl group as CO_2, trapped as $BaCO_3$. Mass-spectrometric analysis of the two carbonate preparations showed the following values:

Total glycine carbon:	2.17 % C^{13}-excess
Carboxyl carbon:	0.01 » C^{13}-excess

The isotope carbon was thus located to the methylene group, having a C^{13}-excess of 4.32 %.

SUMMARY

A new method is given for the synthesis of methylene labelled (C^{13}) glycine, the reaction steps being the preparation of N · N′-diphenyl-cyano-formamidine, glycine-N · N′-diphenyl-amidine and hydrolysis of the latter to glycine.

The overall yield from $KC^{13}N$ is round 50 % and the reaction steps work rapidly and satisfactory.

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Received July 7, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 975–981

On the Formation of Reducing Sugars in Thermophilic Cellulose Fermentation

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The behavior of cellulose fermenting bacteria towards cellobiose and glucose is rather interesting. By checking the bacterial growth in crude cultures with chemicals or by suddenly changing the incubation temperature from 55 to 20° C Pringsheim [1] as early as 1912 was able to obtain these sugars as intermediate products in thermophilic cellulose fermentation. When the fermentation is carried out with crude cultures under normal conditions, however, these sugars seldom appear in the fermented medium, owing to consumption by the subsidiary flora. Not until recently when pure cultures of thermophilic cellulose bacteria became available was it possible to show that these bacteria produce glucose (Imsenecki [2], McBee [3]) or glucose and cellobiose (Enebo [4]) as regular fermentation products which are accumulated in the medium.

The present report deals with the formation of sugars in cellulose fermentation performed without special precautions by a pure culture of a cellulose fermenting thermophile and also with the action of this organism on sugars, chiefly glucose and cellobiose.

A. FORMATION OF REDUCING SUBSTANCES IN THERMOPHILIC CELLULOSE FERMENTATION

Methods

Fermentations were carried out anaerobically at 55° C under reduced CO_2-pressure. The medium consisted of an inorganic nutrient salt solution according to Simola [5] which also contained 2 % Bacto tryptone, 2 % autolyzed baker's yeast, 2 % precipitated chalk and 0.03 % thioglycollic acid. Cellulose wadding was used as cellulose source. Inoculation was always made with 5 % of a pure culture of a thermophilic cellulose bacterium, grown on the same medium (Enebo [6]). For further details concerning the fermentation method see Enebo [7].

Table 1. Reduction values obtained when fermenting a 1 % cellulose suspension. (Incubation for 14 days at 55° C.)

ml 0.1 *N* sodium thiosulfate consumed per 5 ml medium in determination of reduction according to Schoorl					
I	II	III	After fermentation with baker's yeast		
			IV	V	VI
Before hydrolysis	After hydrolysis	Per cent increase	Before hydrolysis	After hydrolysis	Per cent increase
7.22	8.65	19.8	2.44	4.03	65.4

Determinations of reducing substance in the medium were performed according to Schoorl after purification of the solution with lead acetate. Cellobiose and higher saccharides were hydrolyzed by adding conc. HCl until a concentration of 10 % HCl was reached and then keeping the sample at 100° C for 60 minutes. Glucose was removed from mixtures of other sugars by fermentation with baker's yeast.

The increase in reduction after HCl-hydrolysis is, under the conditions mentioned above, ca. 46 % for a pure solution of cellobiose at a concentration corresponding to 2.44 ml 0.1 *N* thiosulfate per 5 ml sample. The low value under III in table 1 thus makes it probable that the fermented medium contains glucose together with higher saccharides. After fermentation with baker's yeast the reduction before hydrolysis has fallen to only one third of the initial value. The increase in reduction after hydrolysis is considerable (VI), indicating that besides the expected cellobiose the solution must contain higher oligosaccharides.

The presence of glucose and cellobiose was shown by the following procedure, based on the different solubility of these sugars in abs. alcohol:

250 ml of fermented medium were distilled *in vacuo* to a volume of 50 ml and then purified by means of lead acetate. The distillation was then continued to a volume of ca. 8 ml. After dilution with water to 10 ml, 90 ml abs. alcohol were added and the precipitate obtained removed by filtering. 100 ml alcohol were added to the filtrate and the mixture concentrated *in vacuo* to a volume of ca. 10 ml. After cooling a crystalline layer was found on the walls of the flask. The crystals were removed, washed with abs. alcohol and dissolved in a few ml of water (fraction I). To the remaining liquor 150 ml alcohol were added and the mixture again distilled to a volume of ca. 10 ml and again a precipitate was obtained. The remaining liquor after addition of water and removal of alcohol formed fraction II.

The method followed produced an enrichment of cellobiose in fraction I and of glucose in fraction II. A Schoorl-test showed that fraction I possessed a reductive power

Table 2. Influence of different fermentation times on the formation of reducing sugars in a 1 % cellulose-suspension.

	ml 0.1 *N* sodium thiosulfate consumed per 5 ml medium in determination of reduction according to Schoorl					
I	II	III	IV	After fermentation with baker's yeast		
				V	VI	VII
Fermentation time (days)	Before hydrolysis	After hydrolysis	Per cent increase	Before hydrolysis	After hydrolysis	Per cent increase
4	1.34	2.11	57.5	0.82	1.54	87.8
8	2.13	2.82	32.4	1.18	1.98	67.8
14	2.54	3.02	18.9	1.12	1.81	61.6

which after hydrolysis increased with ca. 50 per cent. Thus this fraction seemed to contain only cellobiose as reducing sugar.

Phenylosazones of the fractions I and II were prepared according to the direction given by van der Haar [8] and purified by repeated recrystallizations from alcohol-water and acetone-water solutions. The osazone of fraction II was washed with hot water in order to remove any traces of cellobiose-osazone.

The osazone of fraction I had the m. p. 197—198° C (corr.), corresponding to the generally accepted value for cellobiose-osazone [9]. For the osazone of fraction II the m. p. 204—205° C was obtained which is somewhat to low for glucose-osazone (m. p. 210° C [8]). Unfortunately the amount of the osazones were too small to permit a determination of their optical rotation.

Table 2 contains reduction values obtained for different fermentation times. As is shown in column II the total amount of reducing sugars increases during the fermentation. According to the values in the columns III and IV a further increase in reduction is obtained after hydrolysis, indicating the presence of higher sugars in the fermented medium. The percentage increase after hydrolysis becomes lower when the fermentation proceeds, showing that the relative amount of glucose gradually increases. Values corresponding to the original amounts of glucose before hydrolysis are obtained by subtracting the values of column II from those of column V. After 4 days the reduction by glucose thus was $0.52/1.34 = 39$ % of the total reduction. After 14 days the reduction by glucose had increased to $1.42/2.54 = 56$ % of the total reduction. Column VII shows that the higher sugars are slowly converted to lower sugars.

Table 3. Results of fermentation of various sugars by a pure culture of a cellulose decomposing thermophile. Initial carbohydrate concentration 1 %. (Incubation for 10 days at 55° C, mean values of at least 3 experiments.)

Sugar	% of added sugar fermented
Cellobiose *	95
l-Arabinose	46
d-Xylose	45
d-Glucose	33
d-Fructose	31
Maltose	28
d-Mannose	17
Sucrose	0

Even after 14 days the solution contains higher sugars other than cellobiose, as the increase in reduction after hydrolysis is much greater than is calculated for the latter. (For an amount of pure cellobiose corresponding to the value 1.12 ml 0.1 *N* thiosulfate in the Schoorl determination, the increase in thiosulfate consumption after hydrolysis should be ca. 48 % instead of 61.6 % as the table indicates.)

The enrichment of cellobiose and glucose in a cellulose medium on fermentation with a pure culture of a cellulose thermophile indicates that the hydrolytic activity of the bacteria is greater than the fermentative activity. Contrary to the report of McBee [3] these sugars are indeed formed during the fermentation and not only after fermentation has ceased.

B. FERMENTATION OF CELLOBIOSE AND GLUCOSE WITH A CELLULOSE THERMOPHILE

The literature gives contradictory reports concerning the action of cellulose thermophiles on various sugars. Thus the pure culture of Imsenecki [2] was able to ferment glucose and to a slight extent maltose and sucrose. A culture of Rotmistrov [10], also described as pure, easily fermented several sugars. More recently McBee [3], working with provably pure cultures, has obtained fermentation of cellobiose but not of glucose.

* Remaining reduction is mainly caused by glucose, formed by the cellobiase activity of the bacteria.

Table 4. Fermentation of cellobiose and glucose with a pure culture of a cellulose decomposing thermophile. Initial sugar concentration 1 %.

Fermentation time (days)	I. Fermentation of cellobiose % of added sugar consumed	II. Fermentation of glucose % of added sugar consumed
2	43	10
3	78	16
4	90	21
6	94 *	29
8	95 *	34
10	97 *	38

Experimental

The present author has made sugar fermentations with the same pure culture of a cellulose decomposing thermophile that was used for the cellulose fermentations. The method was the same as described under A only with the cellulose changed for sugars (always sterile filtered, not autoclaved). The results of this test are in Table 3.

The table shows that the cellulose decomposing thermophile is not directed to cellulose only as carbohydrate source, as was once proposed. Contrary to the behavior of certain mesophilic cellulose decomposing bacteria their growth is not inhibited by comparatively high concentrations of fermentable sugars.

As is shown in Table 4 cellobiose is fermented at a rate which is several times as high as that for glucose.

The preference for cellobiose shown by the bacteria makes it probable that they are especially adapted to this sugar as a final product of the hydrolysis of cellulose. It is possible that this effect has a connection with the chemical structure of cellulose, in which cellobiose as well as glucose may be considered to form the ultimate unit. For some reason the bacteria may prefer to break up every second of the glucosidic linkages and then directly ferment the cellobiose formed. On the other hand the accumulation of glucose in the fermented medium indicates a certain cellobiase activity of the bacteria. As is shown in Table 5 part of the cellobiose, during fermentation of this sugar, is hydrolyzed to glucose.

* See also note to Table 3.

Table 5. Cellobiase activity on fermentation of cellobiose with a pure culture of a cellulose decomposing thermophile. (Incubation for 4.5 days at 55° C.)

Expt. no.	Initial concentration of sugar %	ml 0.1 *N* sodium thiosulfate consumed per 5 ml medium in determination of reduction according to Schoorl					
		Before hydrolysis	After hydrolysis	Per cent increase	After fermentation with baker's yeast		
					Before hydrolysis	After hydrolysis	Per cent increase
1	1.25	8.27	10.70	29.4	4.27	6.07	42.2
2	1.75	17.00	22.03	29.6	12.63	18.17	43.8

After the glucose is removed the increase in reduction after hydrolysis corresponds approximately to the value calculated for cellobiose. Synthesis of higher saccharides does not therefore appear to take place.

As an uninoculated blank with cellobiose kept at 55° C for 10 days showed no hydrolysis the glucose formation must be considered to be an entirely enzymatic effect.

SUMMARY

This paper deals with the formation of lower sugars in thermophilic fermentation of cellulose using a pure culture of a cellulose decomposing bacterium and with fermentation of sugars, especially glucose and cellobiose by the same organism.

By repeated addition of alcohol to the medium from cellulose fermentation and evaporation *in vacuo* to a minute volume, cellobiose and glucose fractions were obtained. The formation of small amounts of saccharides higher than cellobiose could also be shown.

Experiments with fermentations of different sugars with the cellulose bacterium showed that the rate of fermentation was highest for cellobiose, followed by arabinose and xylose (equal), glucose, fructose.

Cellobiose was fermented at a rate, several times as high as that for glucose. During cellobiose-fermentation a certain cellobiase activity was observed.

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Received July 25, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 982–984

β-*nor*-Conidendrin *

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Following the discovery of the antioxidant *nor*-dihydro-guaiaretic acid [1] in *Larrea divaricata*, Fisher, Kyame and Bickford converted conidendrin (sulphite liquors lactone) by demethylation with hydrobromic acid, to a product called *nor*-conidendrin [2]. This product appears to possess promising antioxidising properties. Conidendrin (α-conidendrin) occurs in the wood of several conifers, *e. g. Podocarpus spicatus* [3], several species of the spruce genus [4] and most hemlocks (*Tsuga*) [5]. Large amounts of conidendrin are available from the waste liquors from the sulphite digestion of western hemlock *(Tsuga heterophylla)* and a convenient process of isolation from these liquors has been worked out [6]. This makes conidendrin, previously an 'academic curiosity', a substance of potential technical importance.

The yield of pure *nor*-conidendrin obtained with hydrogen bromide is unsatisfactory. Consequently experiments were instituted to increase the yield by employing milder processes of demethylation. It was found that α-conidendrin could be smoothly demethylated by pyridinium chloride [7] to yield a pure crystalline product of at least 85 per cent yield **. The high melting, dextrorotatory substance had a composition corresponding to the formula $C_{18}H_{16}O_6$. It contained no methoxyl groups but four hydroxyl groups as shown by the formation of a tetra acetyl derivate, which is also dextrorotatory. On methylation with methyl sulphate, a tetramethyl derivative was obtained which showed a zero rotation. This substance is identical with the β-dimethyl sulphite liquors lactone obtained by Holmberg [8] by the isomerisation of the normal methylation product of conidendrin (α-dimethyl sulphite liquors lactone) with sodium ethoxide in ethanol. It follows that the

* Also XIII contribution on the *Constitution of resin phenols and their biogenetic relations*. Part XII, this journal 3 (1949) 896.

** Patent pending.

formation of our *nor*-conidendrin is due to the demethylation of α-conidendrin with simultaneous isomerisation under the influence of the demethylating agent. It is therefore *β-nor*-conidendrin. It is possible that *α-nor*-conidendrin occurs in the mother liquors. The dextrorotatory acetate obviously belongs to the same series of β-conidendrin derivates. Dr. Moyer [6] suggests that the crystalline demethylation products of conidendrin should be termed conidendrols. Thus, *β-nor*-conidendrin would be termed β-conidendrol.

EXPERIMENTAL PART

β-nor-Conidendrin

Conidendrin (5.2 g) and pyridinium chloride (11 g) were heated to 180° for two hours on a salt bath. The hot reaction mixture was poured into 2 *N* hydrochloric acid (80 ml) and the solution extracted continuously with ether over night. After evaporation the residue was dissolved in acetone (250 ml) and filtered through a column of aluminium oxide (5 × 2 cm) to remove some coloured impurities. The acetone was evaporated and the residue (4.7 g. Calc. 4.8 g) recrystallised from water (100 ml). Yield 4.0 g m. p. 245—246°. From the mother liquor a second crop (0.1 g) was obtained by partial evaporation. Total yield 4.1 g or 85 %. The residue is an oil probably containing *α-nor*-conidendrin. Repeated recrystallisations yielded pure substance. M. p. 248—249° (uncorr.)

$[\alpha]_D^{20}$ + 13° (Acetone c = 2)

$C_{18}H_{16}O_6$	Calc.	C	65.8	H	4.92
	Found	»	65.7	»	4.92

β-nor-Conidendrin tetraacetate. This substance was obtained in a quantitative yield by acetylation with acetic anhydride in pyridine. From ethanol crystals of m. p. 174—175°.

$[\alpha]_D^{20}$ + 11° (Acetone c = 2)

$C_{26}H_{24}O_{27}$ Calc. CH_3CO 34.7 Found CH_3CO 34.7

β-nor-Conidendrin tetramethyl ether. This substance was prepared by methylation of β-nor-conidendrin with methyl sulphate and alkali in an inert atmosphere essentially according to the method described by Holmberg [7] for the methylation of β-conidendrin. The crystalline product melted at 139—140°, solidified and melted again at 153°. It showed no optical rotation and was identical with a specimen prepared by Holmberg. (Mix. m. p.)

SUMMARY

α-Conidendrin has been demethylated with a good yield by means of pyridinium chloride. The demethylation is accompanied by an optical inversion and the *nor*-conidendrin obtained belongs to the β-series of conidendrin deriv-

atives as proved by its methylation to a product identical with β-conidendrin dimethyl ether.

We wish to express our thanks to Dr. W. Moyer for a generous gift of conidendrin.

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Received June 20, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 985–990

Short Communications

Urinary Excretion of a Pterin after Administration of Pteroylglutamic Acid

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Koschara's [1] observation that xanthopterin is excreted in human urine has gained in importance after the identification of folic acid with pteroylglutamic acid. We have been able to obtain a significant increase in the excretion of xanthopterin in human urine after oral or intramuscular administration of pteroylglutamic acid. (Fig. 1.)

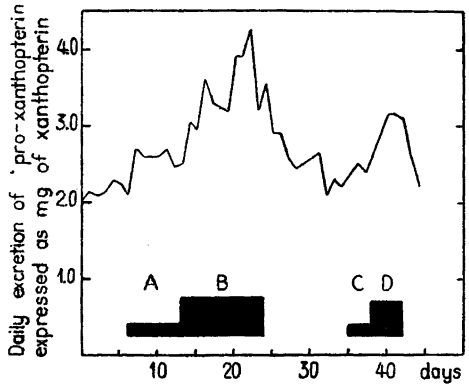

Fig. 1. Administration of pteroylglytamic acid.
A. 15 mg per day orally.
B. 50 mg per day orally.
C. 15 mg per day intramuscularly.
D. 45 mg per day intramuscularly.

Xanthopterin does not occur in a free state in the urine, as revealed by the fact that the fluorescence of untreated urine does not decrease after addition of xanthopterin oxidase, which would convert any free xanthopterin to the non-fluorescent leucopterin. The fluorescence of urine is therefore due to other substances, *e. g.* riboflavin. Xanthopterin can be obtained from the urine by adsorption on coal or superfiltrol and subsequent elution with dilute alkali [3]. In the neutralized eluate it may be determined quantitatively by the enzymatic method previously described [2].

Obviously the compound present in untreated urine is not xanthopterin and we have called it 'pro-xanthopterin'. It is not fluorescent and is stable in the dark between pH 1 and 13. When irradiated with ultraviolet light at pH 7–8 it is converted to xanthopterin and the same transformation takes place by heating to 100° C for about ten minutes. The R_F value of 'pro-xanthopterin' as determined in a paper chromatogram is about half that of xanthopterin in a butanol-acetic acid-water mixture.

At pH values lower than 8–9 xanthopterin is partly and reversibly converted to a non-fluorescent compound, which is not oxidized by xanthopterin oxidase [3]. This compound differs from 'pro-xanthopterin' in the following respects: 1) At pH 10 it is rapidly reconverted to xanthopterin, 2) its stability is unaffected by ultraviolet irradiation and 3) it is on heating transformed to xanthopterin at a much faster rate than is 'pro-xanthopterin'.

Dimethanesulphonyl Disulphide

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Dimethanesulphonyl disulphide is the pseudohalogen corresponding to the pseudohalide, methanethiosulphonate. It is formed when sodium or potassium methanethiosulphonate, suspended in ether, reacts with bromine:

$$2\ CH_3SO_2SNa + Br_2 = \\ = (CH_3SO_2S)_2 + 2\ NaBr \qquad (1)$$

The compound separates from ether as colourless needles or plates, m. p. 61° C. It is insoluble in water, sparingly soluble in methanol and petroleum, and readily soluble in ether, benzene and chloroform. It was recovered unchanged from a 10 % solution in benzene after heating to 80° C for 5 minutes. A corresponding aromatic compound, dibenzenesulphonyl disulphide, undergoes rapid rearrangement if heated in glacial acetic acid [1], [2].

Earlier [3], the term 'thiosulphonatogen' was suggested for compounds of this type. Dimethanesulphonyl disulphide is the first aliphatic thiosulphonatogen to be prepared. Its pseudohalogen properties are displayed in the reversible equilibrium:

$$2\ CH_3SO_2S^- + I_3^- \rightleftharpoons (CH_3SO_2S)_2 + 3\ I^- \qquad (2)$$

the equilibrium constant of which is the same as for the corresponding ethanethiosulphonate equilibrium [4], *viz.*, about unity, at room temperature.

In polythionic compounds there are, generally, two structural possibilities, *viz.*, that of unbranched, zigzag sulphur chains, and that of branched, co-ordinated structures. The reactions (1) and (2) show that dimethanesulphonyl disulphide is built up from two methanethiosulphonate radicals. The unpaired electron of such a radical is situated on the thio sulphur atom of the radical, because of the lower electron affinity of sulphur as compared with oxygen, and therefore, the bond joining the two thiosulphonate groups of dimethanesulphonyl disulphide is between two divalent sulphur atoms. Thus, from chemical considerations, dimethanesulphonyl disulphide has an unbranched sulphur chain structure, like other thio pseudohalogens and disulphides.

Experimental. About 20 % excess, with respect to bromine, of finely powdered, dry sodium or potassium methanethiosulphonate was employed, and 10—15 ml of dry ether per g of thiosulphonate. The suspensium was cooled in ice, and the bromine slowly added. On treatment of the solid particles with a glass rod the bromine colour vanished rapidly. The mixture was heated gently, and filtered with suction through a sintered glass filter. The residue on the filter was treated with warm ether, and the washings added to the filtrate. On partial evaporation and cooling the product separated as small needles or plates, m. p. 61° C (corr.). It is best recrystallized from ether.

'Pro-xanthopterin' is not identical with pteroylglutamic acid or any of its photolytic products [4].

Our thanks are due to Lederle Laboratories Division, American Cyanamid Co., New York, and to Kong Christian Xende Fond for financial support.

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Received October 3, 1949.

The Structure of Dimethanesulphonyl Disulphide

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In the present note, some preliminary results are reported of a crystal structure analysis of dimethanesulphonyl disulphide [1]. This compound is the analogue of potassium tetrathionate, for which lattice dimensions, space group and refractive indices are known [2]. No other data on tetrathionic compounds are reported in literature.

The crystals of dimethanesulphonyl disulphide are in most cases found as colourless, transparent needles or plates elongated in the *a*-axis direction. The most frequent faces of the monoclinic crystals are (010), (021), (001) and (011). The crystals seem to have good cleavage parallel to the planes (011) and (01$\bar{1}$) with less good cleavage parallel to the (010) plane.

0.1029 g substance: 0.4325 g $BaSO_4$.
$(CH_3SO_2S)_2$ (222.3)
Calc. S 57.68. Found S 57.72.

Further experiments on aliphatic thiosulphonates and disulphonyl disulphides, including measurements of their equilibria with iodide-iodine, will be described in a later article.

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4. Foss, O. *Acta Chem. Scand.* **1** (1947) 307.

Received October 30, 1949.

The dimensions of the unit cell were obtained from rotation and goniometer photographs: $a = 5.52 \pm 0.02$ Å, $b = 15.78 \pm 0.02$ Å, $c = 10.05 \pm 0.02$ Å. $\beta = 97.6°$ and $V = 866$ Å^3. The values for the axial lengths correspond to axial ratios of $a : b : c = 0.349 : 1 : 0.637$. The density of the crystals is 1.71 g/cm^3 and the number of $(CH_3SO_2S)_2$ in the unit cell therefore 4. The following reflexions are absent in the X-ray photographs: $0k0$ when k is odd, $00l$ when l is odd, and $h0l$ when l is odd. The *b*-axis is accordingly a twofold screw axis and the (010) plane is a glide plane of symmetry with translation $c/2$. Laue patterns indicate monoclinic holoedry and no other observations contradict this finding. The space group is therefore $C_{2h}^5 - P\frac{2_1}{c}$.

The molecule could possibly have a plane, a centre or twofold axis of symmetry. The space group C_{2h}^5 possesses only glide planes, screw axis and centres. The possibilities of molecular symmetry are therefore reduced to centre only. It has, however, proved impossible to account for the observed reflexion intensities if the centres of the molecules should be located to the symmetry centres of the space group. The molecules must consequently be placed in general positions, and can thus have no strict symmetry of its own. A general point repeats four times in the space group C_{2h}^5. All the molecules must therefore be crystallographically equivalent to each other, since there are only four molecules in the unit cell.

A preliminary analysis has shown that the molecule has an unbranched sulphur chain structure. The main direction of the sulphur chain is nearly parallel to the [011] or [01$\bar{1}$] direction alternatingly. The approximate centres of the four molecules are located very closely to the positions:

$$x\ \tfrac{1}{8}\ \tfrac{1}{8}\ ;\ x\ \tfrac{\bar{1}}{8}\ \tfrac{\bar{1}}{8}\ \text{and}\ x\ \tfrac{7}{8}\ \tfrac{\bar{7}}{8}\ ;\ x\ \tfrac{\bar{7}}{8}\ \tfrac{7}{8}$$

The Structure of Dimethanesulphonyl Disulphide

HARALD SØRUM AND OLAV FOSS

Fysisk Institutt, Norges Tekniske Høgskole, Trondheim, Norway, and Universitetets Kjemiske Institutt, Blindern — Oslo, Norway

In the present note, some preliminary results are reported of a crystal structure analysis of dimethanesulphonyl disulphide [1]. This compound is the analogue of potassium tetrathionate, for which lattice dimensions, space group and refractive indices are known [2]. No other data on tetrathionic compounds are reported in literature.

The crystals of dimethanesulphonyl disulphide are in most cases found as colourless, transparent needles or plates elongated in the *a*-axis direction. The most frequent faces of the monoclinic crystals are (010), (021), (001) and (011). The crystals seem to have good cleavage parallel to the planes (011) and (01$\bar{1}$) with less good cleavage parallel to the (010) plane.

0.1029 g substance: 0.4325 g $BaSO_4$.
$(CH_3SO_2S)_2$ (222.3)
Calc. S 57.68. Found S 57.72.

Further experiments on aliphatic thiosulphonates and disulphonyl disulphides, including measurements of their equilibria with iodide-iodine, will be described in a later article.

1. Otto, R., and Troeger, J. *Ber.* **24** (1891) 1125.
2. Troeger, J., and Hornung, V. *J. prakt. Chem.* [2] **60** (1899) 113.
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Studies on Liver Arginase. II. The Separation of a Single Protein with Arginase Activity

M. SAFWAT MOHAMED*

Biokemiska Institutet, Stockholms Högskola, Stockholm, Sweden

An extensive study of the properties of horse liver arginase revealed that although it is extremely sensitive to pH values lower than 5.0, yet it can withstand unusually high temperatures in the neutral range. It is neither precipitated nor its activity impaired by such treatment. Manganese-treated crude extracts at pH 7.2—7.7, can be heated directly on the flame to a temperature of 80—90° C. A lot of inert proteins closely associated with the enzyme can thus be removed. Moreover, the enzyme activity is unaffected by lead ion in any concentration. The amount of lead ion that can be added to the enzyme extract without causing its precipitation depends upon the concentration of the enzyme protein and previous treatments; such as, addition of manganese and heating. The enzyme is more easily precipitated with lead if previously heated. Unheated liver extracts can be treated with considerably high concentrations of lead (about 8 mg Pb^{++}/ml) without any change in the enzyme which remains in solution. This fact was first mentioned and utilized for the partial purification of arginase by Safwat Mohamed and Greenberg [1]. Their procedure was utilized later by Thompson [2, 3] with some additional steps. The use of the combination of Mn^{++} and phosphate ions as a means of purification of arginase, reported by Thompson, has been tried by the author and was found to entail a considerable loss to the enzyme. The disappearance of the color in Thompson's final extract (K)[3] is due more to dilution than to removal of the coloring matter. Still more loss is to be encountered if the final dilute extract (K) is to be concentrated through the use of acetone or ammonium sulfate.

In the following a brief account is given of the procedure that yields arginase of the highest purity and activity.

a) Three hundred g acetone-dried horse liver, extracted with 3 l distilled water containing 2 g $MnSO_4 \cdot 4H_2O$. Adjust pH to 7.5. Stir continuously for 8 h at room temperature. Centrifuge and discard residue.

b) Supernatent (I) at 3° C is mixed with 0.7 volumes cold acetone with stirring. Let stand at 3° C for 8 h. Centrifuge in cold room. Discard residue.

c) Supernatent, at 3° C, mixed with 0.5 volumes cold acetone with stirring and left at 3° C for 8 h. Centrifuge in cold room and discard supernatent.

d) Precipitate is taken up in distilled water. Extract (II); add 0.5 g $MnSO_4$, adjust pH to 7.5. Heat to 80° C and cool rapidly. Centrifuge and discard precipitate.

e) To supernatent (III) add $Pb(CH_3COO)_2 \cdot 3H_2O$ to give 2.0 mg Pb++/ml. Mix thoroughly and adjust pH to 7.5. Let stand at room temperature for 2 h Centrifuge.

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either of these pairs forming double molecules around the centres of symmetry in (000) and (0 $\frac{1}{2}$ $\frac{1}{2}$) respectively.

This structure is in agreement with interatomic distances, previously reported, with a Patterson synthesis of the 0*kl* data and can account satisfactorily for the relative intensities of the reflexions.

Further data and a more detailed description of this structure will be given in another paper.

1. Foss, O. *Acta Chem. Scand.* **3** (1949) 986.
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Table 1.

Extract	Volume ml	AU/ml	Total AU	mg N/ml	AU/mg N
I	2800	90	252000	4.0	22
II	650	380	251000	2.0	190
III	650	350	228000	0.6	580
IV	650	250	162000	0.4	600
IV+Mn	650	450	292000	0.4	1100
V	50	2100	110000	3.0	700
V+Mn	50	5000	250000	3.0	1650
VI	180	900	160000	1.2	750
VII	70	2000	140000	2.6	780

AU signifies arginase units, a term adopted by Safwat Mohamed and Greenberg [1].

By AU/ml is meant arginase units per ml of extract. AU/mg N = arginase units per mg N in 1 ml of extract.

f) Supernatent (IV) is mixed at 3° C with 0.7 volumes cold acetone. Let stand for 8 h and centrifuge in cold room.

g) Supernatent + 0.5 volumes cold acetone and left at 3° C for 8 h Centrifuge and the greenish blue precipitate is taken up in distilled water (V).

h) The precipitate formed in (e) contains considerable arginase. Take up in phosphate buffer, pH 7.5. Let stand with shaking overnight and centrifuge. Extract (VI) is mixed at 3° C with 1.2 volumes cold acetone, centrifuge after 8 h. Precipitate was taken up in phosphate buffer, pH 7.0 (VII).

Extract (V) contains very active arginase which is strongly activated by Mn^{++}. Electrophoresis at pH 7 and pH 6.0 showed the presence of a single homogeneous protein. The protein was precipitated several times with acetone in the cold (1.2 volumes). A greenish blue protein was always recovered. It dries into a white powder with bluish tinge.

Electrophoresis of (VII) showed the presence of three peaks with arginase forming 85—90 % of the total protein present.

From Table 1 it is evident that part of the arginase is split up, under the effect of heat and Pb^{++} ions, into smaller fragments still very active and can be much further activated by Mn^{++} ions. This fraction (V) was separated in a pure, yet not crystalline, form. In fraction (VI) the remainder of the enzyme, probably intact, could be separated along with two other proteins; arginase forming 85—90 %.

In a later publication a more detailed report will be presented.

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Received October 29, 1949.

On the Reducing Sugars in Sera from Pregnant and Lactating Women

LARS ODIN and IVAR WERNER

Institute of Medical Chemistry, Upsala, Sweden

The statement by Lövgren [1] that sera from pregnant and lactating women contain lactose only and no glucose as determined by his osazone formation method seemed to the present authors so surprising that it was desirable to check its validity.

It is well known that the microscopical identification of osazone crystals can be very difficult. This is especially the case if more than one osazone-forming sugar are simultaneously present or if the mother liquor is contaminated with other crystal-

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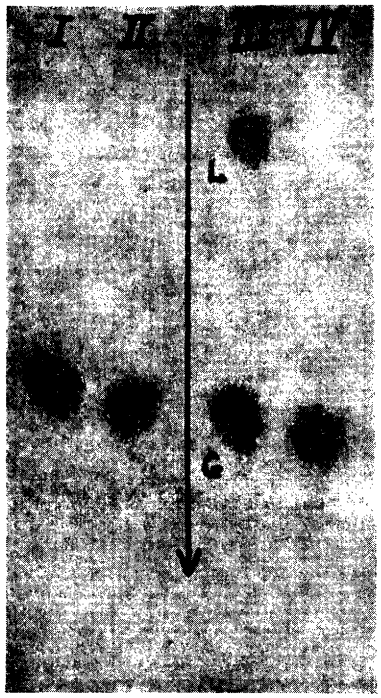


Fig. 1. Chromatogram showing reducing sugar in serum from woman in pregnancy (I), puerperium (II) and lactation (IV). Reference sugars (III): lactose (L) and glucose (G). Solvent: butanol-acetic acid. Developer: $AgNO_3$-ammonia.

lizing or noncrystallizing material (inorganic salts, peptides, lipids *etc.*). Thus instead of using the method of Lövgren we have carried out the analyses for reducing sugar by means of paper partition chromatography.

Method: 1 ml serum was precipitated with 9 ml 95 per cent ethanol and centrifuged. (This concentration of ethanol was shown not to precipitate a 200 mg per cent lactose solution). The supernatant liquid was decanted and evaporated to dryness *in vacuo*. Water was added and the solution desalted in the apparatus used by de Verdier and Ågren [2], and finally brought to a small volume *in vacuo*. A few drops of this solution were applied to the paper strip. For the details of the chromatographic technique see Partridge [3] and Werner and Odin [4].

In tests with lactose added to serum we found that by this method a lactose content in serum of about 5—10 mg per cent could be detected.

30 sera from women in late pregnancy and puerperium and 3 from women who had been lactating for a few months were investigated. *Glucose was found to be the dominating sugar in all these cases.* Only in one of the sera from women in puerperium and one from those in lactation did the chromatograms show traces of lactose. (A typical chromatogram is shown in Fig. 1.) Thus it seems probable that lactose, if at all present, generally does not reach a concentration exceeding 5—10 mg per cent.

It is well known that the urine in cases of pregnancy and lactation may contain lactose in an amount sufficient to give a positive Almén's test. Even that, however, can be explained by a lactose content in serum of less than 5—10 mg per cent.

The presence of lactose in serum in this low concentration can easily be explained by a slight resorption to the blood from the mammary glands. It certainly does not imply any profound alteration in the carbohydrate metabolism as suggested by Lövgren.

1. Lövgren, T. *Svensk Kem. Tid.* **59** (1947) 67.
2. de Verdier, C. H., and Ågren, G. *Acta Chem. Scand.* **2** (1948) 783.
3. Partridge, S. M. *Biochem. J.* **42** (1948) 238.
4. Werner, I., and Odin, L. *Upsala Läkareförens. Förh.* **54** (1949) 69.

Received November 3, 1949.

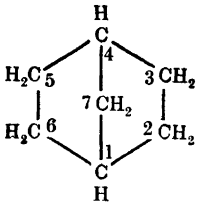
ACTA CHEMICA SCANDINAVICA 3 (1949) 991–1010

Über die *endo-exo*-Isomerie bei Verbindungen vom Camphertypus.* II.** Die Konfiguration des Borneols und Isoborneols

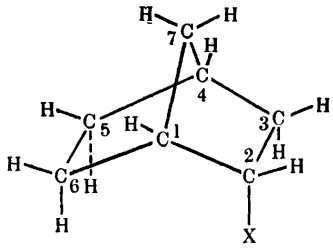
N. J. TOIVONEN, PEKKA HIRSJÄRVI, ASKO MELAJA, AUNE KAINULAINEN, AIRA HALONEN UND ERKKI PULKKINEN

Chemisches Institut der Universität Helsinki, Finnland

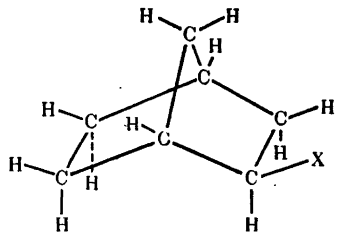
Wie bekannt, treten alle solche Derivate des Bicyclo-[1,2,2]-heptans oder Norcamphans (I),



I



II *endo*-Form



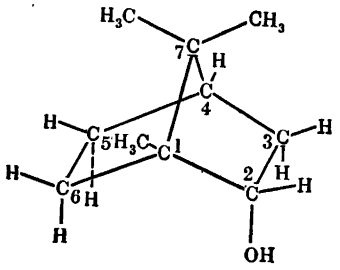
III *exo*-Form

in denen ein Wasserstoffatom an den Kohlenstoffatomen 2,3,5 oder 6 substituiert ist, paarweise in zwei diastereomeren Formen auf. Nach Bredt[1] benennt man diese Art der Isomerie, die durch den räumlichen Bau dieses bicyclischen Systems und, im Grunde genommen, durch die tetraedrische Gruppierung der Substituenten um das Kohlenstoffatom bedingt ist, als *endo-exo*-Isomerie; in der *endo*-Form (II) befindet sich der Substituent (X) innerhalb des durch die beiden Fünfringe gebildeten Winkels, in der *exo*-Form (III) ausserhalb dieses Winkels.

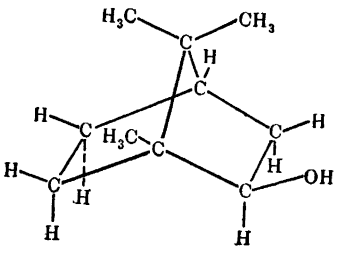
* Als Verbindungen vom Camphertypus werden hier alle Vertreter des Bicyclo-[1,2,2]-heptansystems (I) (= Ringsystem des Camphers) bezeichnet.

** I. Mitt. *Sjätte Nordiska Kemistmötet.* Lund (1947) S. 276.

Die für die ganze Chemie dieser Verbindungen so wichtige Frage, welche von jenen beiden Konfigurationen dem einen, welche dem anderen Glied irgendeines obenerwähnten Verbindungspaares zukommt, ist bisher vor allem und sogar sehr gründlich in Bezug auf die beiden Hydrierungsprodukte des Camphers, Borneol und Isoborneol, erforscht worden. Die diastereomeren Formen dieses Isomerenpaares sind:

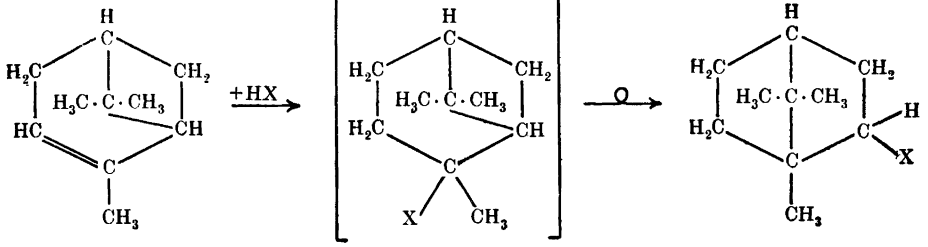


IV Endoborneol



V Exoborneol

Bredt [1] betrachtete das Borneol als *exo*-Form, weil bei der Anlagerung von Säuren an Pinen (VI→VIII), wobei gerade die Ester des Borneols gebildet werden,



VI VII VIII

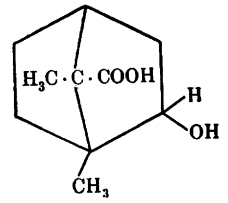
der Säurerest die von dem ebenfalls *exo*-ständigen quartären Brückenkohlenstoff hinterlassene Stelle am Kohlenstoff 2 einnehmen müsste. — Vgl. dazu die Bemerkung von W. Hückel [2].

Zu der entgegengesetzten Auffassung kamen Vavon und Peignier [3] auf Grund ihrer reaktionskinetischen Untersuchungen. Sie hatten u. a. festgestellt, dass die Ester des Isoborneols bedeutend langsamer sowohl gebildet als auch verseift werden als die des Borneols. Das Isoborneol entsprach in dieser Hinsicht den in Nachbarstellung *cis*-alkylierten, das Borneol den *trans*-alkylierten monocyclischen Alkoholen, wonach es sich auch hier um eine sterische Hinderung handelte. Bei der Betrachtung der Raummodelle erwies sich das Hydroxyl in der *exo*-Stellung als mehr — und zwar durch die Brücke mit der geminalen Dimethylgruppe — abgeschirmt als in der *endo*-Stellung, weshalb sie dem Isoborneol die *cis*-(*exo*-), dem Borneol die *trans*-(*endo*-) Konfiguration zuschrieben.

Auf Grund vergleichender, theoretischer Betrachtungen u. a.[r] über die verschiedenen Bildungsbedingungen der paarweise diastereomeren Alkohole sowie über die verschiedene thermische Zersetzungsleichtigkeit derselben und ihrer Ester äusserte sich auch W. Hückel [4] zugunsten der Annahme: Borneol = *endo*-, Isoborneol = *exo*-Form. — Dagegen betonte P. Lipp [5] die räumliche Nähe der Kohlenstoffatome 2 und 6 und die dadurch bedingte sterische Hinderung in dem *endo*-ständigen Hydroxyl. — Die von Lipp bestimmten Parachorwerte des Bornyl- bzw. des Isobornylacetats konnten wegen ihrer Gleichheit kein Material für die Entscheidung der *endo-exo*-Frage beibringen.

Auf die Anwendung von Ramanspektren zur Konfigurations-Ermittlung der Borneole sei hier nur hingewiesen [6].

Auf rein chemischem Wege haben Asahina und Mitarbeiter [7,8] die Frage behandelt. Von den beiden Formen der 2-Oxy-*cis*-π-apocamphan-7-carbonsäure (IX)

IX 

bildet die eine sehr leicht ein Lacton (das sog. Semmler-Barteltsche Lacton), die andere nicht, bzw. erst nach Umlagerung. In der ersteren muss die Hydroxylgruppe also die *exo*-, in der letzteren die *endo*-Stellung (vgl. die üblichen Raummodelle) einnehmen. Das *endo*-Isomere konnte, in der letzten Phase als 2-Acetoxy-*cis*-π-apocamphan-7-aldehydsemicarbazon mit Natriumäthylat auf 150—155° erhitzt (Wolffsche Reduktion) in Borneol übergeführt werden. Das sog. *trans*-π-Apo-isobornylacetat-7-aldehydsemicarbazon, vergleichsweise ebenso behandelt, wurde in Isoborneol übergeführt; das Isoborneol selbst wurde durch Natriumäthylat erst bei 170—180° in Borneol umgewandelt. Auf Grund dieser Ergebnisse war also dem Borneol die *endo*-, dem Isoborneol die *exo*-Konfiguration zuzuschreiben.

Schon im Jahre 1898 hatte W. Biltz [9] festgestellt, dass die kryoskopisch bestimmte Assoziation beim Borneol viel stärker hervortritt als beim Isoborneol. Dies war nach ihm darauf zurückzuführen, dass in dem ersteren die Hydroxylgruppe exponierter (weniger geschützt) als in dem letzteren steht. Weil die beiden Norborneole (II und III, x = OH) nach Untersuchungen von Komppa und Beckmann [10] stark und zwar etwa gleich stark assoziieren, kann nach ihnen für das recht unterschiedliche Verhalten von Borneol und Isoborneol nur die stark abschirmende Wirkung der 1- und 7-ständigen Methylgruppen auf das *exo*-ständige Hydroxyl im Isoborneol verantwortlich sein.

Nach der Synthese des Borneols aus 1,5,5-Trimethylcyclopentadien-1,3 und Vinylacetat kamen auch Alder und Windemuth [11], auf Grund ihrer Untersuchungen über den sterischen Verlauf der betreffenden Dien-synthesen zu der Auffassung, dass dem Borneol die *endo*-Konfiguration zukommt.

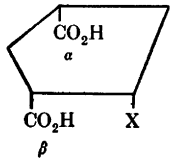
Auch der Einfluss des Lösungsmittels auf die Drehung der Polarisationsebene beim Borneol und Isoborneol und besonders bei deren Methyläthern ist nach W. Hückel [12] am besten zu erklären, wenn man dem Borneol die *endo*-, dem Isoborneol die *exo*-Konfiguration zuschreibt.

Von den bisherigen Untersuchungsergebnissen kann das von Asahina und Mitarbeitern als die sicherste und ziemlich befriedigende Lösung der Frage betrachtet werden. Alle Forscher hat jedoch auch sie nicht überzeugen können [13]. Jedenfalls beschränkt sich die Brauchbarkeit des von ihnen benutzten Verfahrens nur auf den Einzelfall Borneol-Isoborneol bzw. auf Verbindungen, die an dem Brückenkohlenstoff reaktionsfähige Atomgruppen tragen, was ziemlich selten vorkommt.

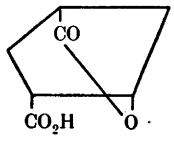
VERFAHREN ZUR KONFIGURATIONS-ERMITTLUNG DER *ENDO-EXO*-ISOMEREN

Es sei deshalb ein Verfahren beschrieben, das sich wohl ziemlich allgemein zur Konfigurations-Ermittlung solcher *endo-exo*-Isomerenpaare anwenden lässt, wo der in Frage stehende Substituent aus einer reaktionsfähigen Atomgruppe wie Hydroxyl, Halogen, Carboxyl, Aminogruppe usw. besteht.

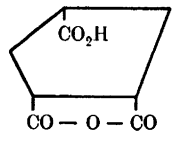
Es gründet sich auf folgende Überlegungen und Schlussfolgerungen über die sterischen Verhältnisse und Reaktionsmöglichkeiten bei den betreffenden Verbindungen (vgl. Formeln II und III sowie X—XIII):



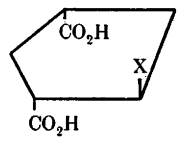
X



XI



XII



XIII

1. In der *endo*-Form (F. II) befindet sich der Substituent X, in Bezug auf den Fünfring 1,2,3,4,7, in *cis*-Stellung zu den Kohlenstoffatomen 5 und 6.

2. Wenn man nun die Bindung zwischen den Kohlenstoffatomen 5 und 6 aufhebt (in gewissen Fällen kommen auch die Bindungen 1—6 bzw. 4—5 in Frage), z. B. durch Oxydation derselben zu Carboxylen (vgl. F. X), so sollte wenigstens das eine von diesen mit dem Substituenten X unter intramolekularer Ringbildung so reagieren können, dass ein Lacton (z. B. XI), ein Säureanhydrid (z. B. XII), ein Lactam usw. gebildet wird. — Um Anhydridbildung zwischen den Carboxylen α und β zu verhüten, muss das eine von ihnen vielleicht zuerst verestert werden.

3. Falls der Substituent in der ursprünglichen Verbindung in *exo*-Stellung (= *trans*-Stellung zu den Kohlenstoffatomen 5 und 6) steht (III), so sollte der genannte Ringschluss bei der entsprechenden Säure (XIII) nach den bisherigen Erfahrungen nicht oder höchstens, in einigen besonderen Fällen, sehr schwer eintreten können.

Bei jedem Schritt der Untersuchung muss man natürlich einer eventuellen Konfigurationsänderung Rechnung tragen und die erforderlichen Operationen am besten mit den beiden Diastereomeren parallel durchführen.

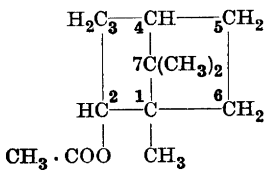
KONFIGURATIONS-ERMITTLUNG VON BORNEOL UND ISOBORNEOL

Abbau zu Cyclopentanderivaten: Diastereomere 5-Oxy-camphersäuren

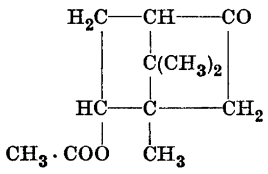
A. Borneolreihe

Als erster geeigneter Schritt zur experimentellen Durchführung des obengeschilderten Arbeitsplans erbot sich die schon von Bredt und Mitarbeitern [14] ausgearbeitete Oxydierung verschiedener Verbindungen vom Camphertypus mit Chromsäure zu den entsprechenden 5-Ketoderivaten.

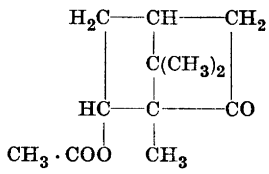
In dieser Weise hatten sie sowohl aktives [14] wie auch racemisches [15] Bornylacetat (XIV) zu 5-Ketobornylacetat (XV) oxydiert. Daneben entsteht nach Asahina und Mitarbeitern [16] auch 6-Ketobornylacetat (XVI). Wir bedienten uns des kristallisierten Gemisches beider Ketoverbindungen, die offenbar, wie nach Watanabe [17] die entsprechenden Ketocampher, unter einander Mischkristalle bilden.



XIV



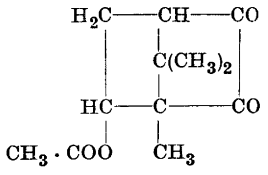
XV



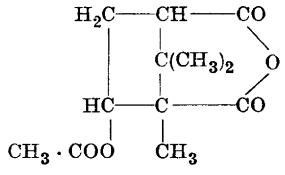
XVI

Als darauffolgende Operation erwies sich (auch andere Wege waren denkbar) die Oxydation der Verbindungen XV und XVI mit Selendioxyd zu 5,6-Diketo-bornylacetat (XVII) als zweckmässig [16].

Die Diketoverbindung oxydierten wir mit Wasserstoffperoxyd in Eisessig zu 5-Acetoxy-camphersäureanhydrid (XVIII) und hydrolysierten diese



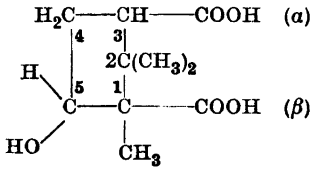
XVII



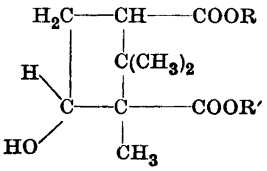
XVIII

Verbindung in alkalischer Lösung zu 5-Oxycamphersäure (XIX). — Diese Oxysäure erwies sich als identisch mit der von uns früher [18] synthetisch dargestellten, niedriger schmelzenden 5-Oxy-*cis*-camphersäure.

Um den β-Methylester (XXI) dieser Oxysäure zu gewinnen, wurde die Säure zuerst, durch Einwirkung von Dimethylsulfat auf ihr Kaliumsalz, zum Dimethylester (XX) umgesetzt und dieser durch partielle Verseifung in den β-Ester (XXI) übergeführt. — Auch der α-Methylester (XXII) wurde durch Einwirkung von Chlorwasserstoff auf die Methanollösung der Säure dargestellt.



XIX



XX: R = R′ = CH_3

XXI: R = H, R′ = CH_3

XXII: R = CH_3, R′ = H

B. Isoborneolreihe

Die Oxydation des Isobornylacetats (XIV) mit Chromsäure zu 5- und 6-Ketoisobornylacetat (XV und XVI), die besondere Massnahmen erfordert und früher nicht gelungen ist, haben wir schon vor einiger Zeit beschrieben [19]. Ganz wie die obengenannten Verbindungen der Borneolreihe wurden schon damals auch die anderen entsprechenden Verbindungen der Isoborneolreihe: 5,6-Diketo-isobornylacetat (XVII), 5-Acetoxy-camphersäureanhydrid (XVIII) und 5-Oxy-camphersäure (XIX) dargestellt. — Die 5-Oxy-camphersäure der Isoborneolreihe erwies sich als identisch mit der von uns früher [18] synthetisch dargestellten, höher schmelzenden 5-Oxy-*cis*-camphersäure.

Später haben wir auch den Dimethylester (XX) und den β-Methylester (XXI) der 5-Oxy-camphersäure der Isoborneolreihe dargestellt (vgl. den experimentellen Teil).

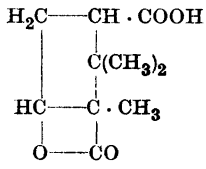
KONFIGURATION DER 5-OXY-CAMPHERSÄUREN DER BORNEOL- UND ISOBORNEOLREIHE

Im folgenden sei zunächst das Verhalten der obenerwähnten Säuren, dann dasjenige ihrer sauren Ester gegen einige anhydrisierende Mittel beschrieben.

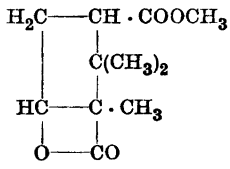
Durch Einwirkung von Acetylchlorid werden die beiden Oxysäuren (XIX) praktisch vollkommen in ihre Acetoxy-anhydride (XVIII) übergeführt.

Gegen Essigsäure-anhydrid verhalten sie sich verschiedentlich. Aus der 5-Oxy-camphersäure der Borneolreihe wird durch dieses Agens sowohl das obengenannte 5-Acetoxy-camphersäureanhydrid (XVIII) als auch die β-Lac-

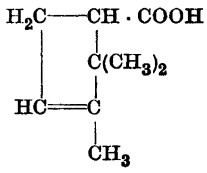
tonsäure (XXIII) gebildet. Diese Säure gibt mit Diazomethan das entsprechende α-Methylester-β-lacton (XXIV); dieselbe Verbindung wird aus dem α-Methylester (XXII) der 5-Oxy-camphersäure der Borneolreihe mit Acetylchlorid erhalten. Beim Erhitzen wird die β-Lactonsäure in Kohlendioxyd und α-Campholytsäure (XXV) gespalten, wodurch ihre Konstitution am sichersten bewiesen wird; eine entsprechende Zersetzungsweise ist übrigens gerade den β-Lactonen charakteristisch.



XXIII

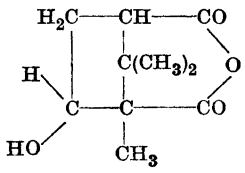


XXIV

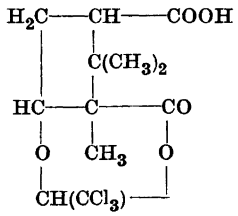


XXV

Durch gleiche Behandlung mit Essigsäureanhydrid wird aus der 5-Oxy-camphersäure der Isoborneolreihe praktisch ausschliesslich das 5-Oxy-camphersäureanhydrid (XXVI) gebildet. Dass die 5-Oxy-Gruppe hier durchaus nicht so leicht wie in der entsprechenden Verbindung der Borneolreihe (vgl. oben) acetyliert wird, ist wohl auf die abschirmende Wirkung der brückenbildenden $C(CH_3)_2$-Gruppe auf das jetzt in »*exo*»-Stellung stehende Hydroxyl zurückzuführen. — Durch Einwirkung von Acetylchlorid auf die Verbindung XXVI erfolgt auch hier die Acetylierung.



XXVI



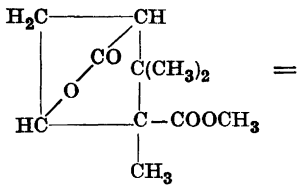
XXVII

Durch Einwirkung von Chloralhydrat und konc. Schwefelsäure wird aus der 5-Oxy-camphersäure der Borneolreihe in praktisch quantitativer Ausbeute ein Chloralid gebildet, dem wohl die Formel XXVII zukommt [20]. Bei alkalischer Verseifung wird aus diesem Chloralid neben Chloroform und Ameisensäure die 5-Oxy-camphersäure der Borneolreihe zurückgebildet.

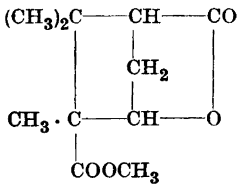
Bei gleicher Behandlung mit Chloralhydrat und Schwefelsäure wird aus der 5-Oxy-camphersäure der Isoborneolreihe kein fassbares Reaktionsprodukt gebildet.

Das Verhalten der Oxysäuren bei Erhitzung, die zu verschiedenen Reaktionsprodukten führt, ist vorläufig nicht gründlich genug untersucht worden.

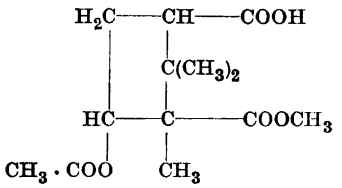
Beim Entwurf der hier befolgten Methode zur Ermittlung der *endo-exo*-Konfiguration wurde vorausgesetzt (vgl. S. 994), dass wenigstens die sauren Ester, die in den beiden diastereomeren Reihen der Abbau-Produkte der *endo-exo*-Isomeren dargestellt werden können, in ihrer Fähigkeit zu Ringschlussreaktionen einen deutlichen Unterschied aufweisen würden. In Übereinstimmung damit wird aus dem β-Methylester (XXI) der 5-Oxy-camphersäure der Borneolreihe durch Einwirkung von Acetyl- oder Thionylchlorid neben sauren Reaktionsprodukten das β-Ester-γ-lacton (XXVIII = XXIX) gebildet.



XXVIII



XXIX



XXX

Dagegen gibt der entsprechende Ester (XXI) der Isoborneolreihe mit Acetyl- bzw. Thionylchlorid kein neutrales Reaktionsprodukt; mit dem ersteren liefert er in der Hauptsache den 5-Acetoxy-camphersäure-β-methylester (XXX), mit dem letzteren nur polymere Estersäuren.

In Gegenwart von Kaliumacetat oder Pyridin wird der β-Methylester auch der Borneolreihe durch Acetylchlorid nur in die entsprechende Acetoxy-Verbindung (XXX) umgesetzt.

Über das Verhalten des α-Methylesters der 5-Oxy-camphersäure der Borneolreihe s. S. 997.

Durch Hydrolyse wurden aus allen obengenannten Umsetzungsprodukten die ursprünglichen Oxysäuren, im Falle der β-Methylester gewöhnlich auch diese Ester zurückgebildet. Die Reaktionen sind also ohne sterische Umlagerung verlaufen.

Von den obenerwähnten Ringschlussreaktionen verdienen einige, u. a. wegen der Seltenheit der β-Lactonbildung direkt aus den β-Oxysäuren, eine etwas nähere Betrachtung.

In einer 5-Oxy-camphersäure, wo sowohl das Hydroxyl als auch die Carboxyle in *cis*-Stellung zueinander stehen, kann der Ringschluss zwischen diesen Gruppen in dreierlei Weise erfolgen. Eine von diesen ist durch die Bildung des 5-Acetoxy-camphersäureanhydrids (XVIII) verwirklicht worden. Diese Verbindung wird aus der 5-Oxy-camphersäure der Borneolreihe durch Acetylchlorid als ausschliessliches, durch Acetanhydrid als vornehmliches Reaktionsprodukt gebildet, was wohl darauf zurückzuführen ist, dass sie praktisch spannungsfrei ist. Die beiden anderen möglichen Produkte, die γ-Lactonsäure (entspr. F. XXVIII bzw. XXIX) und die β-Lactonsäure (XXIII) sind ziemlich stark gespannt; die erstere, weil sie ihrem räumlichen Bau nach ein teilweise heterocyclisches Analogon der carbocyclischen Verbindungen vom Camphertypus darstellt (Gesamtspannung dieses Kohlenstoff-Ringsystems = 77° 22' [21]); die letztere wegen des in ihm enthaltenen Vierrings (Spannung im Kohlenstoff-Vierring = 77° 52'). Dass von diesen etwa gleich stark gespannten Verbindungen bei Einwirkung von Acetanhydrid auf die Oxysäure nur die letztere entsteht, hängt wohl damit zusammen, dass das β-Carboxyl dem Hydroxyl räumlich viel näher steht als das α-Carboxyl. Auf diese, seitens des Fünfrings fixierte Nähe der genannten Atomgruppen, deren Bindungsrichtungen bereits in einer (bzw. beinahe einer) Ebene liegen, ist wohl neben anderen Faktoren zurückzuführen, dass die Bildung des β-Lactons direkt aus einer β-Oxysäure — wovon bisher nur sehr wenige Beispiele vorliegen — in diesem Falle überhaupt und sogar in relativ grossem Betrag erfolgt.

Andererseits wird wohl die γ-Lactonbildung, also die Entstehung des heterocyclischen Ringsystems vom Camphertypus (vgl. F. XXIX) durch die ungünstige Verteilung der Substituenten (Methyl- und Carboxylgruppen) sowohl auf den entstehenden γ-Lactonring als auch auf das ganze bicyclische Ringsystem verzögert; vgl. dazu die Konstitution bzw. die äusserst leicht erfolgende Bildung der Camphansäure. In unserem Fall ist für die Erzielung der γ-Lactonbildung (vgl. oben) die Ausschaltung (Veresterung) des β-Carboxyls und, der bisherigen Versuche gemäss, die Einwirkung von Säurechloriden erforderlich.

Die Ergebnisse der obenbeschriebenen Versuche — die Bildung eines β- und γ-Lactons sowie eines Chloralids in der einen, das Nichterreichen intramolekularer Ringschlussreaktionen in der anderen Verbindungsreihe — zeigen eindeutig, dass in der 5-Oxy-camphersäure der Borneolreihe das Hydroxyl in *cis*-, in der 5-Oxy-camphersäure der Isoborneolreihe in *trans*-Stellung zu den Carboxylen steht.

KONFIGURATION DES BORNEOLS UND ISOBORNEOLS

Um die Konfigurationen des Borneols und Isoborneols auf Grund der Konfigurationen der aus ihnen dargestellten 5-Oxy-camphersäuren festlegen zu können, bedarf es noch der Feststellung, dass bei den durchgeführten Operationen keine Änderung in der räumlichen Anordnung der Kohlenstoffatome und der Hydroxyle eingetreten ist. Dies gilt vor allem für die β-Carboxyle (F. XIX) bzw. Kohlenstoffatome 6 (F. XIV), weil hier kein für die Umlagerung notwendiger α-Wasserstoff vorhanden ist, und demzufolge auch für die α-Carboxyle bzw. Kohlenstoffe 5 (F. XIV), die die ganze Zeit ihre *cis*-Stellung zu den β-Carboxylen beibehalten haben (Anhydridbildung). Ebensowenig können die Hydroxyle ihre räumliche Lage geändert haben, weil dies einen vollständigen Übergang der beiden Verbindungsreihen ineinander bedeuten würde.

Schon im Borneol befindet sich somit das Hydroxyl in *cis*-Stellung zu den Kohlenstoffatomen 5 und 6, also in der *endo*-Stellung, im Isoborneol in *trans*-Stellung zu diesen Kohlenstoffatomen, also in der *exo*-Stellung. Mit anderen Worten: *das Borneol ist Endo-, das Isoborneol Exoborneol.*

Berücksichtigt man noch die von uns früher [18] ausgeführte Totalsynthese der beiden obenbeschriebenen 5-Oxy-camphersäuren, so wird durch alle diese Reaktionsserien ihrerseits auch die eigentliche Struktur des Borneols und Isoborneols nochmals bewiesen.

Die Konstitution des Isoborneols ist ja bis in die jüngste Zeit in Frage gestellt gewesen [22a]. — Auch die Frage über die Existenz eines neuen, durch Hydratation des Bornylens entstehenden stereoisomeren Borneols, des sog. »Endoborneols», ist seit 1926 erörtert worden [22b]. Nach Schawrygin [23] ist diese Substanz augenscheinlich ein Gemisch von Alkoholen der Campher- und Epicampherreihe. Auch nach unseren Versuchen (Spezialarbeit von Keijo Aunio 1947) ist die genannte Substanz nicht einheitlich; sie besteht vermutlich aus Mischkristallen der letztgenannten Alkohole.

EXPERIMENTELLER TEIL *

A. Borneolreihe

5- und 6-Keto-bornylacetat (XV, XVI). Reiner, inaktiver Campher (nötigenfalls durch Behandeln mit Chromtrioxyd in Eisessiglösung gereinigt [24]) wurde mit Natrium in Alkohollösung reduziert, das nebenbei gebildete Isoborneol durch Kochen des Reduktionsproduktes mit Zinkchlorid in Benzollösung zerstört [25], und das reine *r*-Borneol

* Bei den Versuchen sind auch die Herren Veijo Mattinen und Kai Noramies beteiligt gewesen. Die Analysen sind von Herrn Kalervo Salo ausgeführt worden.
Die Schmelzpunkte wurden im Rothschen Apparat bestimmt.

(Schmp. 207°) mit Essigsäureanhydrid bei 145°[14] in Bornylacetat übergeführt. Die Oxydation desselben mit Chromtrioxyd zu Keto-bornyl-acetat wurde in Eisessig bei 140°[14], in Eisessig und Acetanhydrid bei Zimmertemperatur [15] oder in Eisessig und Acetanhydrid bei 80°[16] ausgeführt.

Das beim Fraktionieren des Reaktionsproduktes erhaltene Ketobornylacetat-Gemisch (Siedep. 145—148°/15 mm, gewöhnlich etwa 30 % d. Th.) wurde bald kristallinisch und schmolz, nach Umkristallisieren aus Petroläther, bei 51—52°. Über die Zusammensetzung desselben vgl. S. 995.

$C_{12}H_{18}O_3$ (210,1)	Ber.	C	68,53	H	8,63
	Gef.	»	68,45	»	8,47

5,6-Diketobornylacetat (XVII) wurde durch Erhitzen des Gemisches von 5- und 6-Ketobornylacetat (10 g) mit Selendioxyd (5,3 g = 1 Mol) im Ölbad dargestellt. Die Reaktion begann deutlich bei 150—155° und war nach einer halben Stunde zu Ende. Das Gemisch wurde mit Äther und Wasser digeriert und das Selen als schwarzes, schweres Pulver abgeschieden. Die ätherische Lösung wurde mit Sodalösung gewaschen und mit Natriumsulfat getrocknet. Nach dem Verjagen des Äthers wurde der Rückstand, 7,4 g, aus Ligroin und dann aus Alkohol umkristallisiert. Gelbe, dünne Prismen, Schmp. 95—96°. Ausb. 6,0 g = 56,2 % d. Th.

$C_{12}H_{16}O_4$ (224,1)	Ber.	C	64,26	H	7,20
	Gef.	»	64,20	»	7,35

Hydrolyse: 5stündiges Kochen von 0,2795 g des Diketobornylacetats in 50 ml Alkohol und 36,3 ml 0,1 *N* NaOH-Lösung. Verbrauch: 12,8 ml Lauge, ber. 12,5 ml.

Später wurde die Oxydation auch in Acetanhydridlösung mit je 2,5 Mol Selendioxyd durchgeführt [16]. Ausb. etwa 36 % d. Th.

5-Acetoxy-camphersäureanhydrid (XVIII). 10 g 5,6-Diketobornylacetat wurden in 60 ml Eisessig gelöst und bei Zimmertemperatur allmählich mit 40 g 30 %igem Wasserstoffperoxyd versetzt. Nach einigen Stunden war die Lösung farblos geworden und eine reichliche Menge farbloser Kristalle sich abgeschieden. Durch allmähliches Zusetzen von Wasser wurde das Anhydrid in schönen Kristallen gefällt. Ausbeute 9 g, Schmp. 124°. Durch Umkristallisieren aus Eisessig oder Alkohol — glänzende, dünne, meistens rechteckige Blätter — wurde der Schmp. nicht verändert.

$C_{12}H_{16}O_5$ (240,1)	Ber.	C	59,97	H	6,72
	Gef.	»	59,83	»	6,93

5-Oxy-camphersäure (XIX) der Borneolreihe und Derivate

38 g Acetoxy-camphersäureanhydrid wurden in 10 %iger Kaliumhydroxydlösung (4 Mol KOH) im kochenden Wasserbad 10 Stunden erhitzt. Nach 3maligem Auswaschen mit Äther wurde die Lösung mit verd. Schwefelsäure angesäuert und ausgeäthert. Diese Ätherlösung wurde einmal mit etwas Wasser gewaschen, mit Natriumsulfat getrocknet, der Äther abdestilliert und der kristalline Rückstand, 34 g, aus Wasser um-

kristallisiert (28,7 g). Nach nochmaligem Umlösen aus Wasser schöne Rhomboide, Schmp. 194° (Zers.).

0,2045 g Sbst. verbrauchten 18,7 ml 0,1 *N* NaOH.

$C_{10}H_{16}O_5$ (216,1)	Ber.	C	55,53	H	7,46	Äquiv.-Gew.	108,1
	Gef.	»	55,48	»	7,34	» »	109,3

Mit Acetylchlorid in der Kälte oder bei Siedetemperatur behandelt gibt diese 5-Oxy-camphersäure in vollständiger Ausbeute dasselbe 5-Acetoxy-anhydrid (S. 1001), aus dem sie durch Hydrolyse dargestellt worden war.

Die obige 5-Oxy-camphersäure (der Borneolreihe) erweist sich in allen Beziehungen als identisch mit der früher [18] synthetisch dargestellten, niedriger (bei 194°, Zers.) schmelzenden 5-Oxy-*cis*-camphersäure. Mischschmp.: keine Depression. — Ganz gleich verhalten sich auch die Acetoxy-anhydride beider Oxysäuren.

Dimethylester (XX). 23,09 g (1 Mol) 5-Oxy-camphersäure wurden allmählich in eine Lösung von 13,1 g (2,2 Mol) Kaliumhydroxyd in 30 ml Methanol gelöst. 30,8 g (2,3 Mol) Dimethylsulfat wurden in kleinen Portionen zugesetzt, die beim lebhaften Schütteln immer warm gewordene Lösung abgekühlt, eine neue Portion Dimethylsulfat zugesetzt u. s. w. Nach dem Stehen über Nacht bei Zimmertemperatur wurde das Methanol i. V. abdestilliert, etwas Wasser zugesetzt und die Lösung sodaalkalisch gemacht. Der gebildete Ester wurde in Äther aufgenommen und die Lösung mit Natriumsulfat getrocknet. Der Rückstand nach dem Abdestillieren des Äthers kristallisierte i. V. allmählich vollständig; 22,5 g, Schmp. 45—48°. Aus Benzol oder Äther-Petroläther (1 : 1) sechsseitige Tafeln, bei langsamer Bildung auch dicke, etwas schiefwinkelige Kuben, Schmp. 48—50°.

$C_{12}H_{20}O_5$ (244,2)	Ber.	C	58,99	H	8,26
	Gef.	»	58,79	»	7,99

Beim Versuch, den Dimethylester i. V. zu destillieren, wurde der Kolbeninhalt unter Gasentwicklung zersetzt und zum grössten Teil in eine dicke, dunkle, undestillierbare, nach dem Erkalten glasartige Masse verwandelt. Das in ziemlich kleiner Menge erhaltene Destillat, Siedep. 116—124°/6,5 mm, war gegen alkalische Permanganatlösung stark ungesättigt.

5-Acetoxy-camphersäure-dimethylester wurde durch 24stündige Einwirkung von überschüssigem Acetylchlorid auf den obigen Dimethylester erhalten. Aus Eisessig umgelöst Kristalle, die denen des Dimethylesters sehr ähneln. Schmp. 78—79°.

$C_{14}H_{22}O_6$ (286,2)	Ber.	C	58,70	H	7,75
	Gef.	»	58,62	»	7,65

β-Methylester (XXI). Darstellung:

A. Eine Lösung von 5,2 g Dimethylester, 1,1 g (0,9 Mol pro 1 Mol Dimethylester) Kaliumhydroxyd, 1,1 g Wasser und 40 ml Methanol wurde 6 Stunden auf dem Wasserbad gekocht. Nach Verdünnen mit Wasser, Abdestillieren des Methanols i. V., Entfernen des neutralen Esters mit Äther, Ansäuern mit Schwefelsäure, Extrahieren mit Äther, Trocknen mit Natriumsulfat und Abdestillieren des Äthers wurde der saure Ester (4,7 g) zum grössten Teil kristallisiert erhalten.

B. 8 g Dimethylester und 1,25 g (0,95 Mol) Natriumhydroxyd in 30 ml Wasser wurden mit Methanol auf 100 ml aufgefüllt und bei Zimmertemperatur stehen gelassen. Nach 12 Stunden waren von dem Alkali 35 %, nach 40 Stunden 65 %, nach 120 Stunden ca. 100 % verbraucht. Das Methanol wurde i. V. abdestilliert, die neutralen Bestandteile mit Äther entfernt und der β-Ester, dessen Kristallisationsfähigkeit durch verwandte Verbindungen (Oxysäure, α-Ester u. dgl.) sehr erschwert wird, mit Salzsäure fraktioniert ausgefällt.

Der β-Methylester kristallisiert aus Äther-Petroläther oder auch aus Wasser oder 20 %iger Salzsäure in sehr charakteristischen, dreiseitigen, stark doppelbrechenden Pyramiden. Schmp. 104°.

0,1915 g Sbst. verbrauchten 8,30 ml 0,1 *N* NaOH

$C_{11}H_{18}O_5$ (230,1)	Ber.	C	57,35	H	7,88	Äquiv.-Gew.	230,1
	Gef.	»	57,19	»	7,64	» »	230,7

α-Methylester (XXII). Eine Lösung von 2 g der Oxysäure in 10 ml Methanol wurde mit trocknem Chlorwasserstoff gesättigt, 1 Stunde stehen gelassen und dann während 1 Stunde auf dem Wasserbad erwärmt. Das Methanol wurde i. V. abdestilliert, der Rückstand in Äther gelöst und diese Lösung zuerst mit Soda-, dann noch mit etwas verdünnter Natriumhydroxydlösung ausgeschüttelt. Die vereinigten alkalischen Lösungen wurden mit Äther gewaschen, mit Schwefelsäure angesäuert, mehrere Male mit Äther extrahiert, die Ätherlösung mit Natriumsulfat getrocknet und der Äther zuletzt i. V. entfernt. Der Rückstand, 1,7 g, kristallisierte bald vollkommen. Aus methanolhaltigem Wasser schöne, wohlausgebildete, flächenreiche Prismen, Schmp. 121—122°.

0,0485 g Sbst. verbrauchten 2,08 ml 0,1 *N* NaOH

$C_{11}H_{18}O_5$ (230,1)	Ber.	C	57,35	H	7,88	Äquiv.-Gew.	230,1
	Gef.	»	57,45	»	7,92	» »	233,1

Einwirkung von Acetanhydrid auf die 5-Oxy-camphersäure der Borneolreihe: 5-Acetoxy-camphersäure-anhydrid (XVIII) und β-Lacton der 5-Oxy-camphersäure (XXIII)

3,05 g Oxysäure wurden bei Zimmertemperatur in 30 ml Acetanhydrid gelöst. Nach 3 Tagen wurde das Acetanhydrid i. V. abdestilliert. Der feste Rückstand wurde in viel Äther gelöst und durch Behandeln mit gesättigter Natriumhydrocarbonatlösung in einen neutralen und einen sauren Teil zerlegt.

Die neutrale Substanz, 1,90 g, kristallisierte aus Äther in langen Nadeln, Schmp. 123°, und erwies sich als das *5-Acetoxy-camphersäure-anhydrid (XVIII) der Borneolreihe* (Mischschmp.).

β-Lactonsäure (XXIII). Die saure Substanz, 0,82 g, wurde zuerst aus Äther, worin sie sehr schwerlöslich ist — in 100 ml siedenden Äther lösen sich nur ca. 0,5 g der Lactonsäure — und darauf aus Essigester umkristallisiert. Lange Nadeln und Prismen, zersetzt sich bei schneller Erhitzung bei 209—210° unter Bildung einer klaren Schmelze. In Sodalösung gegen Kaliumpermanganat beständig.

0,0235 g Sbst. verbrauchten 1,16 ml 0,1 *N* NaOH.

$C_{10}H_{14}O_4$ (198,1)	Ber.	C	60,59	H	7,12	Äquiv.-Gew.	198,1
	Gef.	»	60,77	»	6,89	» »	202,6

Zwecks Hydrolyse wurde eine Lösung von 0,1158 g der Lactonsäure in 2,35 ml 1 *N* NaOH 5 Stunden auf dem Wasserbad erwärmt, wobei 1,18 (ber. 1,17) ml der Lauge verbraucht wurden. Die in Äther aufgenommene Säure kristallisierte nach dem Abdampfen des Äthers gänzlich und schmolz bei 190—193° (Zers.). Mit der 5-Oxy-camphersäure der Borneolreihe gemischt: keine Depression des Schmelzpunktes.

Methylester der β-Lactonsäure (XXIV). Eine Lösung von 0,5007 g der Lactonsäure in 10 ml Aceton wurde unter Kühlung mit Eis allmählich mit einer 0,113 N Diazomethan-Ätherlösung versetzt, wobei die Reaktion nur langsam erfolgte. Die Lösung — insgesamt waren 33 ml (ber. 23 ml) Diazomethanlösung zugesetzt worden — wurde 5 Tage im Eisschrank stehen gelassen, wonach die sauren Bestandteile mit Sodalösung entfernt wurden. Aus der mit Natriumsulfat getrockneten Lösung wurden Äther und Aceton, zuletzt i. V., abdestilliert. Nach Verweilen i. Hochv. schmolz der kristalline Rückstand, 0.496 g, bei 97—99°. Aus Äther oder 75 %igem Methanol kurze, dicke, rhombische Prismen, Schmp. 101—102°. Zersetzt sich bei ca. 160°.

0,1894 Sbst. in 18,56 g Benzol (E = 5,12). Δ = 0,229°.

$C_{11}H_{16}O_4$	Ber.	C	62,23	H	7,60	Mol.-Gew.		212,1
	Gef.	»	62,18, 62,40	»	7,37, 7,49	»	»	228,1

Einwirkung von Acetylchlorid auf den α-Methylester der 5-Oxy-camphersäure: β-Lacton des 5-Oxy-camphersäure-α-methylesters (XXIV). 0,18 g des α-Methylesters wurden in 2 ml Acetylchlorid gelöst und die Lösung über Nacht stehen gelassen. Das Acetylchlorid wurde im Abzug verjagt, der Rückstand in wenig Methanol gelöst, die Lösung mit Natriumhydroxyd alkalisch gemacht (Phenolpht.), mit Wasser verdünnt und mit Äther extrahiert. Der Rückstand nach dem Abdestillieren des Äthers (0,06 g) wurde aus Äther umgelöst, Schmp. 100—101°. Nach Mischschmp. und Kristallform identisch mit dem aus der β-Lactonsäure mittels Diazomethans erhaltenen Ester.

Pyrogene Zersetzung der β-Lactonsäure: α-Campholytsäure (XXV). Die β-Lactonsäure wurde in einem schmalen Kolben im Paraffinölbad erhitzt. Die Zersetzung begann bei etwa 190°; zuletzt wurde die Temperatur bis 210° gesteigert. Durch den Kolben und weiter durch eine mit ihm verbundene, mit Bariumhydroxydlösung beschickte Vorlage wurde trockener Stickstoff geleitet und der grösste Teil des gebildeten Kohlendioxyds als Bariumcarbonat abgefangen. An dem oberen Teil des Kolbens setzte sich ein dickes Öl ab, das nach dem Kaltwerden bald kristallisierte. Die Substanz wurde mit Petroläther, worin sie sich leicht löst, von sublimierter β-Lactonsäure, und durch Lösen in Natriumhydrocarbonat von neutralen Beimengungen befreit. Aus Petroläther oder aus Wasser lange, dünne, blättrige Nadeln. Schmilzt bei 40,5°, welchen Schmelzpunkt auch Noyes und Nickell [26] für die racemische α-Campholytsäure angeben.

0,0404 g Sbst. verbrauchten 2,57 ml 0,1 *N* NaOH.

$C_9H_{14}O_2$ (154,1)	Ber.	C	70,08	H	9,16	Äquiv.-Gew.		154,1
	Gef.	»	70,11	»	9,12	»	»	157,2

Vergleichsweise wurde die α-Campholytsäure durch Elektrolyse des rac. Camphersäure-α-äthylesters nach Walker [27] dargestellt. Aus Wasser Nadeln, die nach dem Trocknen mit Petroläther gewaschen wurden. Schmp. 38—39°. Mit der aus der β-Lactonsäure erhaltenen Säure gemischt keine Depression des Schmelzpunktes.

Einwirkung von Chloralhydrat und Schwefelsäure auf die 5-Oxy-camphersäure: Chloralid der 5-Oxy-camphersäure (XXVII). 0,27 g 5-Oxy-camphersäure wurden mit 0,20 g (1,2 Mol) Chloralhydrat innig zusammengerieben und in 0,5 ml Schwefelsäure gelöst, wobei eine deutliche Wärmeentwicklung wahrzunehmen war. Nach 1,5 Stunden wurden 10 ml Wasser zugesetzt und die sofort ausgeschiedene, schwere, farblose Fällung nach dem Erkalten abgesaugt und mit Wasser gewaschen. Ausbeute 0,39 g (ber. 0,43 g), Schmp. 210—224° (Zers.). Aus Chloroform umgelöst kleine Nädelchen, Schmp. 227—229° (Zers.).

0,0690 g verbrauchten 0,200 ml 0,1 *N* NaOH.

$C_{12}H_{15}O_5Cl_3$	(345,5)	Ber.	C	41,68	H	4,36	Cl 30,80	Äquiv.-Gew.	345,5
		Gef.	»	41,60	»	4,22	» 30,85	» »	345,0

Das Chloralid löst sich leicht in Alkohol, ziemlich leicht in Äther und Chloroform, sehr schwer in Petroläther, Ligroin und Wasser.

Bei der Hydrolyse des Chloralids mit kalter, 0,1 *N* alkoholischer Natronlauge, wobei 3 Mole Natriumhydroxyd verbraucht wurden, entstanden Chloroform, Ameisensäure und die 5-Oxy-camphersäure der Borneolreihe (Kristallform, Mischschmp.).

Einwirkung von Acetyl- bzw. Thionylchlorid auf den β-Methylester: γ-Lacton des 5-Oxy-camphersäure-β-methylesters (XXVIII)

Bei verschiedenen Versuchen wurde der β-Methylester in eine 5 bis 10fache Menge Acetyl- bzw. Thionylchlorid gelöst, wobei eine deutliche Gasentwicklung wahrzunehmen war. Die Lösungen wurden entweder 1 bis 2 Tage stehen gelassen oder 1 bis 2 Stunden auf dem Wasserbad gekocht, wonach das Acetyl- bzw. Thionylchlorid im Luftstrom verjagt oder i. V. abdestilliert wurde. Der gewöhnlich kristalline Rückstand wurde am besten, zur Entfernung der zum Teil sehr schwach sauren Reaktionsprodukte (polymere Estersäuren), in wenig Methanol gelöst; die Lösung wurde mit Natriumhydroxyd schwach alkalisch gemacht (Phenolpht.) und mit Wasser verdünnt, wonach das Esterlacton ausgeäthert wurde. — Auch durch Sublimation i. V. kann man es in ziemlich reinem Zustande von den schwerflüchtigen höher molekularen Produkten befreien.

Die Ausbeute an Esterlacton schwankte in obigen Versuchen zwischen 25 und 50 % d. Th. Verwendung von indifferenten Verdünnungsmitteln (Äther, Chloroform) bei der Reaktion verringerte die Ausbeute. Durch Chlorwasserstoff in Benzollösung oder Schwefelsäure in Ätherlösung wurde die Bildung des Esterlactons nicht bewirkt.

Das Esterlacton löst sich sehr leicht in Benzol, Essigester, Dioxan, Methyl- und Äthylalkohol, leicht in Äther, sehr schwer in Petroläther. Kristallisiert aus Äther in langgestreckten rhombischen Blättchen und Prismen, aus methanolhaltigem Wasser (sehr langsam) in schönen, wohlgebildeten Prismen und Blättern von briefkuvertähnlichem aber schiefwinkligem Habitus. Schmp. 111°.

0,1016 g Sbst. in 21,31 g Benzol ($E = 5{,}12$). $\Delta = 0{,}121°$.

$C_{11}H_{16}O_4$	Ber.	C	62,23	H	7,60	Mol.-Gew.	212,1
	Gef.	»	62,43, 62,53	»	7,61, 7,60	» »	202,0

Durch alkalische Verseifung in wässrig-methanolischer, verdünnter, in Bezug auf Esterlacton und Natriumhydroxyd nahezu äquimolarer Lösung wurde aus dem Ester-

lacton der β-Methylester der 5-Oxy-camphersäure der Borneolreihe zurückgebildet (Kristallform, Mischschmp.). Dasselbe Produkt wurde durch einstündiges Erwärmen des Esterlactons mit 20 %iger Salzsäure auf dem Wasserbad gewonnen.

Einwirkung von Acetylchlorid auf den β-Methylester in Gegenwart von Kaliumacetat oder Pyridin: 5-Acetoxy-camphersäure-β-methylester (XXX). I. Zu einer Lösung von 100 mg β-Methylester und 250 mg Kaliumacetat in 1 ml Eisessig wurden 2 ml Acetylchlorid unter Umschwenken zugetropft; sofortige Abscheidung von Kaliumchlorid. Nach 2 Tagen wurde die Lösung i. V. unter gelindem Erwärmen zur Trockne gebracht. Nach Zusatz von etwas Wasser und Methanol kristallisierten aus der Lösung allmählich schöne lange Nadeln, 51 mg, später noch 27 mg.

II. 100 mg β-Methylester wurden in 160 mg Pyridin gelöst und unter Umschwenken mit 2 ml Acetylchlorid versetzt, wobei eine dicke Fällung gebildet wurde. Nach 3 Tagen wurde das Acetylchlorid im Abzug verjagt, wobei die Fällung allmählich verschwand. Weitere Behandlung i. V. usw. wie oben; zuletzt feine Nadeln, 64 mg. Nach Kristallform und Mischschmp. identisch mit der Verbindung aus Darst. I.

Schmp. nach Umlösen aus methanolhaltigem Wasser 110°.

0,0484 g Sbst. verbrauchten 1,74 ml 0,1 *N* NaOH.

$C_{13}H_{20}O_6$ (272,2)	Ber.	C	57,30	H	7,41	Äquiv.-Gew.	272,2
	Gef.	»	57,08	»	7,47	» »	278,2

Durch 5stündiges Erwärmen mit etwas mehr als der berechneten Menge 0,1 *N* Natriumhydroxydlösung wurde die Verbindung zum 5-Oxy-camphersäure-β-methylester der Borneolreihe hydrolysiert (Kristallform, Mischschmp.).

B. Isoborneolreihe

Die in der vorläufigen Mitteilung [19] beschriebenen Versuche seien hier nur an einigen Punkten ergänzt werden.

Keto-isobornylacetat, wohl ein Gemisch der 5- und 6-Ketoverbindungen (XV und XVI). — Zur Gewinnung von Isoborneol wurde das aus reinem *r*-Campher durch Natrium-Alkohol-Reduktion erhaltene Borneol-Gemisch mit Kaliumbisulfat [28] im Graphitbad zu Camphen dehydratisiert, das letztere sorgfältig gereinigt und unter Zusatz einiger Tropfen Schwefelsäure in Isobornyloxalat [29] (Schmp. nach mehrfachem Umkristallisieren aus Alkohol 113—114°) übergeführt. Das daraus durch Hydrolyse mit alkoholischer Kalilauge erhaltene Isoborneol (Schmp. 215—216°) wurde mit Acetanhydrid unter Zusatz von etwas Pyridin in Isobornylacetat umgesetzt.

Der grösste Teil des zur Chromsäure-Oxydation benutzten Isobornylacetats wurde jedoch durch Hydratation des auf obengenannte Weise erhaltenen Camphens nach der Bertram-Walbaumschen Methode dargestellt. — Das erhaltene Gemisch der Keto-isobornylacetate war immer flüssig.

5,6-Diketo-isobornylacetat (XVII), Schmp. 103—104°. Bezüglich dieser Verbindung sei nur auf die frühere Beschreibung [19] hingewiesen.

5-Acetoxy-camphersäure-anhydrid (XVIII), Schmp. 115—116°, meistens dicke, rhombische Blätter. Aus der vorigen Verbindung mittels Wasserstoffperoxyd in Eisessiglösung.

5-Oxy-camphersäure (XIX) der Isoborneolreihe und Derivate

Die Darstellung dieser Säure durch Hydrolyse der vorigen Verbindung ist schon früher [19] beschrieben worden. Sie kristallisiert aus essigsäurehaltigem Wasser in schönen Prismen, Schmp. 207—208° (Zers.). Nach Misch-Schmp. und Kristallform identisch mit der synthetisch [18] dargestellten höher schmelzenden 5-Oxy-*cis*-camphersäure.

Auch die aus diesen Oxycamphersäuren mit Acetylchlorid dargestellten Acetoxyanhydride erwiesen sich nach Kristallform und Misch-Schmelzpunkt miteinander und mit dem obengenannten Acetoxy-anhydrid identisch.

Dimethylester (XX) wurde wie der entsprechende Ester der Borneolreihe (S. 1002) dargestellt. Aus 12 g Oxysäure wurden so 11,2 g Ester gewonnen. Ziemlich dünne Flüssigkeit mit schwachem, angenehmem Geruch. Siedep. 158—160°/6 mm.

$C_{12}H_{20}O_5$ (244,2)	Ber.	C	58,99	H	8,26
	Gef.	»	58,64	»	8,11

Derselbe Ester wurde auch durch Behandlung der Oxysäure mit Diazomethan erhalten.

Das *Acetoxyderivat des Dimethylesters* wurde wie die entsprechende Verbindung der Borneolreihe (S. 1002) dargestellt und i. Hochv. zur Gewichtskonstanz gebracht. Aus 0,517 g Ester 0,593 g (ber. 0,592 g) Acetoxyverbindung. Ziemlich dünne, geruchlose Flüssigkeit.

$C_{14}H_{22}O_6$ (286,2)	Ber.	C	58,70	H	7,75
	Gef.	»	58,53	»	7,95

β-Methylester (XXI). 3 g (1 Mol) Dimethylester wurden mit 10 ml 2 *N* Natronlauge (1,63 Mol NaOH) eifrig geschüttelt; zur Erleichterung der Reaktion waren 0,5 ml Methanol zugesetzt worden. Nach 1 Stunde war beinahe alles gelöst und von dem Alkali 1,06 Mol verbraucht worden (Titrierung mit Salzsäure). Die sodaalkalisch gemachte Lösung wurde mit Äther gewaschen, der gelöste Äther entfernt und der saure Ester mit Salzsäure fraktioniert ausgefällt. Die sehr einheitlichen Kristalle der ersten Fraktion, 1,953 g, schmolzen bei 133°, die folgenden nur ein wenig niedriger.

Aus Wasser schöne rhombische Blättchen, Schmp. wie oben.

0,0418 g Sbst. verbrauchten 1,807 ml 0,1 *N* NaOH.

$C_{11}H_{18}O_5$ (230,1)	Ber.	C	57,35	H	7,88	Äquiv.-Gew.	230,1
	Gef.	»	57,49	»	7,92	» »	231,3

Einwirkung von Acetanhydrid auf die Oxysäure: 5-Oxy-camphersäure-anhydrid (XXVI). 1,0 g Oxysäure wurden mit 14 ml Acetanhydrid versetzt. Nach Zusatz von 2 ml Äther war alles nach 3 Stunden in Lösung gegangen. Nach 36 Stunden wurde das Acetanhydrid i. V. abdestilliert, der Rückstand in Äther gelöst und mit Natriumhydrocarbonatlösung gewaschen. Aus der letzteren wurde nur eine Spur einer sauren Substanz gewonnen, die nicht näher untersucht wurde. Das neutrale Hauptprodukt (0,83 g, Schmp. 203—205°) wurde aus Benzol umkristallisiert. Dicke Prismen mit sechsseitigem Umriss, zersetzen sich bei 204° unter Bildung einer klaren Schmelze. Geschmack brennend, wie auch bei den 5-Acetoxy-camphersäure-anhydriden sowohl der Borneol- als besonders der Isoborneolreihe.

$C_{10}H_{14}O_4$ (198,1)	Ber.	C	60,59	H	7,12
	Gef.	»	60,60	»	7,26

Ein Teil der Substanz wurde in überschüssigem Acetylchlorid gelöst. Nach mehrtägiger Einwirkung wurde das Acetylchlorid abdunsten gelassen, wobei das Reaktionsprodukt in schönen Prismen kristallisierte. Schmp. 114—115°. Mischschmp. mit dem 5-Acetoxy-camphersäure-anhydrid der Isoborneolreihe: keine Depression des Schmelzpunktes.

Einwirkung von Chloralhydrat und Schwefelsäure auf die Oxysäure. 1 g Oxysäure wurde mit 0,9 g Chloralhydrat innig zusammengerieben und mit 2 ml konc. Schwefelsäure vermischt. Die Kristalle lösten sich nur sehr langsam und es konnte keine Wärme-Entwicklung beobachtet werden. Nach 2 Stunden wurden 40 ml Wasser zugesetzt. Es entstand eine trübe Lösung, aus der auch nach längerer Zeit keine definierbare Substanz abgeschieden werden konnte; eine solche war auch nicht durch Extrahieren mit Äther zu gewinnen.

Einwirkung von Acetylchlorid auf den β-Methylester: 5-Acetoxy-camphersäure-β-methylester (XXX). 0,501 g des β-Methylesters wurden mit 5 ml Acetylchlorid versetzt, wobei die Kristalle sich nur allmählich unter schwacher Gasentwicklung lösten. Nach 2 Tagen wurde das Acetylchlorid abdunsten gelassen. Der kristalline Rückstand wurde i. V. getrocknet (Gewicht 0,592 g) und mit Natriumhydrocarbonatlösung geknetet, wobei der grösste Teil in Lösung ging. Die beim Ansäuern der Lösung ausgeschiedenen Kristalle wurden aus methanolhaltigem Wasser umkristallisiert: dünne Blätter vom Schmp. 152—153°.

0,2374 g Sbst. verbrauchten 0,880 ml 1 *N* NaOH.

$C_{13}H_{20}O_6$ (272,2)	Ber.	C	57,30	H	7,41	Äquiv.-Gew.	272,2
	Gef.	»	57,55	»	7,50	» »	269,7

Die titrierte Lösung wurde mit weiteren 1,100 ml 1 *N* Natriumhydroxydlösung versetzt und einige Stunden auf dem Wasserbad erwärmt. Der gesamte Verbrauch an Lauge 1,730 ml, ber. für 2 Mol Natriumhydroxyd 1,744 ml. Aus der Lösung wurde der ursprüngliche 5-Oxy-camphersäure-β-methylester der Isoborneolreihe ausgeschieden und identifiziert.

Der in Natriumhydrocarbonatlösung ungelöste Teil des Reaktionsproduktes (vgl. oben) wog nach dem Waschen mit Wasser und Trocknen i. V. 0,08 g und stellte eine weiche, schmierige Masse dar. In wenig methanolhaltigem Wasser suspendiert und mit einigen Tropfen Natriumhydroxydlösung versetzt löste sich die Substanz vollkommen und zeigte übrigens alle Eigenschaften einer schwachen höhermolekularen Estersäure.

Einwirkung von Thionylchlorid auf den β-Methylester: Polymere Estersäuren. 0,519 g des obigen β-Methylesters wurden ganz wie der entsprechende Ester der Borneolreihe mit Thionylchlorid, in das er sich unter Gasentwicklung löste, behandelt. Das i. V. getrocknete mikrokristalline Reaktionsprodukt, 0,529 g, das in Natriumcarbonat- und Natriumhydroxydlösung sehr schwer löslich war — die Natriumsalze z. B. des polymeren δ-Valerolactons sind nach Carothers und Mitarb. [30] in Wasser unlöslich, lösen sich aber in Aceton — wurde in wenig Aceton gelöst, mit Natriumhydroxydlösung schwach alkalisch (Phenolpht.) gemacht (Verbrauch der 2 *N* Lauge 0,72 ml) und unter weiterem Zusatz von etwas Aceton mit Wasser verdünnt. Aus dieser Lösung konnten durch Aus-

schütteln mit Äther nur 7 mg einer schmierigen, unkristallisierbaren Substanz extrahiert werden.

Die durch Ansäuern der alkalischen Lösung ausgefällte saure Substanz löst sich leicht in Aceton, Alkohol, Äther und Benzol. Beim Eindunsten dieser Lösungen auf dem Uhrglas werden verschiedenfarbige Filme gebildet. Schmelzintervall der mikrokristallinen Substanz etwa 120—160°. Frei von Halogen und Schwefel.

Nach der Titrierung (Äquiv.-Gew. ca. 835) und Verbrennung (59,8 % C, 7,8 % H) ist diese Substanz durch intermolekulare Veresterung von hauptsächlich 2 bis 3 Molekülen, nach den allgemeinen Eigenschaften auch von mehreren Molekülen des β-Methylesters gebildet worden. — Durch langsam erfolgende Hydrolyse wird aus ihr die 5-Oxy-camphersäure der Isoborneolreihe zurückgebildet (Kristallform, Mischschmp.).

ZUSAMMENFASSUNG

Es wird ein Verfahren beschrieben, das sich ziemlich allgemein zur Bestimmung der *endo-exo*-Konfiguration solcher Verbindungen vom Campher-typus benutzen lassen dürfte, wo der in Frage stehende Substituent eine reaktionsfähige Atomgruppe wie Hydroxyl, Halogen, Carboxyl, Aminogruppe o. dgl. darstellt. Nach diesem Verfahren sind jetzt die schon früher viel erforschten Konfigurationen des Borneols und Isoborneols ermittelt worden. Zu diesem Zweck wurden die genannten Verbindungen unter Erhaltung der gegenseitigen räumlichen Anordnung der Hydroxylgruppe und der Kohlenstoffatome je zu einer 5-Oxy-camphersäure oxydiert. Aus der experimentell ermittelten Fähigkeit bzw. Unfähigkeit zu zweckdienlichen Ringschlussreaktionen ergibt sich für die aus Borneol gebildete Säure die *cis*-, für die aus Isoborneol gebildete Säure die *trans*-Stellung des Hydroxyls zu den Carboxylen und, infolgedessen, für Borneol die *endo*-, für Isoborneol die *exo*-Konfiguration.

Weil die obengenannten Oxy-camphersäuren sich als identisch mit den früher durch eine Totalsynthese erhaltenen 5-Oxy-camphersäuren erwiesen und ihre Struktur auf Grund dieser Synthese feststeht, ist dadurch auch die Konstitution des Borneols und Isoborneols nochmals bewiesen worden.

Dem *Kemian Keskusliitto* sei für ein Stipendium der wärmste Dank ausgesprochen.

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Eingegangen am 16. August 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1011–1020

Substituted Benzyl Alcohols as Lignin Models. II *

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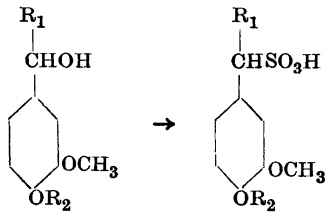
In the investigations of the nature of the reactive groups in lignin responsible for its sulphonation, different model substances play a great rôle. Holmberg [1] showed, in an important paper, that α-phenethyl alcohol reacts with bisulphite under the conditions of the technical sulphite cook: $C_6H_5 \cdot CH(OH) \cdot CH_3 \rightarrow C_6H_5 \cdot CH(SO_3H) \cdot CH_3$. This alcohol resembles lignin not only as regards sulphonation but also in other respects, *e. g.* reaction with thioglycolic acid [2] and with ethanolic hydrochloric acid [3]. For that reason Holmberg considered that a hydroxyl group, activated by a benzene ring in α-position, is of great importance, not only in the reactions of lignin mentioned above, but also in the condensation reactions of lignin. Wacek and Kratzl [4a], however, pointed out that the yield of sulphonic acid from α-phenylpropyl alcohol is low, and therefore consider these alcohols unsuitable as lignin models.

Setting out from Freudenberg's theory of the sulphonation of lignin, Richtzenhain [5] examined the reactions of different benzyl ethers with sulphite cooking acid. The formation of sulphonic acids was far too slow to correspond to the sulphonation of lignin. Investigations, made not only on the benzyl ethers but also on substituted chalkones [4b], however, showed that substituents in the benzene ring have a great influence on the yield of sulphonic acids.

Results to be published by Erdtman, Lindgren and Pettersson [6] show that the sulphonation of 'low sulphonated lignin sulphonic acids' is due to a substitution of hydroxyl groups of outstanding reactivity. For these reasons a series of alcohols which appear to be more lignin-like than α-phenethyl and α-phenylpropyl alcohols have been subjected to sulphite cooking. Vanillyl alcohol (I), veratryl alcohol (II), apocynol (III), and 3,4-dimethoxy-α-phenethyl alcohol (IV) were heated with normal sulphite cooking acids and were found

* Part I, preliminary communication. *Acta Chem. Scand.* 1 (1947) 779.

to react rapidly and quantitatively (compare Table 1) to yield the corresponding sulphonic acids (V—VIII). (A similar reaction is used for making cation exchange resins [7].)



I	$R_1 = H$	$R_2 = H$		V	$R_1 = H$	$R_2 = H$
II	$R_1 = H$	$R_2 = CH_3$		VI	$R_1 = H$	$R_2 = CH_3$
III	$R_1 = CH_3$	$R_2 = H$		VII	$R_1 = CH_3$	$R_2 = H$
IV	$R_1 = CH_3$	$R_2 = CH_3$		VIII	$R_1 = CH_3$	$R_2 = CH_3$

The model substances studied by other researchers are subject to two fundamental objections. They either react far too slowly with bisulphite or they contain groups (carbonyl groups or C = C-groups) which, obviously, do not occur in lignin, at least not to such a degree that they can be responsible for the sulphonation reaction. The alcohols (I—IV), however, are not subject to these objections.

It has been assumed that lignin contains two different, sulphitable groups [8,9]. The one group should be characterized by greater reactivity, such as easier sulphitability, greater condensation power etc. The great reactivity of the alcoholic hydroxyl group in vanillyl alcohol decreases considerably if the phenol group is methylated. Therefore, it has been examined by model tests whether these two groups in lignin could be benzyl alcohol groupings, in the one case activated by a free phenolic hydroxyl group in para position (model vanillyl alcohol), and in the other case by an alkyl ether group in the same position (model veratryl alcohol).

THE RELATION BETWEEN SULPHONATION AND CONDENSATION

Lignin shows a characteristic relation between sulphonation and condensation. It was therefore examined whether it was possible to imitate this relation with the model alcohols. Corey and Maas [10] showed that if dry wood powder is heated in nitrogen, or in toluene, at 130° for six hours, the wood can be delignified as easily as unheated wood by sulphite cooking. If the heating is accomplished in water, however, it is considerably more difficult to dissolve the lignin by sulphite cooking. In fact, the wood treated in this way had to be heated with sulphite cooking acid twice as long as untreated wood to

obtain the same degree of delignification. This may be explained in the following way: there are two sulphitable groups in lignin, group α and group β. When heated in water only group α condenses. None of the groups condense if wood is heated dry or in toluene.

Vanillyl alcohol behaves in the same way as group α, and veratryl alcohol like group β. Vanillyl alcohol did not polymerize when heated in a toluene solution or in air, and the alcohol was easily recovered in the form of crystals. In water vanillyl alcohol polymerized to a product which did not dissolve when heated with sulphite cooking acid. Veratryl alcohol did not polymerize even when heated with water.

HEATING OF LIGNIN AND MODEL SUBSTANCES WITH SULPHITE SOLUTIONS OF DIFFERENT pH

Hägglund and Johnson[9] have investigated the sulphonation of wood using sulphite solutions of different acidities. It appeared that lignin is easily sulphonated by a sulphite solution of pH about 5 to a lignin sulphonic acid

Table 1. The yield of suuphonic acid at various cooking times and various acidities. The temperature was always 135°.

	5 % SO_2					
	0.7 % NaOH pH = 1.5		4.5 % NaOH pH = 6.4		8.7 % NaOH pH = 11–12	
	Time hour	Yield %	Time hour	Yield %	Time hour	Yield %
Vanillyl alcohol	1/2	100	1/2 1	100 100	1/2 1	95 95 *
Veratryl alcohol	1/2 1 1/6	80 95	1 16 1/2	45 70	1 1/2 26	25 50
3,4-Dimethoxy-phenethyl alcohol	1/2 1	95 100	15	70	2 1/4 24	20 30

* 0.5 g of pyridinium salt of V isolated (65 % yield).

containing on the average one sulphur atom per three to four methoxyl groups. Further sulphonation is very difficult to bring about. That may also be explained by considering two sulphitable groups in lignin, only one of which can be easily sulphonated by sulphite solutions of a high pH. Therefore it was of interest to examine the sulphonation of the model alcohols with sulphite solutions of different acidities, (see Table 1).

From this table it can be seen that the rate of sulphonation of vanillyl alcohol was high even with sulphite solutions of high pH, but that the rate of sulphonation of veratryl alcohol and 3,4-dimethoxy-α-phenethyl alcohol was low at a high pH. (By cooking with a sulphur dioxide solution containing no sodium hydroxide, non-reproducible yields of sulphonic acid and polymerized alcohol were obtained.)

HEATING OF LIGNIN AND MODEL SUBSTANCES WITH SULPHITE SOLUTIONS IN THE PRESENCE OF A REACTIVE PHENOL

If wood is heated with technical sulphite cooking acid (pH = 1.5—2) in the presence of a reactive phenol (*e. g.* resorcinol or pinosylvin) the lignin reacts with the phenol to form an insoluble product [8, 11]. If, however, lignin is heated with sodium bisulphite solution (pH = 4.5) in the presence of resorcinol, it is sulphonated to a certain degree and, afterwards, it can be dissolved by an ordinary sulphite cooking procedure (Graham's method). These observations were explained by assuming that there are two sulphitable groups in the lignin [8]: group A and group B, which have the following properties:

Group A.
1) Is sulphonated easily with sulphite solution at pH 1.5—2 and at pH 4.5.
2) Reacts more quickly with phenols than with bisulphite at pH 1.5—2.
3) Reacts more quickly with bisulphite than with phenols at pH 4.5.

Group B.
1) Is sulphonated easily with sulphite solution at pH 1.5—2.
2) Does not react at all or slowly with sulphite solution at pH 4.5.
3) Reacts more quickly with bisulphite than with phenols at pH 1.5—2.
4) Does not react at all or slowly with phenols at pH 4.5.

In model tests it appeared as if the vanillyl alcohol behaved like group A. By heating with a normal sulphite cooking acid (pH = 1.5—2) in the presence of resorcinol, an insoluble condensation product and a small amount of sulphonic

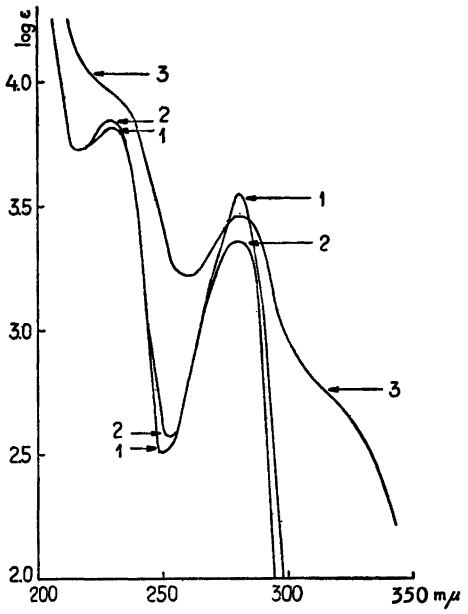


Fig. 1. Ultra-violet spectra.

1. *Vanillyl alcohol (in ethyl alcohol).*
2. *Guaiacyl-methane sulphonic acid (in water).*
3. *Ligninsulphonic acid (in water)*[12].

acid were obtained. When heated with sodium bisulphite solution (pH = 4.5), however, the vanillyl alcohol only yielded sulphonic acid.

On the other hand, veratryl alcohol did not behave like group B, as it condensed with resorcinol in normal sulphite cooking acid.

SAFROLE GLYCOL

It is necessary that the hydroxyl group be in the α-position to the benzene ring for reaction with bisulphite. When safrole glycol (3,4-methylenedioxy-1^2, 1^3-dihydroxy-1-propyl-benzene) was heated with sulphite cooking acid it was recovered unchanged. The alcohol was recovered unchanged even after heating with ethanolic hydrochloric acid under such conditions when α-phenethyl alcohol reacts to form ethyl α-phenethyl ether. Thus in both these respects safrole glycol is not a suitable lignin model.

The ultra-violet spectra of vanillyl alcohol and guaiacyl-methane sulphonic acid (V) have been measured (Fig. 1). Like the spectrum of lignin, they show maxima at 230 and at 280 mμ but, they show no maximum at 320 mμ.

During the course of these experiments it was observed that when sulphur dioxide was led into a water solution of any of the alcohols (I—IV) the solution turned yellow. The colour appeared even in ether or benzene solutions. The colour is evidently due to the formation of an addition product of sulphur dioxide and the alcohol. This addition product is very labile. When the sulphur dioxide was removed by leading nitrogen through the solution, the colour rapidly disappeared. Obviously this addition product has nothing to do with the sulphonation reaction, since the sulphonic acids (V—VIII) as well as even simple phenols produced this colour.

EXPERIMENTAL

Sulphite cooking of benzyl alcohols (I—IV)

1 g of alcohol (I—IV) was heated with 60 ml of sulphite cooking acid (1 % calcium oxide, 6—8 % total sulphur dioxide) for three hours at 135°. The alcohols were thereby completely dissolved. In order to remove free sulphur dioxide, nitrogen was passed through the solution until the smell of sulphur dioxide disappeared. The solutions were extracted with ether, and the extracts dried over sodium sulphate and evaporated. No residues were obtained. The solutions were passed through a column of cation exchange resin (Wofatit K) in the hydrogen state in order to exchange the cations for hydrogen ions. The acid solutions were neutralized by suspending barium carbonate in the solution, centrifuged, and evaporated to dryness. The crude barium salts of the sulphonic acids (V—VIII) crystallized when the residues were treated with ether or acetone. The barium salts were purified by

1) dissolving the products in aqueous methanol, filtering and precipitating the barium salts with ether (V, VI, VII)

2) re-crystallizing the product from 98 % alcohol (VIII).

The yield of crude products was quantitative.

Sulphonic acids (V—VIII) were characterized as pyridinium salts. (The benzylthiouronium salts were too soluble in water and therefore unsuitable.) The barium sulphonates were dissolved in water, and the barium ions were exchanged for hydrogen ions by means

Pyridinium salts of	Melting point	Sulphur, %	
		Calc.	Found
Guaiacyl-methane sulphonic acid (V)	189—190°	10.8	10.6
Veratryl-methane sulphonic acid (VI)	149°	10.3	9.9
α-Guaiacyl-ethane sulphonic acid (VII)	164—164.5°	10.3	10.3
α-Veratryl-ethane sulphonic acid (VIII)	148°	9.8	9.9

of cation exchange resin. The solutions were neutralized with pyridine and evaporated in a vacuum to dryness. The pyridinium salts obtained were purified by

1) re-crystallizing from methanol (V)
2) re-crystallizing from pyridine (VI)
3) re-precipitating the methanol solution with ether (VII, VIII).

Heating of vanillyl alcohol in air, toluene and water

Vanillyl alcohol was heated in a drying oven for six hours at 130°. The alcohol melted to a brown oil. This oil was dissolved in hot benzene. On cooling the solution, vanillyl alcohol crystallized.

1 g of vanillyl alcohol was heated for six hours at 130° in a sealed tube with, in one case, 50 ml of toluene, and in the other, with 50 ml of water. In both cases the alcohol dissolved in the hot liquid. After two hours, polymerized vanillyl alcohol began to precipitate from the water solution. After six hours all vanillyl alcohol was precipitated. The polymerized vanillyl alcohol could not be dissolved by heating with sulphite cooking acid at 130° for three hours.

In toluene solution, however, the vanillyl alcohol was still unchanged even after six hours. On cooling the toluene solution, the vanillyl alcohol crystallized.

Veratryl phenylcarbamate

1 g of veratryl alcohol was mixed with 1 g of phenyl isocyanate and the mixture was heated for ten minutes on a steam bath. The phenylcarbamate was re-crystallized from benzene. M. p. 118°.

$C_{14}H_{11}O_2N(OCH_3)_2$	Calc.	N	4.9	OCH_3	21.6
	Found	»	4.7	»	21.5

Heating of veratryl alcohol in toluene and water

1 g of veratryl alcohol was heated with 20 ml of distilled water for six hours at 130°. 40 ml of sulphite cooking acid (1.1 % sodium hydroxide and 8.5 % sulphur dioxide) were added, and the solution was heated for five hours at 135°. The solution was extracted with ether; the extract contained 0.1 g of an oil.

The water solution was evaporated to dryness and the residue was extracted with hot alcohol. The alcohol extract contained 1.32 g of impure sodium salt of veratryl-methane sulphonic acid (OCH_3: found 21.6 %, calc. 24.4 %). The product contained 78 % of the methoxyl groups of the veratryl alcohol employed in the experiment. It was recrystallized from alcohol. The product now contained 24.4 % OCH_3.

0.5 g of veratryl alcohol was heated with 20 ml of toluene for 18 hours at 130°. 0.4 g of phenyl isocyanate was added and the carbamate was synthesized in the manner mentioned above. 0.6 g of a product m. p. 115.5° was obtained. After re-crystallization from benzene m. p. 118°. Mixed m. p. with veratryl phenylcarbamate 118°.

Heating of vanillyl alcohol with sulphite cooking acid and resorcinol

1 g of vanillyl alcohol was mixed in a tube with 0.71 g of resorcinol (1 mole resorcinol per mole vanillyl alcohol) and 50 ml of sulphite cooking acid (6.6 % sulphur dioxide, 1 % calcium oxide). The mixture was heated slowly to 100° (two hours) in a drying oven. Thereafter the tube was heated further for three quarters of an hour at 130° (this temperature schedule corresponds to that used by Erdtman [8] his studies of the delignification of wood in presence of phenols). After being heated, the tube contained a clear yellow solution and a thick brown oil. On cooling, the solution became cloudy. Nitrogen was passed through the solution in order to remove most of the free sulphur dioxide. The solution was shaken repeatedly with ether. The brown oil which was formed during cooking was also dissolved in the ether. The extract was dried over sodium sulphate and evaporated. 1.6 g of an oil were obtained as a residue.

The cations of the aqueous solution were exchanged for hydrogen ions whereafter the solution was neutralized with barium hydroxide solution. The cloudy (barium sulphate) solution was evaporated in a vacuum to dryness. The residue was extracted with water, the water extract was centrifuged and evaporated to dryness on a water bath. Residue (impure barium salt of guaiacyl-methane sulphonic acid) 0.2 g (yield 12 %).

Heating of vanillyl alcohol with sodium bisulphite solution and resorcinol

1 g of vanillyl alcohol, 0.85 g of resorcinol and 50 ml of 15 % sodium bisulphite solution were heated slowly to 90° (two hours). The temperature was then rapidly increased to 130°, and the tube heated for an hour at that temperature. During the heating the vanillyl alcohol dissolved completely in the bisulphite solution. The solution was shaken with ether, and 0.74 g of resorcinol was obtained from the ether extract.

The sodium ions of the water solution were exchanged for hydrogen ions, nitrogen was passed through the solution until the smell of sulphur dioxide disappeared. The solution was evaporated in a vacuum, neutralized with barium hydroxide and filtered. Barium ions were exchanged for hydrogen ions and the solution was mixed with a few ml of pyridine and evaporated in a vacuum to dryness. The residue was a crystalline substance, which was dissolved in water, filtered, and evaporated on a water bath. The residue consisted of 1.1 g of crystals; m. p. 188°; undepressed by the pyridinium salt of guaiacylmethane sulphonic acid.

Heating of veratryl alcohol with sulphite cooking acid and resorcinol

1 g of veratryl alcohol and 2 g of resorcinol were dissolved in 60 ml of sulphite cooking acid (1 % calcium oxide, 5.1 % sulphur dioxide). The temperature of the solution was increased to 100° during two hours. The temperature was then increased to 120° during half an hour and to 130° during another 1 1/2 hours. During the heating an insoluble oil was formed. On cooling, it yielded crystals contaminated with oily impurities (0.79 g total). Carbon dioxide was passed into the filtrate and a white crystalline substance (0.50 g) was obtained. The latter was re-crystallized from benzene, and 0.1 g of a product was obtained, m. p. 153°. This product contained 25.5 % OCH_3. The compound formed from

one molecule of resorcinol and one molecule of veratryl alcohol by loss of one molecule of water would contain 23.8 % OCH_3. The material was obviously an impure condensation product of these substances.

Heating of vanillyl alcohol, veratryl alcohol, and 3,4-dimethoxy-α-phenethyl alcohol with sulphite solutions of different pH (Table 1)

Mixtures of 0.5 g of veratryl, vanillyl, or 3,4-dimethoxy-α-phenethyl alcohol and 40 ml of a sulphite solution containing 0, 0.7, 4.5, or 8.7 % sodium hydroxide and 5 % total sulphur dioxide; (pH at room temperature 1.1, 1.5, 6.4, or 11—12 respectively) were heated in steel tubes by immersing the tubes in a glycerol bath at 135° for five minutes. The temperature of the sulphite solutions was raised to 135° in about three minutes. The tubes were then brought to rotate in a drying oven for different lengths of time at 135°.

After being heated, the sulphite solutions were extracted three times with ether. The ether extracts were washed with water and evaporated to dryness. The residues were considered to be unsulphonated alcohols. The yields of sulphonic acids were calculated from the weight of the residues.

By cooking with sulphite cooking acids containing no base (pH = 1.1) the alcohols were not recovered. Instead, amorphous condensates of the alcohols were obtained.

Experiments with safrole glycol

1 g of the alcohol was heated with 25 ml of sulphite cooking acid (1 % calcium oxide, 7 % sulphur dioxide) for five hours at 118° and thereafter for ten hours at 135°. After being heated, the cooking acid contained an oil from which crystals of safrole glycol (0.93 g) were obtained.

A solution of safrole glycol in ethanolic hydrochloric acid (2 % HCl) was refluxed for two and a half hours. The solution was poured into water when crystals of safrole glycol were obtained.

SUMMARY

Vanillyl alcohol, veratryl alcohol, apocynol, and 3,4-dimethoxy-α-phenethyl alcohol have been studied as lignin models in the following respects:

a) sulphite cooking at different pH,

b) sulphite cooking in the presence of resorcinol,

c) polymerization reactions.

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Received June 17, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1021–1026

The Reaction between Peroxides and Leucomalachite Green Catalyzed by Heme in the Presence of Organic Solvents

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In a previous paper[1] it was reported that peroxides of fats and fatty acids, in the presence of heme, react with easily oxidizable substances. The reaction was found to be very much accelerated when carried out in an organic solvent. In the quantitative study of the reaction, and for comparison with the corresponding reaction involving hydrogen peroxide instead of organic peroxides, leucomalachite green was used as the easily oxidizable compound. This substance, as well as the malachite green formed in the reaction, are fairly stable compounds, and the colour of malachite green is not much influenced by the pH.

EXPERIMENTS

The reaction between hydrogen peroxide and leucomalachite green has been studied before by other investigators, for instance, Willstätter and Weber[2]. The leucomalachite green used was purified by repeated recrystallisations, as described by these authors. The substance is easily soluble in organic solvents. Aqueous solutions were prepared by saturating 0.05 *N* acetic acid with the substance. With impure products, the solutions were unstable, and the yield of malachite green was not quantitative.

Solutions of hydrogen peroxide were tested quantitatively by titration with potassium permanganate, and fresh dilutions, with redistilled water, were made up before each experiment. Various oils and fatty acids were used as organic peroxides, the peroxide values of which were determined by the method of King *et al.*[3], and by the authors' method[4].

* This work was aided by a grant from *Laurits Andersens Fond.*

In most of the experiments, a solution prepared by dissolving 20 mg hemin * in a mixture of 5 ml pyridine and 10 ml glacial acetic acid was used as a source of heme. All solvents should be rigorously pure, as reported in a previous paper [1].

Most of the experiments were carried out by adding 0.8 ml of a dilution of peroxide to a mixture of 4 ml of the leucomalachite green solution and 0.2 ml heme solution in a test tube. The intensity of the colour was read in the Beckman photometer at 620 mμ or in the Klett-Summerson colorimeter using filter 610. Extinction curves were constructed for malachite green. The results were calculated in terms of milliequivalents of peroxide per kg. One equivalent of malachite green corresponds to 2 moles of peroxide. One milliequivalent per liter of the reaction mixture corresponds to an extinction of 25 in a 1 cm layer.

With hydrogen peroxide in an aqueous medium at room temperature the colour reached its maximum in the course of 30 to 50 minutes. Under these circumstances the yield was quantitative. With 0.1 ml of a 1 : 100 000 dilution of 30 per cent hydrogen peroxide a strong colour was obtained, and even 0.1 ml of a 1 : 1 000 000 dilution could be determined since it gave an extinction of about 0.05 in 1 cm layer in 5 ml total volume. Thus it is possible by this method to determine very small amounts of hydrogen peroxide.

The influence of variations in certain of the experimental conditions on the yield and velocity of the reaction was studied. As mentioned above this problem has been studied by other investigators, and their results could be confirmed. The optimum value for the pH was found to be about 4.1. An increase in the temperature also increased the velocity of the reaction. High concentrations of leucomalachite green favours the reaction velocity. A concentration of heme as given above is suitable. Too high concentrations can result in a decrease in the yield, probably due to a catalatic action of heme. With heme alone, without the presence of pyridine, the velocity of the reaction is very low.

When a peroxidized fat is tested under the same circumstances instead of hydrogen peroxide, no quantitative reaction will take place. On shaking some drops of a highly peroxidized oil with 4 ml of an aqueous leucomalachite green solution plus 0.2 ml heme solution for half an hour, the fluid will assume a green colour which will, however, be very faint as compared with the result of a stoichiometric reaction. It was found necessary, therefore, to cause the reaction to take place in a one phase system by means of a fat solvent. In order to study the influence of a fat solvent on the reaction, the influence of

* We are indebted to F. Hoffmann-La Roche & Co., Inc., Basle, for the kind supply of hemin.

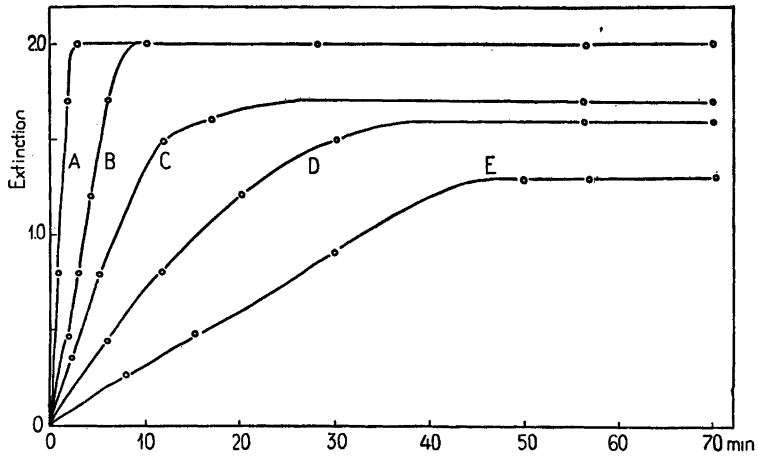

Fig. 1. Heme-catalyzed reactions between hydrogen peroxide and leucomalachite green carried out in mixtures of acetone and water.

Four ml substrate + 0.2 ml hydrogen peroxide solution + 0.2 ml heme solution.
Substrate: Mixtures, in the proportions given below, of 0.4 per cent leucomalachite green solutions, in 0.4 N. aqueous acetic acid and in acetone, respectively.
Hydrogen peroxide solution: A dilution of 5 : 100 000 of a 30 per cent stock solution.
Heme solution: 20 mg heme + 100 ml pyridine + 100 ml glacial acetic acid.
Readings at 615 mμ in the Beckman spectrophotometer.

Curve A: 100 per cent acetone solution,
or 80 » » » + 20 per cent aqueous solution,
or 50 » » » + 50 » » » »
Curve B: 25 » » » + 75 » » » »
» C: 12 » » » + 88 » » » »
» D: 5 » » » + 95 » » » »
» E: 100 » » aqueous solution.

substituting part or all of the water in the hydrogen peroxide reaction by different fat solvents was investigated.

The influence of acetone (or ethyl alcohol) on the reaction between hydrogen peroxide and leucomalachite green can be seen from Fig. 1. The experiments were made in the same manner as described above, and with the same concentrations of active substances, but instead of water, acetone or acetone-water mixtures in various concentrations were used as a solvent for the leucomalachite green. In order to facilitate quick readings of the colours, the experiments were carried out in test tubes which could be put directly into a Klett-Summerson colorimeter where the colour development could be followed continuously. Fig. 1 shows an example of the influence of different concentrations of acetone, which are indicated in the curve.

The curves show that acetone accelerates the reaction greatly. However, the fact that the end-point of the reaction is situated at a much higher colour intensity indicates that the reaction is different from that which occurs when water is absent. When water is present the reaction stops when the hydrogen peroxide has been used up, whereas in the presence of large amounts of acetone the reaction is not stoichiometric.

When the reaction is carried out in acetone there seems to be an induction period of a few seconds during which no formation of colour can be observed. After this period the development of colour accelerates. In water no such induction period can be observed. Therefore, a graphic representation of the resulting colour of the reaction in relation to time will give an S-shaped curve when acetone is used as a solvent, but not when water is used. While the amount of hydrogen peroxide present in the acetone or alcohol reactions does not greatly influence the amount of malachite green formed, it does greatly influence the velocity of the reaction, and especially the length of the induction period. Finally, the reaction in acetone shows a much greater tendency to take place without the presence of peroxides, that is to say, that a solution of heme-pyridine and leucomalachite green is much less stable in acetone, or alcohol, than in water.

As mentioned above, peroxidized fats and fatty acids give a similar reaction with leucomalachite green and pyridine-hemochromogen. Also in this case, the amount of peroxide influences the velocity of the reaction. It is a proof of the identity of the two reactions that the same amount of peroxide, either in the form of hydrogen peroxide or of organic peroxide, gives the same acceleration of the reaction. This can be seen from the experiment reported in Fig. 2 which at the same time examplifies the special characteristics mentioned above of the reaction in an organic solvent.

The experiment was carried out in the following way: In test tubes which could be put directly into a Klett-Summerson colorimeter were placed 4 ml of a 0.06 per cent solution of leucomalachite green in absolute alcohol, and 1 ml of a solution of hydrogen peroxide or peroxidized linseed oil in absolute alcohol having a peroxide content in milliequivalents per liter as indicated in the curves of the graph. Then 0.1 ml pyridine-hemochromogen solution was added, and at the same time the stop-watch was started. The tube was then inserted in the colorimeter and the colour development was followed.

The curves are most easily explained by a chain-reaction which as soon as inhibiting substances are used up, proceeds very rapidly until one of the reacting compounds is also used up. These inhibiting substances can be oxidized by peroxides.

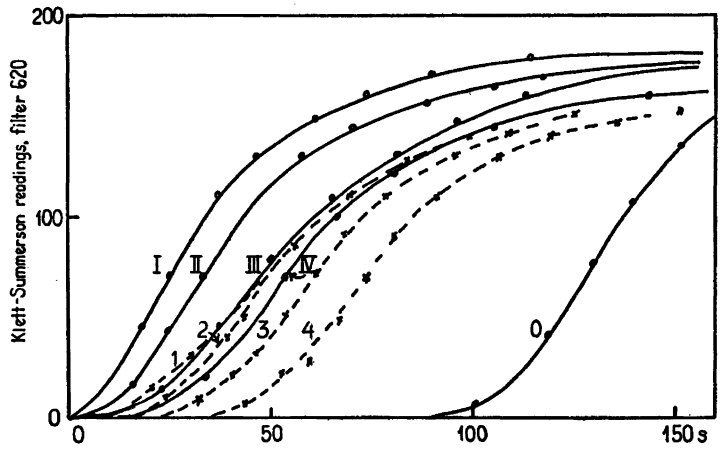

Fig. 2. Heme-catalyzed oxidation of leucomalachite green in alcohol in the presence of inorganic or organic peroxides.

Four ml substrate + 0.9 ml peroxide + 0.1 ml heme solution.
Substrate: 0.06 per cent leucomalachite green in 96 per cent alcohol.
Peroxide: Solutions, of the concentrations given below, of hydrogen peroxide or peroxidized linseed oil in alcohol.
Heme solution: 20 mg hemin + 5 ml pyridine + 10 ml glacial acetic acid.
Milliequivalents of peroxide added:

Curve	*0*	*No peroxide.*		
»	*1*	*0.0012*	*milliequivalents*	*hydrogen peroxide.*
»	*2*	*0.0006*	»	» »
»	*3*	*0.0003*	»	» »
»	*4*	*0.00015*	»	» »
»	*I*	*0.0032*	»	*organic* »
»	*II*	*0.0016*	»	» »
»	*III*	*0.0008*	»	» »
»	*IV*	*0.0004*	»	» »

Atmospheric oxygen is involved in the reaction. This can be seen from some experiments carried out in modified Thunberg-tubes, in which the experiments could be carried out *in vacuo*. They consisted of tubes of such a diameter that they could be put directly in a Klett-Summerson colorimeter for reading. In the bottom of the Thunberg-tube was put leucomalachite green in alcohol plus peroxide, and pyridine-hemochromogen in the small side-bulb. Then the carefully greased glass-stopper was inserted, and the tube was evacuated through a small tube beneath the stopper and provided with a tap which was then closed. The contents of the tube were then mixed, and the tube inserted in the colorimeter. When the reaction in alcohol or acetone

was allowed to take place under such conditions, and the results depicted graphically, no S-shaped curve could be constructed, but a curve similar to that for a reaction in aqueous medium was obtained.

The reaction as a rule does not proceed so that all the leuco-compound is oxidized but stops before, probably because the hemin has been broken down, which can be seen in different ways by the disappearing of the hemochromogen colour. However, by adding more hemochromogen to a test tube wherein a reaction has been carried out until maximal colour, only a small increase in the colour intensity is seen, and the addition of more peroxide also gives rise to only a small increase in the colour intensity. It seems, therefore, as if breakdown products, which inhibit the reaction, have been formed. These may have been formed from the hemin or from the dye, since the dye when the reaction is violent is also destroyed by the formation of a purple pigment.

The presence of chromic acid also accelerates the reaction, whereas ferric chloride or cupric sulfate does not. This or similar reactions can be observed with different sources of peroxides, with different kinds of easily oxidizable substances (leucomalachite green, leucodichlorophenolindophenol, guajac resin), and with heme prepared by Anson and Mirsky's [5] method, or a commercial preparation of hemin. However, the presence of organic solvents such as ethyl or methyl alcohol, acetone, dioxan, *etc.*, is necessary. The nature of the influence of the organic solvent remains to be elucidated.

SUMMARY

Hemin in the presence of organic solvents strongly catalyzes the oxidation of a number of easily oxidizable compounds by oxygen. A similar strong catalysis is not seen in an aqueous solution, but a certain quantity of an organic solvent must be present. The reaction is accelerated by inorganic or organic peroxides.

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Received June 23, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1027–1034

Polarographic Investigation of Proteins in the Brewing Process

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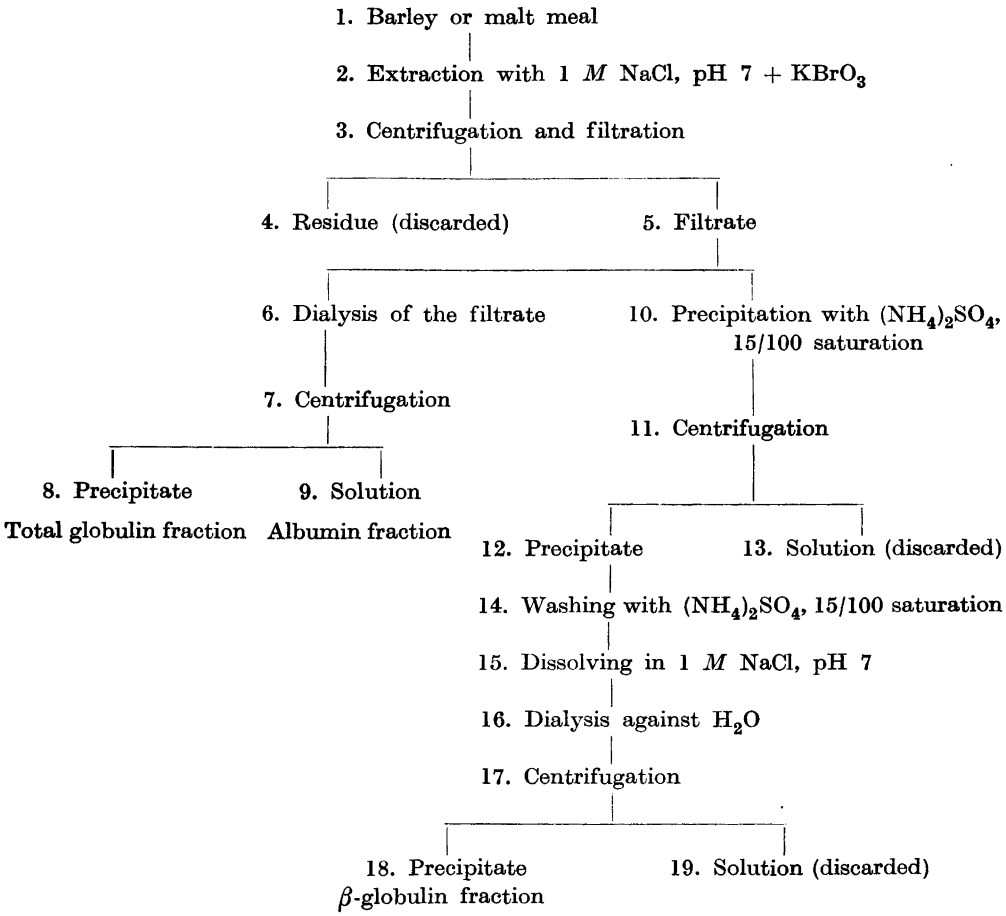
The proteins in beer have a great influence on its chemical stability. Together with tannin, they constitute the hazes formed when beer is chilled or oxidized. Hartong [1] and Helm [2] have pointed out that sulphur plays an important part in the formation of these hazes. We have tried to find a convenient method to study proteins in the brewing process, especially with respect to sulphur in proteins. The polarographic method, applied to the study of proteins by Brdička [3–5], seemed to be suitable for this purpose. Brdicka found that in certain solutions of cobalt ions, proteins give rise to a characteristic polarographic 'double wave', the height of which varies with the concentration of the proteins. He has shown that the effect observed is caused by sulphydryl and disulphidic groups in the protein molecules, and has given an extensive review of protein polarography [6].

The investigations described in this paper have been performed on proteins in barley, malt, wort, and beer. The proteins from barley and malt were divided into three fractions: albumin, total globulins and β-globulin, by methods described below. The different fractions were studied separately. Only albumin fractions were obtained from wort and beer. Two different varieties of malting barleys, Kenia and Heimdal, grown in 1947, were investigated. Some measurements were repeated on barley grown in 1948.

The main objectives of these investigations were to find out if some characteristic differences existed in the polarographic effects of the different protein fractions, and if these effects were influenced by the different stages in the brewing process.

PROTEINS FROM BARLEY

The following scheme of preparation, which is a modification of the schemes given by Quensel [7] and Danielsson and Sandegren [8], has been used to separate the protein fractions from barley. The same scheme has been used for malt.



100 g of barley or malt meal were extracted with 800 ml 1 *M* NaCl, pH 7.0, to which solution had been added 0.1 % of $KBrO_3$ to inactivate the proteolytic enzymes. All dialyses were made at 4° C. The amount of total nitrogen in the different protein fractions was determined by the micro Kjeldahl method. The protein content was obtained from the nitrogen content by multiplying by the factor 6.25. Some of the albumin and β-globulin fractions were in-

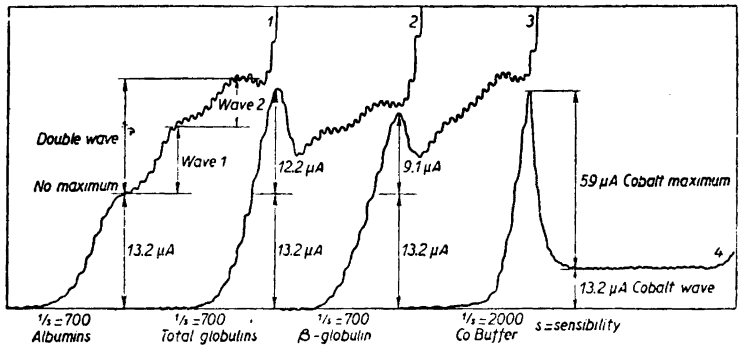


Fig. 1. Polarograms of malt protein fractions. Protein concentration 10 γ/ml.

vestigated by electrophoresis, and it was found that the amount of foreign protein components was very low. The 'total globulin' fraction contained a mixture of four globulin components [cf. 7]. Table 1 shows the approximate percentage distribution of the protein fractions in different barleys. These figures represent, not only the two barley varieties mentioned above, but a greater number of varieties grown in different years and in different places in Sweden.

Table 1. Composition of barley proteins.

Fraction	N, per cent of total N
Albumin	6.0—14.4
Total globulins	2.8—11.0
β-globulin	0.7— 5.0

The polarographic experiments were carried out as follows. The polarograph used was manufactured by Radiometer, Copenhagen, Denmark. The diameter of the cathode capillary was 0.05 mm, and the drop-time 4.1 sec. in distilled water when no e. m. f. was applied. The anode consisted of a saturated calomel electrode. The concentration of the test solution was 4×10^{-3} *N* $CoCl_2$, 0.2 *N* NH_4Cl, 0.2 *N* NH_4OH, and 5—240 γ protein per ml. The total volume of the test solution was 10 ml. The determinations were made at 25° C. The results were generally reproducible within 5 %.

Fig. 1 shows the type of curves obtained. At higher protein concentrations, the waves become less pronounced. At concentrations over 240 γ/ml, it is impossible to distinguish and measure the waves. The albumin fractions give more pronounced waves than the globulins, the curves of which are more

drawn out. When no proteins are present in the test solution, a sharp maximum appears in the cobalt wave before the diffusion current is reached (Fig. 1, curve 4). This maximum can be suppressed by adding small amounts of surface active agents to the solution. The maximum decreases in a given manner, with decreasing surface tension [9]. The results of the measurements of cobalt maxima are given in Table 2. The table shows that the albumin fraction has a considerably greater suppressing effect than the other two fractions. Thus, the albumin is the most surface active of the protein components. The results reported are from the Kenia variety. The Heimdal variety gave essentially the same results.

*Table 2. Suppressing effect of the protein fractions on the cobalt maximum.**

Origin	Concentration of proteins, γ/ml	Height of cobalt maximum, μA					
		Albumin		Total globulins		β-globulin	
Barley	5	0	0	(24)	(19)	(16)	(17)
»	10	0	0	12.0	12.7	9.1	8.8
Malt	5	4.3	4.0	(19)	(19)	(18)	(18)
»	10	0	0	12.2	12.7	9.1	9.6
Wort	5	0	0	—	—	—	—
»	10	0	0	—	—	—	—
Beer	5	0	0	—	—	—	—
»	10	0	0	—	—	—	—

Another important difference between the protein fractions was found when the heights of the polarographic 'double waves' were compared. Each fraction was electrolyzed in concentrations of 5, 10, 20, 40, and 80 γ/ml, and the wave height plotted against the concentration. A typical diagram is given in Fig. 2. The β-globulin always gave a considerably higher wave than the albumin at the same concentration. This is shown in Table 3, which contains the wave heights at a concentration of 80 γ/ml. The activity of the total globulin fraction is somewhat varying. It generally takes an intermediate position between the other two fractions in the range of concentration just mentioned.

* The values in brackets had to be approximately extrapolated, because the cobalt maxima ended outside the polarographic paper. The heights of the cobalt maxima are given in microampéres. From the current corresponding to the top of the maximum is subtracted the cobalt diffusion current obtained from pure cobalt buffer solutions.

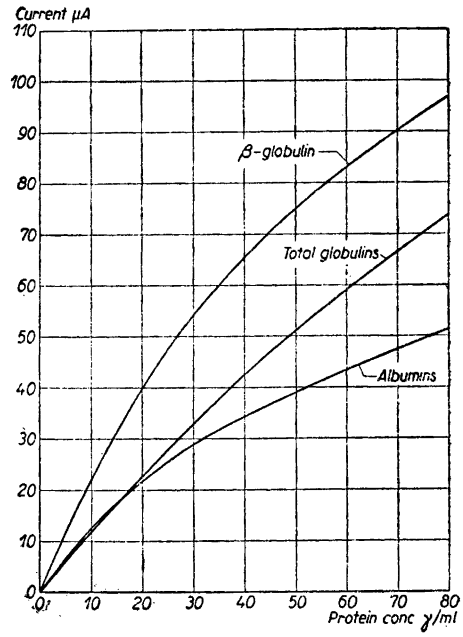


Fig. 2. Variation in the height of the protein 'double wave' with the concentration of proteins from Kenia barley.

*Table 3. Height of protein 'double wave' from different barley protein fractions. Concentration of protein 80 γ/ml.**

Origin	Height of 'double wave', μA		
	Albumin	Total globulins	β-globulin
Kenia, 1947	51.5	73.5	97.0
Heimdal, 1947	48.5	64.5	70.5
Kenia, 1948	46.5	77.0	77.5
Heimdal, 1948	42.0	42.0	53.5

It is evident from the table that the globulins from Kenia barley, especially the β-globulin, are polarographically more active than those of Heimdal barley.

PROTEINS FROM MALT

Part of the barley samples were malted in stockings in malting drums (of the Topf type) at a temperature of 17° C for 7 days. The kilning was

* In the analyses of barley from 1948, 0.1 *N* NH_4Cl and 0.1 *N* NH_4OH have been used in stead of 0.2 *N* (*cf.* page 1029).

performed as for Pilsner malt. The malt proteins were then extracted and fractionated in the same way as the barley proteins.

The surface activity of the malt protein fractions seems to be almost the same as for the barley protein fractions (Table 2).

With respect to polarographic activity, no characteristic differences were observed between the malt protein fractions. Comparison with the proteins of barley, shows that the activity of the albumin is increased about 30 % by malting (Table 4). The activity of the total globulins and the β-globulin is the same as in barley, or in the latter case somewhat lower.

Table 4. Influence of the different stages in the brewing process on the polarographic activity of the albumin fraction. Concentration 80 γ/ml.

Origin	Height of albumin 'double wave', μA			
	Barley	Malt	Wort	Beer
Kenia, 1947	51.5	68.5	56.0	38.5
Heimdal, 1947	48.5	64.0	45.5	35.5

PROTEINS FROM WORT AND BEER

The malt was mashed by the 'Congress method' for extract determinations in malt adopted in Salzburg in 1930. The wort was then boiled for one hour with hops (2.5 g per liter) and filtered. Part of the clear filtrate was dialysed against water. No precipitate appeared, which indicated that no appreciable amount of globulins was present. The dialysed wort, containing only high-molecular water soluble nitrogen compounds, has been called the 'albumin fraction', although the water soluble nitrogen compounds do not consist of the original albumin from malt. They originate from all three malt protein fractions, which are split into less high molecular compounds during mashing and wort boiling. The hopped wort was fermented with bottom fermenting yeast. After filtration, the beer was dialysed. The 'albumin fraction' (water soluble nondialysable nitrogen compounds) was then investigated.

From Table 2 it may be seen that the surface activity of the 'albumin fractions' from wort and beer equals that of barley albumin.

Table 4 shows that mashing, wort boiling, and fermentation decrease the polarographic activity of the 'albumin fraction'. The activity of wort 'albumin' is about the same as that of barley albumin.

DISCUSSION

According to the investigations described above, the albumin of barley is considerably more surface active than the globulin fractions. This agrees with the results obtained by Sandegren and Säverborn [10] during investigations of surface films on water solutions of these substances. Furthermore the albumin fraction retains its surface activity during the brewing process. This has, of course, a great significance for the foam quality of the beer.

It was found that the β-globulin of barley and malt has a higher polarographic activity than the other two fractions. Consequently, it contains greater amounts of reactive sulphydryl and disulphidic groups. It does in fact, contain a higher percentage of sulphur than the other fractions [11]. Thus it seems probable that the degradation products of β-globulin are more rapidly oxidized and precipitated than the other proteins in beer. This conclusion agrees with results from investigations of the chill haze substance. It was found that the protein component of this substance probably originated from the β-globulin of barley [11]. The conclusion is further supported by the fact that proteins in beer, freed from chill haze, have a somewhat lower polarographic activity than proteins in ordinary beer.

The polarographic activity of malt albumin was shown to be about 30 % higher than that of barley albumin. Danielsson and Sandegren [8] have shown that the β-amylase activity of barley and malt is connected with the albumin fraction. They also found that the malt albumin has a considerably higher β-amylase activity than barley albumin. Weill [12] has shown that the β-amylase activity is probably due to prosthetic sulphydryl groups. The fact that the β-amylase activity, as well as the polarographic activity, of the albumin is increased during malting seems to confirm this theory.

SUMMARY

1. The polarographic method has been applied to the investigation of proteins in the brewing process, and found to be very useful in this field.

2. The albumin fraction has the strongest suppressive effect on the cobalt maximum, and thus the highest surface activity of the protein fractions of barley and malt.

3. The β-globulin has the highest polarographic activity of the protein fractions of barley and malt.

4. The polarographic activity of the albumin fraction has been shown to increase during malting and then decrease during mashing, wort boiling, and fermentation.

The authors are indebted to Mr. L. Ljungdahl, who has carefully performed the protein fractionations.

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Received August 2, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1035–1043

On the Use of Monolayer Phase Diagrams for Determining the Composition of Mixtures of Homologous Long Chain Compounds of High Molecular Weight

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The determination of the composition of mixtures of homologous long chain compounds, *e. g.* of the type often found in natural waxes, is usually rather difficult, especially when only small amounts of material are available. As a rule, molecular distillation gives only a partial separation of the components, while crystallization from solvents is quite an ineffective method of separation when dealing with a mixture of closely related homologues of high molecular weight. Chromatographic methods [Cf. 1, 2] may ultimately prove capable of analyzing such mixtures, but the technique is not yet sufficiently developed. Chibnall, Piper and their collaborators, in the course of extensive work on the composition of natural waxes [Cf. e. g. 3, 4], determined the composition of binary and ternary mixtures of long chain compounds by thermal and X-ray methods. The behaviour of a large number of artificial mixtures of known composition was investigated, and the composition of naturally occurring mixtures determined by comparing their behaviour with that of mixtures of known composition.

The object of the present paper is to show that monolayer phase diagrams may be advantageously used for the quantitative analysis of mixtures of homologous long chain compounds of high molecular weight.

In a previous communication [5] we have described the monolayer phase diagram for *n*-docosanoic (behenic) acid. The phase diagram was constructed from force-area curves obtained with an automatically recording Wilhelmy-Dervichian type surface balance with symmetrical compression of the monolayers [6, 5]. The results obtained with this balance have since been checked by means of a recording horizontal balance, employing the 'mikrokator' prin-

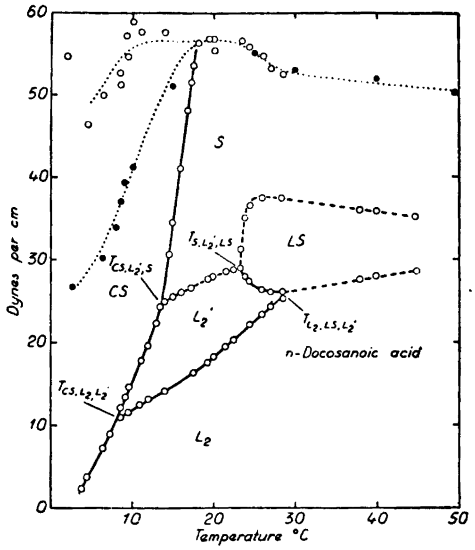

Fig. 1. Phase diagram for n-docosanoic (behenic) acid on 0.01 N hydrochloric acid substrate. Unbroken lines represent first order transitions, and broken lines second order. The stability limit is given as a dotted line. Filled circles represent collapse pressures obtained with a Wilhelmy-Dervichian type balance with symmetrical compression.

ciple [7]. The phase diagram for *n*-docosanoic acid obtained by the use of the latter instrument is reproduced in Fig. 1. Differences between the results given by the vertical and the horizontal balances, outside of experimental errors, are found only for the collapse pressures at low temperatures (CS phase). The lower stability of the monolayers, when the vertical balance is used, is probably due to mechanical breaking of the solid monolayer during the upward movement of the glass slide. The monolayer is not subjected to any strain of this type when the mikrokator balance is used.

The monolayer phase diagram for a long chain acid such as *n*-docosanoic acid shows five different phases and four triple points (a detailed description of the phase diagrams, and a discussion of the symmetry relations between the different phases will be given in another communication) [8]. Of primary importance in the present connection are the changes in the monolayer phase diagrams brought about by an increase in the length of the hydrocarbon chain of the carboxylic acid, and the effects of admixture of homologues. The phase diagrams for monolayers of the C_{20}, C_{22}, and C_{24} normal chain carboxylic acids are shown superimposed on each other in Fig. 2. An increase in chain length brings about a shift of the phase diagrams towards higher temperatures and slightly higher pressures. One CH_2-group causes a shift of about 5°, but the distance between the diagrams decreases with increasing chain-length. The odd-numbered acids (C_{21} and C_{23}) fall in between the evennumbered homologues, no odd-even alternation being observed.

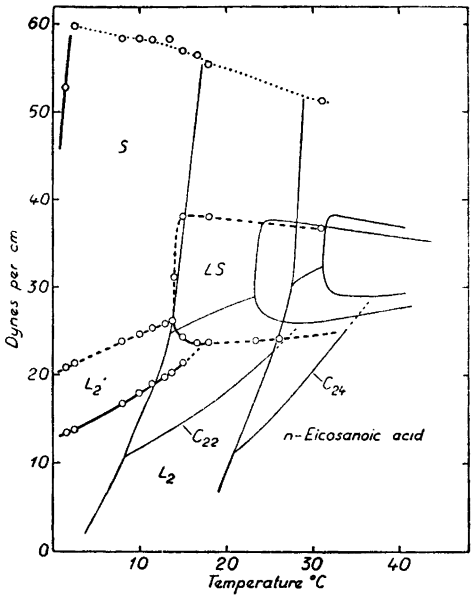

Fig. 2. Monolayer phase diagrams for n-eicosanoic acid, n-docosanoic acid and n-tetracosanoic acid respectively spread on 0.01 N hydrochloric acid substrate.

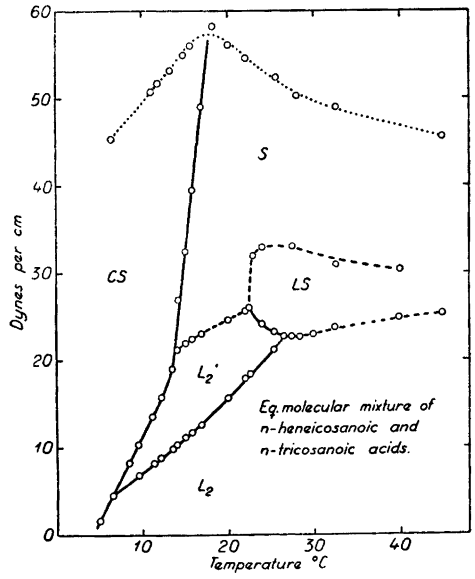

Fig. 3. Phase diagram for the equimolecular mixture of n-heneicosanoic acid and n-tricosanoic acid.

The shifts of the different triple points are not equal, and this changes the form of the diagrams slightly when the chain-lengths are altered. The nature of the phases themselves, however, and the type of transition between them remain unaltered.

Phase diagrams for the monolayers of two artificial binary mixtures, each having a mean molecular weight equal to the molecular weight of the C_{22} acid, are shown in Figs. 3 and 4. When the chain-lengths of the components differ by two CH_2-groups (Fig. 3), the phase diagram is very similar to that of the pure C_{22} acid, but the triple points are shifted towards lower pressures. This shift is most pronounced for the triple point $T_{CS,\ L_2,\ L'_2}$, which lies about 7 dynes per cm lower for the mixture. The shift of this triple point is still more pronounced for the mixture whose phase diagram is shown in Fig. 4. In this case the chain-lengths of the components differ by 4 CH_2-groups, and the triple point in question is now displaced below zero pressure. The three other triple points are also displaced towards lower pressures compared with those of Fig. 3. The phase diagram in Fig. 4 is still of the type found for pure acids, but this is no longer the case when the components of the binary

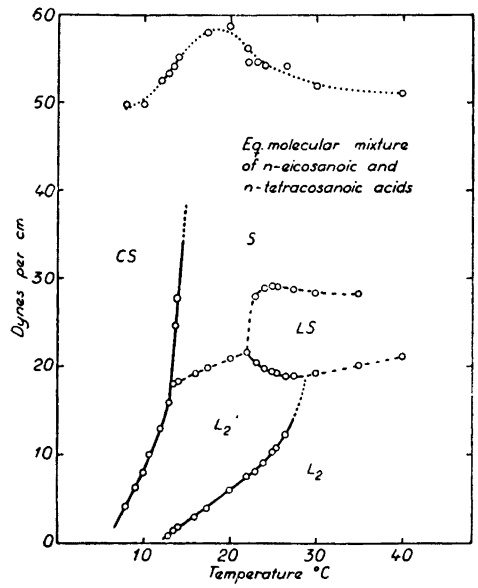

Fig. 4. Phase diagram for the equimolecular mixture of n-eicosanoic acid and n-tetracosanoic acid.

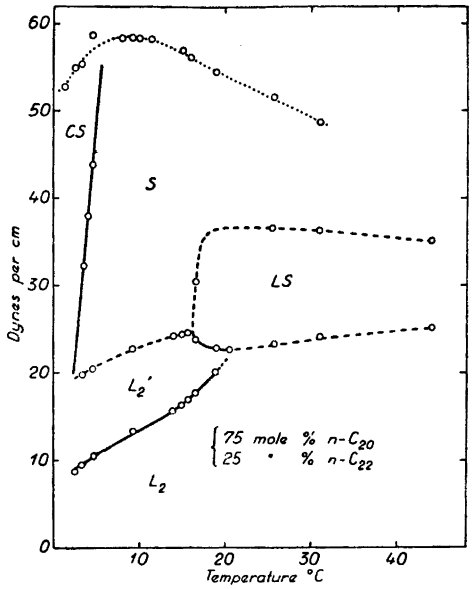

Fig. 5. Phase diagram for mixture containing n-eicosanoic acid and n-docosanoic acid in the molecular proportions 3 : 1.

mixture differ in chain-length by six carbon atoms or more, as in the equimolecular mixtures of *n*-nonadecanoic and *n*-pentacosanoic acids, or *n*-octadecanoic (stearic) and *n*-hexacosanoic acids. The phase transitions now become blurred, and the phase diagrams can no longer be plotted with any degree of accuracy.

Figs. 5—7 show the monolayer phase diagrams for binary mixtures containing *n*-eicosanoic and *n*-docosanoic acids in varying proportions. It appears that there is a gradual shift of the diagram from that of the pure n—C_{20} acid to that of the pure n—C_{22} acid (*cf.* Fig. 2). The relation between the temperature at which a certain phase transition occurs, and the composition of the binary mixture appears to be approximately linear, while the corresponding pressures are slightly lower than those obtained by linear interpolation.

It is evident, from the above results, that the monolayer phase diagram will, in general, be a characteristic property of a given mixture. If a mixture of unknown composition gives a monolayer phase diagram agreeing in detail with that of a mixture of known composition, it may be assumed, with some confidence, that the two mixtures are identical. The chance that two different mixtures give the same result is probably less for the rather complicated monolayer phase diagrams than for thermal and X-ray data.

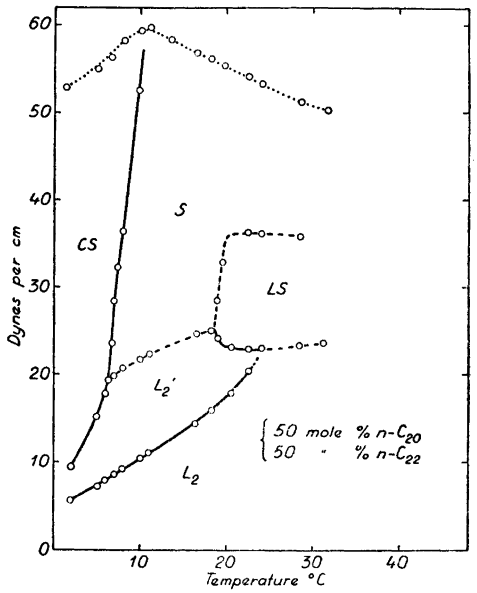

Fig. 6. Phase diagram for the equimolecular mixture of n-eicosanoic acid and n-docosanoic acid.

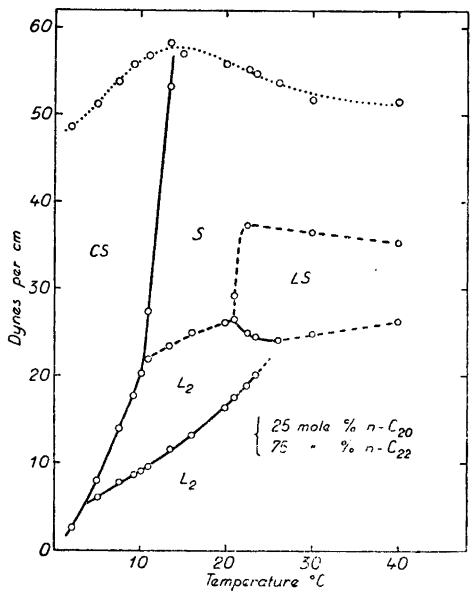

Fig. 7. Phase diagram for mixture containing n-eicosanoic acid and n-docosanoic acid in the molecular proportions 1 : 3.

The new method of analysis has been applied to the quantitative analysis of a mixture of acids obtained by Dr. J. Gripenberg from the heart wood of *Thuja plicata* D. Don. A series of force-area curves for a specimen of this mixture, kindly put at our disposal by Professor H. Erdtman, are reproduced in Fig. 8 a. The curves are of the usual type given by normal chain carboxylic acids of high molecular weight. The various phase transitions occur at higher temperatures, and the transitions $L_2 \rightleftharpoons CS$, $L_2 \rightleftharpoons L_2'$, and $L_2' \rightleftharpoons CS$ are not as distinct as in the case of *n*-docosanoic acid [5]. This indicates that the mean molecular weight of the mixture is higher than the molecular weight of the C_{22} acid. As area values are not needed for the construction of the phase diagrams, a previous knowledge of the mean molecular weight of the mixture is not necessary for the analysis.

The phase diagram for the mixture indicates that the difference in chain lengths of the components cannot be large, as the diagram is very similar in form to that of the *n*-C_{24} acid (Fig. 9). The fact that the diagram for the *Thuja plicata* mixture is shifted towards higher temperatures shows that the mean molecular weight of this mixture is higher than that of the C_{24} acid. This, finding — combined with the fact that there can be no large difference in the

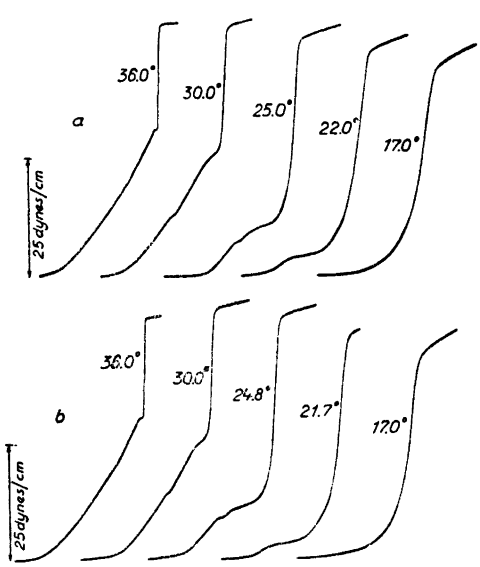

Fig. 8. Force-area curves on 0.01 N hydrochloric acid substrate. a) mixture of acids derived from heart wood of Thuja plicata. b) artificial mixture containing n-tetracosanoic acid and n-hexacosanoic acid in the molecular proportions 7 : 3.

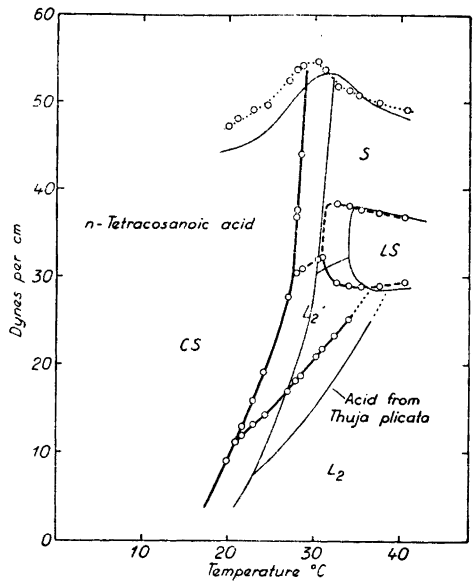

Fig. 9. Comparison between the phase diagram for the mixture of acids derived from Thuja plicata and that given by n-tetracosanoic acid.

chain lengths of the components and the rule that naturally occurring mixtures consist of even-numbered homologues — suggested a mixture of *n*-tetracosanoic acid and *n*-hexacosanoic acid. The phase diagrams of several mixtures of these two acids were therefore determined for comparison. Fig. 10 shows that the diagram for a mixture containing equimolecular proportions lies to the right of that of the natural product, indicating that the mean molecular weight of the latter is lower than that of this mixture. This diagram, together with that of Fig. 11, shows that the mixture from *Thuja plicata* must contain between 50 and 23 % of the higher (C_{26}) acid, and finally Fig. 12 shows that a mixture containing 30 mol-% of the C_{26} and 70 mol-% of the C_{24} acid gives a monolayer phase diagram coinciding with that of the natural product. Fig. 8 b shows a series of force-area curves for the synthetic mixture, which should be compared with those in Fig. 8 a. A very small amount of a third component cannot, of course, be excluded; but appreciable amounts (of the order of 3 to 5 %) should have caused a shift of the triple point $T_{CS,\ L_2,\ L_2'}$

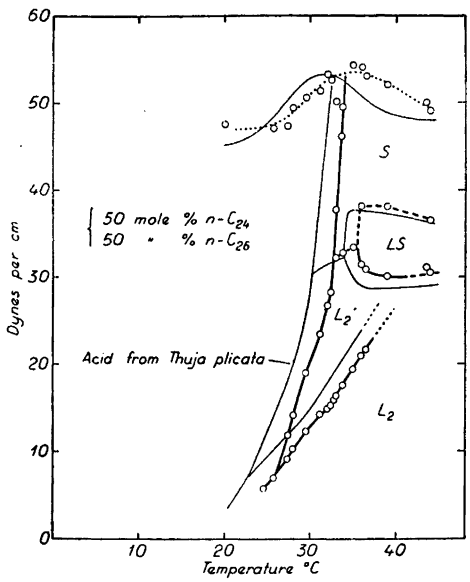

Fig. 10. Comparison between the phase diagram for the mixture of acids derived from T h u j a p l i c a t a and that of an artificial mixture containing equimolecular proportions of n-tetracosanoic acid and n-hexacosanoic acid.

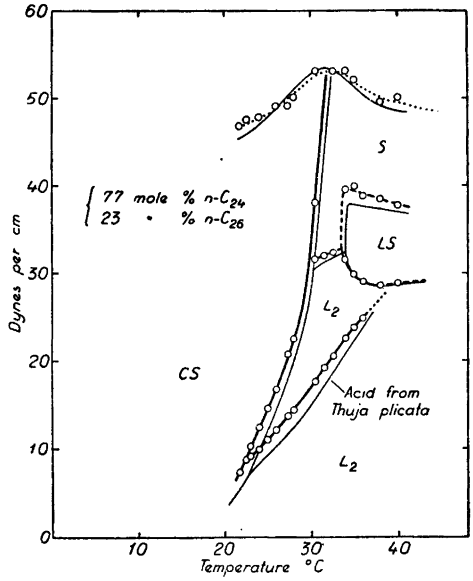

Fig. 11. Phase diagram for mixture of acids derived from T h u j a p l i c a t a compared with that of an artificial mixture containing n-tetracosanoic acid and n-hexacosanoic acid in the molecular proportions 77 : 23.

towards lower pressures. The effect is clearly shown in the phase diagram for a mixture of C_{24}, C_{26} and C_{28} acids reproduced in Fig. 13.

Thermal and X-ray data * confirm the results of the above analysis. The m. p. of the mixture from *Thuja plicata* was found to be 78.2—78.4°. The melt showed a very faint brownish tinge. A synthetic mixture of the *n*-C_{24} and *n*-C_{26} acids containing 70 mol % of the former melted at 78.4—78.7°, and the mixed m. p. with the mixture from *Thuja plicata* was 78.3—78.7°. The melting point curve for the binary system *n*-tetracosanoic acid — *n*-hexacosanoic acid given by Piper, Chibnall and Williams[4] shows that the mixture containing 70 mol % of the lower acid corresponds to the eutectic mixture. This explains the sharp m.p. given by the *Thuja plicata* mixture, as well as the fact that its properties remained unchanged in spite of several recrystallizations.

The long X-ray spacing given by the natural specimen (as received, or after recrystallization from acetone) was 60.1 Å, calculated from a diffraction

* For the technique used *cf. e. g.* ref. 10.

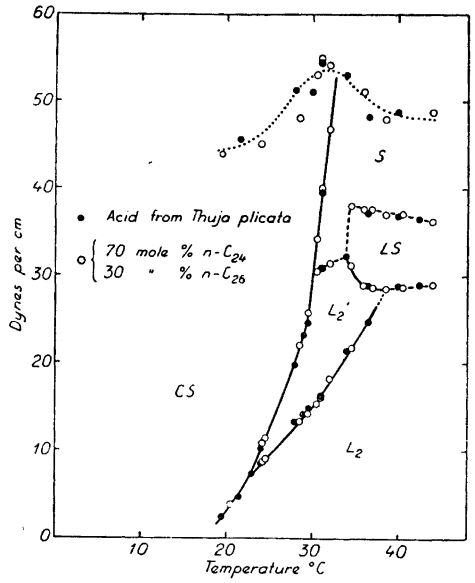

Fig. 12. Comparison between the phase diagram for the mixture of acids derived from T h u j a p l i c a t a and that of an artificial mixture containing n-tetracosanoic acid and n-hexacosanoic acid in the molecular proportions 7 : 3.

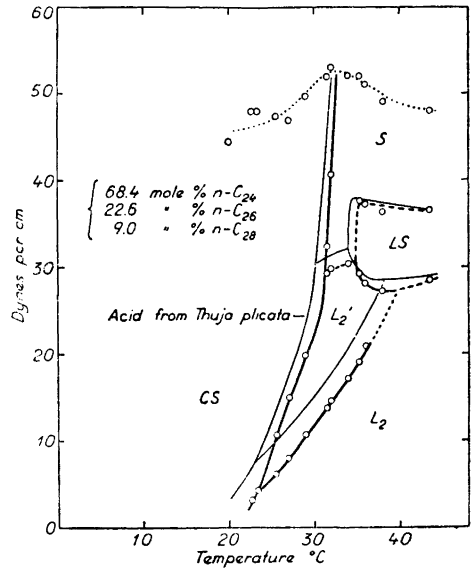

Fig. 13. Comparison between the phase diagram for the mixture of acids derived from T h u j a p l i c a t a and that of an artificial mixture containing n-tetracosanoic acid, n-hexacosanoic acid and n-octacosanoic acid in the molecular proportions 68.4 : 22.6 : 9.

pattern of the type expected for a mixture. The value recorded corresponds to a B spacing [Cf. 4]. A multilayer of the barium salt gave a long spacing of 67.5 Å, calculated from a fairly good photograph. Multilayers of the barium salt of *n*-tetracosanoic acid gave a spacing of 65.3 Å [8], and the value calculated for the barium salt of the n-C_{26} acid is 70.3—70.4 Å. The X-ray data just mentioned supports the results derived from monolayer and thermal data.

Finally, the mean molecular weight of the mixture from *Thuja plicata* was determined by titration. However, owing to the solubility difficulties and the small amount of material available, this determination could not be performed with great accuracy. The value found was 381 ± 5. The value calculated for the eutectic mixture (70 % of the lower acid) is 377.0.

The method of analysis described can be regarded as a micro-method, as the amount of material needed for the analysis is less than 10 mg. For comparison, pure specimens of the appropriate homologues are, of course, necessary.

EXPERIMENTAL

The monolayer phase diagrams in this paper have all been plotted from force-area curves on 0.01 *N* hydrochloric acid substrate obtained with the recording mikrokator balance [7]. Spreading was effected from a solution in benzene-chloroform (9 : 1 by volume) [9].

Materials used. The specimen of the mixture from *Thuja plicata* had been obtained * by alkaline hydrolysis of the neutral fraction of the acetone extract of the heart wood. The alcoholic fission product consisted mainly of a mixture of sterols ('phytosterol'). The free 'acid' obtained by the hydrolysis had been recrystallized 10 times from ethanol, without appreciable change in melting point. The specimen received had a white, microcrystalline appearance.

For comparison, original samples of the normal chain fatty acids described by Francis and Piper [11] were available.

We are indebted to Mrs. Karin Nilsson for skilled assistance in the monolayer work, and to the *Rockefeller Foundation* for financial support.

SUMMARY

It has been shown that monolayer phase diagrams may be used for the quantitative analysis of the composition of mixtures of homologous long-chain compounds of the type often derived from natural sources. The new method of analysis is applied to a mixture of acids from the heart wood of *Thuja plicata*. The analysis requires less than 10 mg of sample.

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Received August 3, 1949.

* We are indebted to Professor Erdtman for this information.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1044–1049

Anaerobic Nitrogen Fixation and Formation of Oxime Nitrogen

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Nitrogen fixation of greatest significance takes place, in nature, in the leguminous root nodules. The legume bacteria are strongly aerobic and the oxygen supply in the root nodules is assured by a special hemoglobin, leghemoglobin, which transports and stores oxygen. In nodules lacking this chromoprotein, no nitrogen fixation has been detected.

Of the free-living nitrogen fixing bacteria, *Azotobacters* are the most rapid and efficient nitrogen-fixers. They, too, are strongly aerobic and respirate intensely.

In nitrogen fixation by Azotobacters and leguminous root nodules, the formation of oxime nitrogen has been noted. Attention must be paid to this fact in explaining the mechanism of nitrogen fixation, and this has given rise to doubt as to whether nitrogen fixation is a pure reduction, occurring, for instance, in the following way (1) acc. to Wieland.

$$N_2 \xrightarrow{H_2} HN = NH \xrightarrow{H_2} H_2N{-}NH_2 \xrightarrow{H_2} NH_3 \qquad (1)$$

Oxime formation in connection with reaction (1) could be explained by assuming that the first reduction product, the di-imide, combines with water whereby hydroxylamine is produced (2), but there is no proof for this kind of reaction.

$$N_2 \xrightarrow{H_2} HN = NH \xrightarrow{H_2O} NH_2OH \qquad (2)$$

Blom believed that hydration of the nitrogen molecule was the first reaction in nitrogen fixation. Reduction of this intermediate would lead to hydroxylamine (reaction 3).

$$N_2 \xrightarrow{H_2O} \begin{matrix} HN & - & NH \\ | & & | \\ HO & & OH \end{matrix} \xrightarrow{H_2} NH_2OH \qquad (3)$$

Later, Virtanen [1] introduced a quite different possibility for the formation of oxime nitrogen. According to this theory the first reaction in nitrogen fixation is oxidation. The transfer of electron from the nitrogen atom to oxygen would be effected by a hemin system as in respiration. The hydration and reduction of nitrous oxide would then lead to hydroxylamine (reaction 4).

$$N_2 \longrightarrow N \longrightarrow N^+ \xrightarrow{O^{--}} N_2O \xrightarrow{H_2O} (NOH)_2 \xrightarrow{H_2} NH_2OH \qquad (4)$$

There is still another route to the formation of hydroxylamine, *viz.*, the oxidation of ammonia. In the nitrification caused by specific bacteria, ammonia is known to be oxidized to nitrite and nitrate. Eggleton [2], Pearsall and Billimoria [3] have concluded that ammonia is also oxidized in green plants. The formation of hydroxylamine from ammonia was noted by Steinberg [4] in cultures of *Aspergillus*. Azotobacter also forms oxime nitrogen from ammonia [5]. If oxime nitrogen arises even in the nitrogen fixation, via ammonia oxidation, hydroxylamine would not be an intermediate and the formation of oxime would in no way invalidate the reaction (1) even if reactions 2 and 3 would not exist.

Virtanen and Miss Järvinen have examined the formation velocity of oxime nitrogen in *Azotobacter* cultures grown in nutrient solutions with either ammonium nitrogen, nitrate nitrogen, or molecular nitrogen as the nitrogen source. Equal amounts of *Azotobacter vinelandii* suspensions were added to each solution. Because of the large number of bacteria, a detectable increase in the nitrogen content occured already after about an hour, and the oxime formation could be followed over a short period. Where the molecular nitrogen and nitrate nitrogen served as the source of nitrogen, oxime nitrogen was detectable much sooner in the cells and solutions than when ammonium phosphate was employed. In general, oxime nitrogen could be found in cells grown on N_2 and NO_3^- already after 60—90 mins., but on NH_4^+ not until after 150—180 min. The cells assimilated ammonium nitrogen as rapidly as or more rapidly than molecular nitrogen. Assimilation of nitrate was slowest. Thus these experiments, which will be reported in detail in another paper, do not support the concept that oxime nitrogen is formed from ammonia in nitrogen fixation by Azotobacters. Hence, reactions 2, 3 or 4 would demand the most attention when interpreting the formation of oxime nitrogen.

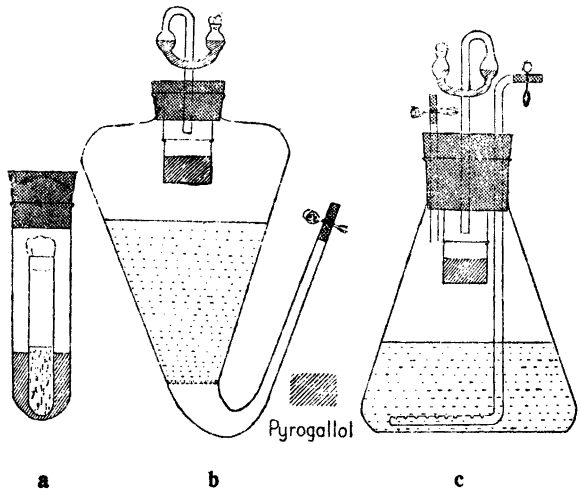

Fig. 1. Apparatuses used for experiments on anaerobic nitrogen fixation.

To ascertain if reactions 2 and 3 may be concerned in the formation of oxime nitrogen, we have examined whether or not oxime nitrogen is produced in fully anaerobic nitrogen fixation. Reaction 4 is, under anaerobic conditions, out of the question. In the following we record our experiments.

EXPERIMENTAL

The culture of *Clostridium butyricum* was obtained from Delft Technische Hogeschool through the courtesy of Prof. A. J. Kluyver. An anaerobic bacterium of the same type was isolated by the authors from the soil. Both organisms fix nitrogen and yield, among other fermentation products, hydrogen and butyric acid. The organism isolated by the authors was called *Clostridium X*. It was isolated in the following manner: 2.5 g leaf mould were suspended in 10 ml water and kept for 20 min at 80° C. 5 ml of the heated suspension were placed in a test tube containing nutrient solution free from combined nitrogen. As soon as a distinct gas evolution was observed 1 ml of the suspension was inoculated into a new nutrient solution (10 ml). The procedure was repeated 6—7 times in succession. Anaerobic conditions were maintained in the test tubes. The lack of combined nitrogen and the anaerobic conditions guaranteed that only the anaerobic fixers were enriched. A microscopically homogeneous culture was obtained.

Nutrient solution and growth conditions. The composition of the nutrient solution was: 0.8 g K_2HPO_4, 0.2 g KH_2PO_4, 0.2 g $MgSO_4$, 0.2 g NaCl, 0.1 g $CaSO_4$, 0.01 g $Fe_2(SO_4)_3$, 0.0252 mg Na_2MoO_4, 10 g $CaCO_3$, 1 l. water. The pH of the solution was 7.3—7.4. The solution resembles that prescribed by Burk [6] for Azotobacter, containing in addition 0.01 p. p. m. Mo, which, according to Jensen [7], promotes optimally nitrogen fixation by Cl. butyricum. $CaCO_3$ was added to neutralize the acids formed in fermentation. The solution contained 1.51 mg N per litre. The temperature in every experiment was about 30°.

In order to establish anaerobic conditions, an alkaline pyrogallol solution was employed in cultivating the bacterium. The pyrogallol solution was kept in a wide test tube closed with a rubber stopper and inside this was placed a test tube closed with cotton wool and containing the bacterial culture (Fig. 1a).

The fermentation experiments proper were carried out in 2 litre Kluyver flasks. The volume of the nutrient solution varied from 1000 to 1250 ml. Anaerobic conditions were accomplished by leading nitrogen gas, which had first passed through two wash bottles filled with alkaline pyrogallol solution through the solution. During sterilization, the Kluyver flask was closed with cotton. The rubber stopper and accessories were sterilized separately and then the cotton replaced by it. The whole system (Fig. 1b) was kept in water at 29—30°. During the experiment, nitrogen gas was occasionally passed through the solution to mix it.

In two experiments a slightly different procedure was employed. A 750 ml wide-mouthed Erlenmeyer flask containing 500 ml nutrient solution was used as a container. This system is illustrated by Fig. 1c.

Determination of oxime nitrogen. Oxime nitrogen was determined according to Blom [8] and Endres[9]. Csáky [10] found that only the > C:NOH group will be determined by this method. Since the concentration of oxime nitrogen could be so low that the reaction for oxime would be negative, the solution was concentrated in many experiments by vacuum distillation. Determination of oxime nitrogen was made from both the original and the concentrated solutions, as can be seen from Table 1.

Table 1. Formation of oxime nitrogen in anaerobic nitrogen fixation.

Organism	Growth days	Nutrient solution		Culture solution at the end of the experiment		Nitrogen fixed		Oxime nitrogen in	
		sugar g/l	nitrogen mg/l	sugar g/l	nitrogen g/l	mg/l	mg/g glucose	culture solution	conc. culture solution
Cl. butyricum	7	9.36	1.51	7.7	5.32	3.81	2.3	0	0 [1]
Cl. X.	9	9.36	1.51	5.1	14.6	13.1	3.1	0	0 [2]
Cl. X.	5	9.36	1.51	0	29.7	28.2	3.0	0	0 [3]
Cl. X.	4	9.36	1.51	4.9	8.8	7.3	1.6	0	0 [4]
Cl. X.	6	9.36	1.51	0	14.1	12.6	1.3	0	0

In one experiment, nitrogen was determined also in the clear centrifugate in order to ascertain the quantity of fixed nitrogen in the cells and in the solution (Table 2).

Table 2. Occurrence of anaerobically fixed nitrogen in nutrient solutions outside cells.

Organism	Growth days	N in nutr.soln. mg/l	Final N in culture soln. mg/l	N in clear centrifugate mg/l	Fixed N in solution %
Cl. X.	6	1.51	14.1	9.07	60

[1] Concentrated to 1/20. [2] 1/10. [3] 1/37. [4] 1/14.

In addition to the experiments described above, another experiment was made with *Clostridium X* in a Kluyver flask containing 99.3 vol. % N_2 and 0.7 vol. % O_2. Clostridium grew still well and used sugar as well as in fully anaerobic conditions. Table 3 shows the decrease of glucose and the formation of oxime in this experiment.

Table 3. Experiment with Clostridium X in low oxygen tension (0.7 vol. % O_2).

Growth, days	Glucose mg/ml	Oxime nitrogen	
		in culture solution	in conc. culture solution
0	20	0	—
3	16.8	0	—
6	9.2	0	—
9	3.3	0	0

Accordingly, *Clostridium* did not form oxime-N even if some oxygen was present. The result suggests that an anaerobic N-fixer is unable to form oxime nitrogen even in low oxygen concentration.

RESULTS AND CONCLUSIONS

The data concerning nitrogen fixation and formation of oxime nitrogen in fully anaerobic nitrogen fixation are summarized in Table 1. As can be seen no traces of oxime nitrogen could be detected even in very concentrated solutions by the sensitive method of Blom. In corresponding experiments with *Azotobacter*, the authors have always found considerable amounts oxime nitrogen. Oxime nitrogen can, in this case, be determined directly from the growth solution, without concentration. Consequently, our results with anaerobic *Clostridium* suggest that oxime nitrogen is not formed in nitrogen fixation under anaerobic conditions although the possibility exists that the oxime formed is reduced too rapidly to be detected. But such a possibility exists likewise in the aerobic nitrogen fixation. Since, moreover, oxime nitrogen was not formed in the *Clostridium* culture even in low oxygen tension (Table 3) formation of oxime nitrogen, according to reactions 2 and 3, whereby hydroxylamine would arise on addition of water to di-imide or nitrogen molecule, does not seem likely. On the other hand, the results obtained justify the conclusion that nitrogen fixation takes place anaerobically via reduction. Whether di-imide and hydrazin are hereby formed as intermediates acc. to reaction 1 is questionable.

The formation of oxime nitrogen, regularly noted in aerobic N-fixation, implies that the first phase in aerobic nitrogen fixation would be oxidative (reaction 4) or that oxime nitrogen arises from hydroxylamine formed from ammonia through oxidation. As was mentioned in the beginning of this paper, the

observations on the formation velocity of oxime nitrogen do not fit easily into the latter concept attractive though it might seem. Provided that oxime nitrogen in aerobic nitrogen fixation could later be explained to result from ammonia oxidation there would be no more objection to assume that nitrogen fixation takes place purely reductively in both aerobic and anaerobic conditions. At the present moment, oxime formation cannot be explained without by-hypotheses in this way.

The difference between the mechanisms of aerobic and anaerobic nitrogen fixation is suggested also by the fact that gaseous hydrogen prevents aerobic but probably not anaerobic nitrogen fixation because H_2 is formed in the fermentation of sugar by *Cl. butyricum*.

SUMMARY

In the anaerobic nitrogen fixation by *Clostridium butyricum* and by another bacterium of the same type, no traces of oxime nitrogen could be found. In the aerobic nitrogen fixation by *Azotobacter* oxime nitrogen is always formed. Theoretical conclusions of the course of nitrogen fixation and of the formation of oxime nitrogen are drawn on the basis of this finding.

Clostridium does not form oxime nitrogen even in low oxygen tension. When the atmosphere contained 0.7 vol. % O_2 and 99.3 vol. % N_2 *Clostridium* still grew well but no traces of oxime-N could be detected.

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Received August 5, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1050–1057

A New Aldehyde Synthesis

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Pure metal hydrides have hitherto not been used as reducing agents, but a mixed hydride, *i. e.* lithium aluminium hydride [1,2] has been used for the preparation of alcohols from organic acid halides with good results. Reduction is obtained at room temperature, and the yields are good, about 80—90 %.

The purpose of this paper is to examine whether it is possible to use metal hydrides for the preparation of aldehydes from organic acid halides, acid anhydrides or thiolesters. This method of preparation will be of special interest in the preparation of unsaturated aldehydes, otherwise so difficult to obtain, as the metal hydrides, in all propability, will not react with the double bond, since lithium aluminium hydride, which is a much stronger reducing agent than the pure hydrides, usually has no effect on the double bond.

The purpose was to examine the reaction with hydrides of lithium, sodium and calcium. It has been shown that only lithium hydride gives good results.

The reaction between acid halides or the other substances used and lithium hydride takes place as follows:

$R-CO-X$	$+ LiH$	$\rightarrow$	$R-CHO$	$+ LiX$	(I)
$(C_2H_5-CO)_2O$	$+ LiH$	$\rightarrow$	C_2H_5-CHO	$+ C_2H_5-COOLi$	(II)
$C_6H_5-CO-S-C_2H_5$	$+ LiH$	$\rightarrow$	C_6H_5-CHO	$+ C_2H_5-S-Li$	(III)
$CH_2Cl-COOH$	$+ LiH$	$\rightarrow$	CH_3-COOH	$+ LiCl$	(IV)

where R is an organic radical (aliphatic or aromatic) and X a chlorine or bromine atom.

As lithium hydride cannot be dissolved in any medium, the reaction is necessarily heterogeneous. The reduction is carried out as follows:

A few hundred milligrams of the organic substance which is to be reduced is mixed in a 50 ml flask with about half a gram of lidhium hydride and

10 ml benzene, toluene or xylene. The mixture is refluxed for some hours, and, after cooling to room temperature, is transferred to a 50 ml bottle through a filter. The best method for the determination of the aldehyde formed was found to be precipitation with methone. To the aldehyde solution in the bottle, 10 ml water, 4 ml of a 10 % sodium chloride solution, and 3 ml of a 5 % alcoholic methone solution is added. The bottle is now vigorously shaken for 12 hours, whereafter the content is poured quantitatively into a small beaker. This beaker is now left at a temperature of 30—40° C until the organic medium has completely volatilized. After filtering, the methone precipitate is washed with a small volume of 50 % alcohol and dried for four hours in a vacuum desiccator over calcium chloride, and is then weighed.

A number of experiments have been carried out with the chlorides, bromides, anhydrides and thiolesters of propionic and benzoic acid as typical examples of respectively aliphatic and aromatic substances.

The yield of aliphatic aldehydes by this method is not good when using acid halides (about 20—25 %). Much better yields are obtained, however, by using the corresponding thiolesters (about 70 %). When using aromatic substances, good yields are obtained by direct use of acid halides (about 55 %).

As to the estimation of the aldehyde formed it can be mentioned that the error is less than 1 %.

EXPERIMENTAL

Preparation of lithium hydride

As lithium hydride was not commercially available, it was necessary to prepare it. Stock reagents of sodium and calcium hydride were used.

The preparation of lithium hydride is a difficult task, as the reaction between lithium and hydrogen is very vigorous and difficult to control. The hydride is obtained by passing a hydrogen current over molten lithium in a nickel boat placed in a steel tube, which, in turn, is placed inside a combustion tube of fused quartz. The pressure must not be more than 550 mm Hg, as it is otherwise impossible to control the reaction. On the other hand, the pressure must not be less than 450 mm Hg, as the metallic lithium tends to volatilize and spread into the whole tube. The temperature must also be kept under control, as the reaction starts very suddenly at about 520—530° C. The preparation of lithium hydride has been previously described by Bode [3], but when carried out as mentioned in the present paper lithium hydride is obtained as a fine powder, very pure, and in a yield of about 95 %.

Determination of aldehydes in hydrophobic solvents

The quantity of aldehyde formed in the hydrophobic medium was very difficult to determine.

First the determination of the aldehyde by titration with a sodium bisulphite solution was tried, but this method was inadequate, as the error was about ± 10 %.

The method of allowing the aldehyde formed to react with 'Girard's reagent' *i. e.* a betaine hydrazide solution [4] was tried. The hydrazone formed is very soluble in water, but insoluble in benzene etc., and therefore dissolves quantitatively in the aqueous phase by shaking. The latter is separated, and a solution of bismuth iodide or mercuric iodide in potassium iodide is added, whereby a complex — insoluble in water — is formed by interaction between a betaine hydrazone ion and a BiI_4^- ion or a HgI_3^- ion. The complex can be weighed directly without recrystallization.

It was shown that when using propionic aldehyde no precipitation with mercuric iodide takes place, whereas addition of potassium bismuth iodide yields a precipitate. On the other hand, mercuric as well as bismuth complex solutions gives a precipitate with benzaldehyde. The estimations were not satisfactory. The amount of aldehyde present could only be determined with an error of ± 10 %.

The method previously mentioned, depending on the interaction between methone (5,5-dimethyl-dihydro-resorcinol) and aldehydes [5–7], has been investigated. The first to investigate this method was Vorländer [5], and it has turned out that it is possible to modify the method so that the error in the estimation of aldehyde is less than 1 %.

Procedure: To 5 ml of the aldehyde solution was added 5 ml of a 10 % sodium chloride solution, 10 ml of water, and 3 ml of a 5 % alcoholic methone solution. (Sodium chloride, according to Vorländer, facilitates the crystallization of the methone aldehyde complex.) The mixture was shaken for 12 hours, and then transferred to a small beaker. The organic solvent volatilizes when the beaker is heated to not more than 40° C. After cooling to room temperature the solution was filtered through an immersion filter tube. The precipitate was washed with 50 % alcohol and was dried over calcium chloride in a vacuum desiccator. The solid residue could now be weighed directly. Recrystallization did not change the m. p.

This method always yields low results, but there is a constant deviation from the true value. More than 100 analyses, with varying quantities of aldehyde (propionic and benzaldehyde) and various organic solvents (benzene, toluene or xylene) in all cases, gave results 11—12 % too low. This method is the best available to determine the quantity of aldehyde, if the found value is increased by 12 %.

In order to find out why the results are low, some experiments were carried out in which the organic liquids were volatilized at various temperatures from a mixture containing a known quantity of the pure substance formed by interaction of methone and propionic aldehyde. It was found, that if the temperature was higher than 50° C, a considerable amount of the substance disappeared together with the vapour. At room temperature, the volatilization of the solvent was extremely slow, even after a week, only half of it had vaporized. At temperatures between 30—40° C, the volatilization took place fairly quickly, and there was no detectable loss of the substance.

A probable reason for the low results might be that the aldehyde methone substance is not quantitatively formed by the interaction between the methone and the aldehyde.

Reaction with hydrides

An attempt was made, at first, to allow lithium hydride to act on propionyl chloride (prepared from propionic acid and silicontetrachloride [12]) in ether. The mixture was shaken for 24 hours at room temperature. As no detectable quantity of aldehyde was formed, the experiment was repeated, with vigorous shaking for 72 hours. No positive

result was obtained in this way either, so heating the mixture for several hours in a waterbath was tried, still without obtaining positive results.

The reaction temperature was now increased by using benzene, toluene or xylene instead of ether, and considerable quantities of aldehyde were formed. The yields after boiling 20 hours, when using benzene as solvent, were 13 %, toluene, 23 %, and xylene, 2 %.

It was evident that a reaction really had taken place between the lithium hydride and the propionyl chloride according to equation I, and now the entire question was to find the best conditions for the reaction.

A number of experiments were now made to find the best reaction conditions. In most cases 0.3 g of the organic substance, 0.5 g hydride, and 10 ml of the medium were used. Some experiments were carried out with a somewhat larger quantity, *i. e.* 0.75 g of the organic substance, 1.0 g hydride, and 20 ml of the solvent.

It was necessary to distill the acyl bromides immediately before the experiments, as otherwise an oil was formed at a later stage in the procedure. The organic solvents (benzene, toluene and xylene) were distilled over metallic sodium.

DISCUSSION

In Tables 1, 2, and 3 are given the experimental results. It can be seen from the tables that the experiments with sodium and calcium hydride in no cases yield good results, while lithium hydride in several cases yields remarkably good results. Better results cannot be obtained by using larger amounts of the reactants.

In Table 1 the experiments with lithium hydride, acyl halides, anhydrides and thiolesters are shown.

The best results from both propionyl chloride and bromide are obtained under the same conditions, namely by the use of toluene and allowing the mixture to boil for 20 hours.

The boiling time of the mixture has of course a certain effect, but not as much as would be expected. In a few experiments, where the mixture was boiled for more than 20 hours, it was impossible to determine the aldehyde formed, because during the very prolonged boiling a decomposition took place, which prevented the methone precipitate from crystallizing. At the same time the mixture became very dark.

As expected when using benzoyl chloride, a higher reaction temperature gives higher yields. When using benzene or toluene, a prolongation of the time of reaction gives a higher yield. On the other hand, when using xylene as medium, the yield is not increased. It seems as if this method is especially useful for the preparation of aromatic aldehydes, as the boiling points of the corresponding acyl halides are so high that the reaction temperature can be increased so much that the reaction can be completed in the course of a few hours with a yield of about 55 %.

Table 1. Reaction between lithium hydride and different organic substances.

Amount of organic substance		(b. p.)	Medium	Hours of boiling	Yield
0.30 g	Propionyl chloride	80° C	Benzene	4	0.5 %
0.75 »	»	»	»	20	13 »
» »	»	»	Toluene	4	13 »
0.30 »	»	»	»	4	17 »
» »	»	»	»	20	18 »
0.75 »	»	»	»	20	24 »
0.30 »	»	»	Xylene	4	1.7 »
» »	»	»	»	4	2.2 »
» »	Propionyl bromide	104° C	Benzene	4	0.5 »
» »	»	»	»	20	0.9 »
» »	»	»	Toluene	4	12 »
» »	»	»	»	20	24 »
0.75 »	»	»	»	20	26 »
0.30 »	»	»	Xylene	4	2.7 »
» »	»	»	»	20	3.7 »
» »	Benzoyl chloride	197° C	Benzene	4	1.0 »
» »	»	»	»	20	10.5 »
» »	»	»	Toluene	4	4.2 »
» »	»	»	»	20	26 »
0.75 »	»	»	»	20	32 »
0.30 »	»	»	Xylene	2	59 »
0.75 »	»	»	»	4	47 »
0.30 »	»	»	»	5	57 »
0.75 »	»	»	»	20	56 »
0.30 »	»	»	»	20	52 »
» »	Propionic anhydride	196° C	Benzene	4	1.5 »
» »	»	»	Toluene	4	3.8 »
» »	»	»	»	20	3.3 »
» »	»	»	Xylene	4	4.4 »
» »	»	»	»	20	4.9 »
0.75 »	»	»	»	4	5.1 »
0.30 »	Ethyl thiolbenzoate	252° C	Benzene	4	39 »
» »	»	»	»	20	69 »
» »	»	»	Toluene	4	44 »
» »	»	»	»	20	49 »
0.75 »	»	»	»	20	51 »
0.30 »	»	»	Xylene	4	43 »
» »	»	»	»	20	44 »

Some experiments with benzoyl bromide have also been carried out, but it was impossible to determine the quantity of aldehyde formed, as an oil, which did not crystallize, was formed from the reaction with methone. From

Table 2. Reaction between sodium and calcium hydride and different organic substances.

Hydride	Amount of organic substance		Medium	Hours of boiling	Yield
NaH	0.30 g	Propionyl chloride	Benzene	20	1.2 %
»	» »	» »	Toluene	20	1.3 »
CaH_2	» »	» »	Benzene	20	0.5 »
»	» »	» »	Toluene	20	1.0 »
NaH	» »	Propionyl bromide	»	4	0.5 »
»	» »	» »	»	20	0.7 »
CaH_2	» »	» »	»	4	0.5 »
»	» »	» »	»	20	0.6 »
NaH	» »	Benzoyl chloride	»	20	1.3 »
»	» »	» »	Xylene	4	1.4 »
»	» »	» »	»	20	1.6 »
CaH_2	» »	» »	»	20	1.1 »
NaH	» »	Ethyl thiolbenzoate	Toluene	20	0.6 »
»	» »	» »	Xylene	20	0.4 »
CaH_2	» »	» »	Toluene	4	0.3 »
»	» »	» »	»	20	0.2 »
»	» »	» »	Xylene	20	0.4 »

the quantity of oil formed one could get an idea of the degree of the reaction and the author presumes that the results are very near the corresponding results obtained when using benzoyl chloride.

As to the experiments with propionic anhydride the yields are very poor; only a very small amount of aldehyde was formed.

The thiolester of benzoic acid was prepared from benzoyl chloride and lead mercaptide in dry ether [8]. As seen from the table, the yields are good. The best (68 %) was obtained by boiling for 20 hours with benzene.

In Table 2 are given the results when using sodium and calcium hydride. Contrary to expectation, the yields in all cases were very low even the experiments with the thiolester gave poor yields. Better results were expected in this case, since the thiolester is very easily transformed into the corresponding aldehyde (by boiling with Raney nickel in 80 % alcohol, a large quantity of aldehyde is formed [9]).

In Table 3 some special experiments are shown. Fumaryl chloride was prepared from maleic anhydride and phthalyl chloride [10]. Fumaryl bromide [11] was prepared by passing dry hydrogen bromide through fumaryl chloride at 120° C. That a reaction really took place between fumaryl chloride (fumaryl bromide) and lithium hydride, could in this case be seen quite clearly, as the solution, after about twenty minutes of boiling, became intensely yellow. The

colour comes from fumaraldehyde (possibly from malealdehyde). The formation of an aldehyde was shown by means of the reaction with phenylhydrazin, as it was not possible to use methone in this case.

A few experiments have also been made, in which monochloroacetic acid was used. After five hours boiling in xylene, about 5 % of the chlorine atoms had been replaced by hydrogen atoms. Free chloride ions were formed by this reaction and they were determined by titration with a 0.1 *N* silver nitrate solution.

Table 3. Reaction between lithium hydride and different substances.

Substance	Medium	Hours of boiling	'Yield'
Fumaryl chloride	Benzene	4	Yellow colour
» »	»	6	» »
Fumaryl bromide	»	4	» »
» »	»	6	» »
Monochloroacetic acid	Xylene	4	4.0 %
» »	»	6	7.0 »

SUMMARY

It has been shown that it is possible to prepare aldehydes from acid halides by reduction with lithium hydride. The reaction gives the best results with aromatic acid halides (approx. 55 % yields). When using aliphatic acid halides the yields are not higher than 25 %, but in some cases the yield can be increased by transforming the acid halide to the corresponding thiolester, which can very easily be reduced by lithium hydride to the aldehyde stage. In this way the total yield, in proportion to the acid halide, will closely approximate that obtained with the aromatic substances.

The author wishes to thank Dr. Phil. K. A. Jensen and Mag. Scient. N. Clauson-Kaas for their interest, advice and helpful discussions during the course of this work and also *Lauritz Andersens Fond* for financial support.

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Received August 26, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1058–1066

The Micro-determination of Water *

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The determination of water content is, in general, no problem with substances that can be heated to over 100° C. On the other hand, substances that decompose upon heating cause difficulty. The procedure generally used for such substances is drying over phosphoric anhydride in a vacuum. In many cases, however, such a moisture determination may take many days. In addition, if there are other volatile substances than water in the sample, both methods are inapplicable. In such cases the Karl Fischer method [1] has found use. It has been adapted by Levy, Murtaugh and Rosenblatt [2] for use in a micro-scale for the determination of water in *e. g.* penicillin. In work with such small amounts of water, the Karl Fischer reagent's greatest disadvantage, its hygroscopic nature, is greatly pronounced. The method, previously suggested by the author [3], in which the Karl Fischer reagent is divided into two negligibly hygroscopic solutions **, should be especially well suited to micro-determinations. In this method, the sample is dissolved or suspended in a solution of sulphur dioxide in pyridine-methanol and titrated with a methanol solution of iodine. In macro-scale these titrations can be carried out in open vessels with negligable error. In micro-determinations, however, the preparation must be accomplished in such a manner that the moisture in the air does not affect the results. In order to achieve still greater accuracy, the method has been further modified so that the hydrogen iodide, formed by reduction of the iodine, is converted into iodic acid by means of bromine. The iodic acid

* This manuscript was completed in October 1947, and submitted to the Royal Institute of Technology, Stockholm.

** Recently, Seaman, McComas, Jr. and Allen [9] have published a paper in which they, with the present author's previous publication [3] as a starting point, propose a procedure for the determination of water. The present author wants to point out, however, that this procedure in all essential points is identical with that previously described [3].

is then determined in the usual way by adding potassium iodide to the acidified solution and titrating the liberated iodine with thiosulfate. For every mole of water 12 equivalents of iodine are formed, or, in other words, 1.5 mg water corresponds to 10 ml 0.1 *N* thiosulfate. This procedure has the advantage that the moisture in the air has to be excluded only during the addition of iodine, while the actual titration can be made in a water solution.

It was, however, necessary first to examine the stoichiometry of the Karl Fischer titration. Fischer, himself, expressed the reaction as:

$$2\ H_2O + I_2 + SO_2 = H_2SO_4 + 2\ HI$$

while Smith, Bryant and Mitchell Jr [4] maintain that sulfuric acid is not formed, but the reaction proceeds as follows:

$$H_2O + I_2 + SO_2 + CH_3OH = 2\ HI + CH_3O \cdot SO_3H$$

In the first case, one mole of iodine consumes two moles of water, while, in the second case, only one mole.

Smith, Bryant and Mitchell Jr., as well as Almy, Griffin and Wilcox [5], who have also studied this problem, have not, in practice, been able to come closer to the theoretical value than a ratio of 1 mole of iodine to 0.7 mole water, due to the occurence of side reactions during the storage of the reagent. Furthermore, part of the discrepancy from the theoretical value is due to the fact that water is taken up from the air. Exactly how much is taken up is difficult to determine by the original Karl Fischer method, and therefore the author's previously mentioned method should give more accurate results, as it reduces, to a large extent, the amount of water absorbed. It is impossible to obtain completely waterfree solutions, but their actual water content can easily be calculated from the water content of the reagents used.

In these experiments two solutions were used. One consisted of a solution of 100 g sulfur dioxide in a mixture of 500 ml pyridine and 500 ml methanol, and the other of 30 g iodine in 1000 ml methanol. The iodine had been dried in a dessicator over phosphoric anhydride. From the water content of the methanol before mixing, the iodine solution was calculated to contain 0.111 mg water per ml solution. The titer of the iodine solution was determined as follows: 20.00 ml of iodine solution was added to a solution of potassium iodide and titrated with 45.23 ml of 0.1047 *N* $Na_2S_2O_3$ solution. The iodine solution was therefore 0.2368 *N*. A confirmatory titration with 15.01 ml iodine solution consumed 33.93 ml of the $Na_2S_2O_3$ solution, wich gave an iodine normality of 0.2366 *N*. The average value was therefore 0.2367 *N*.

Iodine solution was added from a burette to 10 ml of the sulfur dioxide solution, until the color changed from yellow to brown, that is, until the water in the sulfur dioxide solution was consumed. After the addition of 0.0536 g water, 26.80 ml of iodine solution

were needed to cause the same color change. Since the iodine solution contained 0.111 mg of water per ml, a total of 0.0566 g of water had been added. The calculated water content, from the iodine consumption, was 0.0571 g. This was calculated on the basis of two equivalents of iodine per mole water, which is in agreement with Smith, Bryant and Mitchell Jr's formel *.

In a similar test using 4.00 ml sulfur dioxide solution and a 0.0522 g water sample, 26.10 ml of the iodine solution was consumed, which corresponds to a water content of 0.0557 g as compared to the actual content of 0.0551 g. The small differences between the calculated and the actual water contents are probably due to the fact that the iodine also contained some moisture and that moisture is taken up by the solutions during mixing.

The titrated solution was diluted with water and neutralized with 99.10 ml of 0.1023 *N* sodium hydroxide solution, using thymolphtalein as an indicator. A blank, using 4.00 ml sulfur dioxide solution, to which iodine had been added to color change, consumed 68.85 ml of the same sodium hydroxide solution. The difference of 30.25 ml was equivalent to 0.0558 g water.

From these experiments it can be seen that the stoichiometry of the Karl Fischer titration is well defined. In order to increase the accuracy in the micro-determination of water, the hydrogen iodide formed is oxidized, as said before, to iodic acid, which is then determined in the usual manner. Naturally, at the same time, the iodine, which was used to convert the water in the sulfur dioxide solution, is also oxidized, yielding a relatively large and considerably varying amount of iodic acid. In order to eliminate this disadvantage a bromine solution can be used to consume this water instead of the iodine solution. It has been found that the bromine first reacts with the water in the same manner as the iodine, but later it also reacts with the sulfur dioxide pyridine solution. An excess of bromine is consequently not detrimental. On the other hand it is impossible to observe when all the water has been consumed. There is no color change, and even the dead-stop method is inapplicable. If, however, a few micro-drops of iodine solution are added first and then the bromine solution, the hydrogen iodide is oxidized to iodine, when all water has reacted, and the end point can be observed.

Apparatus

Foulk and Bawden's dead-stop method was used to indicate the endpoints. This procedure is based on the fact that if an electrical potential is placed between two electrodes in a solution, in this case in a pyridine and methanol solution of sulphur dioxide, a counter electromotive force is built up, which

* After the completion of this manuscript, Seaman, McComas, Jr. and Allen [9] have reported the same results concerning the stoichiometry of the reaction.

balances the original potential, providing this is small enough. In a water solution this potential should be 10—15 millivolts, but in the Karl Fischer titration it is possible to go up to a value of 0.3 to 0.4 volts and to use a very simple apparatus (see Fig. 1 in (3)). When iodine is added in excess the electrodes are depolarized and the galvanometer is deflected. The titrating vessel consists of a 10 ml flask with two platinum electrodes fused into the glass. Immediately under the neck of the flask there is a side arm containing the sample. This sidearm may be closed with a ground glass stopper. The flask, itself, is equipped with a ground glass stopper having a 1 mm capillary (see D in Fig. 1). The iodine solution is added through the capillary by means of a glass injection syringe with a platinum needle. The syringe should be graduated in 0.1 ml.

Solutions

1) *Sulphur dioxide*, 100 g, is introduced into a mixture of 500 ml pyridine and 500 ml methanol. All chemicals should be as free of water as possible. The methanol is dehydrated in the usual manner with magnesium, and the sulphur dioxide is dried with silica gel. Water-free pyridine is prepared from a sample of known water content by addition of a little more than the calculated amount of acetyl chloride. The precipitate is removed by filtration through a glass filter. The acetic acid formed does not interfere, nor does the dehydroacetic acid formed from the excess acetyl chloride.

2) *Iodine*, 30 g, which has been dried in a desiccator over phosphoric anhydride, is dissolved in absolute methanol and diluted to 1000 ml.

3) *Bromine*, 0.2 ml, is dissolved in 10 ml absolute methanol.

Procedure

One ml of solution 1) is placed in the titration flask and two micro-drops of iodine solution are added. The sample is weighed in a little tube or any other suitable vessel and placed in the side arm of the flask. Both stoppers are inserted and the platinum electrodes connected to the dead-stop apparatus. The bromine solution is added through the capillary tube by means of a pipette having a capillary point, until the galvanometer gives a constant reading. The flask must be shaken constantly since the galvanometer otherwise returns to zero.

In this manner, all water in solution 1) and all water that may have adhered to the walls of the flask is removed. The sample is then added by inclining the flask. Soluble samples are titrated immediately with solution 2) which is added by means of an injection syringe until the galvanometer again deflects. Insoluble samples should be in a finely divided state so that the water can be

easily extracted. The syringe is read to the nearest 0.1 ml and the content of the titration flask is poured into a 300 ml flask and diluted with water to about 150 ml. Then 5 g sodium acetate and 0.25 ml bromine are added and the solution is strongly shaken until the bromine color remains permanent. The excess bromine is removed with a few drops of formic acid. The precipitate of pyridine bromides reacts very slowly with the formic acid. When the solution has become colorless, the remaining red precipitate, which contains only a very small amount of iodine, may be filtered off. One gram of potassium iodide is added to the filtrate, which is acidified and titrated with 0.1 N thiosulfate solution.

Calculations

H_2O (mg) = 1.501 $A \cdot N - B \cdot W$
A = ml thiosulfate
N = normality of thiosulfate
B = ml iodine solution
W = water content of iodine solution in mg/ml.

The water content of the iodine solution may, as mentioned above, be obtained from the water content of the methanol used. Since, however, the water content of the iodine solution may change during storage, the actual value may be determined in the following manner: Iodine solution is added to 5 ml sulfur dioxide solution until a brown color is obtained and then 20 ml methanol of known water content (about 0.2 %) are added. More iodine solution is added until another color change is observed. The titer of the iodine solution is determined by titration with 0.1 N thiosulfate. The water content of the iodine solution is then obtained as the difference between the amount of water added and the amount of water calculated from the titer of the iodine solution.

As can be seen from the above, it is not necessary to know the titer of the iodine solution. The accuracy of the procedure, however, depends on the exactness with which the iodine can be added to cause the change in potential. One drop from an injection syringe can be made very small, about 0.005 ml, depending upon how fine a needle is used. This volume corresponds to about 0.01 mg water. It is scarcely possible by this method to obtain a greater accuracy. As can be seen from the table below, the errors are generally somewhat larger.

Table 1. Micro-titration of water.

Substance	Added amount mg	Added amount mg H_2O	Found mg H_2O	Difference
H_2O	3.92	3.92	3.88	− 0.04
»	4.36	4.36	4.34	− 0.02
»	2.80	2.80	2.82	+ 0.02
NaOAc, $3H_2O$	8.31	3.30	3.26	− 0.04
» »	1.94	0.77	0.80	+ 0.03
$3CdSO_4$, $8H_2O$	2.67	0.50	0.51	+ 0.01

Example

Two micro-drops of iodine solution 2) were added to 1.0 ml of solution 1) and then bromine solution 3) was added until the potential changed, 0.2 ml were used. Water, 4.36 mg, weighed in a capillary tube, was introduced from the side arm of the flask. In reobtaining the change 2.30 ml of iodine solution were consumed. The contents of the flask were then treated as described above and titrated with 30.80 ml 0.1006 *N* sodium thiosulfate solution. This value corresponded to 4.65 mg water (30.80 · 0.1006 · 1.501). Since the water content of the iodine solution was 0.135 mg/ml, the found amount of water was 4.65 − 0.31 = 4.34 mg.

The water content of the iodine solution was determined as follows: 10.00 ml of the solution, added to 10 ml of water containing a few grams of potassium iodide, consumed 23.42 ml 0.1006 *N* sodium thiosulfate. The normality of the solution was therefore 0.2356 *N*. For the titration of 20.00 ml methanol containing 56.94 mg water (2.847 mg/ml) 28.65 ml of the iodine solution were consumed. The water content of this solution was, therefore, as follows:

$$\frac{28.65 \cdot 9.008 \cdot 0.2356 - 56.94}{28.65} = 0.135 \text{ mg/ml}$$

AUTOMATIC TITRATION

As was stated before, the accuracy of the determination is completely dependent upon the exactness with which the amount of the iodine solution equivalent to the water content can be added. In order to facilitate this addition of iodine an apparatus was constructed in which the plunger in the syringe was operated by a motor. Furthermore, the dead-stop apparatus was so arranged that it stopped the supply of iodine solution after all water was consumed.

Description of apparatus

The worm-gear motor A (see Fig. 1), which together with the screw B operates the plunger in the syringe, is equipped with a double wound armature, so it can rotate in both directions. This was used to make the motor to stop instantaneously. If one electrical circuit is left in the system and the other is closed, the motor stops much more rapidly, than if all current is switched off

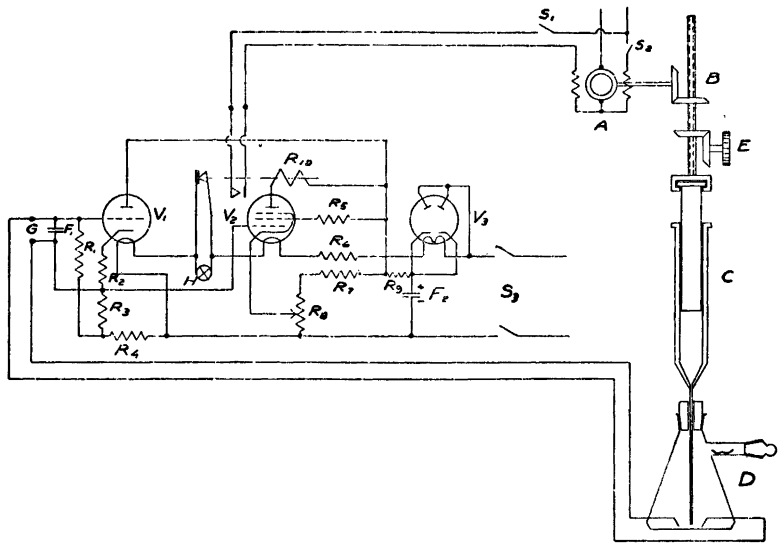

Fig. 1. Apparatus for automatic titration.

A.	*Motor*	R_4.	*Resistance 2500 ohm*
B.	*Plunge operator*	R_5.	» *20 000 ohm*
C.	*Injection syringe*	R_6.	» *500 ohm wire wound*
D.	*Titrating vessel*	R_7.	» *4000* » » »
E.	*Wheel for manual screw operation*	R_8.	» *150* » » »
F_1.	*Condensor 0.1 micro F*	R_0.	*Relay with 400 ohm resistance coil*
F_2.	*Electrolytic condensor 8 micro F*	V_1.	*Tube 6F5*
G.	*Connection contacts*	V_2.	» *25L6*
H.	*Lamp 6V, 0.3A*	V_3.	» *25Z6*
R_1.	*Resistance 100 000 ohm*	S_{1-3}	*Switches*
R_2,R_3,R_9	» *1000 ohm*		

from the armature. By this method there is only a movement of a few degrees after the circuit has been closed. The screw, which has a one mm thread, rotates at a rate of 19 r. p. m. The screw can be uncoupled from the motor and manually operated with the wheel (E), to facilitate the filling of the syringe

The left side of the diagram consists mainly of an electronic relay, *cf* [7] A potential of approximately 0.2 volts exists between the electrodes in the titration vessel as long as all the water has not been consumed. However, as soon as there is an excess of iodine, and the electrodes depolarize, the negative grid potential decreases in the tube V_1. The resulting increase in the plate current in the same tube causes an increased voltage drop in the resistances R_3 and R_4. This voltage drop causes a decreased negative grid

potential in the tube V_2 and thus an increased plate current, which causes the armature of the relay to close the motor circuit. The relay is of the ordinary telephone type with two contacts. One contact closes one of the circuits of the motor and the other opens the short circuit at the lamp H.

Procedure

Titration with this apparatus can be accomplished in the following manner. One ml of sulphur dioxide solution is placed in the flask D and the electrodes are connected to G. Both switches S_1 and S_2 should be open. Two micro-drops of iodine solution are added and then bromine solution until the lamp H lights. The sample is introduced, whereby the relay moves and the light goes out. The needle of the syringe is inserted through the capillary tube into the titration vessel and the switches S_1 and S_2 are closed. The syringe should be inserted far enough so that the needle enters the solution. The motor then forces out the iodine solution as long as the relay is disconnected. After all water has been consumed the relay engages and the motor stops. This generally tends to occur first, just before the end-point is reached, due to a local excess of iodine. However, after this has been consumed the motor starts again. This generally occurs a few times before the motor stops definitely at the true endpoint. For determination of water in substances that are insoluble in the titrating liquids, it is convenient to use magnetic stirring and to let the apparatus add the iodine solution as the water is extracted from the sample. To prevent the syringe from leaking it is good to moisture the plunger with a drop of iodine solution. When the motor has stopped definitely, the contents of the vessel are removed and treated in the aforementioned manner.

By use of the automatic procedure it is possible to omit the determination of iodine by oxidation to iodic acid. The volume of iodine solution used can be obtained with great accuracy from the scale on the screw, which moves the plunger in the syringe. As was said before, each thread on the screw is 1 mm. Furthermore, the screw is provided with a scale, which makes it possible to read parts of a revolution. A suitable syringe volume is approximately two milliliters, which, in the syringe used by the author, corresponded to about 40 mm. Each revolution of the screw is therefore equivalent to 0.05 ml, and if twentieth parts of a revolution are read a satisfactorily accurate determination of volume will be obtained. The calibration may be accomplished by weight-delivery experiments or by determination of the plunger's area [8]. The diameter of the plunger should be measured in several directions, and an average value taken. The volumes calculated from these two methods coinside quite well.

The titration can, therefore, be accomplished in the following manner: One ml of sulfur dioxide solution is introduced into the titration vessel, whereafter the iodine solution is added until the relay makes contact. The scale is then read and the sample introduced. The titration is continued until the motor stops, and then the scale is read again. The iodine solution is standardized by titration of a weighed amount of water or measured volume of methanol of known water content. This standardization is conveniently accomplished in a macro-scale.

SUMMARY

A method for the micro-determination of water with a modified Karl Fischer method is proposed. The hydrogen iodide formed by this reaction is oxidized by bromine to iodic acid, which is determined in the usual way by titration, in water solution, with sodium thiosulfate solution. For every mole of water, twelve equivalents of iodine are formed, *i. e.* 1.5 mg water corresponds to 10 ml 0.1 *N* thiosulfate.

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Received August 31, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1067–1076

On the Complex Chemistry of the Uranyl Ion

III. The Complexity of Uranyl Thiocyanate An Extinctiometric Investigation

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In the preceding paper of this series (Ahrland [1], in the following referred to as II) it was shown that the complexity of uranyl salts may be correctly determined extinctiometrically as well as potentiometrically. This was very valuable as many uranyl salts cannot be investigated by anyone of the well-proved potentiometric methods which are in use (for a survey of these, see Fronaeus [2]). On the other hand, the salts are coloured and therefore possible to measure extinctiometrically.

Unfortunately, the extinctiometric method has a very serious limitation: it is impossible to use as soon as polynuclear complexes exist in the solutions. Moreover, the criterion of the existence of such complexes, stated in II, p. 803, is not very reliable. So a polynuclear complex formation may be easily overlooked and 'constants' of no physical meaning calculated. A more reliable criterion is required and the following one, pointed out by Güntelberg [3, p. 76] permits no doubt a surer decision between the existing possibilities.

Güntelberg recommends that measurements should be carried out at several wave-lengths. If the same result is obtained in all cases, it is reliable. The reason is very clearly seen from the formula of Fronaeus[2, p. 90]: while β_1 is independent of the wave-length when the complex formation is mononuclear ($\varepsilon_1' = 0$), the false 'constant' obtained in the presence of dinuclear complexes depends upon the value of the ratio $\varepsilon_1'/\varepsilon_1$; ε_1' and ε_1 being the molar extinctions of the first dinuclear and the first mononuclear complex respectively. The value of this ratio is likely to be changed at a sufficient great change of the wave-length and hence the 'constant' changes, too. In such a way the false 'constants' may be unveiled. One also concludes, however, that the wave-

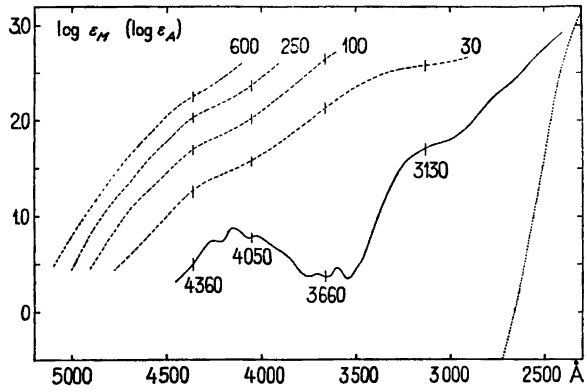

Fig. 1. Extinctions curves of a) uranyl ion (fulldrawn), b) complex solutions with C_M = 33.1 mC, C_A = 30, 100, 250 and 600 mC (dashed) and c) thiocyanate ion (dotted; the molar extinction in this case is $\varepsilon_A = \frac{E}{C_A \cdot d}$).

lengths for measuring must be choosen in such a distance from each other that a perceptible change of the proportion between the ε:s really is likely to occur. Preferably the wave-lengths should be choosen in different bands of the spectrum. In practice, however, the possibilities of a choice are, as a rule, rather restricted.

The present investigation aims to determine the complexity of the uranyl thiocyanate system merely according to the extinctiometric method. The apparatus and the mode of calculation used have been described in II. The calculations are, however, somewhat modified because of the particular properties of the system in question. The modifications will be closer described below.

The solutions measured all contained 100 mC $HClO_4$; from the hydrolysis measurements of the first paper of this series (Ahrland [4], in the following referred to as I) it can be seen that the hydrolysis of UO_2^{2+} may be completely neglected at such a high acidity. The fundamental equations (21) and (22) of II are thus valid here without the somewhat uncertain hydrolysis corrections discussed in II. p. 805. A complex formation between H^+ and SCN^- is not to be feared, as thiocyanic acid belongs to the strongest acids known. This has been proved conductometrically by Ostwald [5] and, recently, potentiometrically by Gorman and Connell [6].

As before, all solutions had the constant ionic strength $I = 1$. $NaClO_4$ was used as the supplementary neutral salt. The determinations were made at 20° C.

Table 1. Direct determined values of $\varepsilon_M - \varepsilon_0$ at given C_A and C_M.

Table 1 A: $\lambda_2 = 4360$ Å.

$d \rightarrow$ cm	0.1		0.3		1		3		10	
C_A mC	C_M mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$	C_M mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$	C_M mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$	C_M mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$	C_M mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$
10					104.5	3.87	28.01	5.22	7.83	5.83
20					60.2	8.98	16.73	10.75	4.90	11.53
30					39.13	14.57	11.74	16.35	3.46	16.70
50			104.4	18.63	24.95	24.92	7.78	26.85		
75			67.0	31.10	16.73	37.49	5.41	39.39		
100			50.4	42.9	13.41	48.9	4.22	50.5		
150			33.73	64.7	9.66	70.2	3.122	71.8		
200	96.0	69.5	26.78	83.7	7.65	88.4				
300	65.4	104.5	19.70	116.5	5.69	121.6				
400	50.9	135.1	15.83	145.1	4.63	149.7				

The *sodium thiocyanate puriss.* used was recrystallised from water by the same method as was mentioned for $NaClO_4$ in I, p. 382. Dried at 150°. The salt was free from Cl^- when tested according to Mann [7]. Analysis according to Volhard gave the equivalent weight 81.3, calc. 81.1. — The *other chemicals* were the same as before.

To select the wave-lengths λ most suitable for the measurements with the accurate light-electric apparatus, extinction curves of solutions with $C_M = 33.1$ mC and $C_A = 30$, 100, 250 and 600 mC were spectrographically determined. In Fig. 1, these curves are compared with that of UO_2^{2+}, obtained from I. The positions of the strong mercury lines which serve as light sources in the light-electric apparatus are also indicated.

Table 1 B. $\lambda_1 = 3660$ Å.

$d \rightarrow$ cm	0.1		0.3		1	
C_A mC	C_M mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$	C_M mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$	C_M mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$
20	95.1	70.8	24.70	93.8	6.73	102.5
30	56.4	121.4	16.08	144.7	4.57	152.0
50	32.16	220.8	9.92	242.9	2.947	249.0

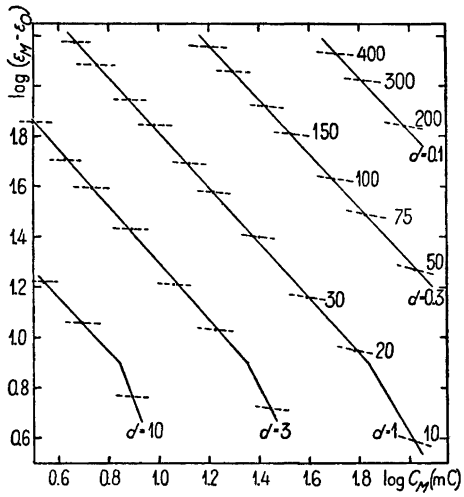

Fig. 2. Log($\varepsilon_M - \varepsilon_0$) as a function of log C_M at different d; λ_2 = 4360 Å. — ●: experimentally determined points. — Full-drawn curves: the established connections between $\varepsilon_M - \varepsilon_0$ and C_M for given d. — Dashed curves: position of points with constant C_A, given to the right.

It can be seen that the effect of the complex formation is especially great in the region of the mercury line λ_1 = 3660 Å. This line was thus selected for measurements, though ε_M of the complex solutions are so high here that C_A cannot be increased over 50 mC in the measurements below. Still one other suitable mercury line should be selected and evidently at lower ε_M, *i. e.* longer

Table 2. $\varepsilon_M - \varepsilon_0$ at given C_A, corrected so as to fit those connections which are established between $\varepsilon_M - \varepsilon_0$ and C_M.

λ→ Å	4360					3660		
d→ cm	0.1	0.3	1	3	10	0.1	0.3	1
C_A mC	$\varepsilon_M - \varepsilon_0$ $C^{-1} \cdot cm^{-1}$							
10			3.87	5.22	5.83			
20			8.93	10.72	11.53	71.1	93.8	102.5
30			14.52	16.33	16.70	121.1	144.7	152.0
50		18.49	25.00	26.92		220.8	242.9	249.0
75		31.12	37.58	39.36				
100		43.05	49.0	50.5				
150		64.7	70.3	71.8				
200	69.5	83.7	88.4					
300	104.5	116.5	121.6					
400	135.1	144.9	149.7					

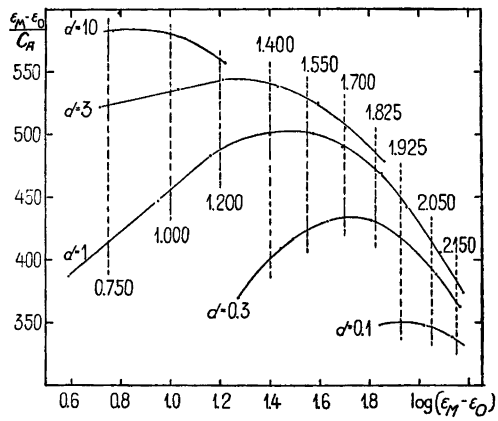

Fig. 3. $(\varepsilon_M - \varepsilon_0)/C_A$ as a function of log $(\varepsilon_M - \varepsilon_0)$ at different d; $\lambda_2 = 4360$ Å. — The curves are cut at ten log $(\varepsilon_M - \varepsilon_0)$, each of them representing a certain constant pair of $(\bar{n}, [A])$.

wave-lengths. The line $\lambda_2 = 4360$ Å was choosen because ε_M has here a suitable size so that C_A may be varied within a wide range; at the same time, the line is fairly distant from 3660 Å.

The extinction curve of the thiocyanate ion was also determined and is given in Fig. 1. In the whole the result agrees with that obtained by v. Kiss and Csokan [8] for potassium thiocyanate. However, at very small ε_A their curve is situated higher than the present one, undoubtedly due to traces of impurities in their salt used. One can see, that the extinction of the thiocyanate ion does not at all affect the measurements at the selected λ.

The thiocyanate curve is of a type characteristic of the halogenides; it rises in the ultra-violet parallel to and between the curves of bromide and iodide (about these, see Fromherz and Menschick [9]). Kept in a glass-stoppered bottle, the 1.00 C sodium thiocyanate solution was completely stable, as judged by the fact that quite the same extinction curve was obtained after one year of storage.

After suitable λ had been selected, the measurements with the light-electric apparatus were carried out. To begin with, the molar extinctions of the uranyl ion ε_0 were determined. At 4360 Å, two solutions were measured: $C_M = 72.8$ mC with $d = 3$ cm and $C_M = 22.14$ mC with $d = 10$ cm. ε_0 was found to be 3.068 and 3.061 respectively; mean 3.065 $C^{-1} \cdot cm^{-1}$, a spread of $\pm$ 1 ‰. Thus Beers law is strictly valid in this case. At 3 660 Å, $C_M = 30.99$ mC was measured with $d = 10$ cm. The value found was $\varepsilon_0 = 2.28$ $C^{-1} \cdot cm^{-1}$.

Then the determinations of ε_M at varying C_A were performed. Here the solutions measured prove to be not at all sensitive to the day-light, what is contrary to the behaviour of chloroacetate (II, p. 804), and organic uranyl salts in general (Gmelin [10]). The extinctions were always reproducible within

Table 3. C_A as a function of C_M at the selected constant values of $\log(\varepsilon_M - \varepsilon_0)$.

Table 3 A. $\lambda_2 = 4360$ Å.

$d\rightarrow$ cm	0.1		0.3		1		3		10	
$\log(\varepsilon_M - \varepsilon_0)$	C_A mC	C_M mC	C_A mC	C_M mC	C_A mC	C_M mC	C_A mC	C_M mC	C_A mC	C_M mC
0.750					13.58	84.9	10.73	27.1	9.65	7.9
1.000					21.93	56.2	18.69	18.7	17.21	5.6
1.200					32.55	36.6	29.14	12.3	28.30	3.7
1.400			62.6	81.3	50.2	23.9	46.4	8.1		
1.550			83.7	58.9	70.8	17.5	67.2	5.9		
1.700			115.5	42.8	102.1	12.7	98.9	4.3		
1.825			155.1	32.7	141.3	9.8	137.8	3.4		
1.925	239.7	79.8	200.8	26.5	187.0	8.0				
2.050	324	60.7	286	20.5	271	6.2				
2.150	422	48.8	386	16.7	369	5.0				

2—3‰ when a solution was prepared anew. Such a control was made for one third of the solutions.

The determined corresponding values of $\varepsilon_M - \varepsilon_0$, C_A and C_M for λ_2 and λ_1 are given in Table 1 A and 1 B. In Fig. 2, the function $\log(\varepsilon_M - \varepsilon_0) = f(\log C_M)$ at $\lambda_2 = 4360$ Å is given for the different values of the parameter d used. Those straight lines are drawn which represent a known connection between the quantities $\varepsilon_M - \varepsilon_0$ and C_M. In the present case, three of the five lines must be broken in order to fit the lowest experimental points as well as it is required. Also for $\lambda_1 = 3660$ Å, such connections are established. From

Table 3 B. $\lambda_1 = 3660$ Å.

$d\rightarrow$ cm	0.1		0.3		1	
$\log(\varepsilon_M - \varepsilon_0)$	C_A mC	C_M mC	C_A mC	C_M mC	C_A mC	C_M mC
1.950	23.60	76.0	19.08	25.7	17.33	7.6
2.150	34.09	49.0	29.35	16.6	27.81	5.0
2.350	50.7	31.5	46.1	10.7	44.8	3.2

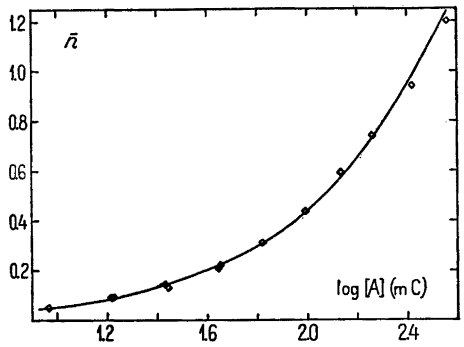

Fig. 4. The complex formation function. — ◇ and ◆: values determined at $\lambda_2 = 4360$ Å and $\lambda_1 = 3660$ Å respectively. — The curve is obtained from the complexity constants finally calculated.

the points of intersection between those lines and the lines of constant C_A (dashed in Fig. 2) new values of $\varepsilon_M - \varepsilon_0$ are obtained which fit the established connection between $\varepsilon_M - \varepsilon_0$ and C_M at the d in question (Table 2). So we have now obtained $\varepsilon_M - \varepsilon_0$ as a function of C_A at different d, with C_M known for every point.

These functions should now be cut at a number of constant $\varepsilon_M - \varepsilon_0$ which each represents a certain constant value of $\bar{n}$ and [A]. To perform these sections with great accuracy at a moderate size of the figure, it is in the present case convenient to transform the functions into

$$\frac{\varepsilon_M - \varepsilon_0}{C_A} = f(\log(\varepsilon_M - \varepsilon_0)) \qquad (1)$$

Table 4. The corresponding values of [A], $\bar{n}$ and $\bar{n}/[A]$ obtained from the figures of Table 3.

$\lambda_2 = 4360$ Å				$\lambda_1 = 3660$ Å			
$\log(\varepsilon_M - \varepsilon_0)$	[A] mC	$\bar{n}$	$\bar{n}/[A]$ C^{-1}	$\log(\varepsilon_M - \varepsilon_0)$	[A] mC	$\bar{n}$	$\bar{n}/[A]$ C^{-1}
0.750	9.29	0.0515	5.55				
1.000	16.8	0.093	5.55	1.950	16.7	0.092	5.5
1.200	27.7	0.130	4.7	2.150	27.1	0.144	5.3
1.400	44.8	0.220	4.9	2.350	44.0	0.208	4.75
1.550	65.4	0.31	4.75				
1.700	96.8	0.435	4.5				
1.825	135.7	0.595	4.4				
1.925	181	0.74	4.1				
2.050	265	0.94	3.55				
2.150	364	1.20	3.3				

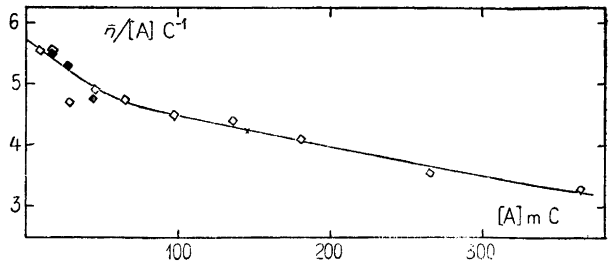

Fig. 5. $\bar{n}/[A]$ *as a function of* $[A]$, *the integration of which gives* $X([A])$ *(Table 5). — The signs relate to the same measurings as in Fig. 4; but the curve is here drawn according to the experimental points.*

The functions so obtained are given for $\lambda_2 = 4360$ Å in Fig. 3. They are cut at ten constant $\log(\varepsilon_M - \varepsilon_0)$, each representing a certain C_A originally used. The corresponding functions of $\lambda_1 = 3660$ Å are cut at three $\log(\varepsilon_M - \varepsilon_0)$. From the points of intersection C_A is obtained and from Fig. 2 one gets the corresponding value of C_M. The results are found in Table 3 A and 3 B. When the pairs of (C_A, C_M) so found are plotted in a diagram, straight lines are obtained within the limits of experimental error. No sign of polynuclear complex formation shows by use of this first criterion, which, however, was

Table 5. $X([A])$, $X_1([A])$ *and* $X_2([A])$ *for given* $[A]$, *giving the complexity constants. — The ligand number and the composition of the system as calculated from these constants.*

$\beta_1 = 5.7 \pm 0.3$ C^{-1} $\quad \beta_2 = 5.5 \pm 1$ C^{-2} $\quad \beta_3 = 15 \pm 5$ C^{-3}

[A] mC	(5a)* $\ln X([A])$	$X([A])$	(7a) $X_1([A])$ C^{-1}	(7b) $X_2([A])$ C^{-2}	(2) $\bar{n}$	(8a) a_0 %	(8b) a_1 %	(8b) a_2 %	(8b) a_3 %
10	0.0559	1.058	5.8						
20	0.1100	1.116	5.8		0.106	89.5	10.5	0	0
30	0.1624	1.176	5.9						
50	0.2629	1.301	6.0	6	0.245	77	22	1	0
75	0.3819	1.465	6.2	6.5					
100	0.4958	1.642	6.4	7	0.44	61	34.5	3.5	1
150	0.7128	2.040	6.9	8					
200	0.9175	2.503	7.5	9	0.785	40	46	9	5
250	1.1102	3.035	8.1	9.5					
350	1.4611	4.311	9.5	11	1.22	23	46.5	15.5	15

* These figures refer to the formulas of II.

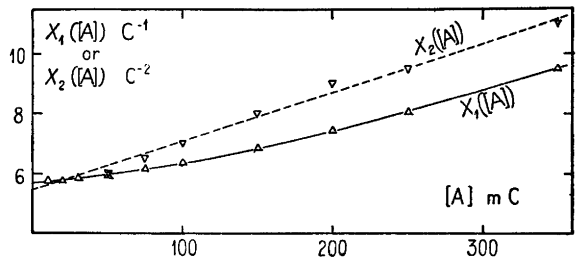

Fig. 6. $X_1([A])$ and X_2 $([A])$ which give the complexity constants.

not very reliable, as was mentioned above. The straight lines have the intercept on the C_A-axis = [A] and the slope = $\bar{n}$, Table 4. The points are further plotted in Fig. 4. It is immediately seen from the complex formation curve so obtained, that the points from the two λ used are situated entirely on the same curve, and thus give the same complexity constants. The criterion of Güntelberg thus also speaks in favour of a mononuclear complex formation.

We may therefore consider the complex formation curve determined as substantial and calculate the constants from it. So the function $\bar{n}/[A] = f([A])$ is formed, Fig. 5, and hence the function $X([A])$ is obtained by integration according to (5 a) of II. From $X([A])$, $X_1([A])$ and $X_2([A])$ are formed according to (7) of II (Table 5) and plotted in Fig. 6. From $X_1([A])$ we obtain $\beta_1 = 5.7 \pm 0.3$ and from $X_2([A])$, which is a straight line, $\beta_2 = 5.5 \pm 1$ and $\beta_3 = 15 \pm 5$, with indicated accidental errors.

With these constants, $\bar{n}$ is calculated at some round [A] according to (2) of II (Table 5) and introduced in Fig. 4 as a fulldrawn curve. It is seen to fit the experimental points very well. The composition of the system at the same [A] is calculated according to (8) of II and also given in Table 5.

The complexity of the uranyl thiocyanate system is thus very weak. This is perhaps a little unexpected as the extinction curve of UO_2^{2+} changes in such a substantial manner by addition of thiocyanate. It is now evident that this remarcable change must be due to an unusually great difference of ε between UO_2^{2+} and its thiocyanate complexes. Even small quantities of these complexes therefore cause great changes in ε_M. The result urges to the greatest caution at qualitative estimations of the stability of complexes on the basis of extinctiometric measurements.

SUMMARY

The complexity of the uranyl thiocyanate system in aqueous solution is extinctiometrically investigated. The measurements are performed at 20° C and at the ionic strength $I = 1$, which is brought about by $NaClO_4$ and 100

mC $HClO_4$. The latter presses the hydrolysis of UO_2^{2+} back to a value which may be neglected.

According to both the criteria applied, no polynuclear complexes seem to exist in this system. The first three complexes of the mononuclear series are proved. Their constants, defined by (6) of I, are at the existing conditions

$$\beta_1 = 5.7 \qquad \beta_2 = 5.5 \qquad \beta_3 = 15$$

My thanks are due to *Försvarets Forskningsanstalt* (FOA), Stockholm, for a financial support.

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Received June 9, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1077–1081

Kinetic Investigations into the Metal Catalysed Autoxidation of Methyl Linoleate

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It has long been known that the oxygen absorption by linseed and other drying oils on exposure to air and the drying process caused by this absorption is accelerated by certain metal salts (so-called siccatives). Among the most important of these salts for practical use are ones involving cobalt, lead and manganese. However, the kinetics of these reactions have been studied only during the last three decades, and then with partly contradictory results. Eibner and Pallauf [1] found that the drying time for linseed oil, reduced with a constant, was inversely proportional to the siccative concentration. Bowles [2] found in a research upon cobalt-siccativated stand oils that the drying time was inversely proportional to the square root of the cobalt concentration, and Lund [3] arrived at similar results in researches upon cobalt and manganese siccatives in linseed oil.

Apart from some experiments on the weight increase on exposure to air of thin oil films on glass (so-called Weger curves) and some introductory oxygen absorption measurements by Lund [3], the earlier investigations were limited to researches upon the relationship between the metal concentration and drying time as determined in a more or less subjective manner. Hereby the drying process was allowed to proceed to a far advanced stage, when the primary phase, the autoxidation, had been followed to a great extent by the secondary one, involving the formation of a tackfree gel of makromolecules.

Our researches have especially aimed to try to establish the reaction mechanism of the metal catalysed autoxidation. It was thereby considered to be necessary to work with a compound of a less complicated composition than linseed oil. As such a compound methyl linoleate was chosen.

The autoxidation has been followed by measuring the oxygen absorption at constant pressure and temperature (25° C) in an apparatus constructed for

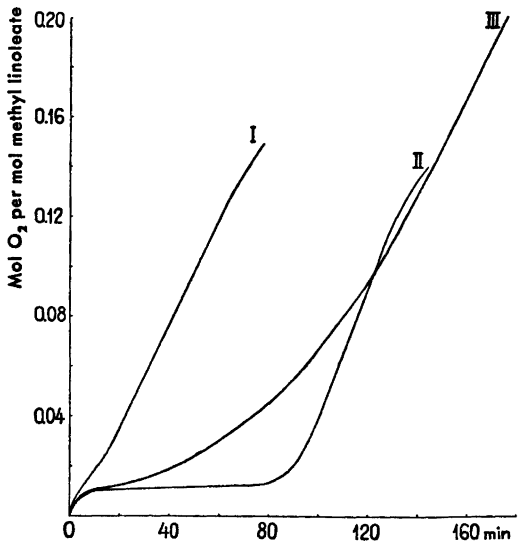

Fig. 1. The oxygen absorption of methyl linoleate as a function of the reaction time in the presence of

I. $[Co] = 2.88 \cdot 10^{-6}$ *mol per g*
II. $[Mn] = 3.09 \cdot 10^{-6}$ » » »
III. $[Pb] = 10.85 \cdot 10^{-6}$ » » »

this purpose and which is essentially similar to the Warburg apparatus. The metal catalysts studied were cobalt, lead and manganese salts of α-ethyl caproic acid. These salts are used as siccatives in the paint industry under the name octoates.

Methyl linoleate from two quite different sources was used and gave identical results. Through special experiments it was demonstrated that the vibration velocity of the reaction vessel (240 strokes/min) was sufficient to insure that the diffusion of oxygen into the reaction mixture would not influence the results.

Our experiments have proved that the reaction velocity, determined by the oxygen absorption per minute, after a variable induction period reaches a value that is constant within the experimental errors (Fig. 1). This constant reaction velocity depends on the metal concentration (Fig. 2) and on the methyl linoleate concentration (Fig. 3) but only slightly on the oxygen pressure within the studied range ($\frac{1}{2}$—1 atm). Through determinations of the peroxide content in the reaction mixture at the end of some experimental series, it has been demonstrated that the absorbed oxygen, calculated from the absorption measurements, is found almost quantitatively as peroxide.

It immediately appears from Fig. 2 that the reaction velocity cannot be proportional to the metal concentration. A mathematical analysis of the present values also shows that proportionality between the velocity and the

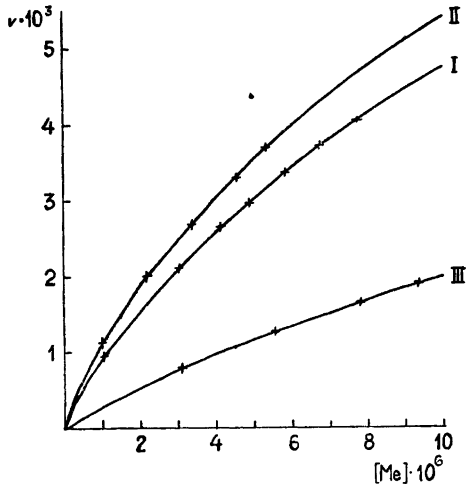

Fig. 2. The dependence of the reaction velocity on the metal concentration in undiluted methyl linoleate.

I. Cobalt
II. Manganese
III. Lead

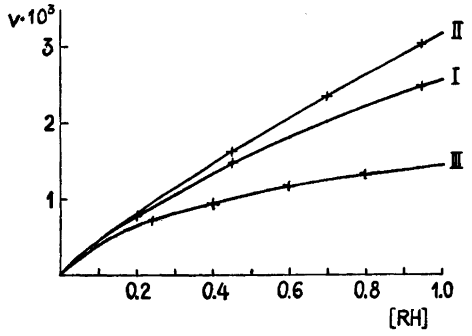

Fig. 3. The dependence of the reaction velocity on the methyl linoleate concentration.

I. $[Co] = 3.8 \cdot 10^{-6}$
II. $[Mn] = 4.0 \cdot 10^{-6}$
III. $[Pb] = 5.8 \cdot 10^{-6}$
$[RH]$ = *the methyl linoleate concentration in parts by weight.*

square root of the metal concentration does not entirely agree with the experimental data. The analysis indicates a parabolic function

$$v^2 + \alpha \cdot v = \beta \cdot [\mathrm{Me}]$$

where v = the oxygen absorption velocity (mol 0_2/mol linoleate and min)
[Me] = the metal concentration (mol/g reaction mixture)
α and β are two factors primarily dependent upon the methyl linoleate concentration, but to a less extent probably also upon the oxygen and peroxide concentrations, but independent of [Me].

It should be observed that lead, in contrast to what is often stated, also catalyses the primary oxygen absorption.

Through researches by Farmer, Bolland, Bergström, and many others it can be regarded as proved that the first comprehensible reaction product in the autoxidation of organic compounds with one or more isolated double bonds is a hydroperoxide with the atom group —OOH at a single carbon atom

and with the double bonds still intact. Bolland and Gee [4] have also made it probable that the reaction follows a chain mechanism with free radicals as chain carriers.

It is fairly reasonable to assume that the metal catalysed autoxidation follows a similar chain mechanism. However, the chain carriers are here probably addition products between the metal and the methyl linoleate and probably with radical character. The following reaction scheme has proved to be able to explain the above-mentioned formula

1. $Me + ROOH \rightarrow MeO_2 + ?$
2. $MeO_2 + RH \rightarrow RMeO_2$
3. $RMeO_2 + RH \rightarrow ROOH + RMe$
4. $RMe + O_2 \rightarrow RMeO_2$
5. $2\ RMeO_2 \rightarrow 2Me +$ inactive end product
6. $RMe + RMeO_2 \rightarrow 2\ Me +$ inactive end product
7. $2\ RMe \rightarrow 2\ Me +$ inactive end product

where

RH symbolizes methyl linoleate
R symbolizes methyl linoleate that has lost one methylenic hydrogen atom
ROOH symbolizes methyl linoleate hydroperoxide

To get theory and experiments to coincide, the metal should be present at the initiation reactions 1. and 2. in an activated state, the concentration of which is but a small portion of the total metal concentration. The reactions 3. and 4. represent the propagation reactions with $RMeO_2$ and RMe as chain carriers. Among the chain termination reactions (5., 6. and 7.) 5. is under the present conditions, high oxygen concentration, so predominant that 6. and 7. can be neglected. The initiation reaction 1. explains the observered induction period.

The researches will be continued in different directions. A full report of the process catalysed by cobalt, lead or manganese will appear within a short time.

SUMMARY

The autoxidation of methyl linoleate in the presence of cobalt, manganese and lead compounds has been examined by measuring the oxygen absorption velocity. It has been shown that these metals catalyse the autoxidation, but the influence of lead is less than that of the others. The autoxidation velocity depends on the metal concentration, the methyl linoleate concentration and — to a less extent — the oxygen pressure. From the experimental data it seems probable that the reaction follows a chain mechanism similar to the one

which prevails at the uncatalysed autoxidation. However, the chain carriers are probably addition products, with radical character, between the metal and the methyl linoleate.

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Received July 7, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1082–1086

Zur Definition der Amidzahl und über Amidzahl-Bestimmungen von Verbindungen mit flüchtiger Basenkomponente und von einigen Phenylurethanen

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Unlängst wurde ein Verfahren zur Bestimmung des Fettsäure- und Basenanteiles in Fettsäureamid-Verbindungen beschrieben[1], das auf der vollständigen Spaltbarkeit derartiger Verbindungen durch Alkali bei erhöhter Temperatur beruht. Im Gegensatz zu der üblichen Verseifungszahl-Bestimmung von Estern, die sich durch $N/2$ methylalkoholische Kalilauge spalten lassen und bei der das überschüssige Alkali zurücktitriert wird, misst man nach dem Verfahren der Amidzahlbestimmung (Verseifung mit äthylenglykolischer Kalilauge!) den Alkalitätszuwachs *, der bei der Verseifung einer Fettsäureamid-Verbindung durch die in Freiheit gesetzte Base entsteht. Die Methode war ursprünglich aus dem Wunsche heraus entstanden, Amidverbindungen höherer Carbonsäuren, die z. T. technisches Interesse besitzen, einer einfachen analytischen Bestimmung zugänglich zu machen. Durch die vorliegende Untersuchung sollte festgestellt werden, ob das Verfahren auch auf kompliziertere Verbindungen übertragen werden kann; in diesem Falle wäre nämlich oft das zeitraubende Aufschlussverfahren nach Kjeldahl zu vermeiden oder in Kombination mit diesem, z. B. in Fragen der Konstitutionsermittlung, Amidstickstoff unter Umständen gegen Gesamtstickstoff in einfacher Weise analytisch abzugrenzen. Über die Möglichkeit zu einer solchen analytischen Kombination lässt zwar das bisher vorliegende Versuchsmaterial eine begründete Aussage noch nicht zu. Mit Sicherheit hat sich jedoch ergeben, dass die Me-

* In speziellen Fällen kann man durch Anwendung eines geeigneten Indikators oder nach dem Abdestillieren der entsprechenden Base, wie bei der Verseifungszahl-Bestimmung, auch das »Seifenalkali» bzw. die »Alkalitätsabnahme» bestimmen, was zu dem gleichen Resultat führen muss.

thode nicht allein auf Fettsäureamidverbindungen, sondern auf eine ganze Reihe anderer chemisch, pharmakologisch und physiologisch wichtiger Verbindungen anwendbar ist.

Bei dieser Erweiterung des Anwendungsbereiches der Methode wäre es unzweckmässig, an der bisherigen Definition des Begriffes »Amidzahl» festzuhalten. Nach der bisherigen Definition gab diese an, wieviele Milligramme Kaliumhydroxyd zur Verseifung der in einem Gramm Substanz enthaltenen Fettsäureamidverbindungen nötig sind. Da, wie erwähnt, nach dem Verfahren der Alkalitätszuwachs bestimmt wird, der durch die in Freiheit gesetzte Base entsteht, würde die Amidzahl nach der bisherigen Definition in dem Augenblick zu einer rechnerischen Grösse werden, wo diese Base mit zwei oder mehreren Säuregruppen in Amidbindung stand, z. B. im Acetimid, Phthalimid, N,N'-Diacyl-äthanolamid u. s. w. In diesen Fällen sind nämlich zur Spaltung von einem Molekül der Amid- bzw. Imidverbindung zwei Moleküle Kaliumhydroxyd erforderlich, während nur ein Molekül einer einsäurigen Base (in den genannten Verbindungen Ammoniak oder Äthanolamin) entsteht. Um nun andere Zusatzdefinitionen, wie z. B. »Imidzahl» o. Ä., zu vermeiden und dem bisher eingeführten Begriff »Amidzahl» den Charakter einer universellen Kennzahl zu geben, wird vorgeschlagen, in allen Fällen nur den der freiwerdenden Base entsprechenden Alkalitätszuwachs, ausgedrückt in Milligrammen Kaliumhydroxyd *, pro Gramm Substanz einer Definition zu Grunde zu legen, wobei es gleichgültig sein soll, welche Base bei der Spaltung frei wird und wieviele Milligramme Kaliumhydroxyd zur Verseifung tatsächlich erforderlich waren. In dieser Weise definiert, gäbe die Amidzahl an, wieviele Milligramme Kaliumhydroxyd der bei der Spaltung von einem Gramm Substanz entstehenden Base äquivalent sind. Die theoretische Amidzahl für Phthalimid errechnet sich beispielsweise nach folgender Überlegung: Bei der alkalischen Verseifung von 1 Mol = 147,1 g Phthalimid entsteht 1 Mol Ammoniak. Dieses ist äquivalent 1 Mol = 56104 mg Kaliumhydroxyd:

$$\text{Amidzahl} = \frac{56104}{147{,}1} = 381{,}4 \text{ (bisher: } \frac{2 \cdot 56104}{147{,}1} = 762{,}8\text{).}$$

* In Anlehnung an die von der Union internationale de chimie 1948 herausgegebenen *Méthodes unifiées pour l'analyse des matières grasses.* — Anstatt auf mg KOH zu beziehen, könnte man auch — für den Fall einer einheitlichen Regelung — (in Übereinstimmung mit der Internationalen Konvention betr. einheitlicher Bezeichnungsweisen für nahrungsmittelchemische Analysen v. 27. 6. 1910 (Paris)) den Alkalitätszuwachs in ml 1 *N*-Lösung ausdrücken und, bezogen auf 100 g Substanz, als Amidzahl bezeichnen.

Bei der Analyse von Amidverbindungen sind je nach der Natur der bei der alkalischen Verseifung freiwerdenden Base folgende Fälle zu unterscheiden: 1. Die Base ist leichtflüchtig (z. B. Ammoniak, Methylamin, Äthylamin). 2. Die Base ist schwerflüchtig, u. U. wasserdampfflüchtig (z. B. Anilin). 3. Es entsteht ein Gemisch von Basen, die sich evtl. in der Flüchtigkeit unterscheiden. 4. Die »Base» ist nicht flüchtig (z. B. Salz einer Aminosäure). — Nachdem durch die Beschreibung der Analyse von Fettsäureäthanolamiden und Acetanilid bereits Beispiele für Verbindungen mit schwerflüchtiger Basenkomponente angeführt worden sind (*l. c.*), haben wir jetzt unter Mitwirkung einer Gruppe von 25 Pharmaziestudenten hauptsächlich den unter 1. genannten Fall experimentell bearbeitet. Zur Untersuchung gelangten Säureamide, Säureimide, Benzylcyanid, Harnstoff und dessen nächste Abkömmlinge, Alloxan, Barbitursäurederivate, Urethane und Kaffein. — Für den Fall 2. haben wir jedoch versucht, die Amidzahl einiger Phenylurethane (freiwerdende Base Anilin!), die bekanntlich zur Charakterisierung von Alkoholen ausgedehnte Anwendung finden, zu bestimmen, um in dieser Kennzahl, die ohne weiteres in Prozent Stickstoff umgerechnet werden kann, eine leicht erhältliche charakteristische Konstante der Phenylurethane von Alkoholen unbekannter Konstitution zu gewinnen. In allen Fällen wurden, wie in der Tabelle 1 gezeigt werden soll, befriedigende Resultate erhalten.

Hervorgehoben sei jedoch die Tatsache, dass für das Kaffein und das Kaffeincitrat wesentlich höhere Amidzahlen gefunden wurden als der Theorie nach Massgabe der vorhandenen echten Amidbindungen entsprechen. Die anomalen Amidzahlen stehen übrigens in Übereinstimmung mit den Feststellungen früherer Autoren, die bei der Alkalieinwirkung auf Kaffein unter den Spaltungsprodukten neben Methylamin auch Ammoniak, also einen weitergehenden Abbau, nachgewiesen hatten. Hier kam es nur darauf an, sinnfällig zu machen, dass die Amidzahlmethode u. U. nicht nur echte Amidbindungen indiziert, was bei Konstitutionsuntersuchungen zu berücksichtigen ist.

EXPERIMENTELLER TEIL

(Unter Mitarbeit von Reidar Lie)

Die Bestimmung der Amidzahl kann, je nach der Natur der zu analysierenden Verbindung, in verschiedener Weise erfolgen. Handelt es sich um eine Verbindung mit flüchtiger Basenkomponente, so wird man diese zweckmässig in einer gewöhnlichen Kjeldahl-Apparatur abdestillieren, in eingestellter Säure absorbieren und für sich titrieren. Bei schwerflüchtiger Basenkomponente verseift man am besten mit etwa 1*N*-äthylenglykolischer Kalilauge unter Rückflusskühlung und titriert den Alkalitätszuwachs (gegenüber einem Blindversuch) direkt im Verseifungsgemisch. Ist die schwerflüchtige Base wasserdampfflüchtig, so kann die Bestimmung in der Kjeldahl-Apparatur in der Weise vorgenommen werden, dass man im Anschluss an die Verseifung die Base nach Zusatz

Tabelle 1. Amidzahl-Bestimmungen von verschiedenen Amidverbindungen.

Nr.	Substanz	Mol. Gew.	Einw. g	Verbr. 0,1 *N* HCl ml	Gesamt-Stickstoff %	»Flüchtiger bzw. titrierbarer Stickstoff» in %		Amidzahl		Beobachter
						Theor.	Gef.	Theor.	Gef.	
1	Acetamid	59,1	0,4992	84,12	23,71	23,71	23,61	949,3	945,4	Boge
2	Benzamid	121,1	1,0072	82,98	11,57	11,57	11,54	463,3	462,2	Paulsen
3	Phenylacetamid	135,2	1,1118	80,48	10,36	10,36	10,14	415,0	406,1	Kjærvik
4	Heptansäureamid	129,2	0,3735	28,88	10,84	10,84	10,83	434,2	433,8	Bjerkevåg
5	Salicylamid	137,1	0,4546	32,29	10,22	10,22	9,95	409,2	398,5	Nötsund
6	Thiobenzamid	137,2	0,8006	53,76	10,21	10,21	9.40	408,9	376,7	Soetorp
7	Succinamid	116,1	0,4932	80,36	24,13	24,13	22,83	1005,4	914,1	Flåtten
8	Oxamid	88,1	0,3554	80,45	31,80	31,80	31,71	1273,6	1270,0	Reimers
9	Phthalamid	164,2	0,7361	89,58	17,06	17,06	17,05	683,4	682,8	Bjerkevåg
10	Succinimid	99,1	0,7818	79,58	14,14	14,14	14,26	566,1	571,1	Steihaug
11	N-Bromsuccinimid	178,0	1,4690	72,21	7,87	7,87	6,89	315,2	275,8	Balke
12	Pthalimid	147,1	0,4735	32,11	9,52	9,52	9,50	381,4	380,5	Balke
13	Benzylcyanid	117,1	0,9595	68,19	11,96	11,96	9,96	479,1	398,7	Saevik
14	Milchsäurenitril	71,1	0,6557	81,71	19,70	19,70	17,46	789,1	699,1	Soetorp
15	Harnstoff	60,1	0,2420	79,46	46,62	46,62	46,00	1867,2	1842,2	Bergane
16	Thioharnstoff	76,1	0,3008	78,64	36,82	36,82	36,63	1474,5	1466,8	Jenssen
17	Nitroharnstoff	105,1	0,3914	38,00	40,19	13,33	13,60	533,8	544,7	Ytre-Arne
18	Guanidincarbonat	180,2	0,1430	47,39	46,64	46,64	46,43	1868,1	1859,3	Kjærvik
19	Nitroguanidin	104,1	0,4023	77,65	54,02	26,92	27,05	1077,9	1082,9	Reimers
20	Biuret	103,1	0.2654	75,08	40,78	40,78	39,64	1632,5	1587,1	Solheim
21	Dicyandiamid	84,1	0,1816	85,04	66,62	66,62	65,60	2668,4	2627,2	Munkhaug
22	Alloxan (verunr.)	142,1	0,5713	68,64	19,72	19,72	16,83	789,6	674,1	Sunde
23	»Prominal»	246,3	0,8561	69,20	11,38	11,38	11,32	455,6	453,5	Nestås
24	»Bromural»	223,1	0,7973	70,97	12,56	12,56	12,47	502,9	499,4	Sunde
25	»Veronal»	184,2	0,8006	85,40	15,21	15,21	14,94	609,2	598,5	Drottning
26	»Aethallynal»	196,2	0,7748	74,84	14,28	14,28	13,53	571,9	541,9	Nötsund
27	»Luminal»	232,2	0,9272	79,54	12,07	12,07	12,02	483,2	481,3	Solheim
28	Urethan	89,1	0,7028	78,57	15,72	15,72	15,66	629,7	627,2	Nesjan
29	N-Methylurethan	103,1	0,8686	80,70	13,59	13,59	13,02	544,2	521,3	Munkhaug
30	Kaffein	194,2	0,3507	60,12	28,85	7,22	24,02	288,9	961,8	Nestås
		194,2	0,3495	59,00	28,85	7,22	23,65	288,9	947,1	»
		194,2	0,3479	59,52	28,85	7,22	23,97	288,9	959,8	»
31	Kaffeincitrat	386,3	0,7607	63,09	14,50	3,63	11,62	145,2	465,3	Roalkvam
32	Trimethylenglykol-bis-phenylurethan	314,3	1,1930	76,1	8,92	8,92	8,94	357,0	357,9	Lie
33	Phenylurethan des 3-Oxytetrahydrofurans	207,2	0,7083	35,4	6,76	6,76	7,00	270,8	280,4	»
		207,2	0,7890	37,2	6,76	6,76	6,61	270,8	264,5	»
		207,2	0,6550	32,0	6,76	6,76	6,84	270,8	274,1	»
34	Carbanilid	212.2	1,1286	104,60	13,20	13,20	12,98	528,8	520,0	

einer hinreichenden Menge Wasser mit Wasserdämpfen überdestilliert und für sich bestimmt. Dieses Vorgehen dürfte bei stark gefärbtem Verseifungsgemisch, das die Erkennung des Indikatorumschlages erschwert, in Betracht kommen. Entstehen bei der Verseifung einer Substanz zwei durch ihre Flüchtigkeit unterschiedene Basen, kann es von Vorteil sein, die eine abzudestillieren und für sich zu titrieren, die andere im Verseifungsgemisch direkt zu bestimmen. Die in diesem Falle notwendigen Veränderungen der Apparatur ergeben sich von selbst.

In der vorliegenden Arbeit wurden von allen Verbindungen, bei deren Verseifung Ammoniak oder eine andere flüchtige Base entsteht, passende Mengen in einer üblichen Kjeldahl-Apparatur mit 50—75 ml starker äthylenglykolischer Kalilauge * durch 2—3-stündiges Kochen verseift und das dabei übergehende Destillat in 100 ml 0,1 *N* Salzsäure absorbiert. Am Schluss der Destillation wurden in den Verseifungskolben 15 ml Wasser eingetragen und dieses zum Ausspülen der Apparatur mit in die Vorlage überdestilliert. Der Inhalt der Vorlage wurde dann mit 0,1 *N* Natronlauge gegen Methylrot titriert. Beim Kaffeincitrat wurde zu Beginn des Versuches ein Kryställchen Kupfersulfat hinzugefügt. — Die Phenylurethane kochte man mit 50 ml 1 *N*-äthylenglykolischer Kalilauge 2 Stunden am Rückflusskühler gleichzeitig mit einem Blindversuch. Nach Spülen des Kühlers und Schliffes mit 10 ml neutralem Alkohol titrierte man gegen Thymolblau mit *N*/2 Salzsäure bis zum Umschlag Gelb-Rot (bestimmter Farbton!). — Bei den verwendeten Substanzen handelte es sich im allgemeinen um reine Laboratoriumspräparate, die jedoch z. T. älteren Datums waren; sie wurden ohne vorheriges Trocknen zur Analyse verwendet. — In der Tabelle 1. wurden diese Substanzen nach laufenden Nummern aufgeführt unter Angabe des Molekulargewichtes und der für jede Bestimmung eingewogenen Menge in Gramm. In den folgenden Rubriken wurde der Verbrauch an 0,1 *N* Salzsäure in Millilitern, der Gesamtstickstoff und der als Base titrierbare Stickstoff in Prozenten, letzterer unter der Rubrik »Flüchtiger bzw. titrierbarer Stickstoff», angegeben. Die beiden letzten Spalten enthalten die betreffenden, nach der neuen Definition berechneten Amidzahlen und die Namen der entsprechenden Beobachter.

ZUSAMMENFASSUNG

Die Amidzahlen bzw. der Stickstoffgehalt einer ganzen Reihe chemisch, pharmakologisch und physiologisch wichtiger Amidverbindungen lassen sich nach dem früher nur für substituierte Fettsäureamid-Verbindungen angegebenen Verfahren titrimetrisch bestimmen. Im Hinblick auf den vergrösserten Anwendungsbereich der Methode wurde eine neue Definition für die »Amidzahl» vorgeschlagen.

Den Herren Professoren A. Jermstad, Bj. Samdahl und Herrn Dr. A. Wickström sind wir für die liebenswürdige Überlassung von Präparaten aus der Sammlung des Pharmazeutischen Institutes zu Dank verpflichtet.

LITERATUR

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Eingegangen am 9. August 1949.

* 300 g Kaliumhydroxyd wurden in 200 ml Wasser gelöst und die Lösung mit Äthylenglykol zu einem Liter aufgefüllt. — Diese Lösung greift in der Hitze Glas sehr stark an; es wird daher empfohlen, eine weniger konzentrierte Lösung zu verwenden.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1087–1093

β-Carbobenzoxylation of DL-αβ-Diaminopropionic Acid and some Acylated Derivatives

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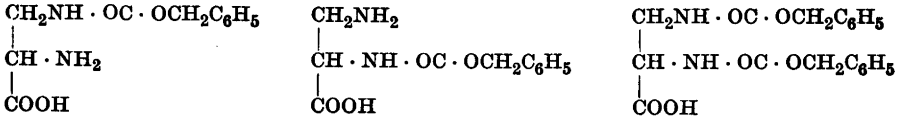
In connection with a synthetic problem in this laboratory, it became of importance to prepare DL-α-amino-β-carbobenzoxyaminopropionic acid (I). A search of the literature revealed that only a few partially acylated derivatives of the diaminopropionic acids are known. Schneider [1] prepared methyl L-α-amino-β-carbobenzoxyaminopropionate by an application of the elegant Bergmann procedure to L-dicarbobenzoxyaminopropionic acid *via* the acid chloride and the corresponding N-carboxy (Leuchs') anhydride. Miyanoki [2], in a study of enzyme substrate models, made a mono-chloroacetyldiaminopropionic acid by direct acylation of the diamino-acid and showed that the chloroacetyl-group had entered the amino-group in the β-position.

Although the Bergmann method leads to the β-isomer in an unambiguous way, the procedure is long and time-consuming. Therefore it became desirable to investigate whether or not it would be possible, in a fair yield, to prepare the β-mono-carbobenzoxylated diaminopropionic acid by a selective one-step acylation of the DL-αβ-diaminopropionic acid.

Greenstein [3] has determined the apparent dissociation constants for αβ-diaminopropionic acid and gives the following pK' values: 1.33 (COOH), 6.80 (α-NH_3^+) and 9.60 (β-NH_3^+). From these figures it is apparent that the ion needed for exclusive β-acylation, *viz.* $CH_2NH_2 \cdot CHNH_3^+ \cdot COO^-$, does not exist at any pH, and that the only possibility of obtaining the β-isomer is by performing the carbobenzoxylation in strongly alkaline solution where the ion $CH_2NH_2 \cdot CHNH_2 \cdot COO^-$ prevails, thereby hoping to obtain chiefly the β-isomer in a competitive reaction. By conducting the acylation in a slightly alkaline solution, however, it should be possible to obtain, in a high yield, the pure α-isomeride (II), because in this pH-region the predominant ionic form is $CH_2NH_3^+ \cdot CHNH_2 \cdot COO^-$. Experimentally this was verified, and the prepa-

ration of DL-α-carbobenzoxyamino-β-aminopropionic acid and some derivatives will be the subject of a future publication.

It was found that when DL-$\alpha\beta$-diaminopropionic acid was treated in strongly alkaline solution (pH>11) with one mole of carbobenzoxy chloride, the main product formed was $\alpha\beta$-dicarbobenzoxyaminopropionic acid which was isolated in a yield of 65-70 %. In addition, there was obtained in 20 % yield a mono-carbobenzoxyderivative which appeared to be quite homogenous and proved on further investigation to be the desired β-isomeride. Careful examination of the mother liquors did not reveal the presence of any of the α-isomeride, some unreacted diaminopropionic acid being the only material that could be isolated. The simultaneous occurrence of only the β-isomeride (I) and the $\alpha\beta$-diacylated acid (III) may be interpreted as a primary attack of the acyl halide on the β-amino group, which according to the titration data possesses the highest basicity, followed by further reaction of the α-amino group in (I) with a second molecule of carbobenzoxy chloride.



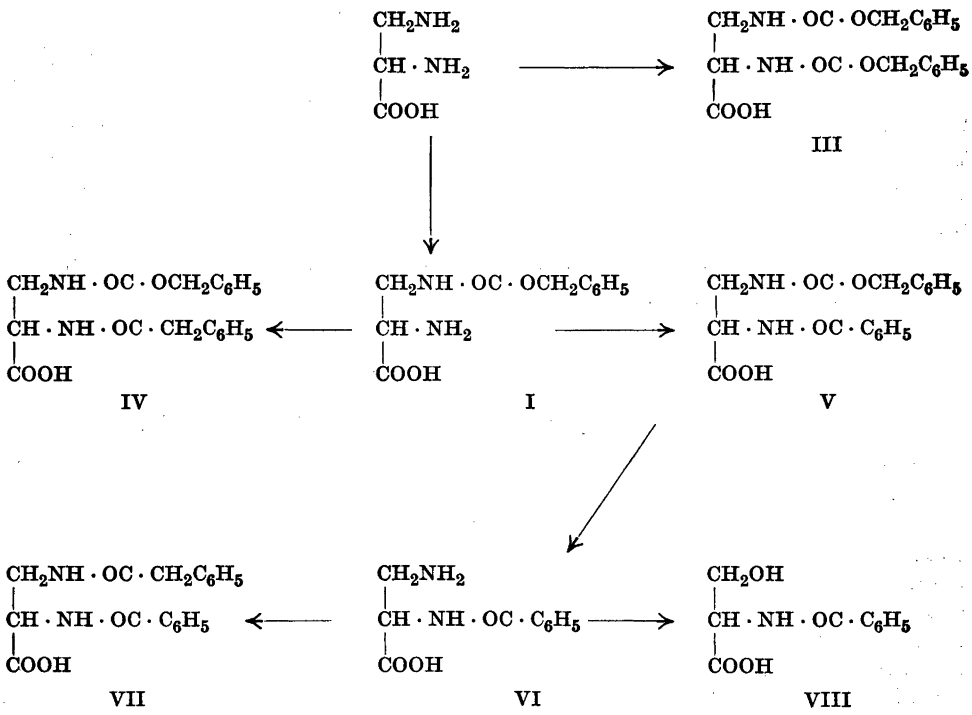
I II III

This is considered to be the most likely explanation, although a simultaneous formation of (I) and (II) followed by a secondary acylation, which in the case of (II) takes place with a velocity considerably higher than for (I), is a mechanism which also satisfies the experimental findings.

This method of preparing the β-isomeride (I) has certain advantages over the Bergmann procedure, which in our hands worked less smoothly in the DL-series than in the L-series, apparently because of the lower solubility of the racemic derivatives. The main product (III) of the carbobenzoxylation can readily be recovered as $\alpha\beta$-diaminopropionic acid by hydrogenolysis with palladized charcoal, and resubjected to carbobenzoxylation.

In order to prove the structure of the mono-carbobenzoxylated $\alpha\beta$-diaminopropionic acid the series of reactions shown in the following flow sheet were carried out.

As mentioned above, carbobenzoxylation in strongly alkaline solution of DL-$\alpha\beta$ diaminopropionic acid yields a mixture of one mono- and the diacylated products. The former (I) was treated with phenacetyl chloride to give the compound (IV) which proved to be identical with DL-α-phenacetylamino-



β-carbobenzoxyaminopropionic acid which the May & Baker group[4] prepared by a completely different route during the penicillin programme*.

Further confirmatory evidence for the correctness in allocating the carbobenzoxy radical to the β-amino group was provided by benzoylation of the mono-carbobenzoxy derivative to give a reaction product (V) which, in turn, was hydrogenolyzed to a mono-benzoyl derivative (VI). By deamination of this substance with barium nitrite in slightly acid solution, a compound was isolated which by comparison with an authentic sample, was proved identical with DL-N-benzoyl-serine (VIII).

Finally DL-α-benzoylamino-β-phenacetylaminopropionic acid (VII) was obtained by phenacetylation of (VI) as a nicely crystallizing substance melting at 165°. In the Penicillin Monograph, the May & Baker group (*l. c.*) describe the isomeric DL-α-phenacetylamino-β-benzoylaminopropionic acid* (m.p. 160—161°) and a mixture of the two compounds melted at 142—145°. Thus by three different sets of reactions, it has been proved that the mono-carbobenzoxy-diaminopropionic acid in question is the β-acylated product.

* A sample for comparison was kindly supplied by Dr. K. N. Langford, May & Baker Ltd.

During the work reported here, the 'ninhydrin' reaction was examined on the different derivatives and it was noticed that under the conditions used — heating aqueous solutions of equimolar amounts in a boiling water bath with a constant amount of the reagent — the results were not very conclusive as to the presence of a free α-amino group. Although the compounds containing such a group gave a more rapid color formation, the substances containing a free β-amino group gave on prolonged heating the same color with about the same intensity. Negative results were, however, observed on the $\alpha\beta$-diacylated derivatives.

EXPERIMENTAL *

DL-$\alpha\beta$-Dicarbobenzoxyaminopropionic acid

To a solution of 7.0 g (37.8 mM) of diaminopropionic acid mono-hydrobromide in 37 ml of 2.07 *N* NaOH (78.2 mM), 75 ml of 2.07 *N* NaOH and 22.8 ml (114 mM) of a solution of carbobenzoxy chloride in toluene (0.85 g/ml) were simultaneously added from two burettes under cooling in ice and vigorous stirring in the course of one hour. During the addition, a crystalline precipitate separated (the sodium salt of the dicarbobenzoxy-aminopropionic acid) which was not completely brought into solution by dilution with 125 ml of water. After all had been added, the reaction mixture was stirred for 30 min in the ice bath and for another half an hour at room temperature. With cooling, 7 ml of conc. hydrochloric acid were cautiously added dropwise to a distinctly acidic reaction. A colorless syrup separated which rapidly crystallized in the refrigerator. The product (m.p. 114—5°) was dried *in vacuo* and weighed 13.4 g (95 % yield). The compound was purified by dissolving in chloroform and adding petroleum ether (b. p. 40—55°). After chilling, a colorless product was obtained. 11.3 g (80 %). M. p. 124°.

The corresponding L-dicarbobenzoxyaminopropionic acid, prepared according to Schneider (*l. c.*), melted at 99—100° and was found in contrast to the DL-compound reported here to be a poorly crystallizing substance. While the optically active compound is readily soluble in carbon tetrachloride and ether, the solubility of the racemic form in these solvents is considerably smaller.

$C_{19}H_{20}O_6N_2$ (372.4)	Calc.	C	61.31	H	5.41	N	7.52
	Found	»	61.35	»	5.30	»	7.31

Carbobenzoxylation of DL-diaminopropionic acid

During 45 min there was added simultaneously to a well stirred and cooled solution of 4.45 g (24.1 mM) of DL-diaminopropionic acid hydrobromide in 50 ml of 0.990 *N* sodium hydroxide, 12.5 ml of an ethereal solution containing 4.25 g (24.9 mM) of carbobenzoxy chloride and 49.0 ml of 0.990 *N* sodium hydroxide. The pH was thereby kept

* All the melting points here reported are uncorrected. Analyses were carried out in this laboratory by Mr. A. Grossmann.

above 11 during the reaction. The precipitate formed was removed by filtration and washed with ice-cold water. It was suspended in water and, with cooling, acidified with concentrated hydrochloric acid. The colorless syrup was readily transformed into a crystalline product by cooling and seeding. After drying *in vacuo*, the compound weighed 2.45 g (m. p. 122—3°) and consisted of practically pure dicarbobenzoxyaminopropionic acid. The filtrate and washings were made very strongly acidic and extracted with three 25 ml portions of chloroform. After drying over sodium sulfate, the chloroform was removed *in vacuo* leaving 1.00 g of an oil which crystallized on standing to almost pure dicarbobenzoxyaminopropionic acid, thereby increasing the total amount of this compound to 3.45 g or 68 % of the theoretical, provided all the acid chloride had been used in the formation of the diacylated compound.

The aqueous phase was freed of chloroform *in vacuo* and brought to pH 7 by careful addition of 1 *N* sodium hydroxide. The separation of crystals started within a few minutes and after cooling overnight in the refrigerator, 764 mg of a colorless crystalline material was collected. M. p. 239—41° with vigorous gas evolution. By concentration of the filtrate, there was obtained a second crop, (252 mg), m. p. 230—2°. The total yield of DL-monocarbobenzoxydiaminopropionic acid was thus 1.016 g or 18.5 % of the theoretically possible. An analytical sample was prepared by recrystallization from hot water. M. p. 239—41° with decomp.

$C_{11}H_{14}O_4N_2$ (238.2)	Calc.	C	55.45	H	5.92	N	11.76
	Found	»	55.33	»	5.87	»	11.75

From the mother liquor a small amount of unreacted diaminopropionic acid in form of the hydrochloride was isolated by way of the mercuric acetate complex.

DL-α-Benzoylamino-β-carbobenzoxyaminopropionic acid

To a solution of 749 mg (3.15 mM) of the monocarbobenzoxyderivative described above in 3.5 ml of 0.990 *N* (3.46 mM) sodium hydroxide, was added gradually 0.40 ml (3.48 mM) of freshly distilled benzoyl chloride and 3.7 ml of 0.990 *N* (3.66 mM) sodium hydroxide with cooling and vigorous shaking. The precipitate formed was transformed into a colorless syrup on acidification. On cooling and scratching, the syrup crystallized to a product which, after drying and repeated extractions with hot petroleum ether, weighed 1.030 g. M. p. 150—1°. Recrystallization from 8 ml of 50 % acetone yielded 959 mg of analytically pure substance, melting at 151°. By concentration of the mother liquor, was obtained an additional 44 mg, thereby increasing the yield to 93 %.

$C_{18}H_{18}O_5N_2$ (342.3)	Calc.	C	63.13	H	5.30	N	8.19
	Found	»	63.01	»	5.23	»	8.33

The compound was readily soluble in ethanol and acetone and slightly soluble in cold water.

DL-α-Benzoylamino-β-aminopropionic acid

A solution of 894 mg of α-benzoylamino-β-carbobenzoxyaminopropionic acid in 20 ml of 50 % methanol to which 0.5 ml of glacial acetic acid and 200 mg of palladized charcoal (5 % Pd) were added, was treated with a fairly rapid stream of hydrogen, while

the reaction mixture was kept at 50°. In the course of the reaction, the methanol was gradually displaced by water in order to keep the reactants in solution. After six hours the carbon dioxide evolution ceased and the catalyst was removed by filtration through celite. By concentration and chilling of the filtrate, the separation of small colorless plates began. The crystals weighed 296 mg and melted with evolution of gaseous products at 227°.

$C_{10}H_{12}O_3N_2$ (208.2)	Calc.	C	57.68	H	5.81	N	13.46
	Found	»	57.65	»	5.80	»	13.46

From the mother liquor there was obtained, by the addition of ethanol, an additional 117 mg of the same grade of purity, the yield thereby reaching 413 mg or 76 %.

The successful decarbobenzoxylation reported here is in marked contrast to the failure noted by the May & Baker group (*l. c.*) in hydrogenolyzing the corresponding α-phenacetylamino-β-carbobenzoxyaminopropionic acid.

DL-α-Benzoylamino-β-phenacetylaminopropionic acid

One hundred and sixteen mg (0.56 mM) of α-benzoyl-diaminopropionic acid dissolved in 0.60 ml of 0.990 *N* (0.59 mM) sodium hydroxide was treated with 0.09 ml (0.67 mM) of freshly distilled phenacetyl chloride and 0.60 ml of 0.990 *N* (0.59 mM) sodium hydroxide with cooling and shaking. The crystalline precipitate formed was dissolved by the addition of a few ml of water, and the solution was decanted from a small amount of sticky material and acidified, with cooling, with hydrochloric acid. The precipitate crystallized in the ice box in contact with a little highly diluted methanol. After washing and drying it weighed 148 mg (81 %) and melted at 163—5°.

Recrystallized from very dilute ethanol it weighed 116 mg, m. p. 165°. The melting point of the isomeric DL-α-phenacetylamino-β-benzoylaminopropionic acid was determined under the same conditions and found to be 160—1°. The mixed melting point of the two compounds was 142—5°.

$C_{18}H_{19}O_4N_2$ (326.3)	Calc.	C	66.27	H	5.56	N	8.59
	Found	»	66.34	»	5.46	»	8.67

DL-α-Phenacetylamino-β-carbobenzoxyaminopropionic acid

A solution of 100 mg (0.42 mM) of α-carbobenzoxy-diaminopropionic acid in 0.5 ml of 0.990 *N* (0.495 mM) sodium hydroxide was treated in the usual way with 0.065 ml (0.49 mM) of pure phenacetyl chloride and 0.5 ml of 0.990 *N* (0.495 mM) sodium hydroxide. After acidification, there was obtained 151 mg of a yellowish oil which tenaciously resisted all attempts of crystallization. Only after a crystalline sample from the laboratories of May & Baker Ltd. became available, and the solution in ethylene chloride was seeded did a deposite of crystalline material result. Several recrystallizations from ethylene chloride-petroleum ether were necessary in order to obtain a well crystallizing sample with the correct m. p. 129—30°. This was not depressed on admixture with an authentic sample from the British laboratory.

DL-*N*-Benzoyl-serine

To a solution of 380 mg of barium nitrite monohydrate (1.54 mM) in 3 ml of water was added 250 mg of α-benzoylamino-β-aminopropionic acid. The suspension was acidified with 1.0 ml of 0.1 *N* hydrochloric acid, and kept very slightly acidic by intermittent addition of 0.1 ml portions of 0.1 *N* hydrochloric acid. The mixture was shaken occasionally and, after having been kept for 3 days at room temperature, the solution was made strongly acidic and extracted thoroughly with ethyl acetate. The organic phase was dried and the solvent removed *in vacuo*. The residue wat recrystallized several times from water, thereby giving 80 mg of a colorless product, m. p. 151°, undepressed on admixture with an authentic sample of DL-N-benzoyl-serine, prepared by benzoylation of DL-serine.

SUMMARY

The preparation of pure DL-β-carbobenzoxyamino-α-aminopropionic acid by selective acylation of DL-αβ-diaminopropionic acid is described.

The structural proof of the position of the carbobenzoxy-radical is given by three different sets of reactions.

This investigation was supported by financial aid from *Det teknisk-videnskabelige Forskningsraad.*

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Received August 17, 1949.

CTAA CHEMICA SCANDINAVICA 3 (1949) 1094—110

Aerobic Microbiological Corrosion of Water Pipes. I

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As is well known, internal corrosion of water pipes is liable to occur in some areas. As often described in literature, such corrosions manifest themselves in the following way:

First, traces of rust are found to appear on the external surface of the pipes, and, subsequently, leakage occurs through an initially small hole in the pipe wall which, however, gradually increases in size.

On the internal side of the pipe wall, numerous crust- or tubercle-shaped dark-brown or ochre-coloured salient spots, of considerable size, can be detected. These prominences are porous and can easily be cut through with a knife. It then appears as if they consist of a dark-coloured to black core in the centre, which is surrounded by different, generally somewhat clearer, layers with a soft rust-coloured layer on the outermost side. The 'tubercle' chiefly consists of hydrated ferric hydroxides; pH 5—6. In the pipe wall, below the tubercle, a crater-shaped hollow is seen in the steel, deepest in the middle, where the pitting of the pipe initially occurs. Generally, concentric rings of less and less corroded parts of the crater appear toward its edge. In galvanized pipes the zink has generally disappeared below the tubercle.

Corrosion is most frequently found at many places in the same pipe and, generally, several or many neighbouring pipes show corrosion. Those parts inside a galvanized pipe which are not occupied by tubercles are, just as uncorroded pipes, covered with a smooth or granular, yellow or ochre-coloured layer, a socalled protective layer, which protects the metal against corrosion. According to Haase[1], this layer consists chiefly of calcareous ferric hydroxides of variable composition, with an admixture of small quantities of Mg and Si.

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A microscopic investigation showed that the tubercles, in addition to large quantities of amorphous ferric hydroxides, contained iron bacteria surrounded by sheaths; and, moreover, sometimes typical filaments of iron hydroxides from *Gallionella ferruginea*. The sheaths-forming bacteria were lying in coiled 'balls', strongly incrusted by iron oxides and hydroxides. When this was dissolved with hydrochloric acid, the bacterium filaments or, more correctly, the empty sheaths, became clearly visible.

When inoculated in sterile tap water with a nail as iron source, a rather strong growth of the thread-forming bacteria can be obtained, which seems to be a *Leptothrix sp.* Thus, there may be a possibility that iron bacteria in one way or another play a part in the corrosion phenomena described above. These bacteria, which cannot corrode metallic iron, are, however, certainly not the direct cause of the corrosion; but, as was first shown by Winogradsky [2], primarily oxidize Fe^{+2} into Fe^{+3} (by means of oxygen dissolved in water), whereby they gain the energy necessary for the assimilation of carbon dioxide and the building up of the organic substances of the cells. This is a process analogous to that in which the green plants use the energy of the sunlight. Thus, iron bacteria are autotrophic organisms; but as they are still scantly explored, it is unknown whether others than *Gallionella* are obligate autotrophic.

In summary, the formation of energy can be expressed by the following equation:

$$2\ Fe^{+2} + (x + 2)\ H_2O + 1/2\ O_2 \rightarrow Fe_2O_3 \cdot xH_2O + 4\ H^+ + Q\ \text{cal}$$

Ferrous ions are taken up in the bacterial cells where they are oxidized to ferric ions which are discharged and gradually hydrolyzed. Subsequently, they coagulate in the surrounding water and are precipitated as hydrated ferric hydroxide.

According to Beger [3], sheaths, without bacteria or with dead bacteria, are able to precipitate ferric hydroxide for a long time, only more slowly than the living bacteria.

In the literature, indications are found of the assumption that iron bacteria may play a part in corrosion. Most frequently, however, the injurious effect of iron bacteria in water pipes became obvious by the fact that their abundant growth made the water muddy (so-called 'red-water') and caused blockage of the narrow pipes (*e. g.* Schorler [4]). Brown [5] described such formations and showed a cross section of an old tubercle. It appears as if its central part consists of a dark core, which is largely iron sulphide, which is surrounded by some layers of a yellowish-red »iron-meal», containing numerous *Gallionella*-

filaments incrusted with ferric hydroxide, chalk, and manganese. The external layer is a mantle of iron hydroxide and chalk with a coating of living *Gallionella*.

Schorler [4] also found that the tubercles initially consist of a loose *Gallionella*-felt, which later disappears and is recrystallized in the form of hexagonal hematite-(Eisenglanz)-like crystals. These crystals grow together into shapeless aggregates, in which no iron bacteria can be distinguished. Ellis [6] mentions that iron bacteria, in water pipes, cause 'slimy streamers, tubercular incrustations', the formation of which is furthered by carbon dioxide, and 'iron incrustation on non-ferrugineous surfaces'. The other physiological and bacteriological theories of this author, which have been strongly critisized by Winogradsky [2], will not be further discussed here.

Hermann [7] observed very large incrustations of iron bacteria *(Gallionella)* in asphalt-coated pipes. In uncoated pipes or in pipes where the asphalt was damaged, deep corrosions (pitting) with superimposed tubercles were found. Inside the tubercle recrystallization to hematite (Eisenglanz) was seen to occur. Thresh, Beale and Suckling [8] state that 'they (iron bacteria) may play some part, probably a subordinate one, in the formation of tubercular incrustations in iron mains. This leads to interference with the flow of the water, and destruction of the pipes'.

Minder [9] reports that by immersing polished iron strips into wells, the growth of iron bacteria is initiated and that corrosion takes place under the crust. Similar phenomena were found to occur in the filter kettle of a closed iron-removing plant. On the wall above the sand were found lens-shaped, rust-coloured deposits, several cm in diameter and up to 1 cm thick. Under these deposits hollows in the iron wall appeared. After drying, the 'lenses' were light, almost like a dry sponge and consisted of a felt of iron bacteria.

Beger [3] assumes that *Gallionella ferruginea* is alone responsible for incrustation in pipes and is contributory to corrosion.

Other authors, however, have found that *Leptothrix ochracea* and *Crenothrix polyspora* also very frequently grow in water pipes.

Haase [1], who thoroughly dealt with these problems, stated that water causing corrosion (pitting) always contained a great deal of organic substances and iron bacteria.

Von Wolzogen Kühr [10] has shown in a very convincing way, that external corrosion of iron pipes buried in the soil may be due to sulfate-reducing bacteria. These are anaerobic and use oxygen from sulphates for oxidation of organic substances and, to a minor degree, also for oxidation of hydrogen for production of energy — at the same time forming hydrogen sulphide.

Starkey [11], furthermore, directs attention to the fact, that the presence of oxygen concentration cells in the soil should be considered. Thus, for instance, a pipe buried in a moist anaerobic area will prove to be anodic in relation to another piece of the same pipe in a well-aerated moist area, where the hydrogen produced is oxidized to water by the oxygen of the air. Iron is dissolved on the anode and is transformed into ferrous hydroxide, and, if sulfate reducing bacteria are present, also into iron sulphide.

We are, of course, here concerned with quite other conditions than these prevailing in water pipes with a more or less continuous current of airsaturated water, and the above mentioned papers on sulphate reducing bacteria have been mentioned only in order to show that corrosion may be due to biological causes.

Bunker [12] (and, also Butlin, Adams and Thomas [13]) assumes that the sulphate-reducing bacteria may be active under the anaerobic conditions under the tubercles, and he has isolated *Vibrio desulfuricans* from such places. It can hardly be doubted that the conditions for growth are favourable here, since there are organic substances from water and destroyed bacterial cells *, a small supply of sulphate-containing water, and rather anaerobic conditions.

A review of the literature thus has shown, that frequently tubercle formations, connected with pitting, occur similar to those cases discussed in the present paper. Iron bacteria were always found — most frequently *Gallionella ferruginea*, but also *Leptothrix ochracea* and others — as constituents of these tubercle formations. All authors agree about the necessity of a cautious statement of the part played by iron bacteria in connection with these phenomena (Knudsen [15], Reddick and Linderman [20], Thomas [21], Brown [22], O'Connel [23], Pillai, Rajagopalan and Subrahmanyan [24]).

The manuals available, dealing with metal corrosion, also pass lightly over this question; thus Kröhnke, Maas and Beck [16] (p. 88) write, that the growth of bacteria is found on the internal walls of water pipes, and at the bottom and on the sides of water reservoirs, partly as 'schlemige Streifen', and partly as 'Verkrustungen' or tubercles. By the activity of the iron bacteria, a danger of obstruction and corrosion arises. In the handbook on corrosion by Bauer, Kröhnke and Masing [17], no notice is taken of microorganisms in connection with corrosion; only the investigations of von Wolzogen Kühr on sulphate-reducing bacteria are mentioned.

Evans [18] states that iron bacteria, no doubt, often are connected with a rapid perforation of the material; their activity ceases at pH 8.2—8.6.

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* According to Butlin and Adams [14] sulphate-reducing bacteria can live under strictly autotrophic conditions.

Hadley [19] writes that the role of iron bacteria in corrosion is still obscure. Their most important contribution to corrosion probably results from the creation of a barrier capable of maintaining oxygen concentration gradients between metal and solution. 'Additional data are admittedly required to clarify the relationship which exists between the iron bacteria, the sulphate-reducing bacteria, and tuberculation.'

The authors believe that iron bacteria are of considerable, primary significance in the development of corrosion and the formation of tubercles. We now shall make an attempt to explain how this interpretation is in agreement with the facts.

MECHANISM OF CORROSION

It is generally assumed, that corrosion in water pipes (only internal corrosion of iron water pipes conducting well-aerated tap-water of normal hardness is discussed here) depends essentially on the existence of short circuited local cells, an assumption whose general validity can hardly be doubted. Differences of opinion only arise in the discussion of details of the mechanism. For a survey of these problems reference is made to the book by Evans [18], from which theories, which are in best agreement with experimental data have been taken. (Only the theory of bacteria playing an essential part seems to be new.)

It is of some importance to know the relative size and distribution of anodic and cathodic areas (anodic: places where the electric current passes from the metal to the liquid, cathodic: places where the current goes in the opposite direction). If cathodic and anodic areas are quite evenly distributed over the whole internal surface of the iron pipe, corrosion everywhere on the surface is possible with an even diminution of the thickness of the wall. This type of corrosion is due to heterogenic structure of the iron surface with evenly distributed non-iron impurities, and is not very dangerous on account of the very low rate of destruction. The mechanism of this type of corrosion is shown in Fig. 1. In the present case, however, it is known by experience, that supply pipes tend to rust locally ('pitting'). This means, of course, that the anodic areas, the places where metallic iron is dissolved according to the equation:

$$Fe \rightarrow Fe^{+2} + 2e \qquad (1)$$

are relatively small.

It can therefore be assumed either that some factors making the metal less noble (more negative) occur in isolated spots, and that the cathodic (nobler, less negative), areas are more extended (which, of course, means, that the current density is great at the anodic spots and small on the cathodic

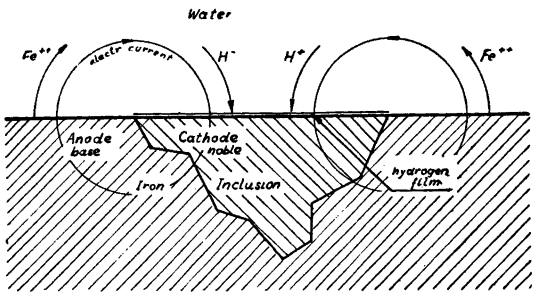

Fig. 1. Scheme of the corrosion in non-aerated water due to the activity of local electrochemical cell.

areas); or that impurities making the metal nobler (more positive) occur over extended areas and that the more negative places occur in isolated spots.

The causes of the differences in electrochemical behaviour may be of various kinds; *e. g.* the well known passivating action of oxidizing agents, *i. a.* oxygen itself may very well play an important role. One might, for instance, imagine that almost the whole inner surface of the tube is passivated (made nobler) by oxygen, but that some spots, for one reason or another, remain active.

However, it is not necessary, at present, to dwell on these different possibilities and theoretical aspects. What is needed is 1) a mechanism which will initiate corrosion at certain spots and 2) a mechanism to make the conditions stationary and favourable to corrosion at the same spot for an extended period of time.

1. The start of the corrosion requires, of course, not only the occurrence of reaction (1) in one place, but also a reaction whereby electrons are used up. In view of the conductivity of the metal this reaction may take place anywhere on the surface and over extended areas. One possibility is the reduction of water:

$$2e + 2H_2O \rightarrow H_2 + 2OH^- \qquad (2a)$$

another the reduction of oxygen

$$2e + 1/2\ O_2 + H_2O \rightarrow 2OH^- \qquad (2b)$$

According to Evans both may occur, but it is obvious that (2b) is by far the most probable.

It is highly probable that they take place through intermediary stages, some of which are identical to both reactions. As such steps one might imagine:

$$H_2O \rightleftharpoons H^+ + OH^-$$
$$e + H^+ \rightarrow H$$

and it must be assumed that the hydrogen atoms are adsorbed on the cathodic areas. From these areas they are removed either by association (formation of H_2) or by reaction with oxygen (formation of H_2O). This latter reaction probably also proceeds through intermediates (HO_2, H_2O_2) but, for the present purpose, it is not necessary to enter into details on this point.

Another point, however, seems rather important. The water contains not only oxygen, but also dissolved carbon dioxide, which must react with the hydroxyl ions according to the well-known scheme. Consequently, we get instead of (2b)

$$2e + 1/2\ O_2 + H_2O + 2CO_2 \rightarrow 2HCO_3^- \ldots\ldots \qquad (2c)$$

Obviously, the corrosion thus displaces the equilibrium

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$$

towards the right. This has the consequence that, if the water originally was saturated or nearly saturated with calcium carbonate, which very often is the case, the corrosion process will cause a supersaturation resulting in a tendency of the water to form scale.

Conclusions: At the start, anodic spots in cooperation with more extended cathodic areas will cause the occurrence of closed electric currents coupled with chemical reactions:

$$\text{anodic:} \quad Fe \rightarrow Fe^{+2} + 2e \qquad (1)$$

$$\text{cathodic:} \quad 2e + \tfrac{1}{2}\ O_2 + H_2O + 2CO_2 \rightarrow 2HCO_3^- \qquad (2c)$$

2) For the reaction to proceed it is necessary to protect the anodic (active) spot against the influence of passivating agents, *e. g.* oxygen. Now, especially if water in the pipe is stationary, this can easily be achieved. Quite apart from the electrochemical process described above the ferrous ions are easily oxidized to ferric ions or, in not too acid solution, to hydrated ferric oxide, the stoichiometrical equation being:

$$2Fe^{+2} + \tfrac{1}{2}\ O_2 + (x + 2)H_2O \rightarrow Fe_2O_3 \cdot xH_2O + 4H^+$$

As the ferrous ions and the oxygen come from either side, a film of colloidal hydrated ferric oxide may be formed around the anodic spot. Inside this film

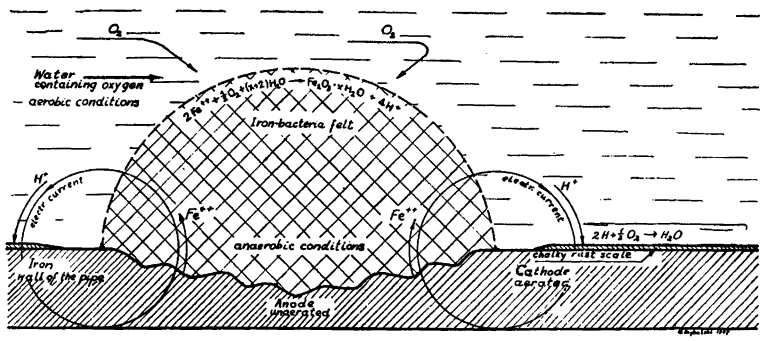

Fig. 2. Scheme of the corrosion in well-aerated, running water after the formation of a »tubercle» (from an iron bacteria »felt») on the internal surface of a water pipe.

the oxygen concentration will remain low on account of the continual flow of ferrous ions released from the active spot, which consumes the oxygen diffusing in from the outside. At the same time, the hydrogen ion concentration remains rather high and, in case such a film can be formed and remain undisturbed, the conditions will be highly favourable for the process to continue. Concerning the cathodic process whose occurrence, of course, is just as necessary as the anodic one, there seems to be no great probability of its inhibition. The surface of the cathodic areas must be expected to be rather easily accessible for dissolved oxygen which, thus, without difficulty can exert its depolarizing influence (removal of hydrogen atoms).

Remembering the low density of the cathodic current, one would expect that only a hard, tight scale with excessively low electric conductivity and high resistance against diffusion might have some effect.

It thus seems possible that a purely inorganic mechanism may be operative, but, if the conditions prevailing around the anodic (active) spots are considered, it soon becomes clear that they are ideal for the growth of certain bacteria and that, on the other hand, a colony of these bacteria will be ideal for maintaining the conditions of corrosion at that spot.

THE THEORY OF THE AEROBIC BACTERIOLOGICAL CORROSION

The types of corrosion described in the introduction to this paper are, no doubt, caused by electrochemical processes. The iron surface is divided into two systems, within and without the tubercles. The water of the supply pipes in question contained about 8 mg/liter of oxygen. The non-tubercle areas are violently washed by this water and consequently aerated. Under the tuber-

cles, however, there is a very slow percolation of the water and consequently weak aeration. Thereby, a short-circuited electric cell is created (fig. 2), in which the aerated parts have a higher potential (more positive) and thereby take over the function of the cathode, while the iron surface under the tubercles has a lower (baser) potential and acts as an anode. Iron goes into solution at the anode ($Fe \rightarrow Fe^{+2} + 2e$), and at the cathode hydrogen is evolved in atomic or molecular form ($H^+ + e \rightarrow H$), and is oxidized by the oxygen contained in the water. At the cathode there is a continuos consumption of hydrogen-ions, and the consequent surplus of hydroxide-ions causes a ‚wall alkalinity' which, however, is limited by the current of water flowing through the pipe. The ferrous-ions going into solution at the anode are immediately hydrolized

$$Fe^{+2} + HOH \rightleftharpoons Fe(OH)^+ + H^+$$
$$Fe(OH)^+ + HOH \rightleftharpoons Fe(OH)_2 + H^+$$

which depends upon the pH in the solution. Ferric-ions, formed by oxidation of ferrous-ions, are also hydrolyzed and to a much higher degree than ferrous-ions (the hydrating of the ions is not considered here). By the hydrolysis, hydroxyl-ions are consumed and the surplus of hydrogen-ions causes an acid reaction, which, as mentioned before, has been ascertained in the tubercles. This acid reaction in itself accelerates corrosion, as: (i) it causes greater electrical conductivity and (ii) renders difficult the formation of carbonate-containing, protective scale of »chalky rust» on the surface of the tubercle so that no increase of the electrical resistance takes place. The resistance of the cathodically formed protective scale has no very great effect because of the large surface of the cathode and the consequently low density of current in comparison with the small corroding anodic surface under the tubercles.

The whole of this phenomenon, however, depends upon the formation of a membrane which renders difficult the percolation of water, and the diffusion of oxygen but without much resistance to the transference of ions. This membrane may also appear in connection with purely inorganic colloidal precipitations of hydrated ferric oxides where the admission of oxygen is rendered difficult by the fact that the diffusing oxygen is consumed by oxidation of ferrous-ions to ferric-ions. The colloidal hydrated ferric oxide, however, is of a granular structure and is mechanically of small resistance. Consequently, it is not always probable that in pipes with running water such membranes could be formed in a purely inorganic way without any other factors. In this connection the iron bacteria should first be considered, as filaments of iron bacteria are always found in tubercles, which is known partly from the literature partly from the authors' investigations of tubercle-forma-

tion. It should be imagined that the iron bacteria settle on the iron surface, chiefly, perhaps, on rough spots (tubercle-formations thus are often found on the welded seam). The bacteria then start multiplying and, as mentioned before, gain energy by oxidation of ferrous- into ferric-ions. Thereby the concentration of oxygen in the immediate vicinity decreases and a local micro-electrochemical cell is formed. On the areas deficient in oxygen, *i. e.* where the bacteria colonies are to be found, ferrous-ions go into solution. This favours the growth of the bacteria.

The iron bacteria will find the best conditions of life in neutral or slightly acid water containing ferrous-ions, oxygen, some free carbon dioxide, and ammonium salts or nitrates. Pipes corrosion shows all the above conditions — on the anodic surface iron is electrochemically dissolved as ferrous-ions, and by their hydrolysis the slight acid reaction appears. Simultaneously free carbon dioxide is formed from carbonates according to the reaction:

$$HCO_3^- + H^+ \rightarrow CO_2 + H_2O$$

Thereby all conditions for the development of bacteria (even good conditions of temperature and absence of light) are present, and the bacteria contribute to the corrosion in three essential ways:

1. Primary formation of micro-differential-aeration cells due to a change in concentration of oxygen in the infected place. This cell may develop further either in a bacteriological way or, perhaps, in a purely inorganic chemical way.

2. Mechanical strengthening of tubercle owing to the 'felt structure' of iron bacteria filaments.

3. Catalytic ferrous-ion oxidation and, consequently, rapid precipitation of hydrated ferric oxide, which further strengthens the anaerobic conditions on the anodic side and, hence, increases the difference of potential between the iron surface under and outside the tubercle, whereby corrosion is increased.

It follows from the theory stated here that all microorganisms which are able to grow under these conditions, to consume oxygen, and form compact colonies, can develop or increase corrosion. As already stated iron-bacteria particularly will find favourable conditions of life in new water pipes and consequently become predominant.

An electrochemical and bacteriological investigation of the consequences of the theory is reported in a following paper.

SUMMARY

In the present paper a description is given of a type of corrosion in water pipes. The corrosion is accompanied by the formation of 'tubercles' consisting

mainly of hydrated iron oxides and iron bacteria felt which lie over the pittings. The electrochemical nature of this corrosion is discussed and a theory advanced according to which microorganisms, especially iron bacteria, accelerate these processes.

It is proposed that

1. through their growth on limited regions of the iron surface the iron bacteria primarily form 'differential aeration cells'. These give rise to a difference of potential and consequently to a corrosion current between the site of the bacterial colony and the surrounding surface of the iron.

2. the felt-like structure of the sheaths of the iron-bacteria causes a mechanical strengthening of the tubercle, which becomes resistant to the flow of water in the main.

3. with increasing thickness of the tubercle the conditions inside the tubercle will be more anaerobic. This increases the difference of potential between the iron surface under and outside the tubercle, and the corrosion is thereby increased, with or without the collaboration of the bacteria.

The authors wish to express their gratitude to Professor J. A. Christiansen, head of the Chemical Laboratory A, and Professor J. L. Mansa, head of the Mechanical Engineering Laboratory of the Technical University of Denmark for their information and helpful discussions during the performance of the present work. Special thanks are due to Professor J. A. Christiansen for his interest and efficient help in our work with the section on mechanism of corrosion. We also wish to thank Dr. K. R. Butlin and Mr. N. Hofman-Bang, C. E. for their kind assistance in translating this paper.

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Received August 26, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1106–1116

Aerobic Microbiological Corrosion of Water Pipes. II

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In a previous paper[1], we have dealt with corrosion and proposed the hypothesis that iron bacteria play a prominent, primary part in this phenomenon of electrochemical nature. In our opinion, their mode of action should be as follows:

1. Primarily, the formation of 'differential aeration cells' by the growth of iron bacteria on limited regions of the iron surface.

2. The felt-like structure of the sheaths of iron bacteria causes a mechanical strengthening of the 'tubercle', which becomes resistant to the flow of water in the main.

3. Increasing anaerobiosis below the tubercles formed by the iron bacteria which are oxygen consuming. Hereby, the anode potential decreases and the potential difference between the anode (under the tubercle) and the cathode (outside the tubercle) and, consequently, the corrosion flow, increase.

In the present investigation an attempt has been made to obtain experimental corroboration of our assumptions.

As a first step towards the verification of the assumption that the corrosion is of bacterial origin, a bacteriological investigation of the tubercle has been carried out. As mentioned in the previous paper, it was observed that those parts of tubercle shells, found in the closest proximity to the iron surface, consisted of amorphous, recrystallized hematite (Eisenglanz) and, towards the surface, of a felt of mostly empty, largely iron-incrusted sheaths of iron bacteria. This is most beautifully seen on younger tubercles (2—3 months old). In Fig. 1 such a felt is depicted in ca. 20 fold magnification. The surface

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Fig. 1. Internal structure of a 3 months old tubercle. The single threads covered with a very thick knotted sheath are easily seen. Magnification ca. 20 x.

of the tubercle is removed. It consists of an ochre-yellow, porous, amorphous, very thin layer of strongly hydrated ferric oxide, which is easily soluble in dilute hydrochloric acid. The iron compound in the recrystallized layer and in the sheaths of the iron bacteria is sparingly soluble in dilute hydrochloric acid.

The bacterium felt shows little electric resistance, but is an effective membrane inhibiting both percolation of water and oxygen diffusion.

In our experimental pipes, iron bacteria grew especially well on bakelite packings, while a continuous flow of tap water was led through the pipes. Here, a velvet-like, rather firmly fixed layer of iron bacteria developed.

Long threads, approx. 1 μ thick and 100—500 μ long, were formed. Most of these were entire, however, many of them were divided into separated cells, 1 $\times$ 10 μ large. The older threads were surrounded by a yellow-brown sheath of hydrated ferric oxide, while the quite new ones lacked these sheaths. With increasing age the sheaths become darker brown and thicker. Incrustation seems to occur in such a way that single grains of hydrated ferric oxide appear on the sheath and, in the course of time, a larger and larger number of grains develops until, finally, the whole surface is covered by a brown, knotted layer (Fig. 2).

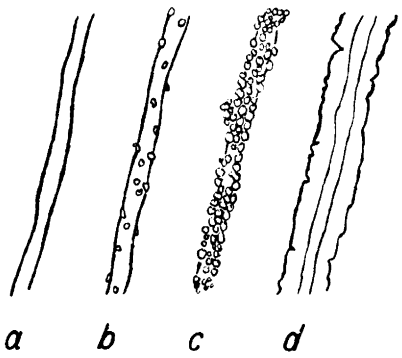

Fig. 2. Scheme of the formation of the sheath. a — uncovered, young threads. b, c — the threads covered with single grains of hydrated hematite. d — completely developed sheath.

Generally, the threads appear to be unbranched, but some are actually ramified. Morphologically, they seem to belong to the *Crenothrix* group, but some types forming ramifications recalled definitely the *Clonothrix* or *Leptothrix* types.

At the present time, however, the classification of iron bacteria is rather doubtful, and it appears probable that the multifarious types should in reality be condensed into a few, since the morphology, the most important grouping basis, may vary considerably according to the exterior conditions (*cf.*, for example, Beger [2], Bergey [3], Pringsheim, [4]).

It was our aim to elucidate whether iron bacteria are the primary cause of tubercle formation and, correspondingly, of corrosion. We have, therefore, investigated whether germ filtration of tap water suppresses these phenomena, and whether inoculation of sterile main water with iron bacteria causes tubercle formation. The experiments were performed in the following way.

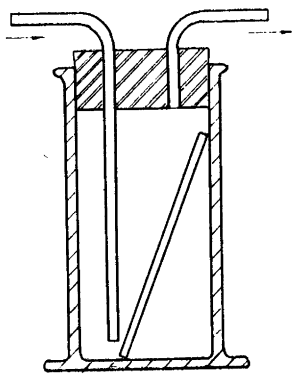

Fig. 3. Glass cylinder with a piece of iron sheet for experiments with sterile and unsterile water. → indicate the flow of water.

Figs. 4 and 5. Iron sheet pieces with well developed tubercles. After removal of the tubercle, the corrosion cavities become visible (on the upper iron piece of Fig. 5).

Main water was directly filtered through a Seitz filter into a level flask. From here, it was led into two parallel series of sterile glass cylinders (200 ml), each containing a square piece of iron sheet, 20 × 100 × 0.5 mm (Fig. 3).

Into the first cylinder of one of the (inoculated) series was mounted a piece of folded filter paper containing a culture of iron bacteria — this caused a very vigorous inoculation. Unfortunately, we have not yet succeeded in producing a completely pure culture of bacteria, but there is no reason to assume that this should affect the results presented here.

After a lapse of 4—6 days, the metal sheet plates in the uninoculated series were covered with a thin, firm, protective layer ('chalky rust').

In the inoculated series, however, small, velvet-like colonies on the iron surface appeared after a few days. They grew rather rapidly into common tubercles with light-yellow, rather smooth and porous surfaces and, after 2—3 months, they were up to approximately 2 cm in diameter and 5 mm high.

In other experiments, non-sterile tap water was led through glass cylinders with iron sheet plates without special previous inoculation. Also in these cases, the growth of the bacteria appeared, even after a presumably very slight inoculation. In the last mentioned experiments, however, single tubercles were observed which were spread over the surface of the iron sheet, while after strong inoculation almost the whole surface was overgrown. Fig. 4 shows the appearance of the tubercles and, in Fig. 5, corrosion is seen after the tubercles were removed from the iron sheet pieces. Fig. 1 is also taken from this experiment.

Hence, it was possible to show that sterile water does not cause tubercle formation and, moreover, that the iron bacteria are the cause of tubercle formation and, correspondingly, of corrosion.

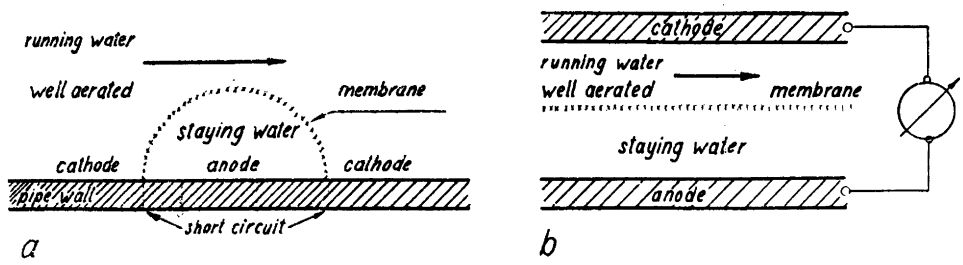

Fig. 6. Schematic comparison of the conditions during pitting with tubercle formation and by artificial differential aeration cell.

In order to further prove the significance of iron bacteria in iron corrosion, a series of experiments were performed in which it was attempted to produce the effect of the tubercles by means of artificially constructed 'differential aeration cells'.

Fig. 6 a shows schematically the conditions prevailing in a water pipe. Under the membrane ('the tubercle') built up by the iron bacteria we have stagnant, practically oxygen-free water, and the underlying part of the iron surface acts as the anode. The iron surface outside the tubercle is continuously in contact with water saturated with air and, therefore, forms the cathode relative to the covered part. The two terminals are short-circuited through the pipe wall.

In order to imitate these conditions, two iron plate terminals were placed parallel to each other and separated by a membrane of hardened filter paper. In this way, the cell formed two compartments, one of which containing stagnant water, while continuous flow of water passed the other compartment of the cell. The two iron plates, short-circuited through a micro-amperemeter, thus formed anode and cathode, respectively. The principle of this arrangement is seen in Fig. 6 b.

Fig. 7 shows the construction of the cell. It consists of four bakelite plates (denoted by 1, 2, 3, 4), 50 × 50 × 10 mm. A hole, 30 mm in diameter, was drilled into the two central plates (2, 3), whereby an internal cylindrical cavity (A · B) was formed.

At the ends of this cavity, two iron plates (a, b) were placed, their position being fixed by the external bakelite plates. The filter paper membrane (c) was placed between the two internal bakelite plates, thus dividing the cell into two compartments (A and B), each 10 mm deep and 30 mm in diameter. The arrangement was held together by iron clamps (not shown in Fig. 7).

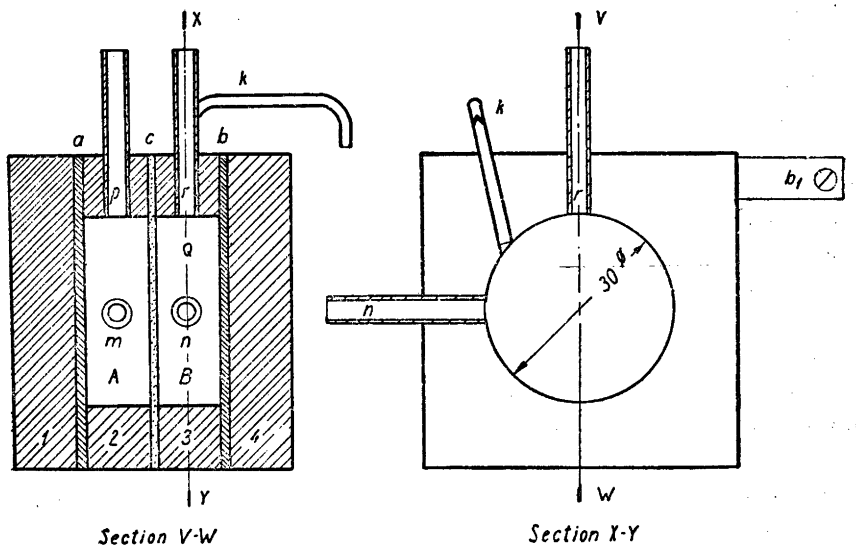

Fig. 7. Differential aeration cell. 1,4 — external bakelite plates. 2,3 — internal bakelite plates; the holes drilled form the real cell compartment A—B. a, b — iron plate electrodes, b_1 — terminals for the electric connection of the iron electrode with the measuring apparatus. c — membrane made of filter paper. m, n — water inlet pipes. p, r — water outlet pipes. k — agar salt bridge for the potentiometric measurements.

Through the bores of the internal bakelite plates, water could pass into and out of the two compartments of the cell through cemented glass tubes (m, n, p, r).

During the experiment proper, water was led through one compartment (A) only, while the other one (B) contained stagnant water. During the corrosion process, the oxygen will rapidly be consumed and the iron plate, therefore, become anodic relative to the other plate which is in continual contact with oxygen containing water. Oxygen diffuses only slowly through the hardened paper and, in the compartment with stagnant water, we shall therefore rather soon find (after some hours) that highly anaerobic conditions prevail near the iron surface, becoming more and more aerobic in the direction of the membrane.

The iron plates were short-circuited except for the time when the measurements were performed. These included corrosion current as a function of time, potential between the iron plates, their individual potentials measured by means of a calomel electrode (saturated KCl), the internal resistance of the cell to d.c. and a.c., and the electric capacity of the cell.

The water flow was 100 ml/min. Each experiment lasted for 15—30 days.

In the first experiments, ordinary main water flowed through the cell. When the cell was opened after the lapse of 3 weeks, vigorous growth of iron bacteria was found to occur on the non-aerated part of the filter paper membrane. The bacteria formed a 1—2 mm thick felt. The non-aerated electrode (anode) was quite bright and homogeneously affected. The weight loss of this electrode approximately agreed with the loss calculated from the measurements of the corrosion current, the weight loss being about 10 % higher than the Coulomb equivalent of the current. The cathode was covered with a compact, thin, yellow layer of 'chalky rust' which stuck firmly. The cathode seemed to be especially well protected under this layer.

The described development of iron bacteria was observed in all cases where untreated main water was led through the cell. This apparatus can, therefore, also be applied to the demonstration of iron bacteria in water.

For the sake of comparison, a series of experiments was carried out under sterile conditions, the water being germ-filtered through Seitz filters and led through two parallel cells of the type shown in Fig. 7. One of the cells was strongly inoculated by introducing the iron bacteria grown in the above mentioned experiments into the anodic part of the cell, the other one remained sterile. The results of these measurements are shown in the diagrams (average of 5 series), and it appears that the bacteria have a rather small, but pronounced effect on the decrease in potential of the non-aerated electrode, which causes a greater potential difference between the electrodes and an increase in the corrosion current (Fig. 8, I).

It could, however, be observed that strong inoculation with iron bacteria very rapidly brought forth a decrease in the electric potential of the non-aerated electrode to the ensuing, rather constant value observed later.

The potential of the aerated electrode decreased much slower owing to the high EMF of the cell at the start of the experiment and, thus, of the vigorous corrosion current (Fig. 8, II).

In the uninoculated cell, however, a rather simultaneous decrease in electric potentials of both electrodes could be observed. The potential difference thus fluctuates around zero and, therefore, in the first hours of the experiment, the corrosion flow shows low positive and negative values * (Fig. 8, II).

This difference in the initial course of corrosion indicates that iron bacteria may be the primary cause of corrosion in new pipes. In regions where the

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* This must be considered the explanation of the undefined direction of current in the beginning of the experiment with 'differential aeration cells' as discussed by Evans [5] who, however, does not explain the phenomenon.

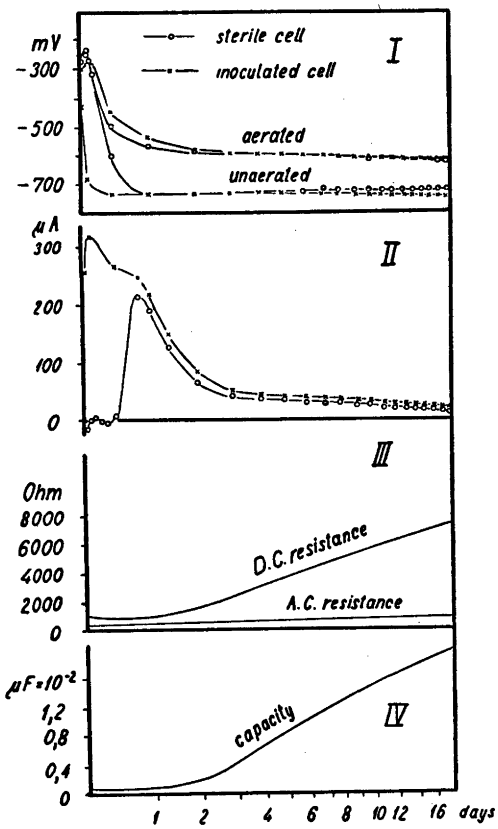

Fig. 8. I. Time-potential curve of the aerated electrode (cathode) and the unaerated electrode (anode) for sterile and inoculated differential aeration cell.

II. Time corrosion current curve.
III. Time d. c. resistance curve.
» a. c. » »
IV. » capacity curve.

The curves III and IV are practically identical for inoculated and uninoculated cells.

bacteria are deposited, the potential of the iron decreases rapidly, whereby primary micro-'differential aeration cells' are formed. Corrosion and formation of 'tubercles' by precipitation of hydrated ferric oxide start momentarily and, simultaneously, also the bacteria colonies grow.

Experiments with sterile water, moreover, showed that, in the course of a week, the potential of the anode was — 720 — 740 mV, measured against a calomel electrode (saturated KCl). This potential deviates, practically spoken, only very little from the potential of the iron in completely O_2-free medium under otherwise similar conditions. *

* After some days the potential of the iron in O_2-free main water was rather constant — 775 mV against a calomel electrode (— 553 mV against normal H_2-electrode). The concentration of ferro-ions was 0.85 millimol/1. These values are in good agreement with the normal potential of the iron, — 441 mV against a normal H_2-electrode.

From these results it becomes clear that the iron bacteria cannot further decrease the potential of the anode. After 'tubercles' have been formed, the iron bacteria practically do not play any part in the progressing corrosion. In our experiments, the iron bacteria caused an average increase in corrosion by ca. 10 % (Fig. 8, I and II).

Thus, there is no possibility of inhibiting corrosion by killing the iron bacteria. However, such a measure — for example, frequent addition of chlorine to the main water — will presumably suppress the formation of new corrosion fields [8, 9].

One way in which inhibition of corrosion might be obtained is to cover the tubercles with a thick protective layer. Hereby, the current density on the anode is largely decreased and the corrosion effectively inhibited.

The normal equilibrium water (with respect to the system $CaCO_3-CO_2-H_2O$, Bauer, Kröhnke, Masing [6] and others), forming protective 'chalky rust' layers in iron pipes, will not form such layers on the tubercle surface because of the hydrolysis of ferric and ferrous ions which gives an acid reaction. The equilibrium (pH) of the water is changed and the water dissolves chalk rather than forming protective layers. Only after alkalizing the water (for example, by addition of lime water or filtration through freshly burnt magnofilters) do the conditions on the surface of the tubercles favour the formation of tight layers of 'chalky rust'.

Preliminary experiments indicated that newly filled magnofilters completely inhibit the formation and growth of 'young tubercles' (Mansa [7]).

We found that fresh magnofilters increase the pH by 0.5—0.8, while a filter with old filling (ca. 6—12 months old) only caused an increase in pH of 0.1. This filter proved to be ineffective in preventing corrosion.

The effect of freshly burnt magnofilters seems to be based not only on the adjustment of the equilibrium state of the water, but also on the alkalization. This involves a supersaturation with chalk which, in the course of time, causes (1) precipitation of a protective layer on the 'tubercle' in a zone where the degree of acidity is suited for the readjustment of the equilibrium of the water, and (2) precipitation of a porous layer of $CaCO_3$ on the wall of the pipe outside the 'tubercles' which may choke up the pipe. Therefore, the alkalization of the water must be performed very cautiously, in the right places, and over the right period of time.

It can easily be shown that water which passed through a fresh magnofilter and is supersaturated with chalk is above the equilibrium curve of the Tillman and Heublein [6] diagram (p. 224). If ca. 50 cm long glass tubes are inserted in both sides of the magnofilter, a thick chalky precipitate is observed on the walls of the tube inserted behind the filter, while the tube leading to the filter is unchanged.

After precipitation of the excess chalk, protective layers on or inside the tubercles can no longer be formed. The pretreatment of the water should, therefore, be performed in the proximity of those parts of the pipe which must be saved, for example by instalment of a magnofilter in the attacked houses for a shorter period of time (some months).

Finally, it should be mentioned that, in parallel experiments with inoculated and uninoculated cells, the internal resistance of the cell to a.c. and d.c.,

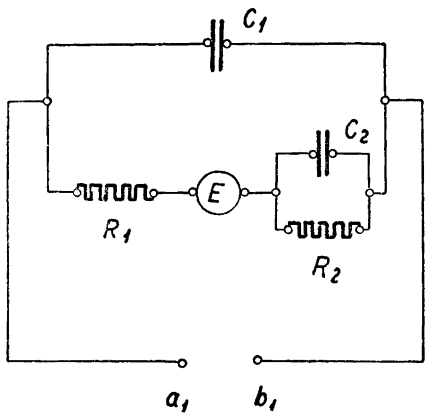

Fig. 9. Electrical scheme for the differential aeration cell (explanation in the text). a_1, b_1 — the terminals of the iron electrodes.

and the electrical capacity, did not show any deviation (Fig. 8, III and IV). However, the results are typical. The initial d.c. resistance* of ca. 800 ohms increased after the lapse of 24 hours and after 8 days it was 7—8 times as high.

At the same time the a.c. resistance ** increased only by ca. 50 %. The capacity of the cell increased in the beginning proportional to the d.c. resistance.

These observations may be interpreted as follows. Let us consider the scheme of Fig. 9. In the beginning, we have only the resistance (R 1) of the water layer between the electrodes. The two iron electrodes, separated by the water layer, form a condensor (C_1). (E) is the electromotive force of the cell. These values change only insignificantly in the course of the experiment.

By the formation of the chalky rust layer more or less covering the cathode surface, two new components (R_2 and C_2) appear which cause the vigorous increase in total resistance and capacity of the cell.

If the chalky rust covered cathode is replaced by a new iron sheet, the initial C_1 and R_1 values are reestablished.

It might be possible by means of this 'differential aeration cell' to measure quantitatively the capacity of the water of forming protective layers. In this case, all dimensions and all experimental conditions must be standardized.

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* The d. c. resistance was calculated from the changes in current caused by an increase in the external resistance of the circuit by 500 ohms. The inner resistance of the micro-amperemeter was 20 ohms.

** The a. c. resistance was measured by means of a Wheatstone bridge. A tube generator with a frequency of 1000 cycles served as a current source. The capacity of the cell was balanced by a variable condensor inserted parallel to the variable decade resistance of the Wheatstone bridge.

SUMMARY

An attempt was made to prove experimentally our previous hypothesis regarding the activity of iron bacteria during corrosion of water pipes. This hypothesis comprised three arguments.

The first and second arguments, the primary formation of the 'differential aeration cell' by iron bacteria and the mechanical reinforcement of the 'tubercle' could be completely corroborated in parallel experiments, using germ filtered water with and without inoculation with iron bacteria and, moreover, by microscopic observations.

The iron bacteria play a decisive part in these phenomena. However, iron bacteria play only a minor part in the establishment of anaerobic conditions under the tubercle during the main period of the corrosion (third argument).

In other words, after the tubercle has formed, corrosion (pitting) proceeds practically independent of the metabolic activity of iron bacteria.

It has been attempted to inhibit further corrosion by changing the equilibrium of the water in such a way that a compact protective layer of 'chalky rust' is formed on the surface of the 'tubercle'.

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Received August 24, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1117–1127

Indigoid Oxidation Products of some *Iso*coumaranone Derivatives

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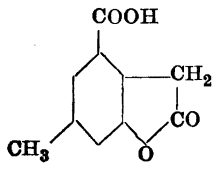
In the course of an investigation of the reaction between maleic anhydride and acetylacetone, one of us [1] prepared the hitherto unknown 6-methyl*iso*-coumaranone-4-carboxylic acid (I). This compound may also be regarded as the lactone of an *o*-hydroxyphenylacetic acid and accordingly on dissolution in sodium hydroxide the lactone ring is opened. When such an alkaline solution was treated with potassium permanganate the methyl group was oxidized to a carboxyl group, and a tricarboxylic acid could be isolated. If, however, substance I was dissolved in a dilute solution of sodium carbonate, no opening of the lactone ring took place, and oxidation with potassium permanganate in the cold in this case gave a blood-red solution from which an intensely red powder, containing only carbon, hydrogen and oxygen, separated on the addition of sulphuric acid. This result seemed so remarkable that the present authors took up the question of the constitution of the red oxidation product.

The red oxidation product (hereafter designated as R) was at first isolated in an amorphous and hygroscopic state. No accurate analysis could therefore be obtained, and the substance was used only for some preliminary experiments. The question naturally arose as to how far the structure of the starting material (I) had been affected by the oxidation. As R was found to be very labile in the presence of alkali it was assumed that the lactone ring was still present after the oxidation. This would also seem to be in accordance with the titrations, as seen from the following:



I

If R was dissolved in cold 0.1 *N* sodium hydroxide and the solution titrated immediately, the following highly varying figures for the equivalent weight were obtained:

E = 130.4, 134.1, 166.0. Calc. for I as a monobasic acid 192.2.

If, however, the solution of R in the alkali was left for some 50 hours at 40° before the titration the values were:

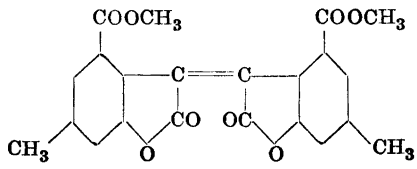
E = 93.0, 92.1, 93.4. Calc. for I as a dibasic acid 96.1.

Another red substance, similar to R, was obtained by oxidation of the methyl ester of I. As the ester was insoluble in aqueous solutions the oxidation had to be carried out in acetone. This new red substance, abbreviated R-Me, could be brought into crystalline form and consequently could be purified sufficiently for analysis. The empirical formula was $C_{11}H_8O_4$ as compared with the formula $C_{11}H_{10}O_4$ for the starting material, the methyl ester of I. The result of the oxidation was therefore that two atoms of hydrogen were removed for each molecule of I. This is also in accordance with the fact that a maximum yield of R-Me (about 60 per cent) was obtained when oxidizing the ester of I with a quantity of potassium permanganate corresponding to the taking up of two redox-equivalents.

Cryoscopic measurements in benzene showed that R-Me had the molecular formula $C_{22}H_{16}O_8$. The molecule of R-Me must therefore be built up of two molecules of the ester of I from which, in all, four atoms of hydrogen had been removed. The following additional facts should also be considered in setting up a structural formula.

1. The carboxyl group is not involved in the coupling.
2. The lactone —O—CO-grouping is present in the oxidation product.
3. The deep red colour of R and R-Me indicates that the new linking contributes to the formation of a chromophoric group.
4. The new linking between the two halves of the molecule is most probably of an unsaturated character, since, by catalytic hydrogenation or by reduction with nascent hydrogen, R-Me took up one molecule of hydrogen giving a colourless substance $C_{22}H_{18}O_8$.

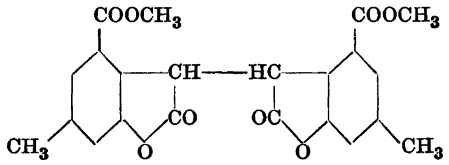
Accordingly formula II is suggested for R-Me. The corresponding free dicarboxylic acid should then most likely represent the original red substance R. It ought to be mentioned that, as in the case of indigo, the formula could as well be written in a *trans*-form.



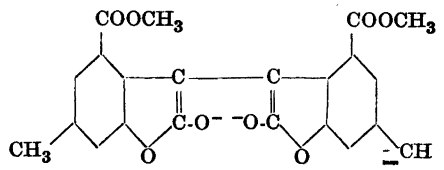
II

The skeleton of this formula is isomeric with that of oxindigo. The two red substances R and R-Me could therefore be designated as 4,4′-dicarboxy-6,6′-dimethyl*iso*oxindigo and 4,4′-dicarbmethoxy-6,6′-dimethyl*iso*oxindigo respectively. According to a proposal by P. Jacobson, referred to by Friedländer [2], such substances could be named as derivatives of bis-(coumaran-3)-indigo.

As mentioned in point 4, R-Me on hydrogenation took up one molecule of hydrogen. The hydrogenated substance would therefore have formula III. When a little sodium hydroxide was added to a solution of III in alcohol or acetone the solution immediately turned brownish-red, the colour disappearing again on acidifying. The reason for this was obviously that a double enolisation took place in alkaline solution as shown in formula IV.

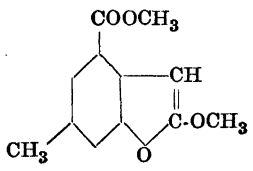


III



IV

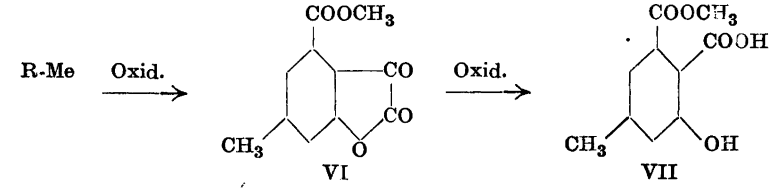
If the brownish-red solution was allowed to stand for a short time the colour turned yellow, probably due to an opening of the enolised lactone rings by the prolonged action of the alkali. The hydrogenated substance itself was partly oxidised again when exposed to the air giving R-Me which was relatively stable in the presence of mineral acid.



V

By the action of diazomethane on the methyl ester of I in the presence of some methanol, the methyl enol ether V was prepared. In this substance the enol grouping is fixed and there should be no possibility of the formation of a double bond between two such molecules. Accordingly no red oxidation product could be obtained from V.

Further oxidation of R-Me gave a substance $C_{10}H_{10}O_5$ which was evidently a derivative of phtalic acid having the formula VII. The coumarandione derivative VI may be expected as an intermediate product of the oxidation, but attempts to isolate it were unsuccessful.



VI VII

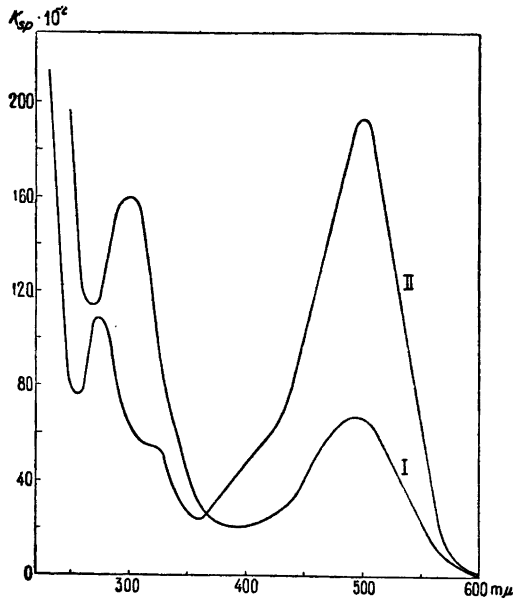
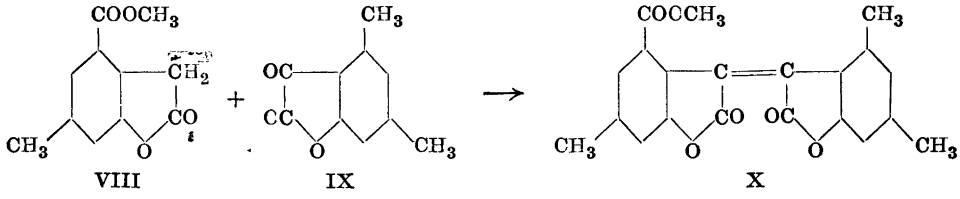


Fig. 1. Light absorption in ethanol.

I 4,4'-Dicarboxy-6,6'-dimethyl i s o-oxindigo (R)
II 4,4'-Dicarbmethoxy-6,6'-dimethyl i s o-oxindigo (R-Me)

The free acid corresponding to VII had a m. p. of 223°, and on sublimation in a vacuum easily split off water giving an anhydride of m. p. 206°. Both these substances gave a strong fluorescein reaction when heated with resorcinol and sulphuric acid. According to the literature [3] 4-methyl-6-hydroxyphtalic acid and its anhydride have m. p. 226—228° and 209° respectively.

The synthesis of a red compound analogous to R-Me was accomplished in the manner used by Stollé and Knebel [4] in their synthesis of tetramethyl-oxindirubin. The methyl 6-methyl*iso*coumaranone-4-carboxylate (VIII) was condensed by means of sulphuric acid with 4,6-dimethylcoumarandione (IX) prepared according to Stollé and Knebel. The condensation product (X), which had the composition $C_{21}H_{16}O_6$, resembled R-Me in colour and behaviour. The light absorption curves of the two substances will be found in Fig. 2.



This synthesis could lead one to the opinion that the oxidation of the methyl ester of I proceeded in such a way that some molecules were first

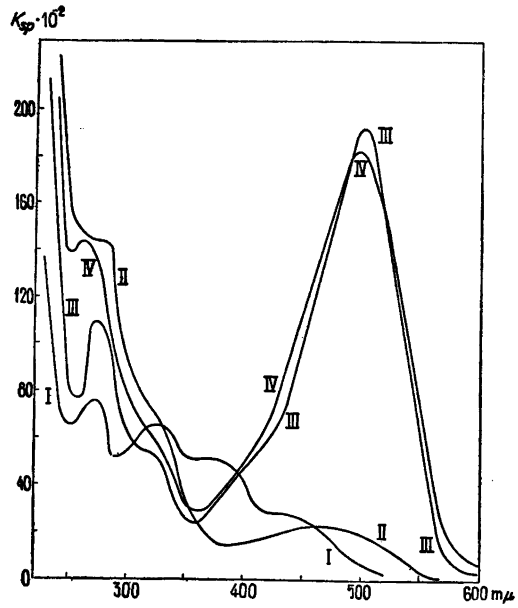


Fig. 2. Light absorption in ethanol.

I I s o oxindigo
II 4,4'-Dimethyl i s o oxindigo
III 4,4'-Dicarbmethoxy-6,6'-dimethyl- i s o oxindigo (R-Me)
IV 4-Carbmethoxy-6,4',6'-trimethyl- i s o oxindigo

oxidized to a coumarandione derivative which then condensed with other non-oxidised molecules. This interpretation is opposed by two facts:

1. The bad yield of the condensation (5—6 per cent) as compared to that of the oxidation (60 per cent).

2. The solution in which the oxidation was carried out was not free from water, and it became alkaline during the oxidation. A coumarandione derivative would certainly, under these conditions, be very rapidly hydrolysed and withdrawn from further reaction.

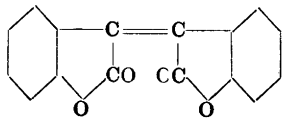
In this connection it ought to be mentioned that the methyl ester of I could be dehydrogenated directly to R-Me. Thus R-Me was obtained in excellent yield when the methyl ester was heated with selenium in a nitrogen atmosphere.

In the foregoing a complete analogy in the structures of the two oxidation products R and R-Me has been assumed, although R was at first obtained only in an amorphous state. Later, however, after experience had been gained in the work with R-Me, even R could be brought into a crystalline form which was no longer hygroscopic. It could therefore easily be purified and analysed. The analysis gave the formula $(C_{10}H_6O_4)_n$, but as the solubility of the substance was very small no determination of the molecular weight by cryoscopic or ebullioscopic methods could be carried out. On reduction of R with nascent hydrogen a colourless, crystalline substance $(C_{10}H_7O_4)_n$ was obtained. It

therefore seems justified in the case of R also to accept $n = 2$. A comparison of the light absorption of R and R-Me will be found in Fig. 1. The great increase in absorption in the visible part of the spectrum when the carboxyl groups are esterified is very remarkable.

Substances similar to R and R-Me were obtained by the oxidation of other *iso*coumaranone derivatives. They are mentioned in the experimental part.

By treatment of *iso*coumaranone with S_2Cl_2 Chovin [5] prepared a yellow-orange substance $C_{16}H_8O_4$ which he assumed to be *iso*oxindigo (XI).



XI

We have repeated his experiments and were able to verify them. As our red oxidation products may be regarded as derivatives of *iso*oxindigo it was of interest to compare the light absorption of them with that of *iso*oxindigo. The results of our measurements will be found in Fig. 2. As can be seen, the introduction of methyl groups in the benzene nuclei affects mainly the absorption in ultraviolet. The introduction of one esterified carboxyl group, however, very markedly increases the absorption in the visible part of the spectrum. This absorption is not increased appreciably by the introduction of further esterified carboxyl groups.

EXPERIMENTAL PART

Preparation of substance R

6-Methyl*iso*coumaranone-4-carboxylic acid (3 g), prepared according to Berner [1], was dissolved in 2 *N* sodium carbonate (20 ml) and diluted to 500 ml. Keeping the solution at about + 1° and stirring vigorously, 70 ml of a 2.5 per cent solution of potassium permanganate were slowly added. After the manganese dioxide had been filtered off a clear blood-red solution was obtained from which R was precipitated as a very fine red powder by the addition of sulphuric acid. This was left over night in a refrigerator after which it could easily be filtered by suction. After washing with distilled water until the filtrate gave no reaction for sulphuric acid, the red powder was dried in a vacuum desiccator. Yield 0.7 g.

The precipitated form of R was amorphous and very hygroscopic. Leaving it in an atmosphere of 50 per cent humidity the weight increased 1.8 per cent in 7 hours. The samples used for the titration experiments already mentioned, were dried directly in small weighing tubes which were fitted with stoppers.

Crystallisation: Amorphous R (1 g) was dissolved in acetone (50 ml), filtered, and water (10 ml) added. When the acetone was allowed to evaporate slowly, a red powder

separated which seen under the microscope was found to consist of small but well developed crystals. After recrystallisation in the same manner, the powder was dried at 80°. It had no definite melting point.

$C_{20}H_{12}O_8$	Calc.	C	63.25	H	3.18
	Found	»	63.18, 63.06	»	3.28, 3.30

Reduction of substance R

Crystallized R (0.5 g) was dissolved in ethanol (50 ml) and zinc dust (2.5 g) and a little 2 *N* sulphuric acid were added. The reaction vessel was closed with a stopper and shaken for 17—18 minutes when the solution still had a light red colour. After filtration some more zinc dust and acid were therefore added. In a couple of minutes the red colour disappeared completely and only a light brownish-yellow colour remained. The solution was again filtered, and, after adding water, the ethanol was evaporated by blowing a current of air on the surface. A colourless crystalline substance began to separate, but the solution gradually became red, obviously due to oxidation by the air. In order to obtain quite colourless crystals, the substance was recrystallized from acetone-water leading a current of nitrogen through the solution. The crystals had no definite m. p., but on heating turned into a red substance which was decomposed.

$(C_{10}H_7O_4)_n$	Calc.	C	62.82	H	3.69
	Found	»	62.71, 62.65	»	3.85, 3.79

Preparation of substance R-Me

6-Methyl*iso*coumaranone-4-carboxylic acid was esterified in the usual way by means of an ethereal solution of diazomethane. The optimum conditions for the oxidation of the ester to R-Me were found to be the following: The methyl ester (1 g) was dissolved in acetone (100 ml) and potassium permanganate (0.5 g) in acetone (100 ml) was added slowly. The solution was stirred mechanically and the temperature kept below 10°. The manganese dioxide was filtered off and washed twice with acetone as it easily adsorbed the red oxidation product. Having added a sufficient quantity of 2 *N* sulphuric acid, the filtrate was evaporated on the water-bath to half volume and cooled. Water was now added until the solution became slightly opaque, and the solution afterwards heated until it was clear again. On standing, small red crystals began to separate. They were filtered off and the filtrate left in an open beaker where the acetone gradually evaporated and more crystals of R-Me were obtained. The yield was about 60 per cent of theory. The substance was recrystallized twice from acetone-water and, after drying, had the unsharp m. p. 205—210°.

$C_{22}H_{16}O_8$ (408.4)	Calc.	C	64.71	H	3.92
	Found	»	64.85, 64.60	»	4.07, 3.96

0.0970, 0.1009 g subst. in 22.8, 20.6 g benzene, Δ 0.053, 0.062° Mol. wt. 411, 405.

This red oxidation product was so insoluble in water that it remained unchanged after boiling for half an hour with 2 *N* sodium hydroxide. If, however, a few drops of alkali were added to a solution of R-Me in ethanol, the red colour disappeared momentarily, and the solution became yellow with a strong fluorescence in green.

Hydrogenation of R-Me

With gaseous hydrogen: A solution of R-Me (4 g) in glacial acetic acid (200 ml), to which platinum oxide (0.4 g) had been added, took up 165 ml hydrogen (1200 mm Hg, 20°) in 5 hours; calc. for one mole hydrogen 152 ml. During the hydrogenation the red colour disappeared and at the end the solution became quite colourless. However, upon evaporating the main part of the glacial acetic acid on the waterbath, the solution again took on a red colour; and after the remaining acetic acid had been removed in a vacuum desiccator over potassium hydroxide the hydrogenated substance was obtained as light red crystals. After being recrystallised twice from acetone, the substance became colourless. When heated, the crystals took on a red colour at about 200°, and they melted at 230—232° giving a dark red liquid. For analysis see below, sample a.

With nascent hydrogen: After shaking a solution of R-Me (0.5 g) in ethanol (50 ml) to which zinc dust (3 g) and 2 *N* sulphuric acid (3 ml) had been added, the red colour disappeared in half a minute. After the filtered solution had been diluted with water small colourless crystals separated. They were recrystallised from acetone-water and had the same m. p. as above. Sample b.

$C_{22}H_{18}O_8$ (410.4)	Calc.	C	64.40		H	4.43
Sample a	Found	»	64.30, 64.27		»	4.56, 4.50
» b	»	»	64.37, 64.45		»	4.42, 4.25

0.0830, 0.0919 g subst. in 21.3, 20.8 g benzene, Δ 0.048, 0.054° Mol. wt. 416, 419.

The hydrogenated substance was entirely insoluble in water. Dissolved in chloroform it did not add bromine. When 2 *N* sodium hydroxide (2 ml) was added to a solution of the hydrogenated substance (0.05 g) in acetone (50 ml), the solution turned brownish-red. A small part of this solution was set aside and in a short time the colour became yellow. The rest of the solution was acidified with sulphuric acid. After some time a red substance congregated on the surface, whereas colourless crystals were formed on the walls and bottom of the beaker. The two substances could therefore be isolated separately.

The red substance on purification gave crystals of the same form as found for R-Me and the m. p. was the same (205—210°), alone and in admixture. The colourless substance was identical with the hydrogenated R-Me. M. p. 229°, alone and in admixture.

Enol-ether of methyl 6-methyl*iso*coumaranone-4-carboxylate

Finely powdered methyl 6-methyl*iso*coumaranone-4-carboxylate (1 g) was added to an ethereal solution of diazomethane (from 4 g of methylnitrosourea) which contained 1 ml methanol. After standing for 12 hours in a refrigerator and 4 days at room temperature, most of the ester had dissolved. The solution was still strongly yellow, but it contained no more diazomethane. On evaporating the ether some unchanged ester gradually separated and was removed. When all the solvent had been evaporated, a yellow viscous residue remained. This was extracted with a small quantity of ether and a small residue of the unchanged ester was left. After evaporation, the new residue was subjected to a second extraction process. Finally the enol-ether was obtained in a quite pure form as a yellow highly viscous syrup. The last traces of solvent were removed by

leaving the syrup for 4 days in a vacuum desiccator (1 mm Hg) above conc. sulphuric acid. The enol-ether rapidly added bromine and simultaneously split off hydrogen bromide.

0.1150, 0.1083 g subst. gave 0.2395, 0.2240 g AgI

$C_{10}H_6O_2(OCH_3)_2$ Calc. CH_3O 28.18 Found 27.50, 27.31

When the enol-ether (1.3 g) dissolved in acetone (50 ml) was oxidized with a 2.5 per cent solution of potassium permanganate in acetone no formation of a red oxidation product took place.

Further oxidation of R-Me

To a solution of methyl 6-methyl*iso*coumaranone-4-carboxylate (0.5 g) in acetone (100 ml) a 2.5 per cent solution of potassium permanganate in acetone was added until the permanganate colour remained. In all 40 ml of the latter were needed. The red colour of the R-Me first formed disappeared with prolonged oxidation. During the last stage of the oxidation the temperature was kept at 45°. Having destroyed the excess of permanganate, the filtered solution was acidified with sulphuric acid, and the acetone evaporated on the water-bath. The remaining aqueous solution was left for a couple of days in a cool place when a small quantity of a brown material separated and was removed by filtration. Upon extraction of the filtered solution with ether a crystalline substance was obtained which after recrystallising twice from benzene-gasoline had m. p. 128—129°. The substance gave a violet colour with ferric chloride.

$C_{10}H_{10}O_5$	Calc.	C 57.15	H 4.81
	Found	» 57.03, 57.10	» 4.86, 4.95

A solution of this substance (0.1 g) in 2 *N* sodium hydroxide (5 ml) was boiled for 10 minutes. After acidifying with sulphuric acid the solution was shaken three times with ether. On evaporation of the dried ethereal solution a crystalline substance was obtained which obviously was the 4-methyl-6-hydroxyphtalic acid. It had m. p. 223° and when sublimated in a high vacuum gave a yellowish anhydride with m. p. 206°. Both substances on heating with resorcinol and conc. sulphuric acid gave the fluorescein reaction. According to Schleussner and Voswinckel [3] 4-methyl-6-hydroxyphtalic acid and its anhydride have m. p. 226—228° and 209° respectively.

Condensation of coumarandione and *iso*coumaranone derivatives

4,6-Dimethylcoumarandione (0.33 g), prepared according to Stollé and Knebel [4] and methyl 6-methyl*iso*coumaranone-4-carboxylate (0.33 g) were mixed and glacial acetic acid (1 ml) added. The mixture was cooled to about — 20° and conc. sulphuric acid (5 ml) added cautiously. After keeping the mixture for 20 hours in a refrigerator it was poured into water and a precipitate was obtained consisting of a red component mixed with some of the starting material. Boiling the dried precipitate with ligroin dissolved the red component and some of the starting material, but upon cooling most of the dimethylcoumarandione separated again and was filtered off. When the filtrate was con-

centrated the red component congregated along the walls of the glass vessel, whereas some more of the unchanged starting material crystallized on the bottom and could easily be removed. The red substance was dissolved in acetone and water added until the solution became opaque. After the solution had been heated till it became clear again it was left for crystallisation. At first a little dimethylcoumarandione separated and was removed by filtration, and then the red substance crystallized. It was recrystallized from acetone-water and dried in a vacuum. M. p. 190—200°. Yield 5—6 per cent of theory.

$C_{21}H_{16}O_6$	Calc.	C	69.21		H	4.43
	Found	»	69.08, 69.16		»	4.58, 4.60

Dehydrogenation with selenium

Methyl 6-methyl*iso*coumaranone-4-carboxylate (1 g) and selenium (0.5 g) were ground together thoroughly in a mortar and then heated in a sealed tube in a nitrogen atmosphere to 180° for 10 hours. When the tube was opened a considerable amount of hydrogen selenide effused. The reaction product was extracted with acetone and a strongly red-coloured solution was obtained. A red crystalline substance was gained from this solution in the same way as described for R-Me. M. p. 205—210°.

$C_{22}H_{16}O_8$	Calc.	C	64.71		H	3.95
	Found	»	64.60, 64.65		»	4.03, 4.15

Oxidation of other *iso*coumaranones

The oxidation was carried out as described for the preparation of R-Me. Therefore only the analytical results are given here.

Methyl *iso*coumaranone-4,6-dicarboxylate, prepared from the corresponding acid [1] by means of diazomethane, gave a red crystalline substance which had no definite melting point.

$C_{22}H_{10}O_{10}(OCH_3)_2$	Calc.	C	58.05		H	3.25	CH_3O	25.00
	Found	»	57.80, 58.10		»	3.40, 3.44	»	24.60

4-Methyl*iso*coumaranone, prepared by stud.mag.scient. E. Augdahl (unpubl.), gave a red crystalline substance which melted from 210 to 245°.

$C_{18}H_{12}O_4$	Calc.	C	73.98		H	4.14
	Found	»	73.86, 73.80		»	4.25, 4.28

Methyl *iso*coumaranone-7-carboxylate, prepared by stud.mag.scient. O. Aubert (unpubl.), gave a red crystalline substance which was extremely labile in the presence of alkali.

$C_{20}H_{12}O_8$	Calc.	C	63.17		H	3.18
	Found	»	63.00, 62.88		»	3.25, 3.30

SUMMARY

The red substance obtained by the oxidation of 6-methyl*iso*coumaranone-4-carboxylic acid with potassium permanganate in sodium carbonate solution was found to be a derivative of *iso*oxindigo. Similar red substances, all crystalline, could be obtained by the oxidation in acetone solution of other *iso*-coumaranones or by the condensation of an *iso*coumaranone with a coumarandione. Dehydrogenation with selenium could be used instead of oxidation with permanganate. The light absorption of the *iso*oxindigo derivatives dissolved in ethanol was measured.

One of us (Berner) wishes to express his appreciation to *Det Videnskapelige Forskningsfond av 1919* for a grant.

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Received August 31, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1128–1132

On the Occurence of Isoguanine in Pig Blood

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2-Hydroxy-6-aminopurine (isoguanine, oxyadenine) was first synthesized by Emil Fischer in 1897 [1].

The first report on its occurence in nature appeared in 1927 when Buell and Perkins [2] claimed to have isolated isoguanine from pig blood in a yield of about 5 mg per liter.

In 1932 Cherbuliez and Bernhard [3] isolated a nucleoside from croton bean (*Croton tiglium* L.) and identified the aglycone as isoguanine and the sugar as D-ribose by the properties of various derivatives. The sugar was then crystallized by Spies and Drake [4], and Spies [5] confirmed that the purine in this nucleoside was isoguanine by converting it to xanthine.

Isoguanine has further been identified by Purrman[12] among the nitrogenous bases in wings of certain butterflies *(Catopsilia)*.

Schütz [7] has reported that he was unable to isolate any isoguanine from pig blood with the procedure of Buell and Perkins [2]. The only purine found was adenine. He also questioned the work of Cherbuliez and Bernhard as to the identity of the purine in the crotonoside but later work has proved these doubts to be unfounded.

The confirmation of the occurence of isoguanine in an animal organism would be of interest in connection with the transformation of adenine into guanine demonstrated to occur in rat by Brown, Roll and Plentl [7] with the aid of isotopically marked purines. We have therefore reinvestigated the composition of the purine fraction in pig blood.

It was found that partition chromatography on starch, as developed for purines by Edman, Hammarsten, Löw and Reichard [9], can be used to separate isoguanine from its mixture with adenine and guanine as exemplified in Fig. 1.

Recently Bendich, Tinker and Brown [10] have described a method to purify and characterize the natural product from croton bean with countercurrent distribution and they have also worked out a new synthesis of isoguanine.

Following the procedure of Buell and Perkins [2] for the preparation of isoguanine from pig blood we have tried to demonstrate the presence of isoguanine in the later stages of their procedure by means of chromatography on starch. With this sensitive method no trace of isoguanine could be demonstrated. The ultimate product was practically pure adenine.

This is in accordance with the findings of Schütz [6] and taken together with the note in the paper by Mitchell and Houlahan [11] it appears improbable that isoguanine has ever been isolated from higher animals.

EXPERIMENTAL

Isoguanine

Isoguanine, prepared from the crotonoside according to Cherbuliez and Bernhard [3], was first used as a reference compound. We are greatly indebted to professor Bernhard for a sample of the crotonoside. After hydrolysis of the natural crotonoside and one recrystallization from sulfuric acid pure isoguanine sulfate was obtained.

$(C_3H_3ON_5)_2H_2SO_4H_2O$ Calc. N 33.5 Found N 33.1

Later a sample of synthetic isoguanine [10] was kindly furnished us by Dr. Bendich. The two samples had identical absorption curves (Fig. 3) and also gave identical x-ray powder diffraction patterns. We are greatly indebted to Dr. Einar Stenhagen, Upsala, for the latter measurements.

Preparation of purines from pig blood

For the preparation of the purine from pig blood the procedure of Buell and Perkins [2] was followed. In two different preparations from 5 l of pig blood the yields of purine in the final step were 15 and 60 mg.

In order to separate isoguanine from other purines expected to contaminate the preparation, starch chromatography was employed. The technic was essentially that of Edman [8] and Edman, Hammarsten, Löw and Reichard [9]. The starch was freed from soluble material absorbing in the ultraviolet through extraction with 80 per cent dioxane-water for 24 hours in a Soxhlet apparatus. Likewise methyl glycol and *n*-butanol were freed from most of the absorbing material by distillation through a Widmer column.

As a model experiment a mixture of adenine, guanine and isoguanine was separated on the starch column. 1.98 mg adenine, 2.05 mg guanine and 1.37

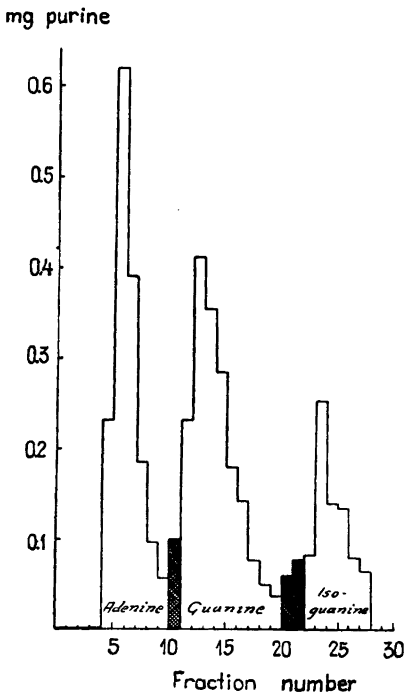

Fig. 1. Chromatography on starch of mixture containing adenine, guanine and isoguanine. Shadowed areas indicate mixed fractions.

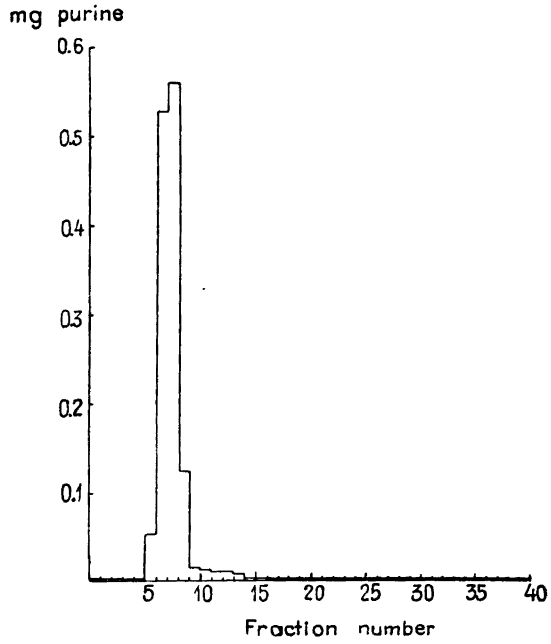

Fig. 2. Chromatography on starch of purine from swine blood.

mg isoguanine were dissolved by heating on the water bath in 0.7 ml methyl glycol and 0.1 ml *N* sodium hydroxide. To this solution was added 9.8 ml *n*-butanol-water (87 % v/v *n*-butanol) and the whole transferred to the top of the starch column. The height of the column was 205 mm and the diameter 24 mm. The chromatogram was developed in a mixture of *n*-butanol /water/ methyl glycol in the proportions 12 : 2 : 1 by volume. The effluents were cut with an interval of one hour, corresponding in this experiment to a fraction volume of about 12 ml. After completion of the chromatography all fractions were evaporated to dryness *in vacuo*, subsequently dissolved in 1 *N* hydrochloric acid and the absorption measured at wave lengthes 248, 262 and 285 mμ. From the extinctions at these wave lengthes coefficients can be formed suitable for the identification of the individual purines. The result of the fractionation can be seen in Fig. 1.

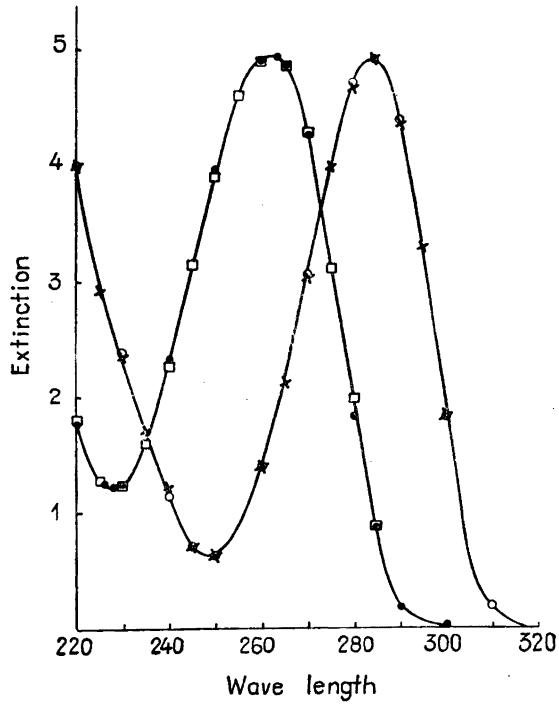

Fig. 3. Ultraviolet absorption curves of a) adenine (Hoffmann—La Roche), $5.6 \cdot 10^{-3}$ %, —□—□—; b) chromatography fraction no. 7, —●—●—; c) isoguanine (synthetic), $6.0 \cdot 10^{-3}$ % —○—○—; d) isoguanine (from crotonoside), $6.1 \cdot 10^{-3}$ %, —×—×—. Solvent N HCl, d = 1 cm.

Under identical conditions a fractionation was carried out on a 2 mg specimen of purine (as hydrochloride) from pig blood. The result is presented in Fig. 2. This experiment was repeated on an independent preparation with the same result. The complete ultraviolet absorption curve of fraction no. 7 was measured and this is compared in Fig. 3 with the corresponding curves for pure adenine (Hoffmann-La Roche) and samples of synthetic and natural isoguanine.

Since it were conceivable that the isoguanine had been lost during the later stages of the purification procedure, it was also tested for isoguanine at an earlier stage. After the precipitation with cupric sulfate and sodium bisulfite the preparation was freed from copper with hydrogen sulfide in hydrochloric acid. The filtrate was evaporated to dryness and a sample was taken for chromatography. The chromatogram showed only one peak. This appeared on the place of adenine and had the quotient $\frac{E\ 262}{E\ 248} = 1.37$ characteristic of adenine.

The x-ray diffraction pattern of the product isolated from blood was identical with that of adenine hydrochloride.

SUMMARY

1. A chromatographic procedure is described whereby small amounts of isoguanine, adenine and guanine can be separated.

2. Using this technic isoguanine could not be found in pig blood.

We gratefully acknowledge the assistance of med. stud. I. Gottfries and B. Haeger. This work has been supported by a grant from *Therese och Johan Anderssons Minne.*

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Received September 6, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1133–1136

Compounds of Thorium and Quadrivalent Uranium with Benzoylacetone and Dibenzoylmethane

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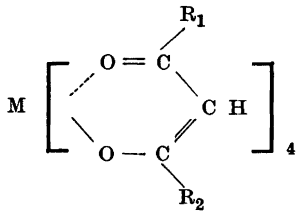
Partly for some attempts to obtain a Szilard-Chalmers concentration of thorium, partly for a magnetochemical investigation, it was of interest to prepare the six complex compounds



where M = Th or U^{IV} and R_1, R_2 = CH_3 or C_6H_5.

A good method used for the preparation of thoriumacetylacetonate is the one described in *Inorganic Syntheses* [1]. Uranium(IV)acetylacetonate was prepared according to Biltz and Clinch [2], with some small modifications.

It does not appear that the other compounds, included in the above formula, have been reported in the literature. This paper describes the preparation of these chelate compounds and some of their properties.

The thorium compounds were prepared by treating a solution of benzoylacetone or dibenzoylmethane in ether, with a solution of thorium nitrate in water, and adding ammonia until the mixture had about pH 7. The uranium compounds could not conveniently be prepared in that way, on account of the rapid oxidation of the quadrivalent uranium. It was, however, possible to synthetize them in a hot, saturated, alkaline solution of diketone in water to which an uranium solution was added. The work with the uranium compounds in solution was carried out in nitrogen atmosphere.

EXPERIMENTAL

Preparation of the diketones *. Acetylacetone and dibenzoylmethane were prepared according to methods given in *Org. Syntheses* [3, 4]. Benzoylacetonate was prepared by condensation of acetylacetate with acetophenone according to Fischer [5].

Preparation of the uranium(IV)solution. Uranium(IV)solution was prepared by electrolytic reduction of uranyl chloride in hydrochloric acid solution, according to a modification of a method described by Rosenheim and Loebel [6]. In order to obtain the reduction to quadrivalent state of uranium it was, however, not necessary to exclude air from the reduction vessel. However, for preservation, the uranium(IV)solution was kept in nitrogen atmosphere.

Analysis of thorium and uranium. The complex compounds were decomposed by strong nitric acid. After evaporation up to dryness the residue was ignited over the blast to constant weight. Thus thorium was determined as ThO_2 and uranium as U_3O_8.

In order to determine the concentration of quadrivalent uranium in the uranium solutions, titration with permanganate was used [7].

Thorium benzoylacetonate

27 g of benzoylacetone was dissolved in 250 ml ether and the solution was added to a solution of 17 g $Th(NO_3)_4$, $4HO_2$ in 40 ml of water. The solutions were shaken after each addition of small portions of 4 *N* ammonia until the complex salt precipitated. 4 *N* ammonia was then added drop by drop until the mixture just became alkaline. When the ether layer had been poured off, the precipitated thorium benzoylacetonate was filtered off on a Büchner funnel, washed with a small portion of ether, and after that with hot water.

When the precipitate had been dried in a vacuum desiccator, it was recrystallized from benzene. M. p. 212—213° C (dec). Yield 18.6 g (69 %).

$C_{40}H_{36}O_8Th$ (876.8)	Calc.	C	54,79	H	4.14	Th	26.47
	Found	»	54.38	»	4.14	»	26.46

Thoriumbenzoylacetonate constitutes a pale-yellow powder, insoluble in water, acetone, ethanol and ether, slightly soluble in benzene, toluene (about 0.4 g/l), aniline and pyridine (about 1 g/l).

Thorium dibenzoylmethane

10 g of dibenzoylmethane was dissolved in 50 ml ether. This solution was added to a solution of 5 g $Th(NO_3)_4$, $4H_2O$ in 15 ml of water. The complex compound was then prepared in the same manner as the benzoylacetonate. Excess of ammonia was avoided. An excess could conveniently be counteracted by a small portion of acetic acid.

The precipitate was recrystallized from benzene. M. p. about 196° C (dec). Yield 5.1 g (49 %).

$C_{60}H_{44}O_8Th$ (1125.1)	Calc.	C	64.04	H	3.94	Th	20.63
	Found	»	64.01	»	3.98	»	20.51

* These preparations were carried out by Mr. K. Halvarson, whom I wish to thank.

Thorium dibenzoylmethane forms yellow crystals, insoluble in water and ethanol, soluble in acetone, ether, benzene (about 50 g/l) and toluene, very soluble in pyridine.

Uranium(IV)acetylacetonate

This compound was prepared according to Biltz and Clinch [2]. However, it was found to be advisable to recrystalize from ether in nitrogen atmosphere. Without this precaution the substance was easily oxidized. Its colour became yellow-green, and the analysis gave too high values of the uranium content. Also by recrystallization from toluene in air, the compound was easily oxidized.

The recrystallizations in nitrogen atmosphere were carried out in a Soxhlet apparatus, where the bulb was provided with a glass inlet tube for the gas from a nitrogen container.

The decomposition of the acetylacetonate seems to begin at about 165° C, and the melting point at about 177° C is vague. The yield of the recrystallized product was 40 %.

It is soluble in acetone, ethanol, ether, benzene, toluene (about 40 g/l) and pyridine.

Uranium(IV)benzoylacetonate

To 150 ml of a solution of UCl_4(0.20 C) and HCl(3.8 C) were added first 17 g of sodium acetate and then with rapid stirring a solution of 15 g NaOH in water. 15 g benzoylacetonate was dissolved at about 65° C in 600 ml water, containing 5 g of NaOH. The stirring was carried on and the solution added to the buffered uranium solution, which was first heated to 65° C. A dark-brown precipitate was formed, which easily clogged together and adhered to the glass walls. It was filtered off on a Büchner funnel and washed with hot water and afterwards with portions of ethanol. The precipitation and the rapid filtration was suitably carried out in air.

The substance was dried in a vacuum desiccator and then recrystallized from benzene in nitrogen atmosphere. M. p. about 210° C (dec). Yield 13.3 g (65 %).

$C_{40}H_{36}O_8U$ (882.7)·	Calc.	C	54.42	H	4.11	U 26.97
	Found	»	54.89	»	4.17	» 26.50

Uranium(IV)benzoylacetonate forms a red-brown powder insoluble in water, very slightly soluble in ethanol, ether and toluene, slightly soluble in benzene and soluble in pyridine. It is easily oxidized by air in solutions.

Uranium(IV)dibenzoylmethane

To 80 ml of a solution of UCl_4 (0.22 C) and HCl(5.1 C) were added, first 10 g of sodium acetate, and then under stirring a solution of 12 g NaOH in water. 15 g dibenzoylmethane was dissolved at about 65° C in 600 ml water containing 4 g NaOH. The preparation was then done in the same way as the preparation of the benzoylacetonate.

The compound was recrystallized from benzene in nitrogen atmosphere. M. p. 198—199° C. The decomposition begins at about 180° C. Yield: 10.4 g (55 %).

$C_{60}H_{44}O_8U$ (1131.0)	Calc.	C	63.71	H	3.92	U 21.05
	Found	»	63.71	»	3.95	» 20.86

Uranium(IV)dibenzoylmethane forms a red-brown powder, insoluble in water, slightly soluble in ethanol, soluble in acetone, ether, benzene and pyridine.

It might be possible to find addition compounds of the described thorium and uranium complex compounds with different solvents. However, attempts to prepare such compounds have not been made.

SUMMARY

Preparations of thorium benzoylacetonate, thorium dibenzoylmethane, uranium(IV)benzoylacetone and uranium(IV)dibenzoylmethane have been described. The method to prepare uranium(IV)acetylacetonate given by Biltz and Clinch has been commented.

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Received September 3, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1137–1146

Antibiotic Substances from the Heart Wood of *Thuja plicata* D. Don

VI.* The Structure of Thujic Acid (Dehydroperillic Acid)

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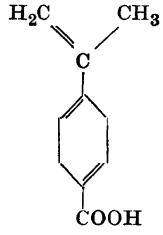
In 1933 Anderson and Sherrard [1] attributed formula I to an acid occuring in the heart-wood of western red cedar (*Thuja plicata* D. Don.).



I

As it contains one double bond more than perillic acid, they termed the acid dehydroperillic acid. Their chief arguments in favour of this formula were the conversion of the acid into cumic acid by boiling with 3 % hydrochloric acid; and the results of ozonisation experiments which yielded, among other compounds, a substance believed to be β, γ-diketovaleric acid.

In this connection it might be mentioned that the author has been unable to duplicate this conversion with 3 % hydrochloric acid as described by Anderson and Sherrard. Boiling with conc. hydrochloric acid in acetic acid solution, however, gave cumic acid in good yield.

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* Part V. Preliminary note. Erdtman, H., and Gripenberg, J. *Nature* **164** (1949) 316.

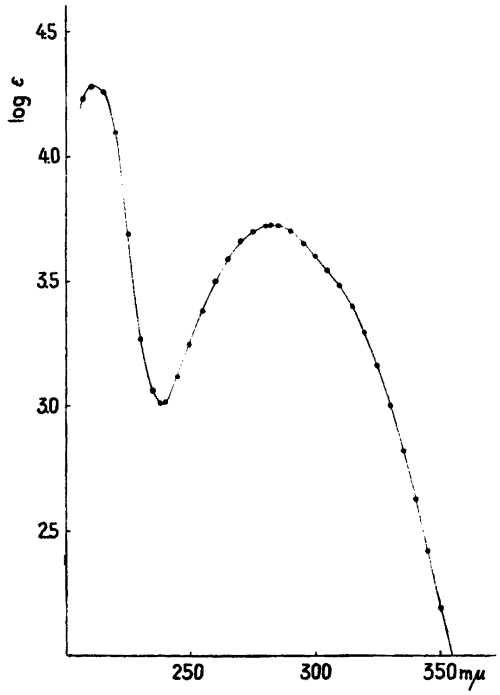


Fig. 1. Absorption spectrum of thujic acid.

In a preliminary communication [2], Erdtman and the present author pointed out that the absorption spectrum of the acid from *Thuja plicata* (Fig. 1) is not compatible with structure I, but indicates a larger conjugated system. Quite recently Hurd and Edwards [3] have also expressed some doubts as to the validity of this structure. Also in agreement with their observations is the failure of 'dehydroperillic acid' to react with maleic anhydride.

A closer investigation showed that the acid possesses a quite different carbon skeleton. The formation of cumic acid is due to a molecular rearrangement. It has therefore been proposed [4] that the name 'dehydroperillic acid' should be abandoned, as there is no structural relationship to perillic acid, and the acid renamed *thujic acid.*

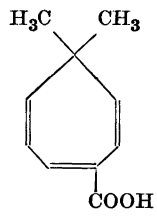
Anderson and Sherrard [1] prepared hexahydrothujic acid by catalytic hydrogenation of thujic acid. The oily product was believed to be a previously unknown liquid isomeride of hexahydrocumic acid of m.p. 94—95°. Cooke and Macbeth[5], however, later prepared this isomeride and characterised it by conversion into the *p*-bromophenacyl ester m.p. 85°. Hexahydrothujic acid, however, yielded a *p*-bromophenacyl ester which was obtained in two dimorphic modifications, one with m.p. 70—71°, the other with m.p. 65—66°.

A mixed melting point determination with the *p*-bromophenacyl ester of hexahydrocumic acid prepared according to Cooke and Macbeth [5] showed strong depression.

Thus hexahydrothujic acid is not identical with either of the two possible forms of hexahydrocumic acid, and hence, cannot have the carbon skeleton of I.

Furthermore, on oxidation with potassium permanganate, thujic acid gave dimethylmalonic acid. This indicates the presence of a *gem*-dimethyl group in the molecule of thujic acid.

Taking into account the formation of dimethylmalonic acid on oxidation, and the facile isomerisation of thujic acid to cumic acid, the following three formulae suggest themselves as possible structures of thujic acid.



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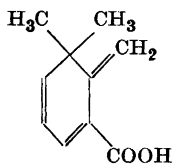
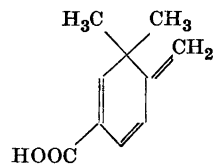


III



IV

Anderson and Sherrard [1] claim the isolation of formaldehyde as a product of ozonisation of thujic acid, but do not mention the yield. This would, tend to indicate either III or IV as the structure of thujic acid. The author, however, has only been able to detect traces of formaldehyde on ozonisation and it is well known that formaldehyde may be obtained in small amounts from many compounds which do not contain methylene groups. The formation of only one hexahydrothujic acid is in harmony with formula II. III and IV would certainly give rise to two diastereoisomerides. Furthermore, C-CH_3-estimations on both thujic acid and hexahydrothujic acid gave only about 0.5 C-CH_3 groups. Hexahydroderivatives of III or IV would be expected to give a value well above 1 C-CH_3-group. Thus, there are strong indications in favour of structure II for thujic acid.

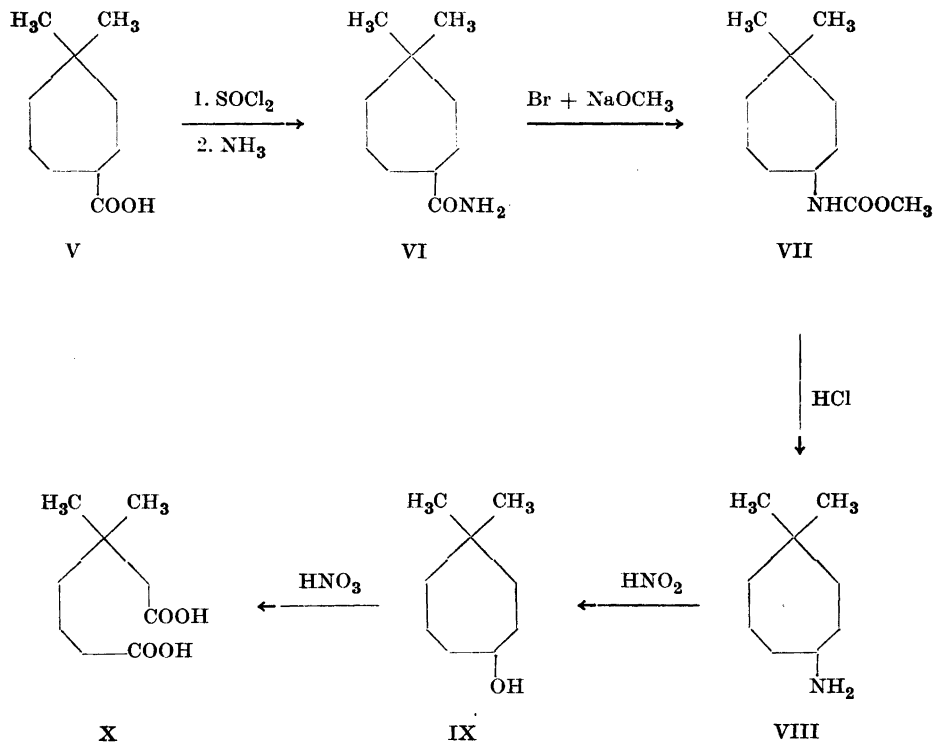
The degradation reactions described below clearly show that this is the correct structure of thujic acid, which consequently is 4,4-dimethylcyclohepta-2, 5, 7-trienecarboxylic acid.

The degradation of thujic acid was first attempted through the tetrahydrothujic acid, which can be obtained from thujic acid by reduction with sodium amalgam in alkaline solution. The yield of tetrahydrothujic acid was,

however, inconsistent. The tetrahydrothujic acid was often accompanied by sparingly soluble, probably high molecular products. Furthermore, preliminary experiments on the degradation of tetrahydrothujic acid were not very encouraging, and this route was therefore abandoned. The position of the remaining double bond in tetrahydrothujic acid remains undetermined.

Hexahydrothujic acid proved to be more suitable for degradation studies and the degradation was carried out along two different lines, both leading to previously known compounds. The steps are outlined in Chart I and II.

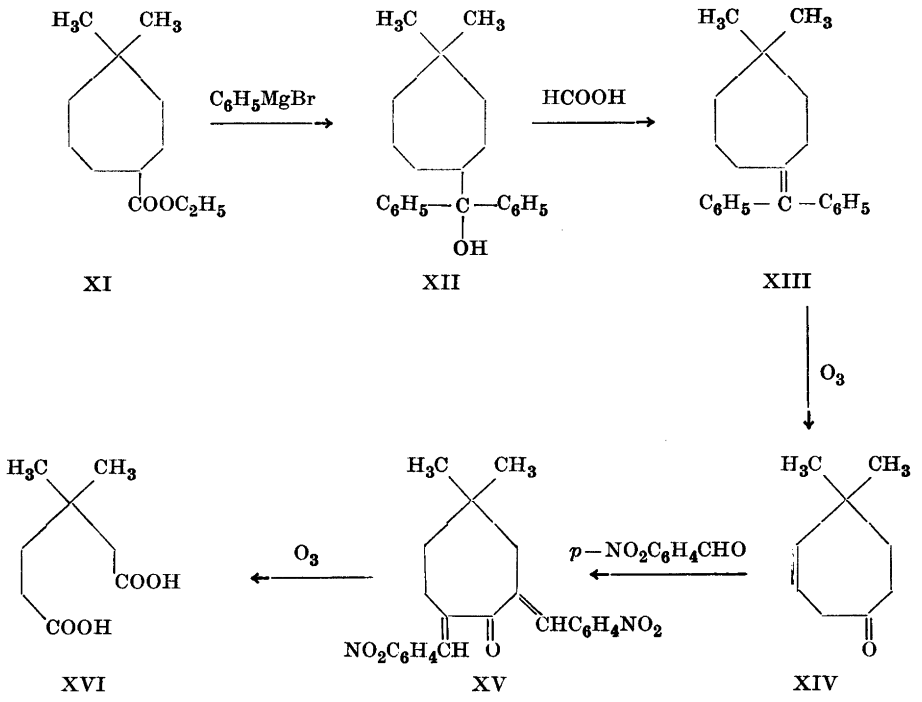
Chart I.



V VI VII VIII IX X

The last step should give a mixture of β, β-dimethylpimelic acid and γ, γ-dimethylpimelic acid. Only one crystalline substance, however, could be isolated, the mother-liquor being a noncrystallisable oil. The former was identified as β, β-dimethylpimelic acid by means of mixed melting point determination with an authenic sample prepared according to Blanc [6].

Chart II.



The last step gave only a rather small amount of an acid, which could not be obtained quite pure. It was, however, identified as β,β-dimethyladipic acid by mixed melting point determination with an authenic sample prepared by oxidation of 4,4-dimethylcyclohexanone.

The isolation of β,β-dimethylpimelic acid and β,β-dimethyladipic acid definitely establishes the structure of hexahydrothujic acid as V and, as there is only one way of arranging three double bonds in this molecule, thujic acid must have structure II.

Hence, it is a higher homologue of cyclohepta-1,3,6-trienecarboxylic acid. Four isomeric cycloheptatrienecarboxylic acids are known and Buchner [7] has attributed this structure to the liquid isomeride prepared by Einhorn and Willstätter [8].

The formula V explains all the reactions of thujic acid, and also the isomerisation to cumic acid. This resembles the transformation of eucarvone into carvacrol [9] and cycloheptatriene into benzylbromide.[10]

It is interesting to note that both the thujaplicins and thujic acid, all occurring in the heart wood of *Thuja plicata* [11], contain seven-membered rings.

Of course there is a great difference between the tropolone ring system of the thujaplicins and the seven-membered ring in thujic acid, but they are probably all biochemically related substances. Thujic acid contrary to the thujaplicins, does not show any 'aromatic' properties. As a matter of fact, thujic acid is rather unstable and decomposes on standing. Anderson and Sherrard noted that thujic acid is slightly toxic to wood-destroying fungi, and this is in agreement with recent findings of E. Rennerfelt, Stockholm.

EXPERIMENTAL

Isolation of thujic acid

The powdered heart wood was extracted with acetone and the extract segregated into acidic, phenolic and neutral fractions as described earlier [11].

The sodium hydrogen carbonate solution was acidified and the precipitated crude thujic acid was collected with ether. After removal of the ether, the crude thujic acid was distilled with steam. The crystalline precipitate in the receiver was filtered, dried and recrystallised from light petroleum. M. p. 88–89°.

$C_{10}H_{12}O_2$	Calc.	1 C–CH_3	9.15
	Found	C–CH_3	6.91

Isomerisation of thujic acid

Thujic acid (0.5 g) was suspended in 10 ml conc. hydrochloric acid, and the solution heated to boiling. Acetic acid was added until a clear solution was obtained. The boiling was continued for half an hour, and the solution then poured into water. The white precipitate was filtered and recrystallised from dilute methanol (m. p. 115–116°). No depression when mixed m. p. was taken with an authenic sample of cumic acid.

Oxidation of thujic acid

Thujic acid (1 g) was dissolved in dilute sodium carbonate solution and potassium permanganate was added until the colour remained. The reaction mixture was then acidified and sulphur dioxide passed in, in order to dissolve the manganese dioxide. The clear colourless solution was extracted with ether. The ether solution yielded 0.2 g of a crystalline substance, which after recrystallisation from ether and sublimation in a vacuum showed a m. p. of 190–192°.

$C_3H_6(COOH)_2$	Calc.	Eqv. wt.	66.0
	Found	» »	66.3

Upon heating, carbon dioxide was evolved. The remaining oily acid was converted into the *p*-bromophenacyl ester, m. p. 75–76°, undepressed on admixture of an authentic sample of isobutyric acid p-bromophenacyl ester.

Tetrahydrothujic acid

Thujic acid (4 g) was dissolved in sodium hydrogen carbonate solution. The solution was heated on a waterbath to about 70–80° and 4 % sodium amalgam (75 g) was in-

troduced in small portions. The heating was continued, with frequent shaking, for eight hours. The solution was decanted from the mercury, acidified, and extracted with ether. After evaporation of the latter, 4 g of an oil was obtained, which soon partly crystallised. The crystals were filtered and recrystallised from dilute methanol. The tetrahydrothujic acid thus obtained had m. p. 65—66°. After standing for some months the crystals were converted into a thick colourless oil.

$C_{10}H_{16}O_2$	Calc.	C	71.34	H	9.61	
	Found	»	71.49	»	9.61	(W. K.)

The *p*-bromophenacyl ester was prepared in the usual way and after recrystallisation from dilute methanol had a m. p. of 74—75°.

$C_{18}H_{21}O_3Br$	Calc.	C	59.16	H	5.81	
	Found	»	58.93	»	5.74	(W. K.)

The benzylisothiouronium salt was prepared in the usual way and after recrystallisation from alcohol had a m. p. of 167—168°.

$C_{18}H_{26}O_2N_2S$	Calc.	C	64.61	H	7.86	
	Found	»	64.04	»	7.90	(W. K.)

Hydrogenation of tetrahydrothujic acid

Tetrahydrothujic acid (0.3 g) was hydrogenated in alcohol solution over a platinum oxide catalyst. The uptake of hydrogen amounted to 40 ml (calculated for 1 mol 40 ml). The hydrogenated acid was obtained as an oil, which was converted into the *p*-bromophenacyl ester, m. p. 70—71°. This gave no depression with the *p*-bromophenacyl ester of hexahydrothujic acid described below.

Hexahydrothujic acid (V)

Thujic acid (1 g) was hydrogenated in alcohol over platinum oxide catalyst. 395 ml of hydrogen (calculated for 3 moles 410 ml) were absorbed. The alcohol was removed in a vacuum and the oily product distilled. B. p. 150—152°/14 mm, n_D^{20} 1.4671. It could not be made to crystallise.

$C_{10}H_{18}O_2$	Calc.	C	70.50	H	10.68	$C-CH_3$	8.82	
	Found	»	70.19	»	10.53	»	4.03	(W. K.)

The *p*-bromophenacyl ester was obtained in two dimorphic modifications, one long needles (m. p. 70—71°), and the other leaflets (m. p. 65—66°).

$C_{18}H_{23}O_3Br$	Calc.	C	58.83	H	6.33	
	Found	»	59.07	»	6.34	(W. K.)

The benzylisothiouronium salt had m. p. 159—160° after recrystallisation from alcohol-ethyl acetate.

$C_{18}H_{28}O_2N_2S$	Calc.	C	64.23	H	8.41	
	Found	»	64.33	»	8.38	(W. K.)

The amide (VI) was obtained by heating the hexahydrothujic acid with thionyl chloride, removing the excess thionyl chloride by distillation, and pouring the residue into conc. ammonia. Recrystallisation from dilute alcohol yielded leaflets m. p. 147.5—148.5°.

The ethyl ester (XI) was obtained by boiling the alcoholic solution obtained in the hydrogenation with a few drops of conc. sulphuric acid. B. p. 107—107.5°/11 mm.

4,4-Dimethylcycloheptylamine (VIII)

Hexahydrothujic amide (2.7 g) was added to a solution of sodium (0.75 g) in 40 ml methanol. Bromine (2.52 g) was then added dropwise. The mixture was warmed for a few minutes on a water bath, made slightly acidic with acetic acid, and the methanol evaporated. Water was added and the urethane (VII) extracted with ether. It was obtained as an oil which was, without further purification, hydrolysed with conc. hydrochloric acid on a water bath. A small amount of a nonbasic material was removed by extraction with ether and the hydrochloric acid solution was evaporated to dryness, when the hydrochloride of 4,4-dimethylcycloheptylamine (2.2 g) was obtained. The free base was an oil which was characterised as its benzoate, colourless crystals from ligroin m. p. 114—115°.

$C_{16}H_{23}ON$	Calc.	C	78.51	H	9.47	
	Found	»	78.83	»	9.52	(W. K.)

β,β-Dimethylpimelic acid (X)

4,4-Dimethylcycloheptylamine hydrochloride (1.1 g) was dissolved in a small amount of water and sodium nitrite (0.36 g) was added. The solution was heated on a water bath until the evolution of gas ceased. The solution separated into two layers. The alcohol (IX) formed was collected with ether. On removal of the ether, 0.45 g of the alcohol was obtained as an oil. This was oxidised, without further purification by heating with conc. nitric acid. The nitric acid was evaporated in a vacuum. There remained a thick colourless oil, which partly crystallised. The crystals were collected by filtration (0.1 g) and recrystallised from light petroleum — ether. M. p. 101—102°. Mixed m. p. with authentic β,β-dimethylpimelic acid [6] of m. p. 102—104° was 102—103°.

$C_9H_{16}O_4$	Calc.	C	57.38	H	8.59	
	Found	»	57.48	»	8.68	(K. S.)

1-Diphenylmethylene-4,4-dimethylcycloheptane (XIII)

Ethyl hexahydrothujate (5 g) was added dropwise to a Grignard-reagent prepared from bromobenzene (12 g) and magnesium (1.75 g) in anhydrous ether. After the addition of the ester, the mixture was boiled for two hours and then allowed to stand over night. It was then poured on ice and acidified with sulphuric acid. The reaction product was extracted with ether, the ether removed, and the remaining oil (probably mostly XII)

boiled for two hours with 85 % formic acid. The mixture was then poured into water and extracted with ether. The ether extract was washed with sodium hydrogen carbonate solution. Evaporation of the ether left a thick yellow oil, which soon partly crystallised. The crystals were filtered and recrystallised from alcohol. Yield 5.1 g, m. p. 89—90°.

$C_{22}H_{26}$	Calc.	C	90.94	H	9.06	
	Found	»	90.96	»	8.80	(K. S.)

4,4-Dimethylcycloheptanone (XIV)

1-Diphenylmethylene-4-4,dimethylcycloheptane (4 g) was ozonised in chloroform solution. The chloroform was evaporated in a vacuum and the ozonide decomposed by boiling with water. The 4,4-dimethylcycloheptanone was separated by steam distillation from benzophenone, which is only slightly volatile with steam. Extraction of the distillate with ether yielded 0.6 g of the ketone, B. p. 60—66°/10 mm. The 4,4-dimethylcycloheptanone was characterised as its semicarbazone. This was first obtained as long needles m. p. 191—192°, but on further recrystallisation from methanol leaflets were obtained m. p. 173—174°. Thereafter only the lower melting form could be obtained.

$C_{10}H_{19}ON_3$	Calc.	C	60.85	H	9.73	
	Found	»	60.94	»	9.66	(K. S.)

Bis-*p*-nitrobenzylidene-4,4-dimethylcycloheptanone (XV)

4,4-Dimethylcycloheptanone (0.3 g) was dissolved in alcohol and *p*-nitrobenzaldehyde (0.7 g) was added. The mixture was warmed slightly in order to dissolve all of the aldehyde. A few drops of a sodium methoxide solution were added. After four days, the crystals deposited were collected (0.7 g) and recrystallised from alcohol-ethyl acetate. This resulted in small yellow needles m. p. 158—160°.

$C_{23}H_{22}O_5N_2$	Calc.	C	67.95	H	5.47	
	Found	»	67.51	»	5.63	(K. S.)

β,β-Dimethyladipic acid (XVI).

The bis-*p*-nitrobenzylidene-4,4-dimethylcycloheptanone (0.5 g) was ozonised in chloroform solution until the yellow colour had disappeared. The chloroform was evaporated in a vacuum and the remaining oil boiled with water. Sodium hydrogen carbonate was added and the insoluble *p*-nitrobenzaldehyde was removed by filtration. The aqueous solution was acidified and a small amount of *p*-nitrobenzoic acid removed. The mother liquor was extracted with ether, the ether evaporated, and the residue treated with water. An additional amount of *p*-nitrobenzoic acid could thus be removed. The aqueous solution was evaporated to dryness, leaving an oil which soon crystallised. This was recrystallised from a mixture of ether and light petroleum and small needles m. p. 82—83° were obtained. The yield was 0.02 g. Mixed with authentic β,β-dimethyladipic acid of m. p. 85—86° it melted 84—85°.

SUMMARY

The 'dehydroperillic acid' of Anderson and Sherrard [1] has been showe to be 4,4-dimethylcyclohepta-2,5,7-trienecarboxylic acid, and the namn thujic acid is proposed for this acid.

The author would like to express his appreciation to *Statens Tekniska Forskningsråd* for grants, which have defrayed the costs of the part of this investigation carried out at Stockholm. Some early experiments on the isolation of thujic acid and the thujaplicins were financially supported by *AB Kärnbolaget*, Stockholm.

The analyses were carried out by W. Kirsten, Upsala, (W. K.) and K. Salo, Helsingfors, (K. S.).

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Received September 3, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1147–1152

Constituents of Pine Heartwood

XIV.* The Heartwood of *Pinus monticola* Dougl.

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Pinus monticola, 'Western White Pine', is endemic to the west coast of North America. It belongs to the section *Haploxylon* and is closely related to *P. strobus*. The heartwood constituents of *P. strobus* and of another *Haploxylon* pine, *P. cembra*, have been investigated by Erdtman [1, 2]. These pines contain a few substances which have also been found in *Diploxylon* pines (pinosylvin monomethyl ether, pinocembrin and pinobanksin), as well as other substances which do not seem to occur in *Diploxylon* pines. Thus, both *P. strobus* and *P. cembra* contain pinitol (monomethyl ether of *d*-inositol), chrysin (5,7-dihydroxyflavone) and tectochrysin (5-hydroxy-7-methoxyflavone). In addition, *P. strobus* was found to contain two new substances, known as pinostrobin (5-hydroxy-7-methoxyflavanone) and strobopinin, which seems to be a C-methyl dihydroxyflavanone. The position of the methyl and the hydroxyl groups has not yet been definitely determined.

The heartwood of *P. monticola* was extracted with ether and acetone in the same way as that described for *P. montana* [3]. The ether extract (2.5 % of the heartwood) took the form of a dark brown syrup which did not crystallise. A phenolic fraction, amounting to 2 % of the extract (or 0.05 % of the heartwood) could be prepared from the latter by the method referred to previously [3]. Since this quantity was large when compared with the amounts of pure phenols isolated from the acetone extract, it was deemed advisable to investigate the ether extract too. The phenolic products (along with some resinous material) were precipitated from the extract with light petroleum, which dissolves the resin acids. The precipitate was then treated in the same way as the acetone extract. The sodium bicarbonate and sodium carbonate fractions yielded no

* XIII. *Acta Chem. Scand.* 3 (1949) 770.

crystalline products, but the 0.2 % sodium hydroxide fraction contained a small quantity of chrysin and large amounts of resinous products. Pinosylvin monomethyl ether (about one sixth of the quantity isolated from the acetone extract) and a very small quantity of tectochrysin were isolated from the 4 % sodium hydroxide fraction.

The acetone extract was divided into fractions in the usual manner (see Part IX[3]). The water-soluble part contained pinitol and *l*-arabinose, part of which was isolated by precipitation as *p*-bromophenylhydrazone. This quantity was too small to allow complete purification, but the identity of the sugar appears to be clearly established.

The quantity of 'membrane substances' (0.15 % of the heartwood) was greater than in most pines hitherto investigated, although a comparatively large part of the phenols could be extracted by ether. The sodium carbonate fraction contained a small quantity of chrysin, which was identified by acetylation and mixed melting-point of the acetate. The 0.2 % sodium hydroxide fraction yielded an additional quantity of chrysin together with small amounts of a pale yellow crystalline compound with m. p. 224—226° and $[\alpha]_D^{20}$—66° (pyridine, $c = 1.6$). This compound proved to be identical with strobopinin from *P. strobus*. Erdtman[1] reports m.p. 225—227° and $[\alpha]_D^{20}$—60.5° (methanol, $c = 0.5$). (Pyridine was preferred by the present author on account of the fact that the solubility in methanol is too low to allow accurate determinations when only small amounts of the substance are available.)

The 4 % sodium hydroxide fraction contained tectochrysin (which was precipitated as its sodium salt) and pinosylvin monomethyl ether.

The following yields were obtained from 8.4 kg of air-dried heartwood:

Ether extract	206 g	(2.5 %)	
'Membrane substances'	12.7 g	(0.15 %)	
Pinitol	10.9 g	(0.13 %)	
l-Arabinose about	0.02 g		
Chrysin	0.3 g	(0.004 %)	
Strobopinin	0.2 g	(0.002 %)	
Tectochrysin	0.9 g	(0.01 %)	
Pinosylvin monomethyl ether	3.9 g	(0.05 %)	(0.5 g of which came from the ether extract)
Neutral fraction of acetone extract	10.0 g	(0.12 %)	

The yields of phenolic substances were extremely low, but it is evident that *P. monticola* differs from *Diploxylon* pines in the occurence of pinitol and of flavones (chrysin, tectochrysin). Its close relationship to *P. strobus* is demonstrated by the presence of strobopinin in both these pines. It may be men-

tioned here that strobopinin has also been isolated from the heartwood of *P. Lambertiana*, which is closely related to *P. strobus* and *P. monticola* (will be published in a forthcoming paper). In addition to the typical *Haploxylon* heartwood constituents, *P. monticola* also contains pinosylvin monomethyl ether and *l*-arabinose, which seem to occur in almost all pines. It is true that *l*-arabinose has not been isolated from *P. strobus* and *P. cembra*, but this is apparently due to the fact that no search has been made for it.

EXPERIMENTAL

The wood used for the investigation was supplied by Dr. A. B. Anderson, Portland, Oregon, U.S.A.

The heartwood gave a brick-red colour when stained with diazotised benzidine solution. 8.4 kg of air-dried fine-ground heartwood were extracted with ether for 24 hours and then with acetone for 48 hours. The ether extract (206 g) took the form of a brown syrup, which did not crystallise even after several months. 23 g of this syrup were treated with 200 ml of light petroleum, which dissolved 16 g. The solution was decanted, and the sticky brown residue extracted by boiling water (200 + 300 ml). The aqueous extract was cooled and shaken with ether. The ether solution was evaporated, leaving 0.49 g of a light brown resinous product. As this experiment indicates that the ether extract may contain rather much phenolic products, the whole extract was investigated.

Investigation of the ether extract

The entire remainder of the ether extract (183 g) was treated with 500 ml of light petroleum. The solution was separated from the residue by decanting and the solvent removed by evaporation, leaving 132 g of a light yellow viscous oil. It gave a thick pale yellow precipitate with cyclohexylamine in acetone solution. This reagent is known to form insoluble salts with resin acids [4]. The fraction soluble in light petroleum was not investigated further.

The insoluble residue was dissolved in ether (200 ml) and shaken with saturated sodium bicarbonate, saturated sodium carbonate, 0.2 % sodium hydroxide and 4 % sodium hydroxide solutions (2 × 100 ml of each). The fractions are referred to as EB, EC, EH_1 and EH_2 respectively. Each fraction was acidified and extracted with ether. The ether solutions were dried over anhydrous sodium sulphate and the ether evaporated.

EB yielded a comparatively large quantity of a brown non-crystalline resinous product. It was not investigated further.

EC yielded only a small quantity of a brown non-crystalline solid.

EH_1 was a brown syrup (about 10 g). It did not crystallise, but a small part of it could be extracted by boiling water. The aqueous extract was shaken with ether and the ether evaporated. The yellow sticky residue left a small quantity of an insoluble product when stirred with methanol. This product (about 20 mg) proved to be crude chrysin, m. p. 255—263°. It was combined with the chrysin coming from the acetone extract.

EH_2 was a brown suryp, similar to EH_1. Very little could be extracted from it by boiling water, but when the insoluble residue was vacuum-distilled, the distillate showed

some tendency to crystallise. It was stirred with ether and the insoluble crystalline residue removed by filtration. The filtrate was evaporated and the ether treatment repeated twice. Thus, two low-melting fractions (m. p. about 110°) and one high-melting fraction (m. p. 147 – 152°) were obtained. The low-melting fractions were vacuum-distilled and recrystallised from 50 % acetic acid, yielding 0.5 g of crude pinosylvin monomethyl ether, which was combined with the corresponding fraction from the acetone extract. 20 mg of crude tectochrysin (m. p. 157 – 160°) were isolated from the high-melting fraction after sublimation in a vacuum and recrystallisation from ligroin.

Investigation of the acetone extract

After the acetone extract had been left standing for some days, a small quantity of a colourless crystalline precipitate had formed. It was collected and recrystallised twice from ethanol, yielding 0.2 g of pinitol. It melted at 183 – 186° and gave no m. p. depression with pinitol from *P. Lambertiana*. $[\alpha]_D^{20}$ + 64.7° ± 0.5° (water, c = 5.4). It did not reduce Fehling's solution.

On the removal of the acetone from the extract by distillation, an aqueous solution (= W, about 100 ml) and a brown resinous product remained. They were separated and the resin treated with ether. The ether-insoluble 'membrane substances' were separated by filtration and stirred with 300 ml of cold water to remove pinitol and sugars. This water was then combined with W and washed with a little ether which was combined with the ether solution of the resinous product. The ether solution was divided into fractions in the same way as described for the ether extract. The fractions are referred to as B, C, H_1 and H_2, respectively. The remaining ether solution (neutral fraction) was concentrated to a brown turpentine-smelling oil (10 g). It was not investigated further.

W: The aqueous solution reduced Fehling's solution and gave a faint pentose colour reaction with phloroglucinol and hydrochloric acid. It was evaporated to dryness in a vacuum. The remaining yellowish-brown syrup (16 g) was probably a mixture of pinitol and arabinose. After two recrystallisations from ethanol it yielded 10.7 g of pinitol which was combined with the pinitol found before. The mother liquors were then concentrated to a small volume. On cooling, crystalline precipitates formed which reduced Fehling's solution. Since it seemed almost impossible to separate arabinose from an excess of pinitol by recrystallisation, the separation of the whole mixture was not undertaken, and only 0.3 g of it was precipitated with *p*-bromophenylhydrazine in dilute acetic acid solution. A pale yellow crystalline precipitate soon formed. It was separated and treated with benzaldehyde in 50 % ethanol solution on a water bath for 30 minutes to liberate the sugar again from the hydrazone. The benzaldehyde and its hydrazone were then removed by ether extraction, and the remaining water solution evaporated, yielding an almost colourless syrup. After two recrystallisations from ethanol, a colourless crystalline product was obtained (20 mg), melting at 153 – 155°. $[\alpha]_D^{20}$ + 99° ± 2° (equilibrium rotation in water, c = 1.6). Reported for *l*-arabinose: m. p. 159°, $[\alpha]_D^{20}$ + 105.5°. Since mannose, fucose and arabinose are the only sugars that give precipitates with *p*-bromophenylhydrazine in the cold, the identity of the arabinose is definitely established.

B was a brown viscous oil (2 g). It was not investigated further.

C yielded a brown syrupy product which showed some tendency to crystallise. When it was stirred with a few ml of methanol, a yellowish insoluble product was formed, which

was separated by filtration. Evaporation of the methanol from the filtrate and repeated stirring with methanol yielded an additional quantity of insoluble product. This product was recrystallised from glacial acetic acid, yielding brownish yellow crystals melting at 268—274°. After sublimation in a vacuum and two recrystallisations from acetic acid a yellow crystalline product (0.3 g), m. p. 273—275°, was obtained. (Reported for chrysin: 275°). Part of this quantity (0.15 g) was acetylated with acetic anhydride and a few drops of pyridine. After a few hours, a colourless precipitate was separated from the mixture and was recrystallised twice from ethanol. A colourless fibrous crystalline product was obtained, m. p. 193—194°. It gave no melting-point depression when mixed with an authentic specimen of chrysin diacetate. Erdtman reports m. p. 194—196° for chrysin diacetate [1], but earlier investigators obtained lower values [5].

H_1 was a light brown syrupy product which did not crystallise. It was stirred with a few ml of methanol, and the insoluble residue separated. After one recrystallisation from glacial acetic acid it yielded a small quantity of chrysin, m. p. 274—276°, which was added to the chrysin from C. The filtrate was concentrated to a viscous oil and the methanol treatment repeated. An almost colourless insoluble precipitate, melting at 207—213°, was separated from the solution. It was recrystallised from 50 % acetic acid four times, yielding 0.15 g of pale yellow needles, m. p. 224—226°, no depression of the m. p. when mixed with strobopinin from *P. strobus*. $[\alpha]_D^{20} - 66° \pm 1°$ (pyridine, $c = 1.6$). It was possible to collect about 0.1 g of less pure strobopinin from the mother liquors.

The methanol filtrate was evaporated again, yielding a brown syrup, but no further crystalline products could be obtained from it by treatment with methanol or with ether. Extraction with boiling water yielded only a few mg of chrysin.

H_2: When the ether solution was shaken with 4 % sodium hydroxide, a yellow crystalline precipitate was formed (H_{21}). This was separated from the solution and treated with dilute sulphuric acid, yielding a pale yellow crystalline product. After one recrystallisation from chloroform-ligroin and two from ligroin, yellow crystals (0.8 g), melting at 163—165° were obtained. When acetylated with acetic anhydride-pyridine, this substance yielded a crystalline colourless acetate, m. p. 154—156°. Mixed m. p. with an authentic specimen of tectochrysin acetate 153—155°.

The sodium hydroxide solution was acidified and extracted with ether. The ether solution was dried over anhydrous sodium sulphate and the ether removed by distillation. The residue (H_{22}) was a brown viscous oil, which soon crystallised to a great extent. The crystals (m. p. 115—120°) were collected and distilled in a vacuum. The distillate, which crystallised on cooling, was recrystallised from 50 % acetic acid. Yield, 3.0 g of colourless crystals, m. p. 119—121°, mixed m. p. with pinosylvin monomethyl ether 120—122°, It was possible to collect 0.4 g of less pure product from the mother liquors.

SUMMARY

The heartwood of *Pinus monticola* Dougl. has been investigated. Pinitol, *l*-arabinose, chrysin (5,7-dihydroxyflavone), strobopinin (probably a C-methyl dihydroxyflavanone), tectochrysin (5-hydroxy-7-methoxyflavone) and pinosylvin monomethyl ether were isolated from it.

The author is indebted to Mrs. B. Strömgren for skilful assistance with the experimental work, and to Dr. A. B. Anderson, Portland, Oregon, U.S.A for supplying the wood. The investigation was facilitated by a grant from *Fonden för Skoglig Forskning*.

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Received September 7, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1153–1169

Action of Strong Acids on Acetylated Glucosides

III.* Strong Acids and Aliphatic Glucoside Tetraacetates in Acetic Anhydride-Acetic Acid Solutions

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This paper deals with the conditions under which acetylated β-glucosides can be transformed into the corresponding α-glucosides. The catalysts used hitherto are stannic chloride [1], titanium tetrachloride [2], hydrogen bromide-mercuric bromide [3], and boron trifluoride [4]. None of these catalysts are suitable for a kinetic study of transglycosidation. Three of them are known to catalyze reactions of the Friedel-Crafts type, as is also the case with sulfuric acid. Reactions, catalyzed by sulfuric acid, however, might be expected to take place in uncolored, homogeneous solutions, and its action on acetylated glucosides has been investigated in solutions of acetic anhydride-acetic acid.

Relatively few kinetic investigations have been carried out previously in this field. Jungius [5] studied the transformation of β-glucose pentaacetate into the equilibrium mixture of α- and β-pentaacetates, catalyzed by zinc chloride in acetic anhydride, and found the reaction to be of the first order. Hann and Hudson [6] extended the experiments to a glycoside, methyl α-mannoside tetraacetate. The latter was transformed into α-mannose pentaacetate in a mixture of sulfuric acid, acetic anhydride and acetic acid. They found that the reaction is of the first order. Finally, Freudenberg and Soff [7] acetolyzed a number of glucosides and found that the composition of the final product is not constant. In addition to α- and β-glucose pentaacetate, varying amounts of glucose heptaacetate were found.

* Part II. *Acta Chem. Scand.* 2 (1948) 534.

SELECTION OF THE EXPERIMENTAL CONDITIONS

The experiments of Jungius using zinc chloride as a catalyst, were first reproduced. The reaction was of the first order, but the velocity constant differed considerably from that obtained by Jungius. It was found that the velocity was dependent to a great extent upon the small amounts of acetic acid present in the anhydride or formed when the reagents were not perfectly anhydrous. It was possible to obtain higher values than those recorded by Jungius when working with very pure acetic anhydride under anhydrous conditions. On the addition of small amounts of acetic acid the velocity decreased considerably. Difficulty was experienced in obtaining reproducible results, and the experiments with zinc chloride were abandoned. In its place mixtures of acetic anhydride, acetic acid and strong acids were employed. The addition of acetic acid to the system is advantageous, as the system becomes buffered with respect to small amounts of moisture. The transformation of β-glucose pentaacetate into the equilibrium mixture was investigated with sulfuric acid as catalyst, but in this case also it was impossible to get perfectly reproducible results. The activity of the catalyst decreases with time, owing to the formation of sulfoacetic acid. With perchloric acid the velocity also descreases with time, but to a lesser extent. At the same time the solution turns yellow and then brown, rendering polarimetric measurements impossible. On account of these complications I have abstained from measuring exact velocity constants and have compared all reactions with a standard reaction, the transformation of β-glucose pentaacetate into the equilibrium mixture. Thus in all kinetic runs, a parallel run has been made with β-glucose pentaacetate in the same catalyst solution. Since two different catalytically active components appear to be present in the solution here (as will be clear from the following) the method is not entirely correct, and the results must be regarded as somewhat uncertain. This method, nevertheless, appears to offer the best solution of the problem of obtaining comparable results.

SULFURIC ACID AS CATALYST

If the rotation of ethyl β-glucoside tetraacetate in a solution of sulfuric acid in acetic anhydride-acetic acid is observed, the following course of events will be seen to take place (Fig. 1). The rotation increases rapidly, passes through a maximum and then slowly decreases to a constant value, which is definitly less than that calculated under the assumption that only glucose pentaacetate is formed. On treating the α-glucoside in the same way, the rotation decreases rapidly to about the same value as that for the maximum mentioned above, and the values will then coincide with those for the β-gluco-

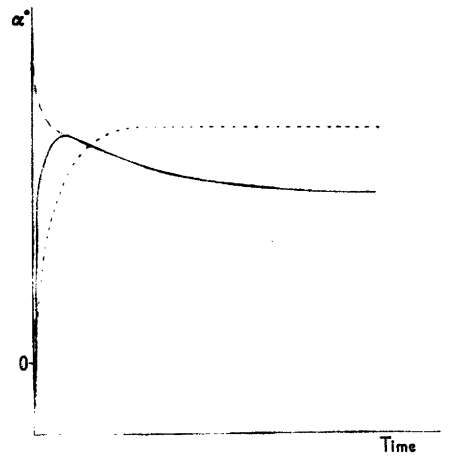


Fig. 1. Transglycosidation and acetolysis of the tetraacetates of α- and β-ethyl glucoside.

——— *Ethyl β-glucoside tetraacetate*
- - - - *Ethyl α-glucoside tetraacetate*
····· *β-Glucose pentaacetate*

side. This sequence of events can be explained in the following way. First the glucoside is transformed into a mixture of the α- and β-glucosides, in which the α-form predominates (90 per cent). At the same time an acetolysis takes place although far more slowly. The final product contains α/β- glucose pentaacetate and glucose heptaacetate. These assumptions have been verified by experiments. The α-glucosides of ethyl, *iso*propyl, and *tertiary* butyl alcohol have been isolated from the reaction mixtures in good yields by the following procedure. When the solution of the β-glucoside showed maximum rotation, it was poured into ice water. From the resulting solution the crude α-glucoside could be separated and purified by recrystallization. The presence of glucose heptaacetate in the final product was demonstrated by acetyl group determinations. The percentage of glucose heptaacetate calculated from these analyses agreed fairly closely with that calculated from the final rotation (Table 1).

Table 1. Products of the sulfuric acid catalyzed reaction.

Glucoside tetraacetate	% α-glucoside isolated under optimum conditions	% glucose heptaacetate in final product, calc. from	
		final rotation	acetyl group determination
α-Methyl	—	5	8
β-Ethyl	60	36	27
β-*iso*Propyl	70	82	90
β-*Tert.*butyl	30	44	55

By varying the concentration of sulfuric acid the different steps can be studied separately. For the first, rapid reaction a low concentration of sulfuric acid is chosen, but for the acetolysis which is somewhat slow, a much higher concentration is preferable. All kinetic runs, except where especially mentioned, were carried out in a mixture of acetic anhydride-acetic acid, 10 : 3 by volume. The velocity constants (calculated for a first-order reaction and expressed in Briggs logarithms) for the transglycosidation of ethyl and *iso*-propyl-β-glucosides and for the standard reaction with different concentrations of sulfuric acid are given in Table 2.

Table 2. Variation of the sulfuric acid concentration.

Glucoside tetraacetate	$C_{H_2SO_4}$	$k_{standard}$	$k_{standard}/C_{H_2SO_4}$	$k_{transglyc.}$	$k_{transglyc.}/k_{standard}$	$k_{ethyl}/k_{iso\text{-}propyl}$	Maximum rotation
α-Ethyl	0.698	0.019	0.027	0.12	6.3	—	5.11°
»	0.356	0.012	0.034	0.096	8.0	—	5.16
»	0.177	0.0040	0.023	0.056	14.0	—	5.18
»	0.094	0.0026	0.028	0.033	12.7	—	5.24
»	0.052	0.0011	0.021	0.019	17.3	—	5.24
»	0.027	0.00070	0.026	0.011	15.7	—	5.31
α-*iso*-Propyl	0.182	0.0059	0.032	0.254	43	3.1	5.52
»	0.092	0.0028	0.030	0.143	51	4.0	5.49
»	0.045	0.0011	0.024	0.064	58	3.4	5.51
»	0.031	0.00078	0.025	0.046	59	3.9	5.55
»	0.015	0.00035	0.023	0.021	60	—	5.54

The data in Table 2 show that:

1. The reproducibility of the experiments is not very high. The values $k_{standard}/C_{H_2SO_4}$ do not vary consistently but seem to be disordered. This is probably due to the formation of different amounts of sulfoacetic acid, and the velocity constant may be regarded as a measure of the sulfuric acid present in the solution.

2. The ratio $k_{transglyc.}/k_{standard}$ increases consistently with a decreasing concentration of sulfuric acid. A plausible explanation of this may be found in the fact that the catalytically active agent is different in the two reactions, as is further discussed below. Another fact, which also indicates the presence of two different catalysts, is that the maximum rotation increases with a decreasing concentration of sulfuric acid. If $k_{acetolysis}/C_{H_2SO_4}$ is constant just as $k_{standard}/C_{H_2SO_4}$, this variation of the maximum rotation is readily understandable.

3. The values of $k_{isopropyl}/k_{ethyl}$ are disorded. The two glucosides being transformed by the same mechanism, the values should be constant.

For methyl glucoside the rates for transglycosidation and acetolysis are of the same magnitude and are also roughly equal to that for the standard reaction. This complicates the kinetic analysis. Since there is no great difference between the velocities, it is clear that the maximum obtained for the other β-glucosides, which is dependent upon the accumulation of the α-glucoside, does not appear in the case of methyl-β-glucoside. Freudenberg and Soff found a minimum for methyl α-glucoside tetraacetate. The other α-glucosides do not show such a minimum, owing to the fact that the acetolysis is so slow, that the β-glucose pentaacetate, formed under Walden's inversion from the α-glucoside, does not accumulate but is quickly transformed into the equilibrium mixture.

In all the runs 0.500 g of glucoside in 20 ml of a solution of sulfuric acid in acetic anhydride-acetic acid 10 : 3 was employed. The rotation was measured at 20.0° in 2 dm tubes. The concentration of sulfuric acid differed for the determination of the two constants for each glucoside (0.1 *C* for the transglycosidation and 1.5 *C* for the acetolysis). The final rotations were measured in the runs with the stronger catalyst. The final rotation for glucose pentaacetate is + 4.90° under these conditions.

The rate of transglycosidation is greater for the *iso*propyl than for the ethyl glucoside. As a general rule the glucosides of secondary alcohols are transformed much more readily than those of primary alcohols. From the maximum rotation the ratio between α- and β-glucoside in equilibrium can be calculated as 9 : 1, in close agreement with the value found by Piel and Purves [8], for the transglycosidation of benzyl β-glucoside tetraacetate with titanium tetrachloride in chloroform. The percentage of glucose tetraacetate in the final product also increases in the series: methyl < *prim.*alkyl < *sec.*-alkyl, as indicated by the final rotation, decreasing in the same series. The results for the different glucosides are summarized in Table 3.

The β-glucoside of *tertiary* butanol differs from the others. Here the course is more complicated. It has been proved that the transformation into the α-glucoside takes place very rapidly. With small amounts of sulfuric acid as a catalyst, the rotation first passes through a maximum, then through a minimum and finally reaches a constant value. This might be explained as follows: First, the α-glucoside, which is responsible for the maximum, accumulates owing to the rapid transglycosidation. The acetolysis also takes place rapidly, although not so fast as the transglycosidation, and after a while β-glucose pentaacetate begins to accumulate, giving a minimum in the rotation. The final mixture consists as usual of α- and β-glucose pentaacetate and glucose heptaacetate. The percentage of the latter is much lower than in the case of

Table 3. Velocity constants for transglycosidation and acetolysis of some acetylated alkyl glucosides.

Glucoside tetraacetate	$k_{transglyc.}$	$k_{acetolysis}$	Final rotation a_D^{20}
α-Methyl	~ 1	~ 1	+ 4.96°
β-Methyl	~ 1	~ 1	4.25
α-Ethyl	13	0.11	3.40
β-Ethyl	15	0.07	3.53
β-*n*-Propyl	14	0.08	2.71
β-*n*-Butyl	14	0.05	2.65
β-*iso*-Butyl	10	0.05	2.24
α-*iso*-Propyl	50	0.08	1.25
β-*iso*-Propyl	50	0.08	1.25
β-Pentyl (3)	29	0.04	1.10
β-Cyclopentyl	44	0.05	1.14
β-Cyclohexyl	61	0.06	0.90
β-Cycloheptyl	70	0.08	1.12

secondary glucosides, which may be connected with the fast acetolysis. With very small concentrations of sulfuric acid, smaller than the concentration of the glucoside, the reaction stops after some time. If more sulfuric acid is added, the reaction will start again. A probable explanation is that the sulfuric acid is consumed in the formation of butyl sulfuric acid.

The effect of the solvent upon these reactions has also been studied. The results are given in Tables 4, 5, and 6.

Table 4. α/β-Transformations in solutions of different acetic acid concentration. $C_{H_2SO_4} = 0.196$.

Substance	% Ac_2O	% AcOH	k	$k_{relative}$
β-Glucose pentaacetate	100	0	0.17	9.4
»	80	20	0.0052	2.9
»	60	40	0.0030	1.7
»	40	60	0.0018	1
Ethyl β-glucoside tetraacetate	100	0	0.19	29
»	80	20	0.090	14
»	60	40	0.025	3.8
»	40	60	0.0065	1

Table 5. The effect of an inert solvent upon the α/β-transformation.

$C_{H_2SO_4}$	Substance	% Ac_2O	% CCl_4	k
0.147	β-Glucose pentaacetate	100	0	0.0030
»	»	80	20	0.0030
»	»	60	40	0.0027
0.040	Ethyl β-glucoside tetraacetate	100	0	0.084
»	»	80	20	0.086
»	»	60	40	0.092

Table 6. Acetolysis of ethyl β-glucoside tetraacetate in solutions of different acetic acid concentrations. $C_{H_2SO_4} = 1.2$.

% Ac_2O	% AcOH	k	Final rotation
100	0	0.027	3.00
80	20	0.0044	3.42
60	40	0.0017	3.84

As usual, the values are somewhat inaccurate owing to the formation of sulfoacetic acid, but they show clearly how the velocity decreases with an increasing concentration of acetic acid and that the addition of an inert solvent does not appreciably alter the velocity. The transglycosidation is more affected by the acetic acid than is the standard reaction. The different values of the final rotation in Table 6 depend on differences in composition and cannot be explained by solvent effects upon the rotation.

PERCHLORIC ACID AS A CATALYST

In addition to sulfuric acid, some other acids such as perchloric acid, boron trifluoride and *p*-toluene sulfonic acid have been investigated. Of these, *p*-toluene sulfonic acid is too weak to permit a study of the reaction. The two remaining acids behave somewhat similarly, and since perchloric acid is easier to work with, this acid has been used in most experiments. The use of both acids is accompanied by the disadvantage that they react with the solvent, producing a yellow to brown coloration, that often renders polarimetric readings impossible. On account of this 'humification' only fast reactions can be followed to equilibrium. Consequently, the reaction of *tert.*butyl β-glucoside tetraacetate has been studied more thoroughly. The final product in this case

is glucose pentaacetate. The final rotation found is in perfect agreement with that calculated under the assumption that only glucose pentaacetate is formed, and α-pentaacetate has been isolated in a good yield from the reaction mixture. The rotation shows no maximum or minimum during the reaction. Efforts to calculate the velocity constant under the assumption that the reaction proceeds as follows:

tert. butyl β-glucoside tetraacetate → α-glucose pentaacetate → α/β glucose pentaacetate

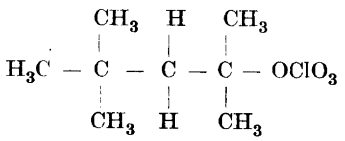
have failed, indicating that the reaction is more complicated. Most probably transglycosidation occurs simultaneously with the other reactions and has a comparable rate. This view is supported by the fact that a maximum was obtained when *iso*propyl β-glucoside tetraacetate was treated with a solution of 5 ml of perchloric acid (about 70 per cent) in 50 ml acetic anhydride. In the same experiment the final rotation was very low, indicating the presence of glucose heptaacetate. The kinetic analysis of the runs with *tert.*butyl β-glucoside shows that the velocity of the acetolysis is approximately of the same order as that of the standard reaction. The velocity of the standard reaction is directly proportional to the concentration of perchloric acid. (Table 7.)

Table 7. Transformation of β-glucose pentaacetate with perchloric acid as catalyst.

C_{HClO_4}	k	k/C_{HClO_4}
0.88	0.7	0.8
0.44	0.23	0.52
0.22	0.11	0.50
0.11	0.055	0.50

During some of the experiments light-yellow crystals were deposited from the reaction solution. The crystals were separated and proved to be explosive. They were practically insoluble in acetic anhydride and chloroform but soluble in a mixture of acetone and water, the solution becoming strongly acid. This points to an organic perchlorate. The equivalent weight of the substance was determined at 213 by titrating the acetone-water solution with standard sodium hydroxide.

The substance might possibly be *iso*octyl perchlorate.



formed from the *tert.*butyl groups via *iso*butene and *iso*octene.

Variation of the solvent gives the same results with perchloric acid as with sulfuric acid.

The methyl, ethyl and *iso*propyl β-glucosides have also been examined. In no case could the final rotation be observed, and the rotation showed no maximum when moderate concentrations of perchloric acid in the usual solvent mixture were used. (For very strong solutions see above.)

DISCUSSION

The behaviour of strong acids in acetic anhydride, aceticacid mixtures has been reviewed and further investigated by Mackenzie and Winter [9a]. The latter have also studied the kinetics of the Thiele acetylation [9b]. The following conclusions may be drawn from these papers. From conductivity measurements it is shown that perchloric acid is a strong and sulfuric acid a weak electrolyte in acetic acid. The acidity can be measured with the acid of the chloranil electrode or by the indicator method and defined by $(pH)^{AcOH}$ or by Hammet's acidity function. Thus $(pH)^{AcOH}$ for 1.0 *C.* solutions of trichloroacetic, sulfuric and perchloric acid in acetic acid is — 0.83, — 3.23 and — 4.4 respectively. When acetic anhydride is added to the system, $(pH)^{AcOH}$ decreases. This decrease is rather small for perchloric acid but very great for sulfuric acid, $(pH)^{AcOH}$ having a value as low as — 12.9 for a 0.7 *C* solution of sulfuric acid in 30 per cent acetic anhydride. Solutions with such extremely low $(pH)^{AcOH}$ values are called 'superacidic'. There are probably two acid ions in the 'superacidic' solution. $AcOH_2^+$ and Ac^+. The latter would probably be responsible for the extremely low $(pH)^{AcOH}$ values *. The existence of two different acid catalysts is also proved by the kinetic experiments. When the Thiele acetylation of benzoquinone is performed with perchloric acid as catalyst, the rate of the reaction increases with the concentration of the acid. The $(pH)^{AcOH}$ of the solution of course decreases simultaneously. The same is true when sulfuric acid is used as catalyst, but perchloric acid is the stronger catalyst. Yet, for solutions of the same molarity, $(pH)^{AcOH}$ is about six units lower in the solution of sulfuric acid. Mackenzie and Winter also investigate the effect of the solvent upon the Thiele acetylation. Their results are more accurate, but in other respects they correspond well with those found in the present investigation.

The results of the latter can be explained by the following hypothesis: Let it be assumed that the sulfuric acid chiefly occurs as the mixed anhydride, acetyl sulfuric acid. This catalyzes the standard reaction and also the normal

* This is not actually stated in the paper of Mackenzie and Winter.

acetolysis of the glucoside to glucose pentaacetate. The velocity of these reactions should be directly proportional to the concentration of sulfuric acid, as is also found for the standard reaction. The acetyl sulfuric acid, however, cannot catalyze the transglycosidation. It dissociates to a small extent according to the reaction:

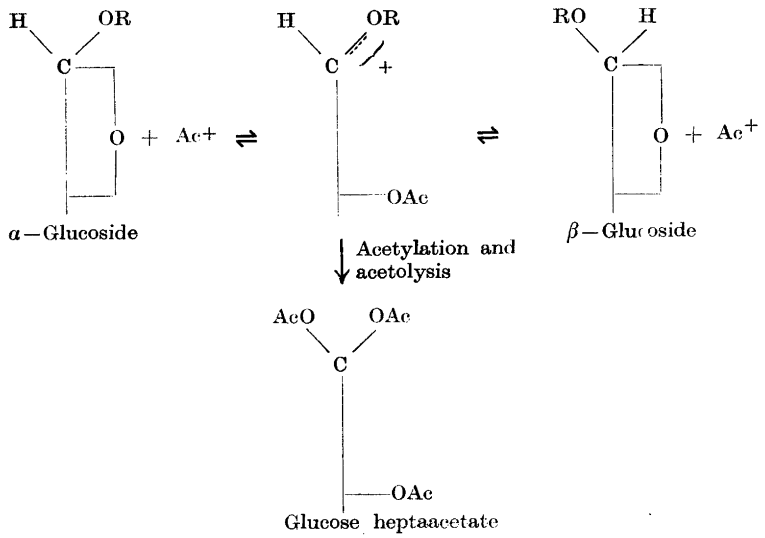
$$AcOSO_3H \rightleftharpoons Ac^+ + HSO_4^-$$

The acetyl cation, responsible for the 'superacidity', is a very strong acid, that catalyzes the transglycosidation similarly to titanium tetrachloride or boron trifluoride. The degree of dissociation decreases with increasing concentration of acetylsulfuric acid, and therefore the ratio $k_{\text{transglyc.}}/C_{H_2SO_4}$ must also decrease.

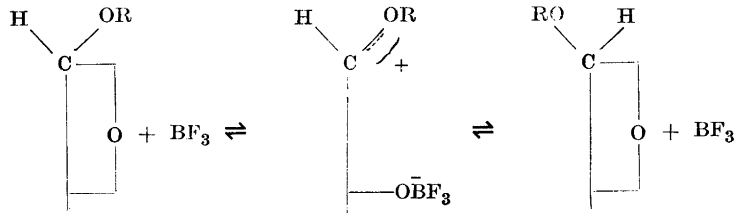
The perchloric acid is completely dissociated, and therefore the catalyst must be the $AcOH_2^+$ ion. The standard reaction and the acetolysis are catalyzed more strongly by perchloric acid than by sulfuric acid. (Tables 2 and 7.) The superacidity and the concentration of the acetyl cation, however, are very small, and consequently the rate of the transglycosidation is low.

Before any mechanism for the transglycosidation can be discussed, it must be ascertained whether the reaction in intramolecular. This seems most probable. The yield of the reaction being high, it would be difficult to understand how all the alkoxyl groups, if once free, could find their way back again in this strongly acetylating medium. That the reaction really is intramolecular has been proved for the analogous catalysis with titanium tetrachloride in chloroform, where no consecutive reactions occur. A mixture of isopropyl β-glucoside tetraacetate and ethyl β-cellobioside heptaacetate was treated with titanium tetrachloride in absolute chloroform. From the reaction mixture only isopropyl α-glucoside tetraacetate and ethyl α-cellobioside heptaacetate could be isolated and in good yields, showing that the reaction must be intramolecular.

From Table 3 it is apparent that a striking connection exists between the rate of the transglycosidation and the yield of glucose heptaacetate in the final product. The faster transglycosidation takes place, the greater will be the quantity of glucose heptaacetate found in the final product. This indicates that transglycosidation and the formation of heptaacetate have one step in common. A plausible explanation is that the reactions proceed in the sequence:



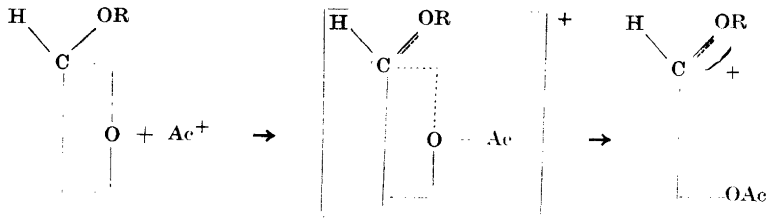
With neutral acids, for example boron trifluoride in an inert solvent, the mechanism would be



The effect of the aglucon upon the rate of transglycosidation is also predictable. A — I substituent (electron repellant) will increase the — *E* activity of the oxygen, which increases in the series:

$$-O-CH_3 < O-CH_2-CH_3 < O\overset{CH_3}{\underset{CH_3}{CH}} < O-\overset{CH_3}{\underset{CH_3}{C}}-CH_3$$

The greater the —*E* activity of the alkoxyl, the lower will be the energy of the intermediate cation and also of the transition state leading to that cation.



Thus both the velocity of formation and the concentration of the intermediate cation will be favoured by a high —I activity of the alkyl group. This means that the rate of transglycosidation and the yield of glucose heptaacetate will increase in the series: methyl < *prim.*alkyl < *sec.*alkyl < *tert.*alkyl *. This is the sequence found by experiment, with the exception that the yield of glucose heptaacetate is too small in the case of the *tert.*butyl glucoside, probably on account of the very rapid normal acetolysis. Unpublished experiments show that glucosides of alcohols, substituted with halogen, that is to say with +I substituents, are transglycosidated at a much lower rate than the unsubstituted glucosides.

An attack of the acid upon the other acetalic oxygen will be counteracted by the electron attraction of the oxygen atoms on the carbon atoms 4 and 6.

EXPERIMENTAL

Materials

Acetic acid, acetic anhydride and carbon tetrachloride of laboratory reagent grade were distilled under the exclusion of moisture, and the middle fraction (60 %) collected. C. P. sulfuric acid was taken as 98 % pure. C. P. perchloric acid (70 %) was standardized by titration. The synthesis of the glucosides investigated is described in an earlier communication [10].

Procedure

The reaction mixture consisted of the solvent, acetic anhydride and acetic acid in the ratio 10 : 3 (except when solvent effects were investigated), of the sugar derivative in low concentration (C = 0.05) and an acid catalyst. The acid was dissolved in the chilled solvent and the solution allowed to stand in the dark room, electrically thermostatted to 20.0 ± 0.2°. After about half-an-hour, 20 ml of the solution were added to 0.5 g of fine-ground glucose derivative. Dissolution was complete in a few seconds. The mixture was then transferred to a 2 dm polarimeter tube and the rotation was determined at appropriate intervals. The initial rotation was determined by dissolving the substance in

* The yield of heptaacetate also depends upon the velocity of 'acetylation and acetolysis', which is here assumed to be independent of the nature of the alkyl group.

the solvent without any catalyst. For consecutive reactions, the maximum value could be taken as the final value for the first reaction where the latter was much faster than the second reaction. For the second reaction the choice of an appropriate initial value for the calculation of the first order velocity constant presented no difficulties. In each run, except when solvent effects were studied, a parallel run was made with β-glucose pentaacetate. When the solvent effects were studied, the acid catalyst was dissolved in pure acetic anhydride and the different dilutions effected immediately before the runs. All the three or four runs in a series were made at the same time.

Typical runs

Table 8. Transglycosidation of ethyl β-glucoside tetraacetate.

β-Glucose pentaacetate and ethyl β-glucoside tetraacetate, 0.500 g of each, dissolved in 20 ml of 0.178 C sulfuric acid in acetic anhydride-acetic acid, 10 : 3. t = 20° C. Rotations determined in 2 dm tubes. (The table gives only a part of the observed values.)

Time min.	α-Glucose pentaacetate		Ethyl β-glucoside tetraacetate	
	α°_{D}	k	α°_{D}	k
0	0.37		− 1.20	
2	0.49	0.0057	+ 0.29	0.058
4	0.52	36	1.38	56
6	0.66	48	2.16	54
8	0.70	41	2.83	54
10	0.75	38	3.35	54
12	0.85	40	3.79	55
14	0.95	42	4.14	56
16	1.00	40	4.42	58
18	1.03	38	4.71	63
20	1.11	39	4.81	62
30	1.46	40	5.18	
40	1.78	40	5.18	
50	2.11	42	5.18	
60	2.35	41	5.16	
70	2.57	41	5.13	
	4.93			
	Mean value 0.0040		Mean value 0.056	

It has not been possible to interpret the observed values for the *tert.*butyl glucoside by any simple assumptions. The values $(\alpha_D)_{calc}$, in Table 10 are calculated under the assumption that the reaction proceeds as follows:

$$\beta\text{-glucoside} \xrightarrow{k_1} \beta\text{-pentaacetate} \xrightarrow{k_2} \alpha/\beta\text{-pentaacetate}.$$

Table 9. Acetolysis of n-propyl β-glucoside tetraacetate.

β-Glucoside pentaacetate and n-propyl β-glucoside tetraacetate, 0.500 g of each, dissolved in 20 ml of 1.4 C sulfuric acid in acetic anhydride-acetic acid, 10 : 3. t = 20° C. Rotation determined in 2 dm tubes.

Time min	β-Glucose pentaacetate		n-Propyl β-glucoside tetraacetate	
	a°_D	k	a°_D	k
0	0.37		− 1.14	
2	1.20	0.044	+ 2.40	
4	1.99	48	3.86	
6	2.57	48	4.39	
8	3.10	50	4.64	
10	3.50	50	4.70	
12	3.75	49	4.73	
15	4.08	50	4.70	
27	4.83		4.48 *	
37	4.91		4.32	0.0041
47	4.93		4.18	40
60	4.93		4.00	42
75	4.93		3.86	39
90			3.74	37
120			3.50	38
150			3.26	41
215			3.00	42
490	4.93		2.71	
	4.93		2.71	
	Mean value 0.049		Mean value 0.0040	

$k_1 = k_2 = 0.024$. They deviate consistently over the whole range, however, so that the assumption cannot be valid. The reaction must be of a more complex nature, probably including a transglycosidation. An evaluation would be very difficult to carry out and of little value. The final rotation, + 4.62° agrees closely with the value + 4.60°, calculated under the assumption that α- and β-glucose pentaacetate are the only final products.

iso-Octyl perchlorate

At the end of the experiment described above, pale yellow crystals appeared in the reaction mixture. These crystals were collected on a filter and washed with a small amount of acetic anhydride. They were almost insoluble in acetic anhydride and chloroform but easily soluble in a mixture of water and acetone. From this solvent the sub-

* Chosen as initial value.

Table 10. Acetolysis of tert.butyl β-glucoside tetraacetate with perchloric acid as catalyst.

β-Glucose pentaacetate and tert.butyl β-glucoside tetraacetate, 0.500 g of each, dissolved in 20 ml of 0.11 C perchloric acid in acetic anhydride-acetic acid, 10 : 3. t = 20° C. Rotations determined in 2 dm tubes. (The table gives only a part of the observed values.)

Time min	β-Glucose pentaacetate		*Tert.* butyl β-glucoside tetraacetate	
	α°_D	k	$(\alpha^{\circ}_D)_{Found.}$	$(\alpha^{\circ}_D)_{Calc}$
0	0.37		− 0.84	
2	0.79	0.022	+ 0.79	− 0.20
4	1.17	22	1.11	+ 0.39
6	1.56	23	1.36	0.89
8	1.87	23	1.59	1.34
10	2.19	23	1.72	1.73
12	2.45	23	1.96	
14	2.65	23	2.11	2.40
16	2.86	23	2.24	
18	3.05	23	2.39	2.80
20	3.28	24	2.54	
30	3.98	25	3.12	
40	4.41	27	3.50	4.31
50	4.62		3.75	
60	4.74		3.99	
75	4.76		4.14	
90	4.76		4.30	
115			4.46	
145			4.62	
			4.62	

Mean value 0.024

stance could not be recovered and the solution became strongly acid. The crystals had no melting point but decomposed violently when heated. The presence of halogene was proved by the Beilstein test. These facts indicate an organic perchlorate. The solution of the substance in acetone-water was titrated with sodium hydroxide and the equivalent weight of the substance determined at 213. It is probable that the substance is isooctyl perchlorate $C_8H_{17}ClO_4$

(212.7)	Calc.	C	45.1	H	8.06	Cl 16.7
	Found*	»	43.3	»	5.00	» 15.7

* Since the substance is explosive, the results of the combustrin analyses can not be stressed upon very much. The equivalent weight determination, howeves, is perfectly reproducible.

Isolation of the α-glucoside from the reaction mixture

Ethyl β-glucoside tetraacetate (2 g) was dissolved in 0.2 *C* sulfuric acid in acetic anhydride-acetic acid 10 : 3 (30 ml). The rotation was followed in the polarimeter and when maximum rotation was observed, the solution was poured into ice water (200 ml) containing sodium acetate (5 g). A very small amount of crystals were separated. The mixture was extracted with ether, the ether solution washed with dilute sodium carbonate and water, dried over calcium chloride and concentrated. The residue was recrystallized from ethanol. Yield, 1.25 g. M. p. 60—61° *. One further recrystallization from ethanol yielded the pure ethyl α-glucoside tetraacetate, melting at 61—62°.

iso Propyl α-glucoside tetraacetate (2 g) was treated analogously, but in this case crystals of the α-glucoside separated in the ice water solution in a good yield (1.4 g). M. p. 81—82°. Two recrystallizations from ethanol yielded the pure substance. M. p. 85—86°.

*tert.*Butyl β-glucoside tetraacetate (1.2 g) was dissolved in 0.07 *C* sulfuric acid in acetic anhydride-acetic acid, 10 : 3 (50 ml). After ten minutes the mixture was poured into ice water (500 ml) containing sodium acetate (5 g). This solution was extracted with ether (2 × 100 ml). The ether solution was diluted with light petroleum (150 ml), washed with sodium carbonate solution and finally with water (4 × 350 ml), dried over calcium chloride and concentrated. The residue was recrystallized from light petroleum. Yield 0.35 g. M. p. 60—62°. Further recrystallizations from light petroleum yielded pure *tert.* butyl α-glucoside tetraacetate. M. p. 69—70°.

Investigation of the products of acetolysis

Methyl α-glucoside tetraacetate and the β-glucoside tetraacetates of ethyl, *iso*propyl and *tert.*butyl alcohol (1 g of each) were dissolved in 1.4 *C* sulfuric acid in acetic anhydride-acetic acid, 10 : 3 (20 ml). After 24 hours the solutions were poured into ice water (500 ml) with sodium acetate (10 g). The mixtures were extracted with ether (3 × 100 ml), the ether solutions washed with sodium carbonate solution and with water, dried over calcium chloride and concentrated. The residues were dried in a vacuum over phosphorus pentoxide, paraffin and potassium hydroxide. They were analyzed with respect to acetyl groups. The values found were: methyl 55.6 %, ethyl 56.4 %, *iso*propyl 60.5 %, and *tert.*butyl 58.4 %. The percentage of glucose heptaacetate calculated from these values compared with that calculated from the final rotation of the solutions, will be found in table 1.

Simultaneous transformation of *iso*propyl β-glucoside and ethyl β-cellobioside

A solution of *iso*propyl β-glucoside tetraacetate (1 g), ethyl β-cellobioside heptaacetate (1 g) and titanium tetrachloride (1 g) in absolute chloroform (60 ml) was heated for 90 minutes on the steam bath. When cold, the mixture was shaken with bicarbonate solution, washed with water, dried over calcium chloride, and concentrated in a vacuum. The residue was recrystallized from ethanol (20 ml). 0.75 g of white crystals separated. M. p. 176—178°. After one further recrystallization from ethanol the melting point was

* All melting points uncorrected.

177—179°, alone or in admixture with an authentic sample of ethyl α-cellobioside heptaacetate.

The mother liquor from the first recrystallization was diluted with water (20 ml). A small amount of precipitate was formed which was discarded. When the solution was further diluted with water (150 ml) crystals of *iso*propyl α-glucoside tetraacetate separated. Yield 0.66 g. M. p. 84.5—85.5°. Mixed m. p. with an authentic sample 85—86°.

SUMMARY

The transglycosidation and acetolysis of some alkyl glucoside tetraacetates, catalyzed by strong acids, has been investigated in acetic anhydride-acetic acid solutions. The results are summarized below.

1. The rate of transglycosidation increases in the series:
methyl < *primary* alkyl < *secondary* alkyl < *tertiary* alkyl.

2. The yield of glucose heptaacetate by the acetolysis increases in the series methyl < *prim.*alkyl < *sec.*alkyl.

3. Two different acid catalysts seem to be present in a solution of sulfuric acid in acetic anhydride-acetic acid. Only the stronger of these, probably the acetyl cation, can catalyze the transglycosidation.

4. A reasonable mechanism for the transglycosidation, which also explains the formation of glucose heptaacetate, is given.

5. A new compound, probably isooctyl perchlorate, has been isolated when *tert.* butyl β-glucoside tetraacetate was acetolyzed with perchloric acid as catalyst.

The author wishes to thank *Statens Naturvetenskapliga Forskningsråd* for financial support and Mr. L. Asp for skilful assistance.

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Received September 20, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1170–1177

An Interferometric Method for Recording the Refractive Index Derivative in Concentration Gradients

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The main purpose of the optical methods used for the measurement of concentration gradients in centrifuges and in instruments for the study of electrophoresis and adsorption is to measure the positions of and the concentration increments across the boundaries. In the classical sedimentation studies of Svedberg and his collaborators [1] this was performed by the light absorption method, and the same technique was used later by Tiselius [2] in electrophoresis. When combined with microdensitometry, this method gave a tracing of the concentration as a function of the cell coordinate. Thus the concentrations could be obtained simply by measuring distances on the microphotograms.

The light absorption method was superseded by a group of methods based on the deflection of light in the refractive index gradients accompanying the boundaries. A common feature of these methods is that they give primarily the refractive index derivative and, by a proportionality factor, the concentration derivative. Consequently, the concentrations themselves must be derived by integrations. The reason why this round-about procedure was found superior to the more direct method used earlier was the fact that the derivative methods made the localization of the boundaries easier and that they proved capable of resolving much more effectively overlapping boundaries. In addition, in the evaluation of diffusion experiements both the concentration and its derivative are required. A differentiation is on the whole much more difficult to carry out with precision than an integration, and microphotograms are especially unsuitable in this respect. To the group of derivative methods belong the scale method (Lamm [3]), the Schlieren scann-

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ing method (Longsworth [4]) and the cylindrical lens method (Philpot [5], Svensson [6]).

Lately, there is an increasing interest in interferometric methods for physico-chemical measurements. Tiselius and Claesson [7] adopted the Rayleigh interferometer in adsorption analysis. Kegeles and Gosting [8] and Longsworth [9], as well as Coulson, Cox, Ogston, and Philpot [10] have introduced the old and nearly forgotten Gouy interference method [11] for diffusion measurements. Calvet and Chevalerias [12] have also devised an interferometric method for the study of diffusion. Chambers and Hartline [13] described the use of the Fabry-Perot interferometer in electrophoresis, and Labhart and Staub [14] have devised a micro-electrophoresis method using the Jamin interferometer. Last year Philpot [15] published a cell-focusing interferometer of the Rayleigh type with the aid of which a direct record of the n versus x curve can be photographed.

Since the interferometric methods depend upon differences in optical path lengths, which are proportional to the refractive index, they are related to the old light-absorption method in that they give primarily the concentration itself and that the derivative has to be computed by differentiation. An exception is the Gouy interferometer. This is based upon the interference of light pencils of the same angular deflection and is thus capable of giving direct information of both the concentration and its derivative. This is very advantageous since the diffusion experiment can be computed without integration or differentiation. However, the method cannot be applied for recording boundary systems, and the interpretation of skew diffusion boundaries is difficult.

It is evident that the principle of direct measurement of both the concentration and its derivative is very valuable especially in diffusion measurements but also for boundary systems in the ultracentrifuge, in the electrophoresis apparatus, and in adsorption analysis instruments, in cases of poorly resolved boundaries. The advantage of this principle for sedimentation equilibrium studies was realized by Kegeles [16], who constructed a double-prismatic cell to get a record of the concentration while the scale method simultaneously gave its derivative.

To devise a method capable of recording both the refractive index and its derivative as functions of the cell coordinate is simpler if both curves are obtained by the same optical principles. This is not possible with the methods based on light deflection, but it is possible with the aid of interferometry.

One possible way of solving this problem is to modify the cellfocusing interferometer described by Philpot (*l. c.*). The essential features of this interferometer, which was studied simultaneously in this laboratory, are given in Fig. 1. A is a vertical slit illuminated by monochromatic light and situated

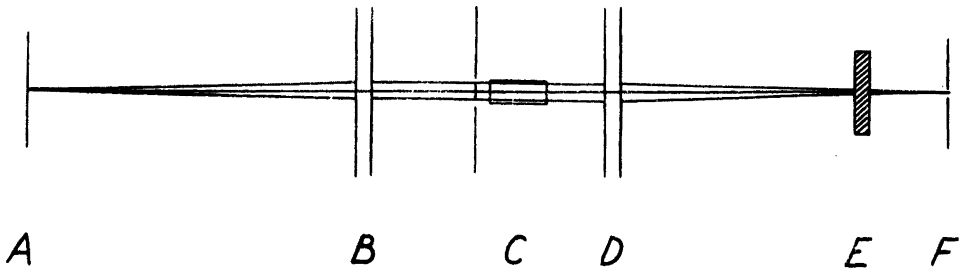

Fig. 1. The Rayleigh interferometer with a cylindrical lens for focusing the cell.

in the focal plane of the astronomical objective B. D is another astronomical objective which gives an image of the slit in its focal plane F. Between the lenses we have the double cell C with one chamber for the solvent and one for the diffusion column.

A detailed description of this twin cell will be given later in this paper. The cylindrical lens E with a horizontal axis gives, in elevation, an image of the cell in the plane F. This lens thus causes the image of every point of the slit A to spread out to a vertical line in which every vertical coordinate corresponds to a certain vertical coordinate in the cell. In the plane F, the vertical line gets a certain lateral extension due to the diffraction of light in the narrow cells C. The breadth of the line can be increased at the cost of light intensity if narrow vertical slits are placed close to the cells. Within this central diffraction band, interference fringes appear which arise from the interference between light pencils coming from the diffusion chamber and pencils from the solvent chamber. The number of the interference fringes depends upon the distance between the chambers. The separation wall between them must be rather thin to make it possible to observe and photograph the fringes. In our twin cell the wall is 3 mm thick.

If both cells have constant refractive indices throughout, the interference fringes will run vertically through the entire diffraction band in the plane F. If a diffusion boundary is present in one of the chambers, the fringes will become tilted in this region as shown in Fig. 2. In fact, each fringe will describe a curve which is identical with the course of the refractive index through the cell. However, due to the limited extension of the central diffraction band, one and the same fringe cannot be traced through the whole boundary unless the concentration increment is very low. To get the n versus x curve, therefore, one has to measure the position of each maximum or minimum or both along the central line of the diffraction band and to plot these readings against the number of the fringes (Fig. 4).

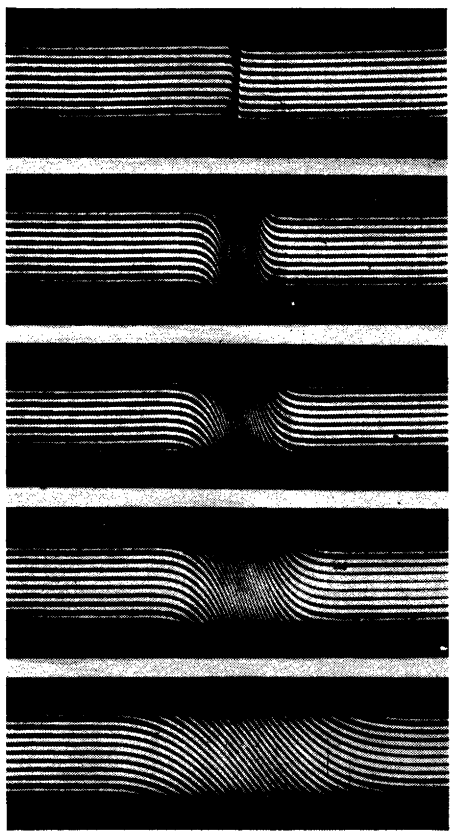

Fig. 2. Interference pictures obtained with the aid of the modified Rayleigh interferometer, showing the diffusion of an 0.2 per cent solution of sucrose against water.

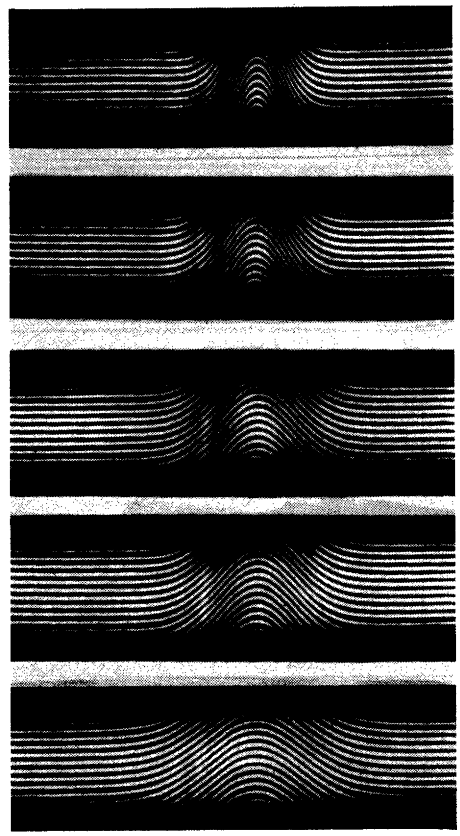

Fig. 3. Interference pictures obtained with the aid of the modified Rayleigh interferometer using two identical diffusion boundaries, slightly shifted with respect to each other, of an 0.2 per cent sucrose solution against water in the twin cell.

The modification which is necessary to get the derivative instead of the function itself is to allow two identical diffusion processes to take place simultaneously in the two chambers at slightly different heights. A suitable height difference can be obtained by pressing in solvent from the top and drawing out solution from the bottom or *vice versa* in one of the compartments. This small transport of the boundary must be done very slowly.

If the boundaries were exactly at the same height and if the two diffusion processes were identical, the interference fringes would run exactly vertically

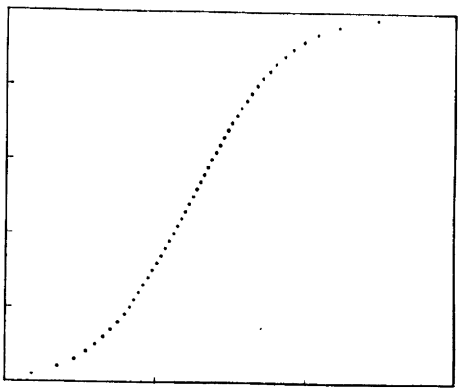

Fig. 4. Plot of the number of the fringes against their positions in one of the exposures of Fig. 2.

throughout the whole diffraction band. However, due to the small shift of one of the boundaries, every two interfering rays will pass through two points in the cell with slightly different positions with respect to the boundaries. Therefore the interference fringes in the plane F, Fig. 1, will describe a curve which is nearly identical with that of the refractive index derivative. Fig. 3 shows some pictures obtained in this way.

It is evident that the method just described does not give the exact derivative, but a quantity $\Delta n/\Delta x$ which approximates to the true derivative as Δx, the shift between the boundaries, decreases. However, with too small a shift between the boundaries the sensitivity becomes insufficient. The distance which one boundary can be transported from the other without introducing significant errors depends of course on the time during which the boundaries have diffused. In the later stages of the process, the shift can be increased.

Errors of the same kind are inherent in the scale method and in the inclined slit method. In the former method, the quantity Δx is defined by that portion of the cell which is passed by light from the particular scale line., and this portion in turn depends on the relative aperture of the camera. In the inclined slit method, every ray also passes through a certain volume fraction due to its curvature, and the quantity Δx corresponds to the thickness of this volume element. The essential difference between the old methods and this interferometric method is that Δx varies from point to point in the former procedures, whereas it is constant in the latter.

The wave-optical treatment of the Gouy fringes led Kegeles and Gosting [8] (*l. c.*) to the conclusion that the ray-optical theories hitherto used in the interpretation of diffusion experiments is insufficient. If a correction is not applied for the 'quarter-wave anomaly', the inclined slit method will give too low diffusion curves and too high diffusion constants. In the scale

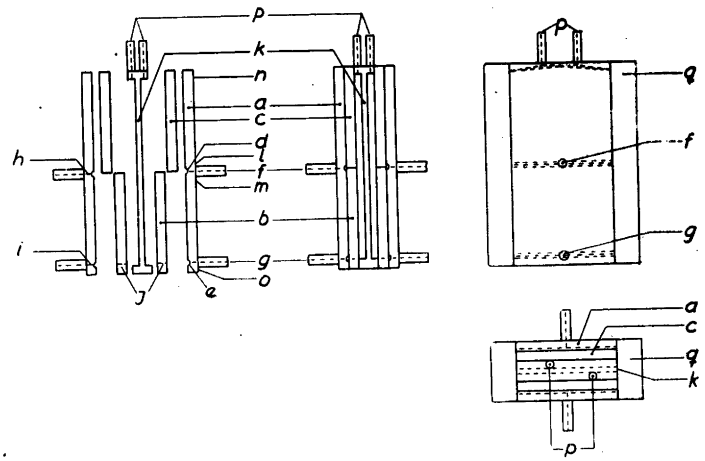

Fig. 5. Twin diffusion cell.

method the error was believed to be smaller. Since the interferometric derivative curve is obtained in a substantially different manner, it can be suspected that this error will be different. Possibly it is absent, but this cannot be stated definitely until a thorough theoretical and experimental investigation of the interferometric method has been carried out.

The necessity of making two identical boundaries slightly shifted with respect to each other is a definite disadvantage already in diffusion measurements, and still more so in more complicated instruments with several boundaries. The experiments described here, however, are only preliminary ones to show the possibility of recording the derivative of refractive index by interferometry. In a following article a purely optical differentiation of the refractive index function will be described, where one single boundary or boundary system can be used.

DESCRIPTION OF THE DIFFUSION CELL

The diffusion cell constructed for this study is shown schematically in Fig. 5. The side and separation walls are made of stainless steel, the front and back walls are plano-parallel glasses. Every side wall is composed of three metal pieces *a*, *b*, and *c*, each of them 3 mm thick. *d* and *e* are half-cylindrical grooves running horizontally along the whole cell. The capillary tubes *f* and *g* are soldered centrally on the metal plates and are connected to the centres of the grooves by the drilled holes *h* and *i*. In the plate *b* a number of very small holes are drilled through at *i*, the level of which is the same as the groove *e*. The central metal plate *k* forming the separation wall is formed as an *I* and carries two capillary tubes on its top which are connected to the two cells by small drilled holes.

The twin cell is put together as follows. First the plate *c* is fixed to the plate *a* by two screws situated at *l*. After placing two hairs on top of plate *b*, this plate is pressed against *c* and fixed to *a* by two screws at *m*. The hairs are now removed, and a slit about 0.04 mm wide is thus formed between *b* and *c* in the middle of the cell, where the groove *d* is also situated. The same procedure is followed with the other plates named *a*, *b*, and *c*. At last the two side walls and the separation wall are fixed together by eight longer screws situated at *n* and *o*. To avoid leakages it is necessary either to grease the metal surfaces before they are screwed together or to use packings. After all metal pieces have been put together, their front and back surfaces are treated mechanically in a milling machine to make them as smooth and plane as possible. Finally the glass pieces are put in position and clamped against the metal surfaces in a suitable cell support. Again, packings are necessary to get the cell tight.

In the cell support, the six capillaries *f*, *g*, and *p* are attached to glass capillaries leading through stop-cocks to six glass containers. When an experiment is to be started, the cells are first filled with solvent which is pressed in through the bottom capillaries. In this way all air is removed from the cells and from the metal and glass tubes. The solution to be investigated can then be pressed into the cell the same way. By the action of the groove *e* and the drilled holes *i*, a minimum of mixing with the solvent is guaranteed. When the boundary has risen to the middle portion of the cell, pressure is applied also from the top container with solvent, and both the solution and the solvent are allowed to escape through the narrow slit between the plates *b* and *c*. A very sharp starting boundary is then readily obtained. The diffusion starts when the stop-cock connected to *f* is closed. The two other stop-cocks are closed immediately thereafter.

The author got the idea of this method of making diffusion boundaries in work with preparative electrophoresis. The apparatus constructed for this purpose [17] had suction capillaries attached to the side walls of the U-tube. On removing fractions through these capillaries it was observed that an extremely sharp boundary was formed at the site of the capillary. The first diffusion cells were also constructed with simple suction capillaries. The reason why they were abandoned in favour of a horizontal slit was that the slit could be made narrower and that the sharpening effect could be extended to the whole cell. Sharpening at one point works fairly well in cells about 10 mm thick. It was also used by Kahn and Polson [8]. The cell described here, however, is 50 mm thick to make it useful for very dilute solutions. It is easily understood that suction at one point in such a cell would not be adequate. Diffusion cells where the boundaries are formed by the flowing junction technique were used by Coulson, Cox, Ogston, and Philpot (*l. c.*). Their first cell is very similar to that described here, but simpler. They did not give any information of the dimension of the slit or how it was made.

Any rigorous and critical tests with this diffusion cell have not been carried out so far.

SUMMARY

The relative merits of the derivative and integral methods for recording concentration gradients in boundary systems have been reviewed with the conclusion that the ideal record is a combination of both. Since a record of the concentration is easily obtained with the aid of interferometry it was found

worth while to try a modification of this procedure capable of giving a record of the concentration derivative. The modification is characterized by interference between two rays passing through the concentration gradient at slightly different levels. The two interferometric methods can easely be combined to give a simultaneous record of the concentration and its derivative.

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Received April 25, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1178–1180

Short Communications

The Splitting of the Porphyrin-protein Bonds in Cytochrome c

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Unlike many other iron-porphyrin proteids, *e. g.* hemoglobin, myoglobin, horse radish peroxidase, and catalase, cytochrome c is not divided into its protein part and prosthetic group by acid acetone. This may be attributed to the unique arrangement with cysteine-sulphur bridges from the side chains 2 and 4 of the porphyrin to the protein [1]. Because of that it has not been possible to examine cytochrome c by means of some of the methods which have proved to be valuable for the studies of heme-linked groups in those other proteids.

Salts of some heavy metals have been used to cleave thioether bonds in other compounds [2,3]. Cytochrome c has been found to be affected in the same way by these salts, of which the silver salts seem to be the best. At faintly acid reaction and slightly elevated temperature the iron porphyrin is liberated, so that it can be extracted with ether-acetic acid or stays in the liquid phase upon the addition of an excess of acid acetone. The heat of activation is roughly constant (about 18 000 cal/mole) between + 20° C and + 80° C.

The prosthetic group can be crystallized from aquous butanol-acetic acid. It gives a pyridine hemochrome, which is spectroscopically indistinguishable from that of hematohemin and after its conversion to the free porphyrin by means of the pyruvic acid method [4], it agrees with hematoporphyrin as regards hydrochloric acid number and spectrum.

The protein part is easily soluble in water after its previous precipitation with acetone in the cold. It is electrophoretically homogenous. A comparison of the titration curves of the intact cytochrome c and its protein part shows that the former consumes three equivalents more per mole below pH 4 than does the latter. Between pH 4 and 6, however, the protein residue takes up 2 equivalents more. The results seem to support the theory that histidines occupy two of the six coordination possibilities of the iron atom [5].

A detailed report as well as some experiments on the position of the thio-ether bonds in the side chains 2 and 4 will be published in this journal. A micromethod for the determination of cytochrome c in tissues has also been worked out on the basis of these experiments and will be published.

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Received November 22, 1949.

On the Solubilization of Steroid Hormones by Association Colloids

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For many purposes it would be advantageous if it were possible to prepare aqueous solutions of the fat-soluble steroid hormones. Hence many attempts have been made either to prepare stable high-disperse suspensions of the hormones in water or to increase the power of water to dissolve these substances [1–7]. By taking advantage of the solubilizing power of association colloids we have succeeded in preparing clear stable aqueous solutions of several steroid hormones. By the end of 1947 aqueous solutions of desoxycorticosterone were obtained and half a year later we found that a similar result was possible in a varying degree in the case of testosterone, α-estradiol and desoxycorticosterone acetate. Difficulty in obtaining sufficient quantities of the pure hormones has prevented us from carrying out our investigations as originally planned. Some of the results thus far obtained are presented in the following.

Hormones can be brought into solution in a manner quite similar to that described earlier for the carcinogenic hydrocarbons [8], *viz.*, by shaking them with association colloid solutions of appropriate concentration. Heating speeds up the process of solubilization. The following association colloids have thus far been studied with respect to their solubilization power: sodium oleate, sodium myristyl sulphate, sodium cholate, sodium desoxycholate, sodium dehydrocholate, sodium glycocholate, and an alkyl aryl polyether alcohol (Triton NE). The solubilities of testosterone, testosterone propionate, estrone, α-estradiol, desoxycorticosterone, and desoxycorticosterone acetate, and also hexestrol were investigated. For example, the following solutions were prepared:

Solutions of testosterone:
5 mg hormone per ml 10 % sodium oleate solution;
10 mg hormone per ml 20 % sodium myristyl sulphate solution;
2 mg hormone per ml 20 % sodium cholate solution.

Solutions of testosterone propionate:
15 mg hormone per ml 10 % sodium oleate solution;
35 mg hormone per ml 20 % sodium myristyl sulphate solution;
10 mg hormone per ml 5 % sodium myristyl sulphate solution.

Solutions of estrone:
1.3 mg hormone per ml 20 % sodium myristyl sulphate solution.

Solutions of α-estradiol:
0.7 mg hormone per ml 10 % sodium oleate solution;
0.8 mg hormone per ml 10 % sodium myristyl sulphate solution.

Solutions of desoxycorticosterone:
14 mg hormone per ml 20 % sodium cholate solution;
10 mg hormone per ml 10 % sodium cholate solution.

Solutions of desoxycorticosterone acetate:
1.5 mg hormone per ml 10 % sodium oleate solution;
1.8 mg hormone per ml 10 % sodium myristyl sulphate solution.

Solutions of hexestrol:
12 mg hormone per ml 10 % sodium oleate solution;
5 mg hormone per ml 10 % sodium myristyl sulphate solution;
12 mg hormone per ml 10 % sodium cholate solution.

All of these solutions are clear and stable, and withstand, for example, boiling without any separation of the hormone taking place. Solubilization is possible only when the colloid concentration exceeds the critical concentration for micelle

formation, and the amount of hormone dissolved increases with further increase in the concentration of the micellar substance. On greater dilution the hormone usually separates out sooner or later, and always when the critical concentration is approached. In this respect the various colloids differ considerably.

The association colloid solutions of the hormones give a small contact angle with lipoid surfaces. They therefore easily wet the skin and the mucous membranes, penetrate them, and transport the solubilized hormone into the tissues and cells. In this manner it is thus possible to transfer considerable amounts of hormones into the organism. Some of these solutions can be introduced by subcutaneous or intravenous injection. The investigations concerned with the latter aspect are, however, still incomplete.

The investigations are being continued.

At the beginning of our investigations we were furnished with the hormone substances required by F. Paulsen, M. D., Director of Research at the Nordiska Organon, Stockholm, and he also obtained information of our methods and results while the work was in progress. We wish to thank Dr. Paulsen.

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Received December 5, 1949.

The Molecular Structure of N,N'-dichloropiperazine

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Structure determinations of simple derivatives of piperazine are of interest in connection with problems related to cyclohexane and other substances containing six-membered rings. The presence of two nitrogen atoms in the ring makes the number of possible configurations attainable by a given molecule greater than it is in the corresponding cyclohexane derivative. In the case of the N,N'-dichlorocompound *three* possible configurations based on the "chair" form of the ring have to be considered: the $\varkappa,\varkappa$, the $\varkappa,\varepsilon$ and the ε,ε configuration [1].

We have measured the dipole moment of the substance in benzene solution, determined the unit cell and space group of the crystalline form and finally carried out an electron diffraction investigation of the vapour, based on the sector method.

Measurements of the dielectric constant of benzene solutions strongly indicate that the dipole moment is zero.

The crystals are monoclinic with the lattice constants:

$$a = 5.63 \text{ Å}, \; b = 5.47 \text{ Å}, \; c = 10.98 \text{ Å}, \; \beta = 94°$$

The space group is $C_{2h}^5 - P2_1/c$. The unit cell contains *two* molecules and the molecules must therefore exhibit a center of symmetry in the crystalline state.

It is interesting to compare the crystallographic data with those found in the case of the 1,4-dibromocyclohexane of m. p. 112° and the corresponding diiodo-compound of m. p. 142° given in a paper published in 1932 [2]. There can be little poubt as to the isomorphism of these

formation, and the amount of hormone dissolved increases with further increase in the concentration of the micellar substance. On greater dilution the hormone usually separates out sooner or later, and always when the critical concentration is approached. In this respect the various colloids differ considerably.

The association colloid solutions of the hormones give a small contact angle with lipoid surfaces. They therefore easily wet the skin and the mucous membranes, penetrate them, and transport the solubilized hormone into the tissues and cells. In this manner it is thus possible to transfer considerable amounts of hormones into the organism. Some of these solutions can be introduced by subcutaneous or intravenous injection. The investigations concerned with the latter aspect are, however, still incomplete.

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Structure determinations of simple derivatives of piperazine are of interest in connection with problems related to cyclohexane and other substances containing six-membered rings. The presence of two nitrogen atoms in the ring makes the number of possible configurations attainable by a given molecule greater than it is in the corresponding cyclohexane derivative. In the case of the N,N'-dichlorocompound *three* possible configurations based on the "chair" form of the ring have to be considered: the $\varkappa,\varkappa$, the $\varkappa,\varepsilon$ and the ε,ε configuration [1].

We have measured the dipole moment of the substance in benzene solution, determined the unit cell and space group of the crystalline form and finally carried out an electron diffraction investigation of the vapour, based on the sector method.

Measurements of the dielectric constant of benzene solutions strongly indicate that the dipole moment is zero.

The crystals are monoclinic with the lattice constants:

$$a = 5.63 \text{ Å}, \; b = 5.47 \text{ Å}, \; c = 10.98 \text{ Å}, \; \beta = 94°$$

The space group is $C_{2h}^5 - P2_1/c$. The unit cell contains *two* molecules and the molecules must therefore exhibit a center of symmetry in the crystalline state.

It is interesting to compare the crystallographic data with those found in the case of the 1,4-dibromocyclohexane of m. p. 112° and the corresponding diiodo-compound of m. p. 142° given in a paper published in 1932 [2]. There can be little poubt as to the isomorphism of these

substances and it seems probable that the corresponding 1,4-dichlorocyclohexane of m. p. 101° has a corresponding crystal structure. In the case of the diiodo-compound the position of the iodine atoms in the unit cell was determined in 1932, and the results of an electron diffraction investigation of the vapours of all three 1,4-dihalogenocyclohexanes in question published in 1938 [3] showed that even in the gaseus state the $\varkappa,\varkappa$ configuration is the most stable one. In benzene solution the dipole moments are all zero [4].

Before proceeding to a complete X-ray analysis of the crystals of N,N'-dichloropiperazine we have carried out an electron diffraction investigation of the vapour using the sector method. The $\frac{\sigma(r)}{r}$-curve obtained (Fig. 1) leaves not doubt as to the correctness of the assumption that the molecular configuration corresponds very closely to that of the cyclohexane derivatives mentioned above. The position of the vertical arrows in Fig. 1 give the theoretical r-values and their height the weight factors of the inter-nuclear distances present in a molecule based on strictly tetrahedral angles and on bond distances of the expected lengths: C—C = 1.54 Å. C—N = 1.47 Å and N—Cl = 1.70 Å.

The weight factors employed are given as:

$$\frac{n\ Z_1 Z_2}{r}$$

n being the number of distances of a certain kind, Z_1 and Z_2 the atomic numbers and r the atomic distance. More correct values of the weight factors may be evaluated when exact "normal curves" corresponding to given distances are at hand. So much may allready be said, that the corrected values of the weight factors will explain the experimental $\frac{\sigma(r)}{r}$-curve even better than those given in Fig. 1.

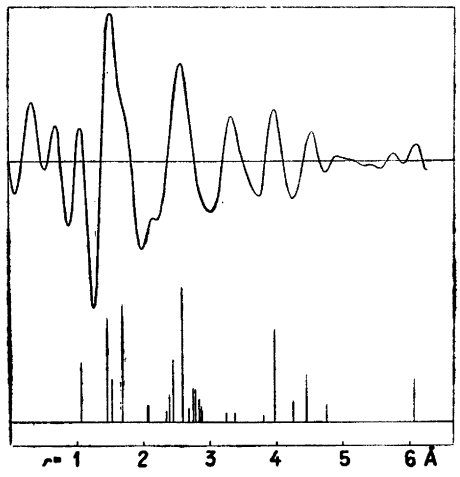

Fig. 1. $\frac{\sigma(r)}{r}$ *-curve of N,N'-dichloropiperazine.*

1. Hassel, O. *Research* (in publication).
2. Halmøy, E., and Hassel, O. *Z. physik. Chem.* **B 16** (1932) 234.
3. Gudmundsen, J. G., and Hassel, O. *Z. physik. Chemi.* **B 40** (1938) 326.
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Received December 3, 1949.

New Books

Vitamins and Hormones, Advances in Research and Applications. Edited by Robert S. Harris and Kenneth V. Thimann. Volume V (1947) and VI (1948) Academic Press, Inc., New York.

It is no wonder that the preceeding volumes of this series have been so favorably received by scientists working in the vitamin and hormone fields. These fields are widening rapidly, and the number of papers dealing with the chemical, biological and clinical properties of vitamins and hormones increases steadily. However, it is not only due to the fact that it is humanly impossible for a single individual to keep conversant with the enormous original literature that this series of monographs has met with approval, but mainly that the reviews given in the 'Vitamins and Hormones' have been found to be up to date, complete and reliable. They give, in a brief form, surveys of questions of actual interest, written by specialists with critical outlook. Volume V contains the following chapters: The synthesis of vitamin A and related products, by N. A. Milas; Physiological availability of the vitamins, by D. Melnick and B. L. Oser; Thiamine and peripheral neurophysiology, by A. von Muralt; The physiological effects of the pteroylglutamates in man with particular reference to the pteroylglutamic acid (PGA), by W. J. Darby; The vitamin requirements of chicks, by H. R. Bird; Vitamin requirements of the mouse, by H. P. Morris; The properties of the growth and adrenocorticotropic hormones, by C. H. Li and H. M. Evans; Effect of exogeneous estrogens on the male mammal, by C. W. Emmens and A. S. Parkes; The biology of antithyroid agents, by H. A. Charipper and A. S. Gordon; The use of androgens in women, by A. C. Carter, E. J. Cohen and E. Shorr; The clinical uses of testosterone in the male, by C. G. Heller and W. O. Maddock.

Volume VI contains a cumulative index of volumes I through V. Surveys published in this volume are: The chemistry and biological action of pteroylglutamic acid and related compounds, by B. L. Hutchings and J. H. Mowat; Vitamin K, by Henrik Dam (København); Nutritional requirements of the cotton rat and hamster, by B. S. Schweigert; Vitamins as pharmacologic agents, by H. Molitor and G. A. Emerson; The assessment of human nutriture, by H. M. Sinclair; Vitamins in microorganisms—distribution and quantitative synthesis, by J. M. van Lanen and F. W. Tanner, Jr; The B vitamins as plant hormones, by J. Bonner and H. Bonner; The influence of the adrenal cortex on the metabolism of water and electrolytes, by E. C. Kendall. These titles give an idea of the magnitude of the field covered by the 'Vitamins and Hormones'; it seems rather superfluous to state that they are extremely valuable to anyone interested in vitamin and hormone questions.

K. Myrbäck

This issue has been prepared to commemorate J. N. Brønsted.

It contains three obituaries, a bibliography including Brønsted's scientific publications, but not his numerous articles in Danish news-papers, and a list of the scientific distinctions bestowed on him in acknowledgement of his achievements in science.

We have added a few scientific papers, one by Brønsted himself, and three others, all concerning his way of presenting the fundamental principles of thermodynamics which so intensely occupied him in the last decade of his life, and also a paper on an application of his method of isotope separation.

We know that many other scientists besides the contributors to this issue would have been glad to express their feelings towards J. N. Brønsted by sending commemorative papers, but for outer reasons we have been compelled to refrain from an extension of this issue.

The editors wish to express their gratitude to the contributors to this issue as well as to all friends and colleagues of Professor Brønsted who might have wished to commemorate him at this occasion. We also wish to express our thanks to Mrs. L. Brønsted for her help.

For the Editors

J. A. Christiansen

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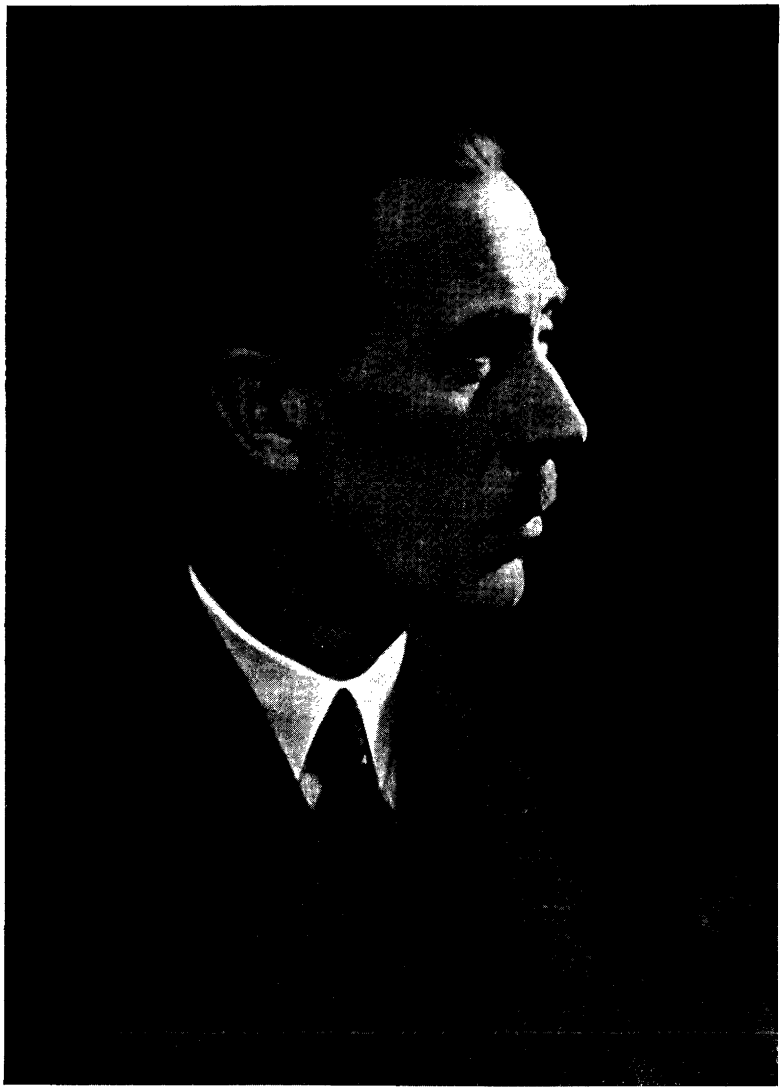

J. M. Brooks

ACTA CHEMICA SCANDINAVICA 3 (1949) 1187–1200

J. N. BRØNSTED MEMORIAL ISSUE

J. N. Brønsted *

22nd February, 1879 — 17th December, 1947

Johannes Nicolaus Brønsted was born on the 22nd February, 1879, at Varde. His father was an engineer of note in 'The Society for Cultivation of Heaths' whose memory lived for a long time in the places where he had worked, and on visiting those parts in later life Brønsted received much evidence of the high esteem in which his father was still held there. Brønsted's mother died shortly after his birth, and his father married again a few years later. His stepmother, who became a widow when the boy was only 14, understood how richly talented he was, and in spite of her modest income saw to it that he received the best possible education.

Until the boy was 12 years old the family lived at one of the farms of the Society for Cultivation of Heaths: Hesselvig Enggaard at the river Skern, where his sense of the beauty of unspoilt nature and his natural faculty of observation were developed. To his death Brønsted knew and loved the few unspoilt and lonely parts which still exist in our populous country, and by letters to the newspapers and correspondence with persons of influence in that matter he fought to protect them from encroachments. This often brought him in opposition even to the Society for Cultivation of Heaths. He was also a keen opponent of the kind of preservation of wild nature which aims at attracting visitors to a place thereby spoiling its very charm.

His well known interest in birds and bird-life no doubt also goes back to those years. Later on it was developed by his artistic bent which was encouraged by his connection with Johannes Larsen, the painter.

12 years old the boy moved with his parents to Aarhus and was sent to school there.

I am told that he felt at home in and loved the countryside around Aarhus, which was also in harmony with his natural bent, and that he distinguished himself at school, especially at mathematics. However, his life at Aar-

* Translation of paper read to The Royal Danish Academy of Sciences and Letters on 15th October, 1948 by J. A. Christiansen.

hus came to an end when his father died, only two years after their move to that town. The family, the mother and the two children, Johannes and his sister, moved to Copenhagen. Johannes entered the Metropolitan School and came into the form from which so strikingly many men of great merit were to issue. Among his school-fellows was Niels Bjerrum, one of the sons of Professor J. Bjerrum, the well known oculist. In 1897 he passed his 'Studentereksamen' (corresponds to the School Certificate Examination), commenced his studies on chemical engineering at the Polytechnic Institute of Copenhagen and passed the 1st part of the examination in this subject two years later. Then he passed on to studying at the University and took his 'Magister scientiarum' (m. sc.) degree of Chemistry in 1902, three weeks after Niels Bjerrum. A 'Magister', especially of chemistry, was at the time a rare bird, so rare indeed that Brønsted for many years afterwards was still called the 'Magister' among his friends. While he studied for his degree he had many other interests besides chemistry, and all through his life he continued to cultivate them. Thus he was very fond of song and music and was a performer in the circle of his family and friends. After his graduation he was among other things for a time Valdemar Poulsen's collaborator on the 'telegraphone', and not until 1905 he became assistant at the Chemical Laboratory of the University. But his first experimental works are from 1903. So although he himself said that he was lazy, he evidently was not, probably because scientific work came so easy to him that he had leisure for other interests. At that time he married Louise Brønsted, nee Warberg, chemical engineer. The family settled down a little north of the small town of Birkerød in a little house on a very large site which was for the greater part left in its natural state, sheltered as it were by 'Nordbanen' (the Northern Railway) which is still to-day protecting the lovely unspoilt place from being built in. Mrs. Brønsted's sister was married to Johannes Larsen, the painter, and through him Brønsted came into touch with artists like the Funen painters. Being of an artistic bent, he derived much pleasure from his connection with these circles, and perhaps it was their influence which made him take to painting in the years around the war of 1914—18. For some time during that war the house was lighted by electricity produced by galvanic batteries designed by the master of the house himself. A patent was, by the way, taken out for these batteries. Later on when the family increased to six members, and as communications to Copenhagen were bad, the home was moved in 1923 to Willemoesgade in Town, and from there in 1930 to the house for the head of the new Institute of Physical Chemistry at Blegdamsvej.

In May, 1908, Brønsted defended his thesis: *Affinitetsstudier III* (Studies on Affinity III), six months before Niels Bjerrum's defence of his thesis.

Opponents *ex officio* were Dr. E. Biilmann, recently appointed Professor of Chemistry, and K. Prytz, Professor of Physics at the Polytechnic Institute. Opponent *ex auditorio* was G. A. Hagemann, head of the Polytechnic Institute, who always followed Brønsted's work with great interest. Hagemann was a chemical engineer and a large-scale industrialist. He was well acquainted with Julius Thomsen, the renowned thermo-chemist. The latter was still alive at the time (he died in 1909), and Hagemann's enthusiasm for physical chemistry certainly had its offspring from his interest in Julius Thomsen's work.

On the 17th December of the same year Brønsted was appointed to the new third chair of chemistry at the University of Copenhagen after a competition with Niels Bjerrum before a committee set up by the University. Also to-day we understand that it must have been a difficult choice to make, and actually the report of the committee of the 7th December gives the most laudatory opinions on both applicants. As a matter of curiosity it may be mentioned that the costs of the competition amounted to 71 Kr. 40 Øre (abt. £ 3/10/—).

The Brønsted family had the news of the result in a most dramatic manner when a passing goods-train suddenly stopped at the house at Birkerød, and a uniformed official from the station got off to deliver a telegram from Niels Bjerrum congratulating them on the decision of the committee. This episode probably took place on the 17th December, exactly 39 years before Brønsted's death.

The actual establishment of the new chair had not passed off without friction. It was agreed that the chair was needed, but there was some disagreement as to whether it was to be placed under the University or under the Polytechnic Institute. In the Faculty of the University there was a majority, but not unanimous agreement that it should come within the University. The Polytechnic Institute seems to have wavered in taking its stand. No doubt there has been a general wish to get the chair for that Institute, but G. A. Hagemann, Director of the Institute, strongly advocated in a letter that the new professorship of chemistry should be placed on an equal footing with the existing two, so that it should come under the University but with obligation for the professor to teach the students of the Polytechnic Institute as well. The following quotation, which might serve as motto to his whole letter, testifies to G. A. Hagemann's farsightedness: 'Chemistry and Physics, I was almost going to say Mathematics, know no border line between Science and its Applications, and there is no reason whatever to believe that the future will change this fact.'

The outcome was that Brønsted was appointed Professor of Chemistry in the University with obligation 1) to teach elementary inorganic chemistry to

the students of the Polytechnic Institute, excepting the chemical engineers, and 2) to teach the latter and the students of the University physical chemistry. Not until 1919 was he exempted from the teaching of inorganic chemistry which took much of his time. The premises in the chemical wing of the Polytechnic Institute where he now got his working place were very modest, not to say insufficient, but nevertheless it was here that his scientific work came into full bloom. His own work and his many collaborators, particularly from England and U. S. A., brought his fame far abroad. A visible result of this was that the International Education Board built for the University of Copenhagen an institute of Physical Chemistry, where Brønsted also got his residence. Here in this hospitable home he could unfold his quiet charm in all its aspects. At the same time, of course, he continued his scientific work, which from the middle of the Thirties was concentrated on the exposition of thermodynamics. This work, which for periods absorbed him completely and no doubt wore upon his health, occupied him to his death, but concurrently he worked on other more tangible problems. Only part of the last results obtained did he get time to publish.

Those who in the course of time got to know him closely were much taken with Brønsted's whole personality and the charm he would display in his many-sided interests. To the students and his younger colleagues, who had difficulty in overcoming the feeling of awe he involuntarily inspired, he was more distant. Perhaps it was owing to the stringent logic which he employed in expressing his thoughts and opinions, and his firm belief in the convincing power of logical arguments, also outside scientific circles. The same stringency in logic he would inexorably demand from his collaborators and his opponents in discussions. Occasionally these qualities of his would cause him personal trouble, and possibly they did not always benefit the causes he advocated. Perhaps he liked polemical discussions, but one thing is certain that the causes he stood up for, among other places in the daily press, he had deeply and seriously at heart. In his early years it was especially questions of preservation of the countryside he wrote about, but during and after World War II his interest centred on political questions and particularly the South Slesvig problem. It is well known that he was a violent opponent of the policy that the border of 1920 should be final, and his letters to the newspapers about this problem were no doubt conducive to his election in 1947 to the Lower House of the Danish Parliament by a great number of personal votes. His election was completely unexpected to him and his family. At first they took it as a fine joke but then, true to his scientific habits, he immediately set to work to study Parliamentary Procedure and the matters in which he took a special interest. To his friends it was a grief that he never occupied his seat

in Parliament to which his power of oratory and his competence would alike have been an adornment and a benefit.

Brønsted travelled widely. In his youth he mainly went southwards. But in 1912 he also participated in the International Chemical Congress in New York where he met for the second time Th. W. Richards. Their first meeting had taken place some years before at Hamburg. Brønsted admired Richards both as a man and as a scientist, and the admiration was mutual, which was not without importance for the honourable American grant for the new Institute.

Already during the first World War Brønsted took a certain dislike to our southern neighbours, and as moreover van't Hoff, the colleague from those parts whom he most estimated, had died already in 1911, he especially attached himself to English and American colleagues after the War. He loved England and often went there, particularly for meetings of the Faraday Society. In 1926—1927 he was visiting professor at Yale University in U. S. A. From one of the meetings of the Faraday Society Mrs. Brønsted relates the following: Brønsted was giving an address, and one of his friends was among the audience. There he heard one of the audience say to his neighbour, 'He has read Brønsted', to which the other replied, 'Yes, but he hasn't quite understood him'.

His sympathy for England was easily understandable, for himself he had some of the very qualities which we usually consider the best and most characteristic English qualities, a quiet humour and a deep interest in human affairs. By look he might to a Dane pass for a typical Englishman, and he wrote and spoke English easily and fluently.

During the last World War he never doubted its final issue, even when matters were at their worst, and from the summer of 1945 I remember the heartfelt sincerity with which he welcomed the first English colleague after the many years of isolation.

The number of scientific honours he was awarded in the course of time are legion, the last was the Degree of Doctor of Science, honoris causa, of the University of London, the conferment of which took place during the XIth International Congress of Pure and Applied Chemistry in London in the summer of 1947. He was a member of the Royal Danish Academy of Sciences and Letters from 1914 and of the Danish Academy of Technical Sciences from its establishment in 1937. Finally it should be mentioned that in 1928 he was awarded the Ørsted-Medal at the same time as Niels Bjerrum. But the greatest memorial to his honour was set by himself by his publications which number about 130, including two well known papers issued in the publications of the University of Copenhagen.

We are still in a position to picture fairly well the scientific environment in which Brønsted grew up. In 1901 Julius Thomsen had been succeeded by Emil Petersen as head of the Chemical Laboratory of the University of Copenhagen while S. M. Jørgensen was still head of the Chemical Laboratory of the Polytechnic Institute. Assistants at this Laboratory were: S. P. L. Sørensen (1892—1901), E. Biilmann (1898—1907), and Julius Petersen (1892—1908). Among Brønsted's fellow-students of his own age Niels Bjerrum and Charlotte Louise Warberg, student of chemical engineering, should be mentioned again.

The works on the chemistry of inorganic complex compounds published by S. M. Jørgensen himself and his group have no doubt impressed the young chemist. This appears among other things from the preference he had later on for using such compounds as the object of physico-chemical works. But in accordance with his natural disposition he went his own ways right from the beginning, his very first works being physico-chemical. Physical Chemistry practically was not cultivated in this country for a number of years after J. Thomsen had concluded his thermo-chemical works. It is well known that it was a subject in great progress after the basic works by van't Hoff, W. Ostwald, Arrhenius, and W. Nernst in the Eighties and Nineties, but as far as I know Emil Petersen was the only one in this country to take an active part in the development of the Nineties. The subject: Physical Chemistry constitutes a domain which it is in principle hard to define. But at the time it was rather well defined. Notably it included the application of thermodynamics and electricity to chemical problems, and reaction-kinetics. But these fields were teeming with problems which would obviously have a great attraction to the rising generation of chemists. According to the statements of contemporaries, Arrhenius' dissociation theory from the Eighties came like a revelation. At one blow they got quite new and much simpler possibilities of describing well known phenomena, *e. g.* the analytical precipitation reactions. Add to this that the law of mass action and thermodynamics afforded the possibility of expressing in figures what formerly had to be described in more or less vague terms such as the strengths of acids and the tendency in general of substances to react with each other, the affinity.

Julius Thomsen originally thought that the amount of heat evolved in chemical processes afforded a measure of their affinity, but already about 1900 it had been widely known for a long time that this did not hold true. The true measure of affinity had been found to be the maximum work which a given process will produce, and it was Brønsted's endeavour to develop methods for such determinations to provide means for reaching the end Julius Thomsen had aimed at by his investigations.

In accordance with this programme the object of his first works was to measure the electromotive force of certain galvanic cells, and later on to measure vapour pressures and solubilities. His experiments were always distinguished by precision and the elegance with which by simple and inexpensive means he would overcome the frequent experimental difficulties. But we, who were then young, were perhaps even more impressed by the imagination with which he knew how to construct the objects of his measurements, particularly galvanic cells. I still retain the memory of his description in 1911 of a reversibly working ammonium electrode, probably one of the first lectures I heard in the Danish Chemical Society.

It was characteristic of him that for a number of years he avoided as far as possible to work with cells with diffusion potential. In this way his results had the advantage of considerable precision, but on the other hand he debarred himself from taking part in the particularly fertile development in those years of the application of concentration cells especially to measurements of hydrogen ion concentrations. This development was initiated in this country by Niels Bjerrum and dealt with in detail by S. P. L. Sørensen.

Brønsted was also deeply interested in the theoretical treatment of the problem of affinity. This appears among other things from papers published in 1904 and 1906 in the publications of the Royal Danish Academy of Sciences and Letters. The theoretical problems which occupied not only him but a number of chemists of the time like F. Haber and W. Nernst, was the question of the relation between affinity and heat of reaction, especially the problem (which Brønsted, however, does not state expressly) whether it would be possible to calculate the affinity from purely thermal data. The question is of exceedingly great practical importance. For while the methods for determination of specific heat and evolution of heat are fairly simple, the determination of affinity often requires a refined experimental technique, and moreover it must be varied so to speak from system to system. Add to this that it is only possible to measure affinity if the reaction in question can actually be effected, while a calculation from thermal data may be carried out also for reactions not yet realized. The problem was solved in its broad lines by W. Nernst in 1906 when he put forward his famous theorem. Brønsted was only 27 then. As, however, the number of reliable affinity measurements was still very limited, and further experimental confirmation of the theorem was consequently needed, Brønsted continued his studies on affinity for many years. The last paper in this series (no. 13) appeared in 1921.

It was, by the way, through Nernst that Brønsted's name early became known internationally. In 1904 Brønsted published a work in the publications of the Royal Danish Academy of Sciences and Letters in which

there is among other things a determination of the differences in energy and free energy between rhombic and monoclinic sulphur. In 1906 the work was published in *Zeitschrift für physikalische Chemie,* and in the 5th edition of his famous *Theoretische Chemie* (1907) W. Nernst quoted it as an example of experimental corroboration of his theorem. Unfortunately the friendly connection between Nernst and Brønsted established by this event did not last long. A violent controversy in 1914 bears sufficient testimony to this. The immediate cause was probably that in his thesis: *Affinitetsstudier III* (1908) Brønsted categorically declared in a so-called 'thesis' at the end of his book that the conception set up by Nernst: ideal concentrated solutions had no justification, and in another place in the same book he deals with this conception in similar terms, although less outspokenly. This provoked Nernst to some sharp remarks in the 7th edition (1913) of his above-mentioned book, and Brønsted paid him back in his own coin. The vehemence with which the controversy was carried on by both parties gives a good impression of the two men's passionate desire to find the best possible expressions for experimental facts. The subject of discord is of historical interest, because according to Nernst's own statements it was the special thermodynamic properties of these solutions which led him to the proposition of the above-mentioned theorem. But his definition of 'ideal concentrated solutions' has not prevailed, and it is now, and was already then as mentioned by Brønsted, preferred to define ideal solutions (mixtures) in a different way.

In connection with his studies on affinity, for which purpose he mainly used measurements of electromotive forces and vapour pressures, Brønsted also undertook determinations of the specific heats of certain substances. These measurements required a special technique, and here as always when it was a question of devising, realizing and applying apparatus which could work with the required precision in the simplest possible way, Brønsted to a very great extent took part in the work together with his trusted collaborators, and so avoided the risk of error involved by the employment of less skilled workers. In these years the interest of physicists and chemists was focussed on the determination of specific heats, P. Debye, A. Einstein and W. Nernst having contrived by means of the quantum theory to interpret the course of specific heats as a function of temperature, which course was completely unintelligible to classical statistical mechanics. Brønsted was hampered in his investigations because he had no liquid hydrogen at his disposal, but by means of liquid air he succeeded in continuing his measurements down to temperatures where it was just possible for him to apply the expression deduced by Debye for very low temperatures.

However, his attention was turning towards other problems. As pointed out by Dr. E. Güntelberg, his collaborator since 1913, Brønsted's production holds indications at an early stage that he has noticed the increase of the solubility of certain electrolytes which occurs on addition of salts without a common ion.

During these years it was being realized, especially through Niels Bjerrum's works from 1909 and 1916, that many salts and some other electrolytes must be practically completely dissociated in ions. But it was well known that thermodynamically such solutions deviated considerably from solutions of uncharged molecules. According to proposals from various quarters, and particularly under the influence of previous works by G. N. Lewis, so-called activity coefficients were now introduced. They are concentration functions which multiplied by the known concentrations give the activities defined by Lewis so as to follow the simple laws which hold for uncharged particles in dilute solution. These activity coefficients may be determined empirically, but it was also an attractive task for the theorists to derive their values theoretically for certain simple systems, particularly very dilute electrolytic solutions. Such attempts at theoretical derivations were made by S. R. Milner, N. Bjerrum and O. Klein. Brønsted, ever first and foremost attaching weight to experiments, concentrated his work on the possibilities of the experimental determination of activity coefficients on the very systems which also theoretically were easiest to handle, viz. dilute solutions of electrolytes. His introductory works perhaps did not attract the attention they deserved, in spite of the clarity with which they deal with the problems and discuss the views of previous authors. But the coping-stone on this work was his purely empirical determination together with V. K. La Mer of activity coefficients as functions of charge and ionic strength. Brønsted and La Mer hereby found the very law which shortly before the publication of their work had been found theoretically by P. Debye and E. Hückel. Brønsted was unusually well equipped through his previous work for this achievement, which raised much well deserved admiration in all those interested. In his preliminary works he had shown that the determination of the solubilities of slightly soluble substances in dilute salt solutions was the most suitable way to determine activity coefficients. Further the slightly soluble substances had to be of such a nature that their concentrations could be determined easily and exactly. For this purpose he could draw on his knowledge, inherited from S. M. Jørgensen, of inorganic complex compounds of cobalt, as many of them are only slightly soluble and contain ammonia, which makes the quantitative determination easy. And finally, to establish equilibrium between solution and crystals he could use the same simple method: percolation of the solvent through a

suitable high layer of crystals, as he had used in the work quoted by Nernst, where he determined the solubility of rhombic and monoclinic sulphur.

While those works were in hand Brønsted together with G. Hevesy took up a work from quite a different field, *viz.* an attempt at separating the isotopes of mercury. By means of the molecular distillation, now used so frequently in other fields of chemistry, it was contrived for the first time to attain a separation which could be proved by analysis, when they succeeded in producing mercury the specific gravity of which deviated perceptibly from normal. The results were mentioned in public for the first time by E. Rutherford in his lecture given in the Commemoration Hall of the University of Copenhagen in 1920. By distillation of concentrated hydrochloric acid according to the same principle, a partial separation was also obtained for chlorine.

Probably the task was set by G. Hevesy, but in the report it is easy to discern Brønsted's knack of accomplishing by very simple methods the precision necessary to prove with certainty the extremely small differences in specific gravity and atomic weight to be determined here.

Also the theory of the separation was dealt with in detail on the lines which have nowadays become so important in the production of pure isotopes by distillation and related methods.

In spite of the sensation created by these works they were not to play such a great role to Chemistry in a more restricted sense, as the works on problems from the sphere of reaction-kinetics which Brønsted published in the same fertile years. After the importance of activity coefficients to the phenomena of equilibrium in reactions, particularly between ions, had been established, the question of their influence on the velocity of chemical reactions came to the fore. The problem was solved by Brønsted in 1922 when he proposed the idea, which afterwards seemed so obvious, that regard should be had not only to the activity coefficients of the reacting ions, but also to the activity coefficient of the so-called critical complex formed by the latter. The concept of critical complexes had already been introduced by Arrhenius in 1889, and had been used again in an inspiring paper by R. Marcelin in 1915. Brønsted's assumption was verified through a great number of examples taken from literature. Its appearance released from many quarters a deluge of works, experimental as well as theoretical. In this connection Brønsted himself together with K. J. Pedersen took up a work on the catalytic decomposition of nitramide which led to quite unexpected results. For it appeared that the reaction was catalysed not only by hydroxyl ions, but also by certain anions and other kinds of molecules which it was not customary at the time to term bases. These findings indicated certain regularities: a most interesting relation between the strength of the bases and their catalytic properties, the

complete explanation of which has hardly yet been given. But what was even more important: the work led Brønsted on to the thought that the definitions of acids and bases used up to then were not adequate. In 1923 before the publication of the nitramide work he, therefore, proposed new definitions of these concepts, which are so very important to chemistry, by defining acids as charged or uncharged molecules which may split off protons, and bases as molecules which may take up protons. Quite a similar definition was at the same time proposed by T. M. Lowry, and on account of the many advantages of the new view it was soon accepted. Although the setting up of a new definition was a purely formal matter, it proved, however, to place many problems in a new and much clearer light. Indeed, rarely has a new definition in chemistry entailed such great scientific advances. Also to elementary teaching the new and very simple definitions became of great value.

The fields of work which were opened up by these pioneer works from the beginning of the Twenties gave Brønsted and his numerous Danish and foreign collaborators enough to do for some ten years, but towards the end of this period, in the Thirties, Brønsted began to devote his attention to problems in connection with the newly roused interest of chemists in high molecular substances. Most characteristically one of these works was published in the volume issued in celebration of S. P. L. Sørensen's septuagenarian birthday in 1938, Sørensen's main subject having for a long time been the study of the physicochemical behaviour of the high molecular albumins.

From this group of works it is possible to get a certain although very incomplete insight into Brønsted's method of working. He begins to form more or less intuitively what I would call a semi-quantitative theory for the phenomenon, after which he works out the details by means of experiments planned under guidance of the provisional theory. On the other hand, to my knowledge, he never felt any inclination to dive deeply into the statistical theories which on many points play such a great part in the treatment of the properties of high molecular substances. Perhaps for this reason, but perhaps also because his intuition took him far in advance of his contemporaries, this group of works has not called the same attention as his works from the Twenties in spite of many interesting observations and important general views. Among other things he points out the very remarkable fact that the solubilities, particularly of high molecular substances, show discontinuity when plotted as functions of the composition of the solvent, i. e. that a substance is markedly soluble, *e. g.* in spirits slightly exceeding a certain alcohol content, but insoluble when the alcohol content falls below the same value.

An offspring of these works was a purely thermodynamic study on mixtures of low and high links in the paraffin series, carried out together with J. Koefoed

and published in the communications of the Royal Danish Society of Sciences and Letters (1946). The work, which was to be the last experimental work Brønsted published, is equally distinguished by the purity of the investigated substances, which was attained by means of the apparatus designed by Dr. A. Klit, the elegant methodical way in which the experiments were made, and the simple form in which it was contrived to render the results. As an example of his simple and efficient technique it may be mentioned that he solved the problem of weighing volatile substances only in contact with glass and mercury simply by suspending under the scale of a balance a glass bulb which was connected through a thin and very flexible capillary with the other part of the apparatus so that the presence of the capillary only impaired the exactness of the weighings immaterially.

From the middle of the Thirties till his death Brønsted moreover worked almost passionately on the problem of the best possible formulation of thermodynamics. In 1912 he had written a little text-book on elementary physical chemistry *Outlines of Physical Chemistry*. As an emergency measure it had been mimeographed in several impressions, but in the middle Thirties a new edition had become urgently needed, and this gave rise to Brønsted's work on the formulation of thermodynamics. With its 175 pages the old text-book is very compendious, but accompanied by the lectures it was satisfactory, and for subsequent use its lapidary style was an advantage. In his lectures Brønsted rendered his subject so elegantly that his students very often did not realize how hard it might be until they grappled with the problems afterwards at home. In the main outlines of his presentation of the fundamental principles he mainly followed the usual methods, but working on the new edition of his book, he was increasingly dissatisfied with them, the more so as on studying the classical presentations he found several examples of untenable reasoning. His ideal was to represent thermodynamics on the sole basis of axioms confirmed by experiences concerning macroscopic systems, the very ideal which must necessarily have been that of the founders of classical thermodynamics. In working out his ideas, however, he was gradually differing much from the classics and took a road which recalls Ostwald's approach to the problem, in order to get to the classical expressions in the form given by J. W. Gibbs in 1878. The latter procedure decidedly meant a modernization. For in the decades around the beginning of the new century so-called reversible cyclic processes were used, also in Brønsted's 'Outlines' to deduce thermodynamic relations. To carry out these reversible cyclic processes rather complicated idealised machines very often had to be designed, *i. e.* it was tried to replace Gibbs' mathematical operations by tangible physical ones. The method is intelligible and comparatively easy, particularly for beginners.

As soon as other systems than the very simplest ones are dealt with, it becomes, however, too cumbersome, and text-books were increasingly reverting to Gibbs' form, which Brønsted knew from Gibbs' 'Works'. This book, in Ostwald's translation, had been in his possession from his youth.

It is told about Gibbs that he left a pile of unpublished manuscripts which on examination proved to be manuscripts of lectures on the basic assumptions of thermodynamics worked out anew each year, and actually in his famous work of 1878 these assumptions: the energy principle and the entropy principle are taken as granted, and he does not attempt a presentation of them. In view of this we understand better that, feeling bound to render a logically unassailable representation of the fundamental principles of thermodynamics, Brønsted had to devote so much work, as in actual fact he did, to a task which seemed to his contemporaries thankless.

In his representation he uses certain words, especially the words work and heat in meanings sometimes deviating from the meanings given to them by physicists. This necessarily involved difficulties, and gave rise to very heated controversies, in this country notably in the physics periodical *Fysisk Tidsskrift*. Brønsted's arguments were characterized by the personal responsibility he felt towards the views he considered right or most expedient. If his opponents suggested, as suggest they would, in the argumentation that they acted as representatives of a collective, for example the physicists, he opposed it in caustic terms. The proposition which perhaps most staggered Brønsted's colleagues was that heat cannot be converted into work. The basis of this statement which so sharply shows the break with the classical formulation, is to my impression the following train of thought: It is well known that no work can be derived from one calorimeter, but from a system consisting of two calorimeters of different temperatures a certain amount of work can be gained, a certain quantity of entropy being transported by means of a reversible process from the calorimeter of the higher temperature to that of the lower temperature. As the amount of work gained only depends on the difference in temperature and the transported amount of entropy, but is independent of whether at the same time small positive or negative amounts of heat are supplied to the two calorimeters, it is natural to ascribe a certain potential thermal energy to the system, and it is this potential energy which is decreased through the process by exactly the amount constituted by the work gained. My own impression is that the language into which Brønsted thus tries to translate the more accustomed representations has considerable pedagogical advantages on account of its simplicity, but the text-book, particularly the last edition (1943), was very difficult in approach, notably as regards the introductory chapters. In the commemoration publication of the

University of Copenhagen of November 1946 he presented his views for the last time in a clarified form. Moreover this paper gives a very interesting contribution to the thermodynamic treatment of systems which are not in equilibrium. This field of problems, the theory of which is still rather undeveloped, is of great importance *e. g.* to reaction-kinetics, and so to biology.

It is still left for me to mention that Brønsted's love of living nature has also found expression in his scientific work, characteristically enough in one of his first and in the very last of his works. The former was a comprehensive work with C. Wesenberg-Lund on the hydrography of the lake 'Furesøen', and the latter a short note equally fascinating in subject and form in *Naturens Verden* (*The World of Nature*) which relates of an ingenious 'Regimentation in the Insect World', an observation from his last holidays.

Finally only this: In spite of help from those nearest to him, his family and his collaborators, I feel that I have only most imperfectly been able to give a picture of J. N. Brønsted, his straight, clean-cut and charming personality. His death, which came unexpected on the 17th December, 1947, after a short illness, was a hard blow to his friends and colleagues abroad and in this country, and we shall remember him as one of our great models.

J. A. Christiansen

ACTA CHEMICA SCANDINAVICA 3 (1949) 1201–1204

J. N. BRØNSTED MEMORIAL ISSUE

Johannes Nicolaus Brønsted — An English View-point

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The general aspects of Brønsted's life and scientific work will be dealt with by those who have known him longer, and who are better qualified to judge his position in Danish academic life. I shall therefore confine myself to some of the particular points which have impressed an Englishman who had the good fortune to work with him for several years and to know him intimately: such an account is bound to embody a personal view-point, but I know that many of my impressions are shared by those of my countrymen who also enjoyed the hospitality of his laboratory.

I first met Brønsted when he attended the Faraday Society Conference on 'Strong Electrolytes' held in Oxford in 1927. This was an important occasion to me for other reasons (since my first published scientific work was presented at this meeting), but I still remember the clarity of Brønsted's paper on the activity of electrolytes, his keenness in debate, and his charm on informal occasions. It was partly this impression which led me to take the opportunity of visiting his laboratory in the following year, and the four years which I spent there were of the very greatest pleasure and profit. During the period 1922—37 a large proportion of Brønsted's collaborators were foreigners, largely from England and the United States. His fame outside Denmark rested chiefly at that time on his work on reaction velocities in solution, in particular in the fields of acid-base catalysis and kinetic salt effects. His definition of acids and bases also became well-known, but there was some tendency at first to dismiss it as 'just another definition' without appreciating its great stimulating and co-ordinating effect in the physical chemistry of solutions. The solubility measurements of Brønsted and LaMer rapidly became famous in relation to the Debye-Hückel theory, but it was not generally realised that these measurements represented the continuation of a long series of valuable experimental studies on solubilities. Similarly, few people knew the importance of Brønsted's early work on affinity in laying the foundations of chemical thermo-

dynamics, where his contributions rank with those of Nernst and G. N. Lewis. His series of papers on chemical affinity (now mostly 30—40 years old) constitute a veritable text-book of thermodynamics, and contain a wealth of fertile suggestion. It is interesting to record that a suggestion which he made in 1911 has just been realised by the help of modern experimental methods. He pointed out then that the affinity of formation of naphthalene picrate from its solid components could be obtained from the E. M. F. of a cell of the type

Picrate electrode / solution I / H_2 Pt. Pt H_2 / solution II / picrate electrode

where solution I is saturated with picric acid and solution II with naphthalene + naphthalene picrate. Brønsted chose mercury-mercurous picrate as the picrate electrode, but was unable to make the necessary measurements because the picrate solutions were reduced by hydrogen. We have recently studied cells of the above type with the hydrogen electrodes replaced by glass ones, and have found exactly the same value for the affinity of formation of naphthalene picrate as Brønsted obtained by an indirect method.

Brønsted's absorption in thermodynamics was one of the aspects of that general integrity and intensity of scientific endeavour which impressed itself so forcibly on those who worked under him. One guest worker in his laboratory described it as a place of 'high chemical potential', and the phrase does give something of the tense and personal feeling which Brønsted inspired. His skill as an experimentalist was not immediately apparent because of the simple nature of many of his methods, and it took some time to realise how much skill and discrimination lay behind an apparently simple piece of experimental work. His great strength lay in the choice of the most suitable substance or reaction, and in the planning of a series of experiments to attain the desired end with the greatest economy of measurement. For this reason his researches always had a much wider application than the immediate purpose in hand, and one of the greatest benefits of working with him was the opportunity of sharing his insight into general methods and planning of research. Many of his pupils have modelled their later work on Brønsted's prototypes: for example, a large proportion of subsequent work on activity coefficients from solubility measurements and general acid-base catalysis follows closely on some lead originally given by Brønsted.

It is more difficult to place Brønsted as a theoretical physical chemist in relation to the main developments of his time. His approach was essentially an experimental one, and his treatment of experimental results always had a strongly thermodynamic flavour, in that he sought to derive empirical relations which should be as simple and as general as possible, without reference to

any particular features of the molecules concerned. In fact, Brønsted showed a great reluctance to speculate in terms of molecular models, and a striking indifference to many of the advances made during his lifetime towards a theoretical and experimental knowledge of molecular structure. There are many examples of this attitude in his work: for example, one felt sometimes that he almost regretted the mechanistic explanation provided by Debye and Hückel for the regularities which he had previously observed in the thermodynamic properties of electrolytes, while he would listen politely, but without marked interest, to my own attempts to provide a molecular basis for the 'Brønsted relation' between catalytic power and dissociation constant. Similarly, his famous expression for the primary kinetic salt effect was arrived at by a curious mixture of intuition and experiment, and the theoretical treatment which he gives in his 1922 and 1925 papers has almost a mystical flavour. Once again he was not much interested in later statistical derivations of the expression.

This attitude was naturally at its strongest when dealing with purely thermodynamic matters, and I remember well his indignation when I suggested (only a few months before his death) that a statistical approach was helpful in teaching students about the second law of thermodynamics. Such a suggestion obviously ranked as heresy, and although his recent work on the fundamentals of thermodynamics strongly criticises many traditional view-points, it never departs from a strictly phenomenological treatment. So far his new ideas on the bases of thermodynamics have aroused little interest in England, and those who have studied them regard them as sound but without much general scientific importance. It would indeed be interesting if these highly formal considerations proved ultimately to be fruitful in the same way as the acid-base definition. This might possibly be the case in the treatment of processes in which a steady state is accompanied by an irreversible transfer of matter or energy, and it is interesting to note that Brønsted had started experimental work on this kind of process shortly before he died.

A large proportion of Brønsted's collaborators were from overseas, and his relations with all these visitors were of the best. They were treated with unfailing kindness and consideration, and the subsequent work done by most of them shows the lasting effect of his influence. On first acquaintance he seemed rather reserved, but one soon realised that this represented only an unwillingness to talk carelessly or lightly about scientific matters, arising from his deep personal feelings about scientific truth. Certainly there was no suggestion of any exclusiveness or selfishness about his own ideas, as may be seen from the large number of major themes which were originated by him and later elaborated by his pupils. When he had once embarked on a dis-

cussion his acuteness and remorseless logic were remarkable, and there can be very few occasions on which he was worsted in a scientific argument.

Brønsted was not altogether easy to know as a person, but when once made the acquaintance was a highly rewarding one. In spite of his strong Danish patriotism he had a great deal of sympathy and understanding for other countries, and especially for England. For the people, the literature and the countryside of England he had a keen interest based on a considerable knowledge, and he could speak and write the English language not only with accuracy but with elegance. The war intensified his English affinities, and the passing of those five years served only to strengthen the ties which bound him to English things and English people. He had read even more about English affairs during the period of separation, and had acquired a remarkable knowledge of Anglo-European politics of the 19th and 20th centuries. Fortunately he was able to visit England again just before his death, and I believe that he enjoyed every minute of his stay: not only the centenary celebrations and the International Chemical Congress, but also the quieter times which he and Mrs. Brønsted spent in the homes of their English friends, and by themselves in a remote corner of the English countryside. It was a great shock to hear of his sudden death so soon afterwards, and many people in this country will mourn him, not only because of his contributions to science or for his inspiring personal genius, but also as a fine and lovable person.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1205–1207

J. N. BRØNSTED MEMORIAL ISSUE

A Great Physical Chemist

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What makes a man a great scientist? Deep and searching interest in his problems. Faith in the importance of scientific research. Keen critical sense which enables him to distinguish the relevant from the irrelevant, avoiding at the same time prostration which searching criticism easily produces. To these indispensable qualities many more can be added as that of hard and systematic work. The late Johannes Nicolaus Brønsted had about all these qualities. His life was mainly spent in pursuing problems of physical chemistry, a science which he never ceased to consider just as important as beautiful. He mastered both lines of attack of physico-chemical research, the thermodynamic and the kinetic one.

Some of his most important work deals with the kinetics of reactions in electrolyte solutions on which our present understanding of kinetic salt effects largely rests. It was this work which led his attention to acids and bases and to the idea of general acid-base catalysis. But deep in his heart he was a thermodynamician. He used to say that kinetics is a difficult science which should preferably be left to the physicist, the chemist better restricting himself to the thermodynamical approach of his problems.

He started his career in the first years of this century by contributing to the theory and measurement of affinity changes, and it was the third of this series, written with remarkable lucidity and dealing with binary mixtures, which was presented for his doctor's degree in 1908. Much of his work on affinity had dealt with electrolyte solutions, and in the period 1918—24 these became his chief interest. His work led him to the establishment of several equally simple and important laws. In the thirtieth he was simultaneously engaged in studies on reaction kinetics, especially in non-aqueous solvents, and in the investigation of the effect of molecular size on the thermodynamic properties of hydrocarbons, polymers, and colloids.

In the last phase of his life his interest was focussed more and more on the fundamental basis and formulation of the laws of thermodynamics. He pursued his task, to which he attached the greatest importance, with great zeal and enthusiasm.

Scientific investigation can be pursued on more deductive lines, on the Newtonian pattern, following up a line of thought; it may be followed on more inductive lines as well, starting from a fortuitous observation as that of Becquerel on the photographic action of uranium rays, or that of Hess, observing an increased ionization in the upper layers of the atmosphere. Brønsted's sympathies and admiration were decidedly with the first mentioned type of scientific work. This is demonstrated among others by the following episode.

The writer of these lines had the privilege of associating with Brønsted in investigations on the separation of the isotopes of chlorine. Large volumes of highly concentrated hydrochloric acid were distilled at low temperature and very low pressure. Density measurements revealed that an appreciable separation of the chlorine isotope was obtained. At that time we discussed how far it is worth while to compare the density of the water samples obtained in the separation process as well. As, shortly before, two eminent German scientists, Otto Stern and Vollmer, had carried out a very detailed investigation of the diffusion of water vapour through porous membranes in search for then not yet discovered isotopes of hydrogen and oxygen, and this investigation led to an entirely negative result, we thought it was hopeless to look for such isotopes in our water samples which, as we know now, must have had densities deviating very appreciably from that of normal water. When, many years later, this episode was recalled, Brønsted remarked that a discovery like that of deuterium has to be made by following up a certain line of reasoning, as was Urey's procedure, and not accidentally.

In the course of four decades during which Brønsted directed the laboratory of physical chemistry at the Technical University of Copenhagen, a great number of research students clustered around him. As one of his most eminent pupils, R. P. Bell, mentioned very appropriately in his obituary notice, Brønsted did not suffer fools gladly among his research students, but, we may add, he offered unlimited inspiration to able and striving men, he offered them friendship as well which lasted for life-time.

A group of chemists coming from U. S. A. and working in the very modest premises then at the disposal of Brønsted in the building of the old Technical University have drawn the attention of Dr. Price, minister of the United States in Copenhagen, to the unsatisfactory housing of the physical chemistry institute. Dr. Price, a former University teacher, reported to New York and, as a result of his report, the Rockefeller Foundation decided to erect and

equip a modern institute for Brønsted. He proved to be an excellent architect, building and equipping a laboratory on as practical as esthetic lines. He was, besides being a chemist, an artist and he understood to combine practical requirements with those of esthetics when constructing a hughe number of mostly novel apparatus which could not help impressing visitors of the physical chemistry institute.

Besides being a painter he took a keen interest in other branches of art and in literature as well. He was a great admirer of nature, and especially of birds, visiting in the company of his beloved wife each year another district of his native country, discovering new beautiful spots and new specimens of birds.

Death came to him with merciful suddenness, removing from our midst a great personality and a great chemist who contributed to a remarkable extent to the development of the chemical sciences.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1208 – 1214

J. N. BRØNSTED MEMORIAL ISSUE

Equilibrium and Thermodynamic Functions in a Gravitational Field *

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The simplest systems dealt with in energetics and in the theory of physico-chemical equilibrium are composed of phases which are characterized by homogeneity in the energetic potentials of all pertinent quantities. If for instance the system is a liquid mixture of various chemical components, it will be a (necessary) condition of equilibrium that temperature, pressure, and the chemical potentials of the components have the same values throughout the mixture.

Furthermore, states of heterogeneous equilibrium are dealt with, in which each separate phase complies with the same conditions. These equilibria can be of two kinds: They may be either complete equilibria in which the homogeneity in potentials extends throughout the composed system, or they may be incomplete or partial equilibria, in which differences in one or more of the potentials are present between sharply defined phases. It is a condition for the establishment of the latter kind of equilibria that the system has been constructed in such a way that the free exchange of some of the energetic quantities is forbidden.

There are, however, other cases in which a certain kind of partial equilibrium is present, in spite of the fact that potential differences exist between parts of the system which are not separated from each other by any definite boundaries. Such conditions may prevail in systems of the most varied character. It is possible to divide them into two main groups: Systems in which the state is maintained through the presence of an outer field of force without requiring any further action from the surroundings, and: Systems in

* Translated from the Danish manuscript, left by Prof. Brønsted at his death, by J. Koefoed. For comments and references see the article by Th. Rosenberg, this issue p. 1215.

which the state is of a stationary character, and is existing, therefore, only by virtue of a flow which is maintained at the expense of constant changes in the surroundings.

Both kinds of systems are of great fundamental significance, and they have certain features in common in spite of their typical dissimilarities. In the present paper the first kind only will be dealt with. In another paper [1] the author has already contributed to the study of systems in stationary states, and it is intended to make them the object of more detailed investigation in a later publication.

1. THE THERMODYNAMIC FUNCTIONS IN A GRAVITATIONAL FIELD

The most important example of the energetic consequences of an outer field of force is the conditions in a system which is under the influence of gravitation. The process of transporting mass in a gravitational field has played an essential part in the establishment of the concept of 'work'. If a body of mass m is moved from one gravitational potential Φ_1 to another Φ_2, a loss of work is said to have occurred in a system comprising only the transported body. This loss of work may be characterized as gravitational, and it can be expressed as:

$$A_{\text{grav}} = (\Phi_1 - \Phi_2)\, m \tag{2.1}$$

According to Gibbs the increase in energy of a system consisting of only one chemical component K, entropy S, and volume v, if gravitational forces can be neglected, is:

$$dE = TdS - pdv + \mu dn \tag{2.2}$$

n being the amount of K, and T, p, μ denoting temperature, pressure, and chemical potential, respectively. In the energetic theory this expression is derived from the definition of the energy of a system as the amount of work lost in an outer work reservoir when the system is built up reversibly from the component quantities, supplied from fixed standard reservoirs. We shall stick to this definition of energy, also where the definition of the system is influenced by a gravitational field.

In setting up (2.2) we have assumed energy to be a function of S, v, and n. The gravitational field taken into consideration, the supply of an amount of matter dn to the system will mean an increase in energy dependent on the gravitational potential as well as on the chemical potential, n and m being connected through the equation:

$$m = nM \tag{2.3}$$

where M is a constant characteristic of K, *viz.* the mass of unity of matter. Equation (2.2) is thus seen to need the addition of a term originating from the supply of mass and of the magnitude Φdm. It then becomes:

$$dE = TdS—pdv + \mu dn + \Phi dm \tag{2.4}$$

or:

$$dE = TdS—pdv + (\mu + \Phi M)\ dn \tag{2.5}$$

But the presence of the gravitational field will necessitate a further modification of (2.2): The term Φdm appearing in (2.4) takes into account the influence of the supply of matter to the system, but according to (2.1) it is necessary to add one more term connected to the possible movement of the system in the gravitational field. This term becomes $md\Phi$. Hence the complete expression for the energy will be:

$$dE = TdS—pdv + \mu dn + \Phi dm + md\Phi \tag{2.6}$$

or:

$$dE = TdS—pdv + (\mu + \Phi M)\ dn + nMd\Phi \tag{2.7}$$

or:

$$dE = TdS—pdv + \mu dn + d(\Phi m) \tag{2.8}$$

Integration of this equation gives for the energy:

$$E = TS—pv + \mu n + \Phi m \tag{2.9}$$

or:

$$E = TS—pv + (\mu + M\Phi)\ n \tag{2.10}$$

These equations differ from the usual energy expression in the appearance of the term Φm, or in the exchange of μ with $(\mu + M\Phi)$.

In (2.9) all quantities and potentials are entering in a completely symmetrical manner. In (2.6), however, m and Φ enter in a special way.

The lack of symmetry is due to the fact that T, p, and μ are 'internal potentials' and dependent on the relative amount of quantities in the system, while Φ is an external potential and as such does not depend on the composition of the system.

It is easily seen that the thermodynamic functions F, G, and H in a gravitational field are derived from the usual expressions in the same way as for E, by addition of the term Φm or by exchange of μ with $\mu + \Phi M$. Hence we obtain:

$$F = E—TS = -pv + (\mu + M\Phi)n \tag{2.11}$$

$$G = E—TS + pv = (\mu + M\Phi)n \tag{2.12}$$

$$H = E + pv = TS + (\mu + M\Phi)n \tag{2.13}$$

as well as the corresponding differential equations:

$$dF = -SdT—pdv + (\mu + M\Phi)dn + nMd\Phi \tag{2.14}$$

$$dH = TdS + vdp + (\mu + M\Phi)dn + nMd\Phi \tag{2.15}$$

$$dG = -SdT + vdp + (\mu + M\Phi)dn + nMd\Phi \tag{2.16}$$

and the expression for the chemical potential:

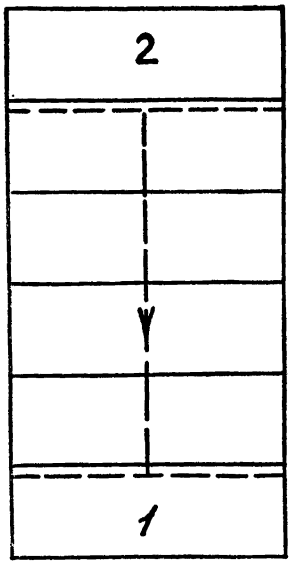
$$\mu + M\Phi = \left(\frac{\partial E}{\partial n}\right)_{S,v,\Phi} = \left(\frac{\partial F}{\partial n}\right)_{T,v,\Phi} = \left(\frac{\partial H}{\partial n}\right)_{S,p,\Phi} = \left(\frac{\partial G}{\partial n}\right)_{T,p,\Phi} \tag{2.17}$$

2. EQUILIBRIUM IN A GRAVITATIONAL FIELD

We shall consider the equilibrium condition in a column of an isotropic fluid of homogeneous temperature enclosed in a cylinder of fixed walls, and with its axis parallel to the direction of gravitation.

This system may be in equilibrium in spite of the presence of potential gradients in the axial direction for the relevant potentials: Chemical potential, pressure and gravitational potential. The reason why these gradients do not give rise to any transport of the corresponding quantities is that the practical definition of the system imposes a coupling on the possible basic processes, preventing them from taking place separately. The conditions are analogous to those in a system consisting of two equally heavy weights suspended from a cord running over a pulley. The lowering of one weight is inevitably connected with the raising of the other.

The active basic processes schematically possible in the fluid system considered, are the transports of matter, volume, and mass. It is not compatible with the existing state of equilibrium that any such process takes place isolatedly. The simplest total process possible is one in which two of these quantities are simultaneously transported. To define such a process completely we shall mark out two partial systems in the column between which the transports are taking place. Let these partial systems be the layers (1)



and (2) of the figure. If only one chemical component is present, their states will be defined by the potentials:

$$\mu_1, \quad p_1, \quad \Phi_1,$$

and:

$$\mu_2, \quad p_2, \quad \Phi_2,$$

respectively, the layers being assumed to be so thin that the potentials inside each of them can be taken as constant. It is a presupposition that in the processes we shall consider, the transported quantities are sufficiently small for the potentials not to change materially on account of the transport.

We shall first assume that matter and mass are transported, while volume is not, and that the transport is taking place from (1) to (2). This process is easily conceiveable. At first sight one might even be inclined to believe that the transport of the chemical component from (1) to (2) would invariably involve a simultaneous transport of its mass. The loss of work in the chemical basic process is:

$$A_{\text{chem}} = (\mu_1 - \mu_2)\delta n \tag{3.1}$$

and that in the gravitational basic process:

$$A_{\text{grav}} = (\Phi_1 - \Phi_2)\delta m \tag{3.2}$$

Applying the work principle to the total process, we hence obtain as the condition of equilibrium in the system:

$$(\mu_1 - \mu_2)\ \delta n + (\Phi_1 - \Phi_2)\delta m = 0 \tag{3.3}$$

Next we shall assume that a volume transport is taking place in the way that the boundary of (1) is moved from b to b'. (1) then loses the volume δv. At the same time the boundary of (2) is shifted from a to a', (2) thereby gaining the same volume δv. Volume (3) of the intermediate part of the column has

remained unchanged, and the spatial process has only consisted in the transport of δv from (1) to (2). Hence the spatial loss of work will be:

$$A_{sp} = -(p_1 - p_2)\delta v \tag{3.4}$$

No transport of matter is connected with this spatial process, but a gravitational process is seen to have taken place, as the mass in (3) has been lowered by a distance $aa' = bb'$.

If, however, the compressibility of the fluid is provisionally assumed to be infinitesimal, this movement in the gravitational field will be identical with the movement from (2) to (1) of the mass δm, contained in the volume δv. Consequently the gravitational loss of work is:

$$A_{grav} = -(\Phi_1 - \Phi_2)\delta m \tag{3.5}$$

From the work principle and on the basis of (3.4) and (3.5) we then obtain:

$$(p_1 - p_2)\delta v + (\Phi_1 - \Phi_2)\delta m = 0 \tag{3.6}$$

Finally, by a procedure equivalent to the successive effectuation of the above described chemical-gravitational and spatial-gravitational processes, it is possible to accomplish a spatial-chemical process, in which volume and matter are moved from (1) to (2) without involving any net transport of mass. For this process the work principle obviously furnishes the expression:

$$(\mu_1 - \mu_2)\delta n - (p_1 - p_2)\delta v = 0 \tag{3.7}$$

If layers (1) and (2) are infinitely close to each other in the column, expressions (3.3), (3.6), and (3.7) are reduced to:

$$d\mu + M d\Phi = 0 \tag{3.8}$$

$$V dp + M d\Phi = 0 \tag{3.9}$$

$$d\mu - V dp = 0 \tag{3.10}$$

when it has been introduced that mass and volume of unity of matter are respectively:

$$M = \frac{\delta m}{\delta n}, \qquad V = \frac{\delta v}{\delta n} \tag{3.11}$$

These equations will, of course, hold regardless of any compressibility of the fluid. For the transport between layers of finite distance the corresponding expressions are obtained for compressible fluids by integrating (3.9) and (3.10), for which procedure it is necessary to know the relation between v and p.

All terms in equations (3.3), (3.6), (3.7), (3.8), (3.9), and (3.10) represent works of different kinds, and in each of these equations they are connected to two coupled basic processes, the equations expressing, in accordance with the work principle, that a sum of such works is zero.

But it is, of course, possible for all three basic processes to take place simultaneously, the chemical loss of work being recovered by equivalent spatial and gravitational gains of work, or in other ways.

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ACTA CHEMICA SCANDINAVICA 3 (1949) 1215–1219

J. N. BRØNSTED MEMORIAL ISSUE

Some Aspects of Brønsted's Energetic Theory[1–10]

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J. N. Brønsted considered his last work on the fundamentals of thermodynamics not only as a logical and consistent presentation of the traditional principles, but undoubtedly also, and primarily, as a possible way to cover new fields. His first papers seemed merely to be polemics on formulation, but to him the terminology of a science was the tools the quality of which will determine whether this science is facing stagnation or further progress. Already in the formulation of the basic concepts of a scientific discipline the solution of a certain part of the pertinent problems is inherent, and the desire to attack a wider field of problems may turn an otherwise satisfactory formulation into a hindrance both to the setting and to the solution of the new problems. And Brønsted did have new questions to ask, the solution of which called for a rearrangement of the bricks, so he tackled the very hard task of making such a rearrangement.

The completeness of the thermodynamic complex of thought, which is generally considered to be one of the most perfect achievements of science, Brønsted rather considered a restraining limitation. He had to attack established dogmas of classical thermodynamics, and also his own theses of yesterday. His remarkable ability to consider familiar problems with completely fresh eyes was once again of the utmost significance.

His latest writings and discussions with his collaborators at the Institute convey to us an idea of the goal towards which his theories were pointing. In the 'Festskrift' of the University of Copenhagen from 1946 he is dealing with certain stationary systems; and in the fragment which is published in this issue, and which is his last manuscript, he has taken the first step to provide a description of equilibria in systems which are not 'potential homogeneous' due to the effect of forces from outside, *i. e.* such systems as include gradients in for instance chemical potentials or pressure. Whereas the treatment of equilibria in such systems have for a long time been incorporated

in thermodynamics, considerable uncertainty has persisted about the relation to thermodynamics of the comparatively few theoretical approaches to the treatment of stationary states. In excluding all systems of non-equilibrium from the domain of thermodynamics, a severe restriction is introduced without obvious necessity, and work covering a great and possibly fruitful field is a priori renounced. Brønsted's work must be considered a preparation for the attack on this field.

One of the more important problems is the formulation of the thermodynamic conditions for stability in stationary systems. A system is called stationary if a state, invariable in time, is forced upon the system by a natural process taking place within the system, *e. g.* a current of matter, entropy ('heat') or other energetic quantities from a higher potential to a lower, the current being maintained through action on the system from outside.

As a definite and simple example we shall consider a tube containing a liquid mixture of uniform temperature and pressure, and consisting of two compounds A and B. A stationary state is maintained by continuous supply of A at one end of the tube and withdrawal of it at the same rate at the other end. If such a process has been going on for some time, the system will presumably attain a state of a certain stability compared to other states which comply with the same conditions of mass transport and of temperature and pressure homogeneity. In the stationary state the system will contain gradients in chemical potential for the diffusing as well as for the non-diffusing component, and the latter must have the same tendency to be transported in all directions whether it be along, against or orthogonal to the gradient in its potential. If the supply and withdrawal of A is stopped, all gradients will disappear, the free energy of the system decreasing to a minimum characteristic of the stability of the equilibrium state.

Thermodynamics has not yet produced a function to measure the relative stability of the stationary state in the same way as Gibbs' functions do for equilibria. There has even been a tendency to consider such a problem as being in principle unapproachable to a thermodynamic analysis, although no evidence has been given why it should not be solvable on a general thermodynamic basis. Similar problems appear with respect to the separate components in the stationary system. Obviously, the usual conditions of homogeneity in the potentials of all components do not apply: The distribution of a component in the various parts of the system, consequently, is not determined only by its chemical potential.

The combination of reversible and irreversible elements present in stationary states shifts the centre of concern from the surroundings into the interior of the very system. It is a characteristic of Brønsted's treatment that weight

is attached to thermodynamic causality in contradistinction to traditional thermodynamics, which aims at nothing more than to keep precise accounts of macroscopical changes and their mathematical relations. Consequently the concept of potentials plays a great rôle in his system.

Brønsted's principal instrument in the analysis of stationary systems is the fundamental equation of Gibbs:

$$SdT - vdp + \sum^{k} n_i d\mu_i = 0 \qquad (1)$$

and this equation is considered an expression for the work connected with the transport of quantity between two adjacent cross-sections in the system. Between two such cross-sections certain processes may take place reversibly even in cases of incomplete equilibrium. Practically all previous treatments of stationary states have been based on this assumption in various formulations, but uncertainty about how to select the reversible elements have made former attempts unsatisfactory. Brønsted applies equation (1) to define the reversible elements: A process will be reversible if it consists in a shift from one of the adjacent cross-sections to the other of a *transport-complex* composed of entropy, volume, and matter in proportions corresponding to the quantities appearing in (1). The isolated movement of the individual quantities is restricted by their coupling into the transport-complex, the same coupling being a condition also for the particular, usually unequal, distribution of the component quantities in the stationary systems.

As (1) will hold also for systems in which the stationary state has not yet been attained, in so far as the potentials will then be defined, it is obvious, however, that the equation will not exhaustively describe the stability of the stationary state. The particular application of (1) is dependent only on continuity in the changes through the system. Hence the shift of the transport-complex will be a reversible process also in non-stationary systems.

Another fundamental principle was outlined by Brønsted in a private discussion on these topics. It can be formulated as follows:

The maintenance of a certain stationary state will be accompanied by the same external 'work effect', *i. e.* the same loss of work per unit time in the surroundings, irrespective of the nature of the external phenomena attached to the maintenance of stationarity.

For the sake of illustration we shall apply the principle to the above-mentioned system: s_A is the amount of matter flowing through any cross-section of the system in unit time, and μ_{A1} and μ_{A2} are the chemical potentials of A at the two end points I and II of the tube. The loss of work in unit time, w_A is then:

$$w_A = (\mu_{A1} - \mu_{A2})\ s_A$$

If now the two end walls of the tube are arranged to be permeable to B and not to A, it will be possible to establish the same stationary state in the tube as before by letting B flow in the direction opposite to that of A's flow in the first case. Then, the loss of work in unit time is:

$$w_B = (\mu_{B2} - \mu_{B1})\ s_B$$

which, according to the above principle, will equal w_A. This loss of work is an entity which is measurable by the changes in the surroundings.

Brønsted, rather jestingly, mentioned this principle as the fourth law of energetics to indicate that according to his view it could not be derived from the other laws. Brønsted did not get time to verify this principle and to put it into a final formulation, and equally unfinished other thoughts remain which he occasionally mentioned in spite of his disinclination to speak about problems which he had not made finally clear to himself. His treatment of the stationary states is left, therefore, at a very preliminary stage, but his way of approach seems to us to be very appropriate to this special problem. The first experiments planned for the verification of the theory also dealt with stationary systems.

The field which it was intended to make accessible to an energetic analysis includes among many other phenomena: galvanic cells, thermocouples, the Soret effect, thermoosmosis, rectifying columns and stationary biological systems. Such subjects have, of course, been treated separately by other workers from various theoretical view-points. They seem particularly suited for approach through statistical thermodynamics, a science which never seems to have appealed very much to Brønsted. It was his opinion that none of the two sciences, statistical and classical formal thermodynamics, had any superiority over the other, and neither can make the other superfluous. No doubt he did appreciate the insight into the basic concepts of thermodynamics afforded by statistical analysis, but he maintained that the mixing up of the two view-points in the very establishment of the fundamental theories would not promote the progress of knowledge and understanding. He found the justification of the existence of thermodynamics as a separate science in the fact that it was able to rest entirely on the fundament developed by itself. The most favourable conditions for a mutually fructifying relation to statistics will be provided by allowing the two sciences to develop separately, either of them along its own lines.

His goal was to describe the separate phenomena under comprehensive view-points, and it is to be hoped that the impulses given by him will promote the solution of problems which are still obscure, and direct attention to phenomena still unknown.

Brønsted had recognized that an adequate treatment of all these problems could only be arrived at on the basis of a general analysis, particularly the simplest potential inhomogeneous systems. He, therefore, intended to treat all important systems of this kind, beginning with equilibrium systems. The above treatise on the state of fluid systems in a gravitational field represents the first part of this programme, and owing to his death it also became the last.

The results he arrives at concerning equilibrium conditions are identical with those of Gibbs's treatment, probably especially well known in Guggenheim's presentation [11]. But as to the terms originating from the presence of a gravitational field, he is led to different thermodynamic expressions. Thus from his derivation it appears, that the fundamental equation (1) is valid in this form also for a system in a gravitational field, *i. e.* this equation does not contain a 'gravitational term'. This may prove significant for further theoretical development.

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ACTA CHEMICA SCANDINAVICA 3 (1949) 1220–1237

J. N. BRØNSTED MEMORIAL ISSUE

L'Energétique de Henry Le Chatelier et celle de J. N. Brønsted

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L'époque troublée durant laquelle le Professeur Brønsted élabora et publia ses travaux sur les fondements de l'énergétique, les empêcha de retenir l'attention autant qu'ils l'auraient mérité. Aujourd'hui, le monde scientifique commence à s'y intéresser vivement. Sa terminologie trés personnelle fait qu'il n'est pas toujours compris. Bien que toutes les critiques qu'on lui adresse ne soient pas également fondées, de sérieuses difficultés demeurent dans son système. C'est une raison de plus pour ses amis et ses admirateurs de regretter sa fin si brusque: nul doute qu'il aurait su défendre sa pensée, la faire comprendre et, au besoin, en retoucher certains aspects.

Le but de cet article n'est pas de développer cette apologie ni surtout cette mise au point, mais il est de faire connaitre un fait qui, tout en ne manquant pas d'importance au point de vue de l'histoire des idées, éclairera quelque peu le débat. Les positions du Professeur Brønsted devant les thèses de la thermodynamique classique et sa façon d'aborder les problèmes paraitront moins insolites quand on saura que l'illustre Le Chatelier professait les mêmes défiances et essayait d'édifier la thermochimie sur les mêmes bases. Que des positions à peu près identiques aient été adoptées indépendamment par des savants éminents qui les utilisèrent comme guides dans l'exécution et l'interprétation de travaux expérimentaux nombreux et de grande valeur, voila un témoignage à ne pas négliger quand on voudra porter un jugement sur leur système. Ajoutons que l'énergétique de Brønsted aura chance d'être mieux comprise quand on aura vu présenter ses principes de base sous une autre forme et dans une terminologie moins déroutante. Enfin, il sera intéressant de noter à côté des similitudes, les divergences et surtout les prolongements que la logique hardie et intransigeante de Brønsted a donnés aux mêmes thèses fondamentales.

Le Chatelier vécut de 1850 à 1936. On consultera utilement l'importante notice que lui consacra P. Pascal dans le Bulletin de la Société Chimique de France, et la bibliographie qui l'accompagne [1]. Les idées générales du savant français doivent se chercher dans les publications suivantes: Deux articles parus en 1894 dans le Journal de Physique théorique et appliquée sous le titre: «Les principes fondamentaux de l'énergétique et leur application aux phénomènes chimiques» [2], puis les quatorzième et quinzième leçons de l'ouvrage: Leçons sur le Carbone professées en 1907 à la Faculté des Sciences de Paris, éditées en 1908 et rééditées en 1926 [3], enfin un article particulièrement clair résumant ceux de 1894, paru dans la Revue des Questions Scientifiques en 1928 sous le titre: «Les Principes fondamentaux de l'Energétique»[4] (cet article a été omis dans la bibliographie dressée par Pascal).

Le Chatelier n'était pas seul à avoir ces tendances, il en appelle volontiers à Mouret. Elles trouvèrent pourtant peu d'écho. Les grands traités français du début du siècle les passent sous silence. C'est le cas notamment de ceux de Duhem et de Poincaré. Et c'est ainsi que cette tentative proche de la sienne ne fut pas connue du Professeur Brønsted, bien qu'il eut dépouillé de nombreux traités de thermodynamique, comme en font foi ses articles. (cf. notamment: On the concept of heat [9].)

Dés le début de ses articles, Le Chatelier indique son intention: «Je ferai complètement abstraction de la notion d'énergie et ferai systématiquement reparaître en son lieu et place la notion d'une réalité bien plus concrète de puissance motrice vers laquelle Sadi Carnot avait d'abord fait converger la nouvelle science de la chaleur. Enfin j'insisterai plus qu'on ne le fait habituellement, sur les analogies de tous les phénomènes naturels: calorifiques, électriques, chimiques, mécaniques» [2, p. 290].

Tous les phénomènes naturels se répartissent entre deux grandes classes qui les distinguent, non d'après leur nature mais d'après leur direction: la classe des phénomènes spontanés et celle des phénomènes provoqués. Ces derniers sont provoqués par le déroulement des premiers. Un système A provoque par son évolution dans un sens, l'évolution du système B dans l'autre sens; par le fait même il perd sa propriété d'évoluer spontanément en agissant sur les autres systèmes; par contre, B acquiert cette propriété. Cette propriété d'un système est sa puissance motrice. Elle décroît dans un système tandis qu'elle croît dans un autre qui lui est couplé. «Sa notion comprend à la fois une idée de causalité et de réciprocité» [2, p. 291]. Il y aurait . . . «identité absolue de signification entre les termes suivants: Puissance motrice (Sadi Carnot); Motivity (Thomson); Available energy (Maxwell); Kraft (Mayer); Freie energy (Helmholtz). Les formules algébriques suivantes: Fonctions caractéristiques (Massieur); Potentiel thermodynamique (Gibbs, Duhem, Natanson etc.) en

expriment la mesure. Enfin l'expression d'énergie et parfois aussi celle de travail sont employées à tort dans le même sens».[2, p. 201, note]

Le Chatelier appelle système simple celui dans lequel se produit une variation de puissance motrice dans un seul sens, et système composé celui qui est constitué par deux systèmes simples couplés entre eux par une machine. L'expérience montre que dans un système simple, une unique variation de puissance motrice comporte deux variations de même type mais de sens opposés, en deux points du système. Une grandeur extensive: masse, volume, charge électrique, entropie, nombre de moles, décroît en un point et croît de la même quantité en un autre. C'est ce qu'exprime une première loi de l'Energétique de Le Chatelier: la Loi de la conservation des capacités de puissance motrice.

Les points où ont lieu ces variations inverses de capacités se distinguent par des valeurs différentes de propriétés de caractère intensif: potentiel gravifique électrique ou chimique, pression, température. On peut dire par analogie avec ce qui se passe au cours des déplacements de masses dans le champ de la pesanteur, que les phénomènes accompagnés de variation de puissance motrice consistent dans le transport d'une grandeur extensive qui se conserve, d'un point à un autre du système, caractérisés par des valeurs différentes de la propriété intensive correspondante.

Pour établir une relation quantitative entre la puissance perdue par un système et celle qui est gagnée par un autre dans une transformation réversible, Le Chatelier fait appel au principe de l'impossibilité du mouvement perpétuel sous la forme que lui avait donnée Carnot: «Il est impossible de créer de rien de la puissance motrice.» La considération de cycles bien choisis permet d'en déduire une série de conclusions: La variation de puissance motrice d'un système ne dépend que des états initial et final de ce système. Si l'on utilise cette puissance motrice à produire du travail mécanique par voie réversible, la perte d'une même quantité de puissance motrice correspondra à la production de la même quantité de travail; ce fait permettra de mesurer et de comparer entre elles les diverses espèces de puissance motrice.

La mesure d'une variation de puissance motrice doit être une fonction déterminée des grandeurs extensives et intensives caractérisant le transport. L'étude détaillée des divers types de puissance motrice révèle que le travail qu'elles peuvent fournir au cours de leurs variations s'exprime, moyennant choix convenable des unités par $\triangle K(P_1—P_2)$ où $\triangle K$ mesure la quantité transportée, et P_1 et P_2 la propriété intensive, le potentiel dans l'état initial 1 et l'état final 2. Les notations que nous utilisons sont déja empruntées à Brønsted.

Un type de puissance motrice spécialement intéressant est celui que possède un systême du fait que deux parties sont à des températures différentes. Ce qui se conserve dans l'évolution réversible d'un tel systême c'est le quotient de la chaleur disparaissant à la température supérieure T_1, par cette température. Le Chatelier démontre cette conservation qui fonde la notion d'entropie, en considérant un cycle de Carnot fonctionnant entre deux températures infiniment voisines T et $T + \mathrm{d}T$. S'appuyant sur des expériences qui ont prouvé que la chaleur absorbée ou dégagée dans le passage isothermique et réversible d'une mole de gaz voisin de l'état parfait, d'une pression p_1 à une pression p_2 était égale à $RT \ln p_1/p_2$, il montre que $\frac{Q_1}{T_1} = -\frac{Q_2}{T_1 + \mathrm{d}T}$ et que le travail mécanique qui peut être obtenu par cette évolution est bien égal à $\frac{Q_1 \mathrm{d}T}{T}$, soit donc $\triangle K(P_1 — P_2)$.

Voici maintenant l'énoncé de la seconde loi de l'énergétique de Le Chatelier: la Loi de la Conservation de la Puissance Motrice:

«La variation de puissance motrice disponible est nulle dans toute transformation réversible

1. d'un systême complexe totalement isolé,

2. d'un systême simple partiellement isolé qui revient à un état final identique à l'état initial». [4, p 390]

Les transformations irréversibles sont gouvernées par un troisième principe, celui de la conservation de l'énergie. Au cours de telles transformations, de la puissance motrice est détruite: un transport spontané de quantités a bien lieu d'un potentiel à un potentiel inférieur, mais un défaut de couplage a comme conséquence qu'aucune autre quantité n'est ailleurs transportée en sens inverse. Dans le cas où c'est de la puissance mécanique ou électrique qui se perd, l'expérience révèle aisément qu'une quantité de chaleur égale à la puissance perdue apparaît dans le systême. Il semble légitime d'admettre qu'il en est ainsi dans tous les cas. Toutefois si l'évolution considérée donne déja lieu à des manifestations thermiques lorsqu'elle est réversible (dilatation d'un gaz, chute d'entropie etc.) il est difficile de constater la production de chaleur due à l'irréversibilité. Mais même alors, on se rend compte que pour ramener un systême simple ayant évolué irréversiblement, au même état qu'après une évolution réversible, il est nécessaire de lui enlever une quantité de chaleur précisément égale à la perte de puissance motrice et évidemment égale aussi au travail qu'aurait fourni l'évolution réversible.

Le Chatelier formule son principe en un long énoncé: «Lorsqu'un systême thermodynamique simple ou complexe subit une transformation réversible ou irréversible, la somme de puissance motrice qu'il communique à l'extérieur

au moyen de machines appropriées et de la chaleur qu'il cède à un calorimètre à travers une paroi conductrice ne dépend que de l'état initial et de l'état final du système, c'est à dire qu'elle est indépendante des états intermédiaires par lesquels le système est passé, des machines mises en oeuvre et du moment où la chaleur a été enlevée au système». [4, p. 403]

Il s'arrête un peu au transport irréversible de chaleur par conduction. La conservation des quantités énergétiques s'observe au cours des transports irréversibles aussi bien que des transports réversibles sauf, en apparence, pour l'énergie thermique. Au cours d'un transport irréversible d'entropie, celle-ci s'accroît, par contre la chaleur se conserve. Voici l'explication: au cours d'un transport réversible d'entropie, celle-ci se conserve et la quantité de chaleur décroît; l'irréversibilité du transport provoque une production d'entropie et donc de chaleur en quantité telle que la chaleur totale enregistrée à la température inférieure égale la chaleur ayant quitté la partie du système à température supérieure.

Le Chatelier indique alors comment la jonction peut se faire entre ses concepts et ceux de la thermodynamique classique. Nous le citons d'après le Journal de Physique. [2, p. 305]

«Puissance motrice des corps isolés .... (On peut) démontrer que l'expression de la variation de la puissance motrice d'un ensemble de corps peut être décomposée en une série de termes se rapportant chacun à l'un des corps et ne dépendant que de l'état initial et de l'état final de chacun d'eux. De telle sorte que, dans le développement de la puissance motrice, chaque corps, pour un changement déterminé qu'il aura éprouvé, interviendra d'une quantité qui sera toujours la même, quels que soient les corps avec lesquels il soit mis en relation et les changements éprouvés par ces corps. On peut donc faire une répartition de la puissance motrice d'un système entre chacun des corps qui le compose, et l'on retombe ainsi sur une expression identique à ce que l'on appelle habituellement l'énergie interne du corps.»

Telles sont les grandes lignes de l'énergétique de Le Chatelier. On aimerait le voir appliquer ses principes à la solution de problèmes, mais il évite l'utilisation exclusive de sa doctrine. C'est dans ses Leçons sur le Carbone que sa méthode est le plus élaborée. [3, p. 345 et sqq] L'objet essentiel de la mécanique chimique est de prévoir le sens des réactions et de déterminer leurs conditions d'équilibre. Une réaction spontanée s'accompagne d'une perte de puissance motrice qui peut du reste être récupérée par un autre système convenablement couplé. Une réaction provoquée s'accompagne au contraire d'accroissement de puissance motrice du système réagissant. En se déroulant dans l'état d'équilibre, elle ne comporte plus pour le système où elle se produit de variation de puissance motrice.

Si une réaction a comme équation: $H_2 + 1/2\ O_2 = H_2O$, la variation de puissance motrice du système pendant qu'elle se produit sera: $\mu dn + 0{,}5\ \mu' dn - \mu'' dn$. Cette expression doit être égale à zéro dans l'état d'équilibre, d'où dans cet état: $\mu + 0{,}5\mu' - \mu'' = 0$. Malheureusement, dit Le Chatelier, nous ne savons rien des potentiels chimiques; il faut par divers artifices, trouver des expressions de la puissance chimique où ne figurent plus les potentiels mais des grandeurs accessibles: la température, les pressions, le volume, les chaleurs de réaction. C'est ce qu'il fait en suivant des voies très classiques. Il aurait été plus satisfaisant de le voir utiliser les potentiels chimiques après les avoir mis en relation de façon générale avec les grandeurs expérimentales. Mais il faut remarquer en outre que sa formule de départ: $\mu dn + 0{,}5\ \mu' dn - \mu'' dn = 0$, ne se raccorde pas directement aux lignes essentielles de son énergétique. Le développement de puissance motrice n'exige-t-il pas des différences de potentiel en deux états du système entre lesquels se fait un transport de quantités? Sa formule devrait comporter pour chaque corps des différences de potentiels au moins infinitésimales, des $d\mu$ au lieu de dn. Si exacte qu'elle soit, elle se rattache aux considérations, accessoires pour lui, relatives à la puissance motrice des corps isolés et à l'énergie interne des systèmes.

Comparons maintenant à l'Energétique de Henry Le Chatelier celle de Jean Nicolas Brønsted. Cette dernière, tout en ayant les mêmes tendances et des principes fondamentaux identiques sous une terminologie différente, est beaucoup plus complète et plus radicale dans ses jugements.

Comme Le Chatelier, Brønsted aime à en appeler à Carnot contre Clausius, [cf. par ex. 7. p. 450] et comme lui, il préfère se passer de l'énergie interne: «The summary and unspecified character of the concept of internal energy makes it useless as a means of interpreting natural phenomena and establishing these natural principles». [9, p. 42]

Les «variations de puissance motrice» qu'éprouve un système deviennent chez lui les «travaux» exécutés dans ce système. La diminution de puissance motrice accompagnant une évolution spontanée devient la mesure du «travail fourni» par le système: $+ \triangle A = (P_1 - P_2) \triangle K$. L'augmentation de puisance motrice accompagnant une évolution provoquée est la mesure du «travail reçu» par le système. Cette mesure a une valeur négative, le potentiel P_1 étant dans ce cas plus petit que P_2.

L'exécution d'un travail signifie une évolution du système et non le résultat extérieur de cette évolution. Un système dans lequel un poids descendrait fournirait du travail, et ce travail serait la chute même du poids; c'est de façon générale le transport même d'une quantité. Le travail est encore fourni

si, par défaut de couplage, le phénomène est irréversible et improductif. Si l'évolution est réversible, un poids différent de celui qui descend pourra être soulevé dans une autre partie du système: ce qui est le travail reçu dans cette partie. La machine à vapeur fournit du travail; ce travail consiste dans la descente d'entropie du générateur au condenseur, les couplages permettront de recevoir à l'extérieur du travail mécanique ou électrique.

Le choix du mot travail est contestable: le mot avait un sens si défini dans une science aussi générale que la mécanique et il était continuellement utilisé dans ce sens par la thermodynamique classique. Que de confusions en perspective! Mais au dire de Brønsted la confusion existait avant lui: Il serait difficile de garder toujours en thermodynamique, son sens mécanique au mot travail. De nombreux auteurs auraient en fait employé ce mot dans des sens variables et impossibles à préciser; certains déconseilleraient même à leurs lecteurs de chercher à en préciser le sens!!. [9 p. 14] Il se permet donc de lui donner un sens précis et adapté aux besoins de l'énergétique, sens auquel il se tiendra. La réalité qu'il désigne est de telle importance qu'elle appelle un nom bref, faisant image, suggérant immédiatement une transformation précise du système. Le mot «travail» a sur l'expression «variation de puissance motrice» l'avantage de ne pas suggérer qu'il s'agit de la variation d'une fonction dont on se désintéresse par ailleurs. Quand des confusions seraient possibles nous dirons «travail brønstédien».

Le Chatelier semble ne pas avoir aperçu les singularités du «travail spatial», il lui accorde peu d'attention. Brønsted fait remarquer que ce travail dont les facteurs conjugués sont le volume et les pressions, ne consiste pas, comme une fausse intuition le ferait croire, dans le passage spontané d'un volume d'une pression plus haute à une pression plus faible, mais au contraire de la plus faible à la plus haute. C'est en effet la partie du systême à basse pression, dont le volume va diminuer de $\triangle V$ tandis que la partie à haute pression accroîtra son volume de ce $\triangle V$. L'expression du travail fourni: $(p_1 — p_2) \triangle V$ serait négative: p_1 pression de départ étant plus petit que p_2; mais précisément les potentiels correspondant au volume ne sont pas p mais $-p$: la pression est un potentiel négatif. [5, p 5] La confusion vient de ce que l'on considère comme potentiel correspondant au volume, la tendance d'un gaz à quitter la partie du système à haute pression, et qu'en effet cette tendance est d'autant plus grande que la pression y est plus grande. Mais la quantité transportée dans cette fuite n'est pas le volume mais la quantité de matière. Le processus considéré est le processus chimique au sens large et non le processus spatial; le potentiel intéressé dans ce processus, le potentiel chimique, est comme on le sait d'autant plus grand que pression et température sont plus grandes.

Un gaz qui se détend réversiblement ou non fournit donc du travail. Le travail élémentaire fourni est $-(p_0 - p)\mathrm{d}V$, où p est la pression du gaz dans le système et p_0 la pression extérieure. Ce n'est que si la détente se fait en repoussant un piston derrière lequel règne une pression gazeuse nulle, que le travail élémentaire prendra la forme pdV. S'il s'agit d'un gaz parfait dont on connait les pressions initiale p_1 et finale p_2, on pourra par intégration calculer le travail fourni au total soit $nRT \ln p_1/p_2$. Ce travail brønstédien est fourni, insistons-y, que la détente soit réversible ou non, à condition qu'elle soit isothermique. On s'étonnera peut-être d'entendre parler d'une détente réversible contre une pression nulle. C'est la presion gazeuse qui doit être nulle; au cours de la détente réversible le piston que le gaz repousse devra évidemment être équilibré par des dispositifs mécaniques qui permettront de recueillir dans une autre partie du système un travail égal à celui que fournit la détente.

La thermodynamique classique suppose généralement que l'enveloppe de ses systèmes est susceptible de se déformer; le système effectue un travail et perd de l'énergie en accroissant son volume aux dépens d'un espace extérieur. Brønsted est obligé de considérer comme partie intégrante de ses systèmes la portion d'espace extérieur dont le volume va s'adjoindre à celui qui était primitivement occupé par le gaz. On rencontrera souvent sous sa plume une curieuse expression telle que «the gaseous system, *i. e.*, the gas + the surrounding vacuum» [Par ex. 8, p. 700]. Si la partie gazeuse du système ne reçoit de ce milieu environnant que du volume, il n'y a pas lieu de se préoccuper de la température et de la composition chimique de ce milieu; seule sa pression a de l'importance. Cela étonne les classiques qui désirent faire l'exact bilan de toutes les formes d'énergie présentes dans leurs systèmes. Mais le travail utile qu'un fluide remplissant une enveloppe peut fournir du fait de sa pression dépend de sa propre pression et de sa température, et pour ce qui concerne l'extérieur exclusivement de la pression.

L'énergétique de Brønsted n'élève pas au rang de principe, l'affirmation de la conservation de la quantité de puissance motrice. Il la considère comme un fait d'expérience. Les considérations de Le Chatelier plus élaborées sur ce point, complètent heureusement son exposé.

Le second principe de Le Chatelier, celui de la conservation de la puissance motrice dans les transformations réversibles, devient le premier principe de Brønsted: son «Principe du Travail»: La somme algébrique des travaux fournis et reçus dans un processus réversible est nulle: $\Sigma \triangle A = 0$.

De même que Le Chatelier voyait des «rapports de causalité et de réciprocité» entre les évolutions couplées, Brønsted voit entre les travaux reliés par son principe, des «relations génétiques». [cf. par ex. 10, p. 16] Les relations établies par la thermodynamique classique sont des relations mathématiques, descripti-

ves plutôt que causales. La chaleur absorbée lors de la dilatation isothermique d'un gaz parfait est égale au travail extérieur fourni. Est-ce l'absorption de chaleur qui fournit le travail? ou la chaleur qui se transforme en travail? On emploie souvent cette dernière expression, mais dans quel sens? Ou encore: La variation d'entropie au cours d'un phénomène irréversible est égale à $\int \mathrm{d}Q/T$, $\mathrm{d}Q$ étant la quantité de chaleur que le système absorberait à chaque température T, s'il passait réversiblement de l'état initial à l'état final caractérisant la transformation irréversible. Il est trop clair que ce n'est pas l'absorption de chaleur dans un processus hypothétique qui est génétiquement responsable de l'accroissement réel d'entropie, ce n'en est que la mesure. Brønsted veut un exposé thermodynamique calqué sur les processus et leurs rapports de causalité. La cause du travail extérieur obtenu dans la dilatation du gaz parfait, c'est cette dilatation même: travail interne au système dont l'élément égale $-(p_1—p_2)\mathrm{d}V$ et qui est responsable du travail extérieur: soulèvement d'un poids ou passage de charges électriques à un potentiel supérieur en quantité telle que le principe du travail soit satisfait.

Signalons encore que l'application de ce principe aux divers types de travaux permettra, par comparaison avec l'un d'entre eux, le processus mécanique, spatial ou électrique par exemple, de déterminer les valeurs de leurs potentiels ou du moins de leurs différences de potentiel. Il sera notamment trés simple de déterminer (ce que ne faisait pas Le Chatelier) la valeur de la différence des potentiels chimiques d'un gaz parfait à deux pressions différentes dont l'une pourra caractériser un état de référence, ou encore la différence des potentiels chimiques entre un constituant gazeux dans un mélange et celui de ce même constituant isolé à la pression du mélange. On détendra le gaz réversiblement et isothermiquement de la pression à laquelle il se trouve jusqu'à la pression standard, on pourra alors le faire pénétrer sans travail dans la partie du système où règne cette pression standard. Le quotient du travail total obtenu, par le nombre de moles soumises à la détente est égal à $(\mu_1—\mu_0)$. On étendrait aisément ces considérations aux gaz non parfaits, aux corps dissous et aux corps quelconques en équilibre avec une phase gazeuse ou liquide.

Une pièce intéressante de l'Energétique de Brønsted et qui manquait à celle de Le Chatelier consiste dans l'étude des «transports neutres». Brønsted entend par là, le transport de quantité entre systêmes ou parties de systêmes se trouvant au même potentiel correspondant à la quantité transportée. Les transports considérés précédemment étaient les «transports actifs». Dans le cas des transports neutres la valeur du travail fourni ou reçu: $(P_1—P_2)\mathrm{d}K$ est nulle, les deux potentiels étant égaux. Ces transports sont réversibles sans

avoir besoin d'être couplés avec des transports en sens inverse, et en tous cas ils ne peuvent être couplés avec des transports actifs ni être déclarés génétiquement responsables de ceux-ci. Ainsi, il ne peut y avoir de lien génétique entre la chaleur absorbée isothermiquement et le travail, pourtant égal, fourni par la dilatation réversible d'un gaz parfait, ou par une pile de concentration dans laquelle des ions sont passés d'une valeur à l'autre de leurs potentiels chimiques. La thermodynamique classique semble admettre la transformation en travail de cette chaleur absorbée. Par contre elle attribue un rôle accessoire et mal déterminé au phénomène proprement causal: rôle de compensation contre lequel s'élève Brønsted et qui déplaisait déja à Le Chatelier [3, p. 363].

Brønsted étonne encore les classiques en affirmant trés fortement: «If a system of pressure p receives a volume $\triangle V$ from an external source, traditional thermodynamics claims an amount of work $p \triangle V$ to be «done» by the system, although this «work» is untransformable . . . As in the case of heat this traditionnal terminology involves the danger of confusing intrinsically incommensurables concepts and is actually to a considerable extent responsible for the inconsistency of classical thermodynamic ideas». [9, p. 76] Le cas typique est celui d'un liquide s'évaporant réversiblement, isothermiquement, à pression constante. Les classiques considèrent que le systême liquide-vapeur fournit un travail pdV; Brønsted le nie. Pour qu'il y ait possibilité de fournir un travail, il faudrait que le systême présentât des différences de potentiel, et pour que ce travail soit pdV, il faudrait d'abord que l'espace extérieur ait été vidé; un dispositif mécanique équilibrant la pression des vapeurs pourrait alors recueillir ce travail.

Les classiques et Brønsted ont raison les uns et les autres, à condition d'entendre chacun le mot travail dans leur propre sens. Le sens du mot travail est beaucoup plus large chez Brønsted que chez les classiques. Voici un cas, le seul sans doute, où il se montre plus étroit, mais il est intéressant de noter que ce travail que Brønsted ne reconnait pas comme tel, est traité par les classiques eux-mêmes avec quelque réserve. Comme l'a fait remarquer Le Chatelier, la diminution de puissance motrice, le travail brønstédien fourni par un systême, mesure la diminution de l'énergie libre du systême, si du moins nous nous limitons aux processus isothermiques. Lorsque les classiques veulent déterminer le travail maximum dA que pourra fournir une évolution isothermique au cours de laquelle la variation d'énergie est $-dU$, ils ont soin de retirer de celle-ci TdS, l'énergie thermique introduite, dirait Brønsted, par transport neutre: $dA = -dF = -(dU - TdS)$. Si en outre le processus s'est déroulé à pression constante en leur fournissant leur travail pdV, ils ne manquent pas de créer une nouvelle fonction G telle que $-dG = dA = -(dU - TdS) - pdV = -(dU - TdS + pdV)$. Le travail ainsi obtenu, dé-

compte fait de celui auquel Brønsted ne veut pas reconnaitre ce titre, est appelé par Lewis et Randall: The net work.[12, p. 157]. Lorsqu'avec l'aide du principe du travail on voudra calculer le travail électrique ou autre couplé avec une transformation à pression constante, on aura soin de négliger dans le travail fourni par celle-ci le $p\mathrm{d}V$. C'est encore à partir de ce «net work» débarassé des travaux parasites que l'on calculera les potentiels chimiques des corps. C'est ainsi par exemple que dans le cas cité de la vaporisation réversible d'un liquide: $(\mu_g - \mu_l)\ \mathrm{d}n = 0$, en même temps que: $\mathrm{d}U + p\mathrm{d}V - T\mathrm{d}S = 0$.

A partir de son principe du travail, Brønsted établit les conditions d'équilibre des transformations physico-chimiques: déduction éludée, somme toute, par Le Chatelier.

Dans l'état d'équilibre interne d'un système, les potentiels entre lesquels une quantité pourrait être transportée sont égaux: le principe du travail est donc inapplicable. Brønsted va s'efforcer de faire apparaître des différences de potentiels, tendant du reste vers zéro, en considérant des états d'équilibre différents mais infiniment voisins. Il met en présence un système homogène I et une portion d'un système homogène II contenant les mêmes quantités que I mais à des potentiels infiniment peu supérieurs: $T + \mathrm{d}T$, $-p - \mathrm{d}p$, $\mu_1 + \mathrm{d}\mu_1$, $\mu_2 + \mathrm{d}\mu_2 \ldots$; la portion de II a été choisie suffisamment petite pour que les potentiels de I ne subissent pas d'altérations. Le système II se dissout spontanément dans I, ses quantités passent des potentiels $T + \mathrm{d}T$ etc. aux potentiels T ... en fournissant un travail brønstédien $\mathrm{d}A = -\Sigma K\mathrm{d}P$. On reprend alors un échantillon de I et on le dissout dans II, on obtient le travail $\mathrm{d}A = +\Sigma K\mathrm{d}P$. «Since these two spontaneous processes are opposite with respect to direction and only infinitesimally different from each other, they deviate only infinitesimally from truly reversible processes». [Réf. 8, p. 703] Appliquant le principe du travail à ce processus réversible, il obtient:

$$-S\mathrm{d}T + V\mathrm{d}p - n_1\mathrm{d}\mu_1 - n_2\mathrm{d}\mu_2 = 0$$

Cette équation est identique à l'une des équations fondamentales de Gibbs, mais elle a ici un tout autre sens. On la retrouvera plus loin avec le sens de Gibbs. C'est à partir de cette équation que Brønsted établit les conditions de divers types d'équilibre. Voici à titre d'exemple la démonstration de la formule de Clapeyron. On trouvera d'autres applications dans l'article du Journal of Physical Chemistry [8].

Soient deux systèmes I et II différant infiniment peu par leurs potentiels, et constitués chacun par un liquide: respectivement L^I et L^{II}, en équilibre avec sa vapeur: G^I et G^{II}. Soient encore S_L, S_G, V_L, V_G l'entropie et le volume d'une mole dans la phase liquide et dans la phase gazeuse. Dans le système

II ces dernières grandeurs devraient être majorées de dS et dV, mais nous négligeons dès à présent ces termes qui figureraient dans les équations suivantes dans des infiniment petits de second ordre: $dSdT$ etc.

On fait passer une mole de la phase L à la phase G du système I mais en passant par le système II. (Ces systèmes sont supposés infiniment grands de sorte que ces transports ne modifient pas les potentiels.) Une mole passant de L^I à L^{II} fournira le travail: $-S_L dT + V_L dp - d\mu$. Aucun travail ne sera fourni lors du passage de L^{II} à G^{II}. Le passage de G^{II} à G^I fournira: $+ S_G dT - V_G dp + d\mu$. Le travail fourni au total dans ce processus dont toutes les étapes sont réversibles sera: $(S_G - S_L)dT - (V_G - V_L)dp = 0$.

d'ou l'on déduit immédiatement: $$T \frac{dp}{dT} = \frac{T(S_G - S_L)}{(V_G - V_L)}$$

Le numérateur du second membre de cette équation représente la chaleur de vaporisation et le dénominateur la différence des volumes moléculaires dans les deux phases.

Notons qu'un bon juge [14, p. 112] a contesté le caractère réversible du transport d'une portion du système I au système II et vice-versa. Il nous semble que son argumentation ne vaudrait que si, comme il parait le supposer, les deux systèmes étaient à des températures T_1 et T_2 différant d'une quantité finie.

Le second principe de Brønsted est celui de la Chaleur. Il correspond exactement au principe de la conservation de l'énergie de Le Chatelier. Au cours des processus irréversibles, des travaux fournis dA ne sont pas couplés avec des travaux reçus dans un autre système ou une autre partie du système: ils sont perdus. Mais une quantité d'entropie dS est produite en quantité telle que $dA = TdS = dQ$. C'est ce qu'il appelle: «la production énergétique de chaleur», phénomène essentiellement irréversible. Cette production énergétique de chaleur ne se manifeste pas nécessairement par des variations de température: ces dernières, phénomènes thermométriques et non énergétiques, ne sont proportionnelles à la quantité de chaleur pénétrant ou produite dans un système, que si la chaleur est la seule quantité qui pènètre dans ce système. La quantité de chaleur produite irréversiblement pourra se mesurer, ainsi que l'avait déja montré Le Chatelier, en ramenant le système du même état initial au même état final, mais réversiblement cette fois, et en observant quelle quantité de chaleur il aura fallu lui donner en plus que dans le cas de l'évolution irréversible. Mais, le principe de la chaleur étant admis, cette chaleur produite se calculera sans imaginer d'évolutions hypothétiques, en faisant la somme algébrique de tous les travaux brønstédiens fournis et reçus

au cours de l'évolution réelle, et en l'égalant à $dQ = TdS$. En faisant cette somme de travaux fournis et perdus, on n'omettra pas les chutes irréversibles d'entropie dans le cas où l'évolution n'est pas isothermique.

Comme déja Le Chatelier, Brønsted interprète le transport irréversible de la chaleur, par un transport d'entropie qui se conserve, à laquelle vient s'ajouter l'entropie produite par l'irréversibilité.

Les transports actifs et la production de chaleur qui sont en relation génétique entre eux, sont dénommés par Brønsted «les phénomènes énergétiques fondamentaux», les transports étant ceux de première espèce et la production de chaleur celui de seconde espèce.

La variation d'entropie d'un système au cours d'un phénomène réel ne se calculera pas en envisageant un processus réversible hypothétique. Elle est égale à $\int dQ/T$ où dQ représente les quantités de chaleur qui ont, soit pénétré dans le système à la température T du système et non à celle des sources extérieures (ces quantités peuvent être + ou —), soit été produites dans le système à température T en quantité égale au travail perdu. (ces quantités sont essentiellement +.) Il est aisé de voir que l'on retrouve ainsi les variations d'entropie considérées par la thermodynamique classique. Cette chaleur produite dans le système avait déja été considérée sous le nom de «chaleur non compensée» par Duhem et elle est largement utilisée par De Donder et ses collaborateurs. [13, p. 38]

Le Chatelier avait appelé son dernier principe celui de la conservation de l'énergie. C'est aussi à son occasion que Brønsted introduit la notion d'énergie. Une même équation réunissant ces réalités si profondément différentes: les variations de puissance motrice et la production de chaleur infertile: $\Sigma\, dA - dQ = 0$, l'invite à introduire un concept s'appliquant à la fois aux deux. Bien qu'il ait fait dériver son «énergétique d'état» de son «énergétique de processus» de plusieurs façons assez différentes [comparez 6, p. 50, et 7, p. 458], nous croyons pouvoir présenter comme suit l'essentiel de sa position, très semblable à celle qu'avait adoptée Le Chatelier.

Pour un système comprenant tous les corps ayant entre eux des interactions, une certaine fonction demeure constante au cours des évolutions irréversibles aussi bien que réversibles. Si l'on introduit dans l'expression $\Sigma\, dA - dQ = 0$, les valeurs des facteurs conjugués, il vient: $\Sigma\ PdK - TdS = 0$, ou puisque TdS est lui même de la forme PdK: $\Sigma PdK = 0 = dU$. La variation totale d'énergie d'un système est donc égale à la somme des divers produits PdK relatifs aux quantités apparaissant dans le système à de certains potentiels moins ceux qui se rapportent aux quantités disparaissant à d'autres potentiels: apparitions et disparitions étant les conséquences soit de création d'entropie, soit de transports actifs, soit de transports neutres. Si l'on découpe dans ce

système une portion quelconque, on considèrera encore la somme des PdK relatifs à cette portion comme mesurant sa variation d'énergie. Il sera particulièrement intéressant d'isoler des régions dans lesquelles les diverses quantités se trouvent chacune à un potentiel uniforme. La frontière pourra passer entre la région où pénètrent des quantités amenées par transports neutres et la région d'où elles viennent. Considérés comme des travaux ces transports ont une valeur nulle: $(P - P)\ \triangle K$, ils n'en enrichissent pas moins la région dans laquelle ils introduisent la quantité $\triangle K$, de l'énergie $P \triangle K$. La variation d'énergie sera donnée par $dU = TdS - pdV + \mu_1 dn_1 + \mu_2 dn_2 + \ldots\ldots$ L'équation étant homogène et du premier degré, on peut poser:

$$U = TS - pV + \mu_1 n_1 + \mu_2 n_2 + \ldots.$$

et $$SdT - Vdp + n_1 d\mu_1 + n_2 d\mu_2 + \ldots = 0$$

Cette dernière équation de forme identique à celle qui a été rencontrée plus haut avec la signification d'un travail, renseigne ici comme chez Gibbs sur les corrélations entre les variations des divers potentiels. Ces variations sont enfermées dans une équation qui précise leurs influences mutuelles, tout comme l'équation $\Sigma PdK - dQ = 0$ enfermait les transports de quantités, la production énergétique de chaleur, avec leurs relations génétiques. Il serait illogique d'établir des relations causales directes entre d'une part les PdK et les KdP d'autre part.

On remarquera que si tous les potentiels ont chacun la même valeur dans toute l'étendue du système considéré, celui-ci est incapable de fournir du travail brønstédien: sa capacité de variation de puissance motrice est nulle. Les exposés classiques admettent qu'il possède encore de l'énergie libre. C'est qu'ils supposent la possibilité de variation de volume, et que cette variation de volume est pour eux un travail pdV qui vient diminuer d'autant l'énergie. Pour Brønsted ce gain de volume pris à l'extérieur à même pression que celle du système, est une introduction dans celui-ci de la quantité dV au potentiel $-p$, ce qui diminue l'énergie du système de pdV sans qu'il y ait eu de travail fourni.

Qu'est-ce au juste que la chaleur chez Brønsted? Il répondra: $dQ = TdS$. De la chaleur apparait dans une partie d'un système dans la mesure où un transport actif ou neutre d'entropie, un processus irréversible quelconque y introduisent ou y créent dS. Les machines thermiques ne consomment pas de la chaleur pour la transformer en travail. Si le travail qu'elles fournissent réversiblement égale $Q_1 - Q_2 = (T_1 - T_2)\ .\ Q_1/T_1$, c'est qu'il y a eu un transport actif de la quantité d'entropie Q/T d'une température à l'autre. Que la chaleur disparaisse en produisant une quantité égale de travail, c'est pour Brønsted

un bilan exact présenté sous une forme causale inexacte. L'exposé classique du fonctionnement de la machine thermique indique cette consommation de chaleur comme responsable du travail; l'abandon de l'entropie à la source froide fait figure de processus compensateur accessoire: pour Brønsted et pour Le Chatelier, il est une pièce essentielle du mécanisme moteur. [7, p. 463; 9, p. 62]

Alors que son système n'avait pas encore sa forme définitive et que l'énergie était encore sa notion de départ, Brønsted présentait d'une façon heureuse sa pensée qui est aussi au fond celle de Le Chatelier [5, p. 1 à 10]: Les divers types d'énergie sont caractérisés par un facteur de capacité et un facteur intensif. un potentiel. Si une forme d'énergie est au même potentiel dans tout le système, elle est dite équipotentielle; si elle s'y trouve à des potentiels différents, on la dit potentielle. La chaleur est l'énergie thermique équipotentielle. Dans les phénomènes réversibles, des énergies potentielles disparaissent moyennant l'apparition de quantités égales d'autres énergies potentielles. L'énergie équipotentielle ne se transforme jamais en potentielle, elle est inutilisable. Mais en sens inverse, un passage est possible: dans les phénomènes irréversibles, de l'énergie potentielle de nature quelconque, thermique y compris, peut dégénérer en énergie équipotentielle et en une seule d'entre celles -ci: la chaleur. La position unique de la chaleur n'est pas, comme on le dit souvent, qu'elle est inutilisable quand on la trouve à température uniforme: il en va de même pour l'énergie électrique, chimique, mécanique ... quand dans le système règne partout le même potentiel électrique ou chimique, la même pression. (A moins qu'on ne veuille utiliser la différence de pression avec l'extérieur, mais c'est sortir de l'hypothèse, on utiliserait alors tout aussi bien la différence de température du système avec l'extérieur.) La position unique de la chaleur est d'être la seule forme d'énergie inutilisable dans laquelle les autres formes d'énergie se dégradent par création d'entropie à la température uniforme du système. En bref, des travaux peuvent dégénérer en chaleur, mais jamais la chaleur ne peut être transformée en travail, les processus compensateurs n'y changeront rien.

C'est précisément sur ce point que portent les principales critiques au système. L'énergétique de Brønsted-Le Chatelier se montre-t-elle ici incompatible avec la thermodynamique classique? Il faudrait alors, si l'on admet la parfaite rigueur, l'entière irréformabilité de celle-ci, montrer très exactement le point où l'énergétique de Brønsted-Le Chatelier quitterait l'objectivité et la logique. Bien que la question doive d'abord être résolue sur le terrain macroscopique où ces auteurs ont voulu rester, on pourra secondairement confronter leurs vues avec les acquisitions considérées comme définitives des théories corpusculaires et de la mécanique statistique. La thermodynamique

classique dira que son énergie interne est indifférentiée et que dans les variations de celle-ci on ne peut distinguer une variation due au changement de volume et une autre due aux échanges de chaleur: lorsqu'un gaz se détend un travail est produit aux dépens de la force vive de ses molécules, tandis que la chaleur amenée isothermiquement la restaure. Les brønstédiens diront que cette énergie cinétique présente divers aspects: un aspect désordonné en relation avec la chaleur absorbée ou produite, et un aspect ordonné en relation avec une répartition des molécules à des températures, des pressions différentes dans différentes parties du systême. La chaleur introduite à température uniforme n'amène aucun accroissement d'ordre. Les travaux, quand ils sont réversibles, transportent l'ordre d'un systême à un autre; quand ils sont irréversibles l'ordre se perd au bénéfice du désordre. Brønsted fait-il autre chose qu'exprimer macroscopiquement ces distinctions et ces relations? Ses découpages sont-ils artificiels? Abuse-t-il de dinstinctions purement verbales à la manière des vieux scholastiques? Son compartimentage est-il légitime? . . . faut-il le proscrire? . . . ou seulement l'assouplir quelque peu? L'équation fondamentale de Gibbs, qui met en relation les variations de potentiel des systêmes n'offre-t-elle pas la possibilité de réaliser cet assouplissement, de diminuer l' étanchéité des compartimentages?

Le Chatelier et Brønsted, ces deux savants éminents, eurent des carrières scientifiques assez semblables. Leur formation intellectuelle fut d'abord à tous deux celle d'ingénieurs. Leur enseignement s'ouvrit, pour Le Chatelier dans la chaire de Chimie de l'Ecole des Mines de Paris, et pour Brønsted dans celle de l'Institut Polytechnique de Copenhague. Tous deux se vouèrent à la thermodynamique et tous deux furent gagnés par Gibbs dont Le Chatelier fit connaitre en France les principales publications. Bien qu'ayant l'un et l'autre attaché leurs noms à des découvertes et des théories de portée générale, ils ne furent pas de purs théoriciens. Cette thermodynamique qu'ils connaissaient si bien, ils l'ont introduite dans leurs laboratoires; durant cinquante ans ils l'ont prise comme guide de leurs travaux journaliers. Tel geste expérimental devait exactement correspondre à la modification de telle variable de leurs formules. Leurs formules devaient représenter aussi clairement que possible les systêmes très concrets qu'ils étudiaient, et les transformations de leurs formules devaient autant que possible être les modèles de l'évolution de leurs systêmes.

Cette tendance bien nette chez Le Chatelier, est plus marquée chez Brønsted. En voici encore deux exemples.

La définition de Gibbs du potentiel chimique est une question bien théorique et qui a fort embarassé les physico-chimistes. Comment peut-il définir le

potentiel chimique d'un constituant par l'augmentation d'énergie du système lorsqu'on vient à y introduire ce constituant sans modifier l'entropie ni le volume du système: $\left(\frac{dU}{dn}\right)_{S,V}$? Le constituant en question n'amène-t-il pas nécessairement avec lui de l'entropie et du volume? Brønsted va répondre en faisant presque une expérience devant nous. Soit e_1, s_1, v_1, p_1, l'énergie, l'entropie, le volume, la pression d'une mole du constituant en question supposé en équilibre avec le système. Il fait pénétrer une mole du constituant dans le système supposé infini: $dU = e_1$, mais de l'autre main (excusez cette accentuation de l'image) il a soin de soutirer s_1 ce qui revient à enlever au système l'énergie Ts_1, ainsi que v_1 ce qui diminue encore son énergie de $-p_1v_1$. L'énergie du système s'est donc accrue au total de: $e_1 - Ts_1 + p_1v_1$; ce qui est une expression intéressante du potentiel cherché[11]. (On constatera que nous avons traité la variation de volume autrement que ne l'a fait Brønsted dans ce travail daté de 1933, époque où il considérait encore pdV comme un travail; mais on se rendra compte que nous n'avons pas faussé la tendance déja marquée alors).

Le second exemple est emprunté à sa dernière publication.[10, p. 90 à 107] Le mécanisme des piles thermo-électriques semble se démonter sous nos yeux. Indépendamment de la chute irréversible de chaleur de la soudure chaude à la soudure froide qui se produit même en circuit ouvert, les effets Thomson et Peltier donnent lieu à des échanges de chaleur tels qu'on peut les considérer comme un transport d'entropie qui se conserve, donc comme un travail thermique fourni. A ce processus spontané va correspondre un travail électrique égal. Comment concrètement vont se conditionner ces deux travaux? Des électrons porteurs de charges électriques, mais aussi d'une certaine quantité d'entropie variable avec le milieu dans lequel ils circulent, vont franchir les soudures, et d'après l'ordre dans lequel ils abordent les deux métaux ils vont absorber de la chaleur ou en céder. Entre les deux soudures ils vont transporter ainsi des quantités différentes d'entropie en descendant d'un côté l'échelle des températures, en la remontant de l'autre. Cela fournira un travail non nul qui devra être équilibré, dans le cas de fonctionnement réversible, par un travail électrique, ce qui suppose l'établissement d'une différence de potentiel. Cet exposé est par trop sommaire, mais il donne une idée de l'étonnante possibilité de faire correspondre les formules de l'énergétique de Brønsted avec les détails d'un mécanisme réel, et même de suggérer ce mécanisme quand, comme ici, il est peu apparent. (Voyez aussi à ce sujet le travail de J. Koefoed, présenté au Colloque de Thermodynamique de Bruxelles[14].)

N'est-ce pas cette facilité d'adaptation de l'énergétique de Le Chatelier et plus encore de celle de Brønsted aux problèmes expérimentaux qui a assuré

la fécondité de leurs laboratoires, d'où les travaux nombreux sortaient encadrés dans des vues théoriques remarquablement au point? Avant que Brønsted n'eut révélé les mécanismes mentaux qui sous-tendaient son travail, les meilleurs thermodynamiciens louaient sa connaissance non-pareille des points les plus délicats de la thermodynamique [12, p. 6]. La valeur de ce travail n'a jamais été mise en question. Si le substrat logique devait s'en révèler inexact, cela poserait un problème des plus curieux d'histoire et de philosophie des sciences. Les thermodynamiciens se doivent d'étudier avec respect et sympathie la façon dont deux grands expérimentateurs ont repensé leurs doctrines en vue de les utiliser, du moins vers quel équilibre ou peut-être vers quelle stylisation de ces doctrines, leur fécond travail durant 50 ans les a conduits.

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Reçu le 4. avril 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1238–1262

J. N. BRØNSTED MEMORIAL ISSUE

Some New Procedures in Thermodynamic Theory Inspired by the Recent Work of J. N. Brønsted *

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During the past decade, Professor J. N. Brønsted has been engaged in developing a new system for presenting the principles and concepts of thermodynamics. Cast into this form he called the subject *Energetics*. Instead of the traditional first and second laws he proposes two new principles; namely, the 'work principle', which is restricted to, and is sufficient for, an exhaustive treatment of all reversible processes, and the 'equivalence principle' which applies similarly to irreversible processes. These principles are expressed in compact analytical form in one equation (4.22) in the following text.

They are introduced as general postulates based upon experience just as the first and second laws of thermodynamics are introduced.

With the aid of the 'work principle' Brønsted achieves in a simple and elegant manner a uniform treatment of all reversible processes on the basis of the concepts of the extensive and the intensive energy factors, and 'work'***. without introducing the concept of heat. More especially all reversible thermal processes may be completely described in terms of temperature and entropy. It is only when proceeding to irreversible processes that phenomena occur which require a concept of 'heat', which embodies some but not all of the characteristics of the heat concept employed in the two classical laws. The

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* Paper read at the Conference on Molecular Interaction sponsored by the New York Academy of Sciences, April 1948. This paper appeared in the Annals of the New York Academy (1949) 605, and it is republished here with the permission of the Academy.

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*** Quotes will be used to designate Brønsted's use of these terms which often differ from their classical meanings.

various forms of the characteristic thermodynamic potentials introduced by Gibbs are defined as functions often more convenient for special applications, but their introduction like that of internal energy is not a necessary part of his system.

The basic ideas of Energetics appeared in two monographs [2,3] in Danish in 1937 and 1939. A brief summary, translated by R. P. Bell, appeared in English in 1940 [4], and also a short paper [5], in which the 'work principle' was used to derive the equilibrium equations for heterogeneous and homogeneous systems. An important monograph [7] clarifying criticisms [6] based upon misunderstandig of this paper [5] was published in 1941 (in English) under the title of 'The Concept of Heat'.

The fundamentals of Energetics were incorporated in the second Danish edition of the textbook of physical chemistry [8]. In his last monograph [9] (in Danish), Brønsted introduces the concept of 'transport complexes' and applies it together with the 'work principle' to the treatment of the reversible aspects (assumed isolable) of steady state processes in thermoelectric, electrochemical and thermal transpiration cells.

BRØNSTED'S ENERGETICS

The following is a brief perspective view of some of the ideas formulated by Brønsted.

Energetics is motivated by the symmetry existing between all of the various extensive energy factors (the quantities) and, likewise, between their conjugate intensive factors (the potentials). For example, in equations like

$$SdT - Vdp + \Sigma\, n_i d\mu_i + \varepsilon d\psi + \ldots = 0$$

we find entropy and temperature, volume and pressure, number of moles and chemical potential, electric charge and potential, *etc.*, always in a conjugate relationship. All of the extensive properties are defined such that they are conserved, except for entropy in the special case of irreversible processes.

Next it is asserted that all the extensive factors, such as volume, mass, electrical charge, *etc.*, and also entropy always tend to occupy states of lowest accessible potential. Natural (spontaneous) and unnatural (imaginary) processes consist of the movement of the extensive factors between states of different potentials. Each individual transport is called a *basic process.*

Such processes involve respectively a positive or a negative *loss of ability to perform useful work.* This 'loss of work' in a process where an amount

δK_i of an extensive property of the i^{th} kind undergoes a transport from one potential to another, is defined as:

$$\delta A_i = (P_{i(1)} - P_{i(2)})\delta K_i$$

Here $P_{i(1)}$ and $P_{i(2)}$ are the potentials, conjugate to the quantity K_i, of the initial and final state of transport, respectively *.

When natural and unnatural basic processes are *coupled* and balanced to within an infinitesimal difference in net potential a reversible process results. Thus, Brønsted asserts that reversible processes are made up of coupled basic processes only, and conversely, that processes consisting of coupled basic processes only are reversible.

The 'work principle' states that in reversible processes the sum δA of the individual δA_i terms (*i. e.*, the sum of all the 'losses of work' in all of the coupled basic processes constituting the total reversible process) is zero:

$$\delta A = \Sigma_i \delta A_i = \Sigma_i (P_{i(1)} - P_{i(2)}) \delta K_i = 0 \qquad (2)$$

In irreversible processes the sum δA is not zero but positive; there is a positive loss of potential work.

The 'equivalence principle' states that:

$$\delta A = T\delta S'' \qquad (3)$$

* Brønsted recognized the necessity of broadening the concept and definition of work beyond the narrow limits it enjoys in the classical presentation in order to achieve the uniform and systematic treatment which he desired for all forms of energy.

In the classical presentation, the element of work DW is an inexact differential defined as

$$DW = PdK \qquad (1)$$

Here d and D are symbols for exact and inexact differentials; P and K are the conjugate potential and quantity factors; thus $DW = pdV$ for volume work.

On the other hand Brønsted's 'loss of (potential) work', δA_i, *always involves the difference between two potentials.* In the reversible case, δA_i becomes a function of state and represents the maximum work the i^{th} natural process can perform upon the unnatural process with which it is coupled (see the following text paragraph). In spite of this similarity δA_i should not be confused with the Helmholtz free energy function bearing the same symbol.

For the transport of a finite amount of quantity between states the potentials of which differ only infinitesimally, the 'loss of work' assumes the form $\delta A = KdP$ but is not in general integrable. Also, in the special case where one of the potentials of a component process can be set equal to zero and the amount of quantity transported is infinitesimal the 'loss of work' assumes the same form and numerical value as the classical Eq. (1) but should not be confused with it.

where $\delta S''$ is the amount of entropy produced in the process. $T'\delta S''$ is consequently the non-compensated heat of Clausius.*

We realize the difficulty of giving a satisfying exposition of Brønsted's fundamental ideas in a few introductory pages; we, therefore, refer the reader to the original articles, of which (4), (5) and (7) are in English, for the logical operational definitions of such topics as internal energy, the absolute temperature scale, entropy and heat in the system of Energetics.

Unfortunately, the very use of the terms 'work' and 'heat' in senses which often differ from the time honored and specific meanings of the classical presentation leads to confusion no matter how carefully they are defined. Also it is not easy to look with favor upon a summary replacement of the well established first and second laws by two new postulates. Only when the advantages of the replacement becomes evident can one expect approval.

When one examines the *direct* experimental evidence supporting the postulates of energetics, one will find that it is not abundant, because the attention of investigators has been directed over the past century to the justification of the two laws in their classical form. Although we believe no one, who will follow through the logical reasoning of Energetics, will question the validity of the postulates on these grounds, nevertheless, the critical reader and the student approaching the subject for the first time are justified in expecting to be led to Energetics from an abundance of direct experimental evidence with which he is familiar.

Consequently, many readers and particularly those who have had access only to the abbreviated presentations available in English have been unsympathetic. Some may have ceased reading before they have had an opportunity to assess the functional value of the ideas embodied in the new principles. As a result many real contributions contained within the manifold of Energetics have been ignored.

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* In order to distinguish reversibly transported (conserved) entropy from irreversibly *produced* entropy, as well as to distinguish reversibly absorbed heat from the non-compensated (irreversibly evolved) heat, Brønsted uses, when necessary, single and double primes, respectively. Thus:

$$\text{Heat reversibly communicated} \quad \delta Q' = T\delta S' \tag{4}$$
$$\text{Heat irreversibly evolved} \quad \delta Q'' = T\delta S'' \tag{5}$$

This notation is adopted in the following text.

The significance of including terms for the irreversibly produced entropy or the non-compensated heat of Clausius — which Brønsted does in his 'equivalence principle' — was pointed out in 1936[1]. It has been stressed recently by Tolman and Fine [10]. See also Eckart [11], Bridgman [12], De Donder and Van Rysselberghe [13]; Prigogine [14] and Leaf [15].

One of the objectives of this paper therefore is to show that the new postulates are completely equivalent to the classical laws, but that they have in addition certain valuable simplifying didactic merits. One of these is that the new system focuses attention upon the physical structure of the concepts and operations rather than upon the mathematical transformations.

Also, Energetics goes beyond Thermodynamics in furnishing a generalized model concerning which a universal statement; namely, our *rule of potentials* (see below) can be enunciated. This does not imply that more information is obtained but only that a concise and unequivocal form of statement results. Our original plans were to describe Brønsted's system in the manner in which he arranged it. Discussions with colleagues, as our manuscript took shape, demonstrated that confusion resulted from a new terminology. This and, in addition, the existence of a natural reluctance to base conclusions upon new postulates until their advantages are clearly evident, have led us to reverse the procedure.

Thus, in this paper, we abstract and emphasize only those facts which represent tangible contributions by fitting them into the established framework of classical thermodynamics to be used as additional tools.* When the reader becomes satisfied that the new treatment is fully equivalent to the old, and through use becomes more confident in its power and simplicity we hope he will be less reluctant to follow Brønsted's procedure and base all of the reasoning on the new postulates thus achieving a further gain in didactic simplicity.

To this end in what follows we have attempted to avoid objectionable terminology, but have retained the advantageous features of the 'spirit' of Energetics. They are listed in the chapter on assessment. In these respects, much of what follows cannot be imputed to Brønsted alone.

CONCEPTS AND DEFINITIONS

A thermodynamic system is defined as a geometric region whose boundaries may be fixed or variable, and which may contain matter, or energy, or both. The suitable description of such a system depends, in part, upon the specification of the amounts of certain components known as the extensive energy factors, which we shall call *quantities*, following Brønsted. Thus, it is customary to say that the system possesses certain amounts of volume, surface, matter, electric charge, entropy, moles of chemical components, *etc.*

* For discussions of the relationship between Brønsted's Energetics and traditional Thermodynamics see Rosenberg [16] and Holtan [17].

Consider an isolated system, *i. e.*, one which cannot receive quantity from, or lose it to, the regions beyond its geometric boundaries. In addition, we shall at first be concerned only with a system in which no chemical reaction is occurring. Any infinitesimal variation which takes place within this system is limited, either to the *redistribution* of quantity among its physically distinct parts or to the *production* * of quantity *within* the system, or both. Call those variations, associated with redistribution, *transport* processes.

The comprehensive description of a thermodynamic system requires the numerical specification of another set of entities, known as *intensities* or *potentials*. In this set we include the familiar parameters, pressure, surface tension, gravitational potential, electrical potential, temperature, and chemical potential, *etc.*, which the reader will recognize are each conjugate respectively to the quantities above. During any infinitesimal change, involving the production and redistribution of quantity the potentials remain, sensibly, constant.

The transport of matter, charge, entropy, and moles of chemical constituent, *etc.*, between parts of the system requires no comment. The situation, in respect to volume and surface, is much the same. However, it is worth while to indicate clearly how these latter transports occur.

Imagine a box (Fig. 1) equipped with a movable partition (cross-hatched) which separates two gases at the pressures p_2 and p_1, respectively; $p_2 > p_1$.

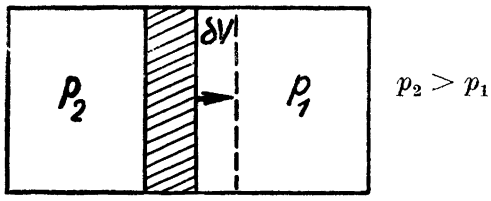

Figure 1.

The partition moves to the right, as indicated by the arrow, and the volume, dV, originally on the right of the partition, appears on the left. In this sense, the volume is transported from the region of lower pressure, p_1, to that of

* The only quantity which can be produced, *i. e.*, which is not conserved, is entropy, in irreversible processes. In the reversible case, entropy and thermal processes can be treated in full conformity with other quantities and processes as emphasized by Brønsted.

higher pressure, p_2; *i. e.*, the potential conjugate to volume is negative pressure. Similarly, we can consider the transport of surface. Consider two films (Fig. 2)

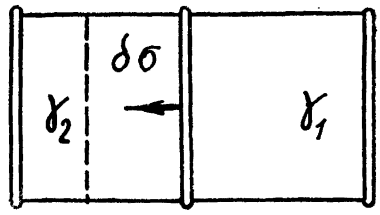

Figure 2.

having the tensions, γ_2 and γ_1; $\gamma_2 > \gamma_1$; which are distended between two fixed wires (extremes) and a wire free to move as the films demand (center). The center wire will move spontaneously to the left, as the arrow indicates, and the surface, $\delta\sigma$, will be transported from left to right, from the region of higher surface tension to that of lower.

THE BRØNSTED PRINCIPLES DERIVED FROM THE FIRST AND SECOND LAWS. THE NON-COMPENSATED HEAT OF CLAUSIUS *

Choose an isolated system which is not the seat of chemical reactions, and divide it into localities, in each of which the *potentials* are *uniform*. By combining the first and second laws of thermodynamics, we follow Gibbs and write for the variation δE_j of the internal energy of the j^{th} locality, during any infinitesimal change:

$$\delta E = T\delta S - p\delta V + \gamma\delta\sigma + \Sigma_i \mu_i \delta n_i + \varphi\delta m + \psi\,\delta\varepsilon \qquad (4.1)$$

In Eq. (4.1) and in all of the following equations to (4.14) we have dropped, for simplicity, the subscript j which should modify every symbol in these equations to specify that it refers to the j^{th} locality.

* Since the non-compensated heat measures the amount of energy that becomes unavailable as useful work in an irreversible process, and is zero for reversible processes, we prefer, for the present, to emphasize this concept also for reversible processes rather than the Brønsted concept 'loss of work' (which is defined differently from the traditional work of thermodynamics). The non-compensated heat as a measure for 'loss of potential work' has been emphasized by Tolman (see Ref. 10 and patents cited there).

Here T = temperature; p = pressure; γ = surface tension
S = entropy; V = volume; n_i = moles of the i^{th} species
m = mass; ε = electric charge; σ = surface

$\mu_i = \dfrac{\partial E}{\partial n_i}$ = chemical potential of a mole of the i^{th} species

$\varphi = \dfrac{\partial E}{\partial m}$ = gravitational potential of a gram of matter

$\psi = \dfrac{\partial E}{\partial \varepsilon}$ = electrical potential of a coulomb of charge.

Now:

$$m_i = n_i M_i \qquad \delta m_i = M_i \delta n_i$$
$$\varepsilon_i = n_i Z_i F \qquad \delta \varepsilon_i = Z_i F \delta n_i$$
$$\Sigma_i \delta m_i = \delta m \qquad \Sigma_i \delta \varepsilon_i = \delta \varepsilon$$

where M_i is the molecular weight, Z_i is the charge per molecule of the i^{th} species, and F is the Faraday constant.

Accordingly (4.1) can be rewritten as

$$\delta E = T\delta S - p\delta V + \gamma\delta\sigma + \Sigma_i (\mu_i + M_i \varphi + Z_i F\psi)\delta n_i \tag{4.2}$$

where the sum $(\mu_i + M_i\varphi + Z_i F\psi)$ can be conveniently replaced by the symbol λ_i, where λ_i is a general component potential for the i^{th} species; *e. g.*, in the electrical case $(\mu_i + Z_i F\psi)$ becomes the now well-known electrochemical potential of Guggenheim [18] which Brønsted adopts and employs effectively in treating galvanic cells [1, 8, 9, 18, 19, 20].

$T\delta S$ represents the heat which could be absorbed by the locality if the variation were conducted reversibly. In order to calculate δE it is therefore demanded that the additional terms in (4.2) which indeed represent work terms, be those which would be obtained if the variation were conducted reversibly. In other words, p, γ, and λ_i must be equilibrium values. If we represent by $\delta Q'$ the heat which would be absorbed if the variation were conducted irreversibly, then we have

$$T\delta S - \delta Q' > 0 \tag{4.3}$$

Accordingly, we write

$$T\delta S = \delta Q' + T\delta S'' \tag{4.4}$$

where, by virtue of (3)

$$T\delta S'' > 0 \tag{4.5}$$

in the irreversible case. In the reversible case equality exists for (4.5) and the double primed quantity vanishes. The term $T\delta S''$ is the so-called non-compensated heat of Clausius represented by $\delta Q''$, while

$$\delta Q' = T\delta S' \tag{4.6}$$

where $\delta S'$ is the entropy which is transported into the locality, through its boundaries. $\delta S''$ represents the entropy *produced within* the j^{th} locality by whatever irreversible phenomena are occurring there.

The variation of entropy, as ordinarily defined (no prime) is a sum given by

$$\delta S = \delta S' + \delta S'' = \frac{\delta Q'}{T} + \frac{\delta Q''}{T} \tag{4.7}$$

For an irreversible variation in the j^{th} locality, the first law gives

$$\delta E = \delta Q' - \delta W' \tag{4.8}$$

where $\delta W'$ is the work performed by the locality upon its surroundings. Substituting for $\delta Q'$ we get

$$\delta E = T\delta S - T\delta S'' - \delta W' \tag{4.9}$$

Eq. (4.9) indicates clearly that the non-compensated heat $T\delta S''$ represents work which is potentially available provided that the variation associated with δE is carried out reversibly. δE and δS have fixed values, being exact differentials, independent of whether or not the change occurs reversibly.

Therefore, the sum of the residual terms

$$-T\delta S'' - \delta W' \tag{4.10}$$

is fixed for the defined variation.

In the limit of reversibility, $\delta S''$ is zero and consequently $\delta W'$ has its maximum value. All of the non-compensated heat can be obtained as useful work in this limit.

Now (4.7) is substituted into (4.2), yielding

$$\delta E = T\delta S' + T\delta S'' - p\delta V + \gamma\delta\sigma + \Sigma_i \lambda_i \delta n_i \tag{4.11}$$

for each j^{th} locality.

To compute the variation of the total internal energy of the isolated system we sum over two types of localities. The first summation is over all of the j localities. Now the system may also contain localities whose quantities are invariant to any general change. Fixed weights or charges are examples. Eq. (4.11) cannot be used for the computation of the variation of the energy connected with the transport of such quantities, since all of its differentials are quantities, and consequently equal to zero. Instead

$$\delta E_k = m_k \delta \varphi_k \tag{4.12}$$

where φ is the gravitational potential and m is the mass, is suitable if we deal with a weight, while

$$\delta E_k = \varepsilon_k \delta \psi_k \tag{4.13}$$

is likewise suitable if we deal with an electric charge. Then the total variation δE in the internal energy of the isolated system is representable as

$$\delta E = \Sigma_j \delta E_j + \Sigma_k m_k \delta \varphi_k + \Sigma_k \varepsilon_k \delta \psi_k \tag{4.14}$$

and can be set equal to zero since the system is isolated. Accordingly from (4.11) we obtain (4.15)

$$\begin{aligned} \delta E = \Sigma_j T_j \delta S_j' + \Sigma_j T_j \delta S_j'' - \Sigma_j p_j \delta V_j + \Sigma_j \gamma_j \delta \sigma_j + \Sigma_j \Sigma_i \lambda_{ij} \delta n_{ij} \\ + \Sigma_k m_k \delta \varphi_k + \Sigma_k \varepsilon_k \delta \psi_k = 0 \end{aligned} \tag{4.15}$$

This equation can be rearranged immediately as follows:

$$\begin{aligned} -\Sigma_j T_j \delta S_j' + \Sigma_j p_j \delta V_j - \Sigma_j \gamma_j \delta \sigma_j - \Sigma_j \Sigma_i \lambda_{ij} \delta n_{ij} - \Sigma_k m_k \delta \varphi_k \\ - \Sigma_k \varepsilon_k \delta \psi_k = \Sigma_j T_j \delta S_j'' \end{aligned} \tag{4.16}$$

Since we have excluded the possibility of chemical reactions, all of the quantities on the left in (4.16) satisfy the condition of conservation for reversible processes in the isolated system. Thus:

$$\Sigma_j \delta S_j' = 0, \quad \Sigma_j \delta V_j = 0, \quad \Sigma_j \delta \sigma_j = 0, \quad \Sigma_j \delta n_{ij} = 0 \tag{4.17}*$$

---

* $\Sigma_j \delta S_j' = 0$ because $\delta S_j'$ is that part of entropy which is transported. $\Sigma_j \delta V_j$ and $\Sigma_j \delta \sigma_j$ can always be set equal to zero by defining, if necessary, transports to and from regions of zero pressure and zero surface tensions. Neither (4.15), (4.16) or (4.17) are affected by these transports because the terms referring to those localities of zero pressure and surface tensions necessarily have zero values. $\Sigma_j \delta n_{ij} = 0$ because we have excluded for the moment the possibility of chemical reactions.

A further rearrangement of the left terms of (4.16) can be effected in the following manner. Consider the sum $\Sigma_j p_j \delta V_j$ and specialize for simplicity to the case where it equals

$$p_1 \delta V_1 + p_2 \delta V_2 + p_3 \delta V_3 \tag{4.18}$$

Then the isolated system is a box, similar to that used in Fig. 1, but having, in this case (Fig. 3), two movable partitions, separating regions having the pressures p_1, p_2, and p_3, respectively.

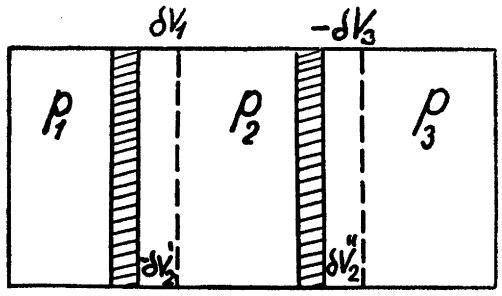

Figure 3.

Now by the conservation of volume

$$\delta V_1 + \delta V_2 + \delta V_3 = 0 \tag{4.19}$$

It is apparent from figure 3, in which $p_1 > p_2 > p_3$, that

$$\begin{aligned} \delta V_2 &= \delta V_2' + \delta V_2'' \\ \delta V_1 &= -\delta V_2' > 0 \\ \delta V_3 &= -\delta V_2'' > 0 \end{aligned} \tag{4.20}$$

so that (4.19) is satisfied. Using the notation of (4.20) we can write for (4.18) the expression

$$p_1 \delta V_1 - p_2 \delta V_1 + p_2 \delta V_2'' - p_3 \delta V_2''$$

or (4.21)

$$(p_1 - p_2)\delta V_1 + (p_2 - p_3)\delta V''_2$$

In other words (4.18) is identical with a sum, each term of which consists of the product of a potential difference, multiplied by the quantity which is transported through the potential difference.

By induction, this result is perfectly general, and can be applied to all of the sums on the left-hand side of (4.16). We thus effect the complete rearrangement, and write finally, the important equation:

$$\delta A = \Sigma_r \delta K_r \, (P_{r(\text{initial})} - P_{r\,(\text{final})}) = \Sigma_j T_j \, \delta S_j'' \geq 0 \qquad (4.22)$$

(4.22) is the analytical form of the *combined* Brønsted principles. The equality applies to reversible processes, *i. e.*, the 'work principle', and the inequality to irreversible processes, *i. e.*, the 'equivalence principle'. δK_r symbolizes a transported quantity, while $P_{r\,(\text{initial})}$ is the conjugate potential in the locality from which and $P_{r\,(\text{final})}$ the conjugate potential in the locality to which the quantity is transported. δK_r is always greater than (or equal to) zero (see Eqs. (4.20)). The potential for volume always appears as negative pressure.

VIRTUAL CHANGES, COUPLING, AND THE RULE OF POTENTIALS

It is to be noted that all of the differentials in (4.22) are specified by the symbol, δ, which designates a virtual variation. The term, virtual, implies that the variation may be of the most general kind, and need not be physically realizeable. Although some of the most important phases of thermodynamic theory deal with the subject of virtual variations, very few authors have succeeded in presenting this subject clearly. The virtual variation is an important adjunct of the Brønsted treatment and hence requires discussion.

Any displacement from equilibrium subject to the same constraints which are imposed upon the system at equilibrium is, strictly speaking, impossible. A real displacement can only occur by the alteration of one or several of the constraints. In this sense, any displacement which a system in equilibrium undergoes is a virtual displacement. Let us confine our attention to displacements which are infinitesimal.

A system in equilibrium can experience two types of infinitesimal virtual displacements. The first type involves a displacement, joining equilibrium states (quasi-static), while the second involves a displacement which originates in equilibrium and terminates in non-equilibrium. The former is the assymptotic limit of some real process, while the latter has no basis in reality whatsoever.

The reader is undoubtedly familiar with many specific examples of quasi-static processes. Examples of this type will be given in the chapter on these processes.

For a concrete example of a *non*-quasi-static displacement, consider a liquid drop, in equilibrium, surrounded by its vapor. It would be impossible,

unless the system were severely altered, to transport isothermally *dn* moles of the drop to the region of its vapor without, at the same time, transporting some of its volume and surface area. On the other hand, there is nothing to prevent us from *imagining* the physically impossible transport, during which the drop simultaneously dilates, so that its volume and surface area remain constant. It is evident that this would represent a displacement passing from an equilibrium to a non-equilibrium state.

In order to obtain a clear understanding of the usefulness of the method of virtual displacements, it is absolutely necessary to have a broader definition of a 'thermodynamic state' than the one ordinarily given. Usually a 'state' means an '*equilibrium* state' whose reproducible properties can be described by a minimum number of macroscopic parameters. Any function of state, *e. g.*, the free energy, has these parameters for arguments.

In a larger sense, a state can be defined as any *reproducible condition* of a system, either in equilibrium or in the process of change. A non-equilibrium state will, in general, require a larger number of parameters for its description than an equilibrium state. In the extreme case the dynamical specification of every microscopic particle in the system may be required. In any event, any function of state, *e. g.*, the free energy, will depend upon a larger number of variables, but will remain a defined function. From the operational point of view, an equilibrium state then becomes a special kind of state defined by the minimum number of parameters. It can be represented by a point in 'state-space', *i. e.*, the space whose coordinates are the parameters defining the state in the most general sense.

An infinitesimal displacement from equilibrium is represented by an infinitesimal path in 'state-space' originating at the point of equilibrium. A number of these paths will satisfy the condition that the temperature and pressure remain constant along them. It is a classical criterion of equilibrium that for an infinitesimal displacement along any one of these isothermal, isobaric paths the Gibbs free energy of the system remains unaltered. This free energy is understood to be defined in the larger sense, so that it remains a defined function of a non-equilibrium state. For the application of this criterion, it is inconsequential whether the displacement is or is not quasi-static. All that is demanded is that it be infinitesimal and that it originate in equilibrium. In particular, it may be of the type illustrated above in connection with the spherical drop.

The point that many fail to grasp is that one does not seek information about the condition of the system along the path of the infinitesimal displacement *but only about the condition at the origin of the path.* Others have difficulty in conceiving the significance of the free energy along a non-quasi-static path

because the description of a 'state' as an equilibrium state has been over-emphasized.

The Gibbs free energy has been chosen as an illustration because of its familiarity. However, all of these implications concerning virtual variations can be transferred in full to Brønsted's 'work principle', (Eq. 4.22), when employed as the criterion of equilibrium. This equation (like other criteria) imposes the demands of thermodynamics upon a system in equilibrium. Very often, however, certain extra-thermodynamic conditions are imposed upon the behavior of the system. When this is true, all of the virtual displacements must be consistent with these conditions.

For example, return to the consideration of the drop. We may impose an extra-thermodynamic condition upon the system represented by the drop and its vapor, namely, the geometric condition which specifies that the transport of volume from the drop to the vapor must occur in such a manner that the spherical shape of the drop is retained. It is then not permissible to carry out a virtual variation during which the volume, δV, is transported without the simultaneous transport of the surface, $\delta\sigma$, because both are connected by the geometrical relation

$$\delta V = \frac{r}{2}\,\delta\sigma \tag{5.1}$$

where r is the radius of the drop.

The equality and inequality (4.22) represents a compact and extremely useful expression of the laws of thermodynamics. In addition, it furnishes a very satisfying model for the internal behavior of a thermodynamic system. These contentions shall be demonstrated in detail.

From the nature of the rearrangement (4.22) it is clear that the potentials conjugate to the different quantities are, in order:

quantity	*potential*
volume	negative pressure
entropy	temperature
surface	surface tension
moles of chemical components	component potential *
mass	gravitational potential
charge	electrical potential

* Note that we are replacing the ordinary Gibbs chemical potential μ by the more general component potential λ defined in the chapter on the derivation of the Brønsted principles.

In mechanics and field theory, potential has the significance of determining the direction of change. That this significance is retained, unaltered, in the above table can easily be shown.

To do this, consider a system undergoing a virtual change which consists of a single transport, such that all of the terms on the left of (4.22) with the exception of one, $\delta K_x(P_{x\,(\text{initial})} - P_{x(\text{final})})$, are zero. Then (4.22) reduces to

$$\delta A = \delta K_x(P_{x(\text{initial})} - P_{x(\text{final})}) = \Sigma_j T_j \delta S_j'' \geq 0 \tag{5.2}$$

In (5.2) as in (4.22) the inequality corresponds to a *natural* irreversible change, *i. e.*, one which does occur spontaneously, and the equality corresponds to a reversible process, *i. e.*, to a displacement of a system in equilibrium. Since δK_x is arbitrary and positive, it follows that the expression in brackets (potential difference) is positively different from zero when a real change takes place. Finally, we observe that the potential difference is zero when no change takes place (when the system is in equilibrium). Therefore, a finite difference of potential bears a one-to-one correspondence to change, while no potential difference corresponds to no change. For this reason, potential difference may be regarded with complete consistency as the motivating factor for change. Taking account of the subscripts (initial) and (final) in (5.2), it is to be observed that all quantities tend to move from a higher to a lower potential. These conclusions which we have derived from the laws of thermodynamics, Brønsted introduces as observations of experience to justify the reasonableness of his principles.

The form (5.2) was achieved by restricting the virtual change to a single transport. But suppose this is not possible, as in the example offered previously, concerning the volume and area of a spherical drop. In that case, the quantities volume and area were *coupled* together so that the movement of one demanded the movement of the other. For such a case, the form (5.2) could not be achieved. Then it could not follow that the potential differences conjugate to the coupled quantities would be required to be zero at equilibrium.

We are thus led (quite rigorously) to a general rule which we shall call the *rule of potentials*, namely, that *all potential differences necessarily vanish at equilibrium except those corresponding to conjugate transported quantities which are coupled to other quantities.* In particular, since chemical components are never coupled so as to defy an *individual virtual transport*, the *component potential* λ_i corresponding to the i^{th} species is *identical in every locality when equilibrium has been attained.*

In the usual presentation of thermodynamics the rule of potentials enunciated above can only be proved by inventing a suitable characteristic function for each case and by setting in motion the machinery of the Lagrange method of undetermined multipliers. In the current presentation it has been obtained *rigorously* and in a *single stroke* by utilizing a satisfactory physical model for the thermodynamic system in which constraints can be described in terms of bonds; '*coupling*'.

This result constitutes part of the evidence for the contention that (4.22) is a compact and useful expression of the laws of thermodynamics and that it furnishes a good model of thermodynamic behavior. We will now proceed to examine the beautiful and consistent description which it provides for the state of internal equilibrium when 'coupling' exists.

A MODEL FOR INTERNAL EQUILIBRIUM

When coupling exists, the potential differences conjugate to the coupled quantities are not necessarily zero. If we write (4.22) for the process involving the reversible transport of these quantities, we retain only the equality, and have:

$$\sum_{\text{coupled}} \delta K_r (P_{r\,(\text{initial})} - P_{r\,(\text{final})}) = 0 \tag{6.1}$$

Physically, the situation in (6.1) can be described as follows. Each of the coupled quantities is invited by its conjugate potential difference to move, but the movement of one quantity, in the direction specified by its conjugate potential difference, compels (because of the bonds between quantities) other quantities to move in directions opposite to those specified by their own potential differences. At equilibrium, all of the opposing tendencies balance and this is signified by the condition (6.1).*

TREATMENT OF QUASI-STATIC PROCESSES

In the first place, it is to be noted that a quasi-static displacement is one along which the system remains in equilibrium. Consequently, all of the potentials in the system are subject to the restrictions of our rule of potentials. In addition, since a quasi-static displacement has a limit-basis in reality, we

* This model of equilibrium (*i. e.*, coupling between basic processes) was applied in Brønsted's last monograph[9] to the treatment of the reversible aspects of steady staet processes; *e. g.*, in the thermoelectric cell he utilizes the coupling between a mole of electrons and the entropy associated with it. Similar procedures were employed for the gas transpiration cell.

shall consider it formally to be a real change and accordingly employ the symbol d rather than δ to symbolize differentials.

The reversible expansion of a gas

Consider a gas (Fig. 4) having the pressure p,

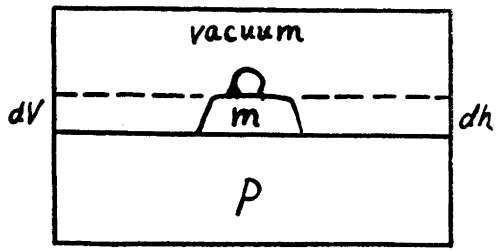

Figure 4.

separated from a vacuum by a partition upon which a weight, m, rests, which is almost but not quite heavy enough to maintain equilibrium. The containing vessel is surrounded by a reservoir of temperature, T. The isolated system which we need to consider consists of the reservoir and the container plus its contents.

Under the prescribed conditions, the partition will move upward the distance dh and the volume dV will be transported from the vacuum to the pressure, p. Corresponding to this transport the loss of spatial potential work is given by

$$dA_{\text{spat}} = -(0 - p)dV = pdV \tag{7.1}$$

There is an accompanying loss of gravitational potential work

$$dA_{\text{grav}} = [gh - g(h + dh)]\ m = -mgdh \tag{7.2}$$

There will also be a flow of entropy between the reservoir and the gas. The variation is quasi-static and the rule of potentials can be applied. Since there is no extra-thermodynamic relation coupling the transport of entropy to other processes, it follows that the potentials conjugate to the entropy, *i. e.*, the temperatures are the same in the gas and in the reservoir. For the thermal loss of potential work we obtain

$$dA_{\text{therm}} = 0 \tag{7.3}$$

However, there is an extra-thermodynamic, geometrical relation between dV and dh, and thus the spatial and gravitational processes *are* coupled. This is consistent with the rule of potentials; the corresponding losses of potential work do not have zero values.

Upon substitution of (7.1), (7.2) and (7.3) in the equality of (4.22), we obtain the result

$$pdV - mgdh = 0 \tag{7.4}$$

Brønsted has commented upon the fact that it is customary to regard the motivation of the quasi-static process just described as originating in the reversible flow of heat from the reservoir to the gas, or what amounts to the same thing, in the transport of entropy. He points out that it is more consistent to regard the transport of volume as the motivating factor since it is here that a finite potential difference exists (see Eq. (7.1)) and because we have considered a potential difference to be the motivating factor for change (see the chapter on virtual changes).

Carnot cycle — Coupling of thermal and mechanical basic processes

As an important example of the direct use of the equality in (4.22) we give Brønsted's treatment of the reversible Carnot engine. Let the heat absorbed by the engine at the upper temperature T_1 be $DQ_1 = T_1\, dS$ and that rejected at the lower temperature T_2 be $DQ_2 = T_2\, dS$:

$$dS = \frac{DQ_1}{T_1} = \frac{DQ_2}{T_2} \tag{7.5}$$

The thermal process in the heat engine thus consists in the reversible transport of an amount of entropy dS between the temperatures T_1 and T_2. The reversible mechanical work dA obtained from the engine may consist in the transport of a weight m from a lower height h to a higher height $h + dh$: $dA_{\text{mechanical}} = -mgdh$. In any case, regardless of the nature of the mechanical work, (4.22) gives:

$$(T_1 - T_2)dS + dA_{\text{mechanical}} = 0 \tag{7.6}$$

or, introducing (7.5):

$$|dA_{\text{mechanical}}| = DQ_1 \frac{T_1 - T_2}{T_1} \tag{7.7}$$

To be consistent, the driving force may be considered to originate in the thermal process, *i. e.*, the tendency of the entropy to go from the higher to the lower temperature. Since this process is coupled and balanced through the engine to a mechanical process, it can only occur reversibly through the simultaneous performance of mechanical work.

The reader is referred to the original articles[4, 7] for Brønsted's objections to the Clausius' interpretation of the Carnot Cycle.*

THE GIBBS-DUHEM EQUATION

In treating problems of equilibrium, it is often necessary to have a differential relation which connects the real variations (symbol d) of the different potentials, rather than the extensive properties in a given phase. The general relation, which we shall call the *generalized* Gibbs-Duhem equation, has the following form:

$$SdT - Vdp + \Sigma_i n_i d\lambda_i = 0 \tag{8.1}$$

Equation (8.1) can be obtained in a simple and straight-forward manner by applying the equality contained in (4.22) to a selected reversible transport.

Consider a phase whose potentials are specified by the pressure, p, the temperature, T, and the *component potential* for the i^{th} species, λ_i. Consider another phase, having the potentials $p + dp$, $T + dT$, and $\lambda_i + d\lambda_i$, which contains the same chemical species as the first phase. Now, combine these two phases in a rigid, adiabatic shell, so that they form an isolated system. Since the potentials in the two phases differ infinitesimally, the transports which now occur do so reversibly. We can thus apply the equality contained in (4.22) to these transports.

$$[(T + dT) - T]\, dS + [p - (p + dp)]\, dV + \Sigma_i\, [(\lambda_i + d\lambda_i) - \lambda_i]\, dn_i = 0 \tag{8.2}$$

or

$$dSdT - dVdp + \Sigma_i\, dn_i d\lambda_i = 0 \tag{8.3}$$

This equation places no restriction upon the amounts of quantity dS, dV and dn_i which are transported, since there is only one dependent variable and we can always choose this to be one of the potential differences, *i. e.*, dT, dp or $d\lambda_i$. By suitably adjusting the amounts of quantity, originally present in the two phases. it is always possible to adjust the transport so that:

* See, also: V. K. La Mer, 'Some current misconceptions of Carnot's Memoir and Cycle.' Paper read before American Physical Society, Jan. 29, 1949; to be published in Am. J. Phys.

$$dS : dV : dn_i = S : V : n_i \tag{8.4}$$

Here S, V, and n_i are the quantities in the first phase. This means that the quantities, transported combine to form a replica of a portion of the first phase. Because of (8.4), (8.2) can be multiplied by a constant to yield (8.1), which is the generalized Gibbs-Duhem equation for the first phase. Since the first phase was arbitrary, (8.1) is applicable to any phase.*

The derivation of (8.1) is again an illustration of the compactness and usefulness of (4.22). In the usual presentation of thermodynamics, it is necessary to invent a function of state and to apply Euler's theorem for homogeneous functions before (8.1) can be derived.

TREATMENT OF EQUILIBRIUM

We are now in position to apply (4.22) to the solution of problems of equilibrium. In a sense, we have already partially solved every conceivable problem of internal equilibrium by the use of (4.22), since we have been able to arrive at the conclusion that the component potentials are uniform throughout the system when equilibrium has been attained. To obtain a more tangible and comprehensive description of the interior of a system at equilibrium, we have only to proceed from this point by the usual methods of thermodynamics taking account of the manner in which the component potentials are related to the other parameters which determine the state of a given locality.

However, we have not exhausted the utility of the equality in (4.22) for in many cases it yields an immediately useful result over and above that pertaining to the equality of the potentials. As an example, compute the difference in pressures inside and outside of a drop having the radius, r. Choose for the isolated system the drop surrounded by its equilibrium vapor contained in a rigid, diathermic shell which is placed in a thermostat. Since the temperature is everywhere uniform, the terms referring to the transport of entropy vanish from (4.22). The same, of course, is true of the transport of material. Writing the equality (4.22) for the transports attending the transport of moles of material from the drop to its vapor, we find that only the terms corresponding to the transport of the 'coupled' quantities volume, δV, and surface, $\delta\sigma$, can have non-zero values. This follows from the rule of potentials. We thus have for (4.22):

$$-(p_1 - p_2)\,\delta V + (\gamma - 0)\,\delta\sigma = 0 \tag{9.1}$$

* Our use of the equality (4.22) for the derivation of the generalized Gibbs-Duhem equation is slightly different from that of Brønsted [5].

Here p_2 is the pressure of the vapor outside the drop, p_1 the pressure inside the drop, θ the surface tension of the hypothetical surface in the vapor, and γ the surface tension of the vapor-drop interface. Substituting (5.1) into (9.1) the familiar formula of Kelvin, specialized to a sphere, follows immediately.

$$p_1 - p_2 = \frac{2\gamma}{r} \tag{9.2}$$

CHEMICAL EQUILIBRIUM

Thus far, systems in which chemical reactions occur have been excluded from consideration. This was done as a matter of convenience only, and does not represent any fundamental insufficiency of the Brønsted treatment. The inclusion of the chemical reaction as a possible source of variation necessitates the introduction of a slight modification in Eq. (4.16).

The rearrangement of (4.16) to yield (4.22) is no longer valid since any particular type of molecular species entering into the reaction is not conserved. It is possible to modify (4.16) so that in place of the mole numbers the numbers of atoms of particular kinds contained in a particular molecular species inhabiting a given phase serve as parameters. It is also possible to use the masses of the various molecular species in this connection. Both atoms and mass are conserved even in the presence of a chemical reaction, and so a rearrangement of the desired type is possible.

However, it is more expedient, for chemical purposes, to define a pseudo quantity $\delta\alpha$, which is also conserved. Let ν_r and ν_p be the stoichiometric coefficients of the r^{th} reactant and p^{th} product in a given chemical reaction of the type:

$$\Sigma \nu_r R_r = \Sigma \nu_p P_p$$

where R_r and P_p are the molecular symbols of the r^{th} reactant and p^{th} product. Let ν_r and ν_p *both be positive*. Then *

$$\delta\alpha_R = \frac{\delta n_r}{\nu_r} \qquad \text{for all } r \tag{10.1}$$

$$\delta\alpha_P = \frac{\delta n_p}{\nu_p} \qquad \text{for all } p \tag{10.2}$$

* It will be notes that α_P, but not α_R is the degree of advancement of reaction employed by De Donder.

From stoichiometric considerations, it is evident that

$$\delta\alpha_P + \delta\alpha_R = 0 \tag{10.3}$$

For simplicity let us restrict our attention to a system in which the mole numbers are varied by a single chemical reaction confined to a single phase. This result can be generalized easily, as the occasion requires.

Then the term, in (4.18), $-\Sigma_j\Sigma_i\lambda_{ij}\delta n_{ij}$, reduces to $-\Sigma_i\lambda_{ij}\delta n_{ij}$ and by virtue of (10.1) and (10.2) this becomes

$$-(\Sigma_r\lambda_r\nu_r\delta\alpha_R + \Sigma_p\lambda_p\nu_p\delta\alpha_p) \tag{10.4}$$

or

$$-(\delta\alpha_R\Sigma_r\lambda_r\nu_r + \delta\alpha_P\Sigma_p\lambda_p\nu_P) \tag{10.5}$$

and by the use of the new conservation condition (10.3), we obtain the form

$$-(\Sigma\lambda_p\nu_p - \Sigma\lambda_r\nu_r)\delta\alpha_P \tag{10.6}$$

If we define

$$\lambda_P = \Sigma\lambda_p\nu_p \tag{10.7}$$

$$\lambda_R = \Sigma\lambda_r\nu_r \tag{10.8}$$

as 'system potentials' for the pseudo-quantity $\delta\alpha_P$, (10.6) indicates that the form (4.22) can be extended to chemical reactions.

Indeed for any reaction proceeding isothermally and isobarically, the work principle now demands that at equilibrium

$$(\lambda_R - \lambda_P)\delta\alpha_P = 0 \tag{10.9}$$

or that the 'system potentials':

$$\lambda_R = \lambda_P \tag{10.10}$$

(10.10) yields the law of mass action when the individual potentials are substituted.

In closing, it is to be noted that the pseudo-quantity can be used as a measure (on stoichiometric grounds) of the rate of transport of the real quantity, mass, from reactants to products.

IRREVERSIBLE PROCESSES

The inequality contained in (4.22) provides a direct means for computing the production of non-compensated heat during an irreversible process, provided that the transports involved are recognizable and that the irreversible process conducts itself in such a way that each stage can be described by what are sensibly equilibrium parameters.

We shall consider one example of this type. A single thermostated phase, the seat of a chemical reaction, but nevertheless in *mechanical* and *thermal* equilibrium, represents a system satisfying the requirements just mentioned. The only transport having a non-zero term will be that corresponding to the transport of the pseudoquantity da_P. The system is out of equilibrium so that entropy is being produced. Eq. (4.22) then reduces to

$$(\lambda_R - \lambda_P)da_P = TdS'' \tag{11.1}$$

If we divide by dt and define the velocity of the reaction, v, as

$$v = \frac{da_P}{dt} \tag{11.2}$$

we obtain

$$(\lambda_R - \lambda_P)v = T\frac{dS''}{dt} \tag{11.3}$$

or

$$\frac{dS''}{dt} = \left(\frac{\lambda_R - \lambda_P}{T}\right)v \tag{11.4}$$

a result given by De Donder.

ASSESSMENT

The favorable points for equation (4.22) follow:

(a) It provides a satisfying model for the internal behavior of an isolated thermodynamic system.

(b) It leads simply and with a minimum of mathematical expenditure to a simple rule of potentials. As a corollary, the general result asserting that the component potentials are uniform at equilibrium is obtained. In the classical discipline the concepts and ideas are not available to make such a concise universal statement.

(c) The generalized Gibbs-Duhem relation is obtained with a minimum of mathematical expenditure.

(d) In cases of coupling (4.22) leads to an immediately useful result, concerning the features of equilibrium in a system where the coupling phenomena exist. By this it is implied that properties, other than the fact that the component potentials are uniform, are described.

(e) In some instances (4.22) affords a direct means of calculating the non-compensated heat evolved in an irreversible change.

Finally, we do not assert that (4.22) is the most convenient form for all thermodynamic purposes. Attention is always focused upon an isolated system, which means any system of physical interest *plus* its environment. In this way some of the detachment which is gained by defining thermodynamic potentials which are functions of the state of some particular non-isolated system is lost. However, by combining both methods of attack, fruitful results are obtained.

SUMMARY

A brief exposition of the salient features of Brønsted's Energetics is given. The complete equivalence of his basic postulates, namely, the 'work' and the 'heat and equivalence principles' in respect to the two laws of classical thermodynamics has been demonstrated by deriving his postulates from these laws. Some of Brønsted's fundamental conceptions, *e.g.*, the existence of a potential difference as the motivating factor for the occurrence of a basic process, balanced coupling of basic processes to produce reversible processes, the localized production of entropy in irreversible processes, *etc.*, emerge as necessary consequences in this derivation.

The compactness and elegance of Brønsted's approach are illustrated by simple examples using his 'work principle' and a new rule of potentials given by us. An assessment of the merits of the system is included.

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J. N. BRØNSTED MEMORIAL ISSUE

(Dedicated to the memory of the great scholar and experimentor, the late Professor J. N. Brønsted)

The Application of Brønsted's Method of Isotope Separation to the Study of the Natural Radioactivity of Potassium

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The late Professor Brønsted has shown a great interest in the application of his method of isotope separation to the determination of the isotope (or isotopes) responsible for the natural radioactivity of potassium. This fact induced the author to present a survey of these investigations.

The first separation of isotopes was carried out by Aston [1]. He started from 100 ml of neon and obtained, by diffusion through ripe clay, after thousands of operations two extreme fractions of 2 to 3 ml, showing the densities 20.15 and 20.28. When continuing the work by means of an automatic apparatus which was expected to permit a much more effective separation, the separation of neon isotopes achieved was only half of that attained previously, although the apparatus performed the mechanical operations of diffusion many thousands of times in a satisfactory manner. This failure was very fortunate since it induced Aston [2] to give up the plan of separating isotopes on a preparative scale and to concentrate on the much more accurate analysis of positive rays in proving the existence of isotopes among the elements in general, which he succeeded in accomplishing to such a remarkable extent. While the proof of the existence of isotopes was established through Aston's work it seemed to be of importance to carry out a separation on a preparative scale and to compare the result with that expected on the basis of theoretic considerations. This work was carried out by Brønsted and assoc.[3]

BRØNSTED'S METHODS OF SEPARATION

Two different methods were introduced. a) Separation by making use of the difference in the evaporation velocities of isotopes (ideal distillation); b) a fraction of the mercury vapour penetrates through narrow openings in the condensation space, where the lighter isotope is found in a relatively larger

amount than in the ordinary mercury (effusion method). Even if we disregard the small difference of the vapour-pressure of two isotopes we can expect evaporation under suitable conditions to lead to a livlier exchange between the lighter than between the heavier atoms in the two phases. The former have, namely, in accordance with their smaller mass (m_1), a velocity (v_1) $\sqrt{\frac{m_2}{m_1}}$ times as great as the latter — the mass and velocity of which we will denote by m_2, and v_2 — respectively, and thus in the time-unit $\sqrt{\frac{m_2}{m_1}}$ times as many molecules of the lighter as of the heavier isotope will be transferred from the liquid into the vaporous stage, and vice versa. As long as evaporation under usual conditions is considered, we are not able to utilize the quicker movements of the lighter molecules to an appreciable extent for the purpose of separating isotopes, because the quicker evaporation in the case of the lighter molecules is just counterbalanced by a quicker re-condensation. We can, however, prevent this effect by suppressing one of the two compensating processes. This is most easily accomplished by allowing the liquid to evaporate in a vacuum and placing a well cooled glass plate over its surface. Now, when the vapour-pressure of the liquid is sufficiently low, each molecule which leaves the liquid will reach the cooled wall before it has had an opportunity of meeting other molecules and being thrown back into the liquid. Having reached the cooled wall, it will be held by it, transferred into the solid state, and hindered in re-evaporation. It follows from the above that in the 'ideal distillate' obtained in this way there will be $\sqrt{\frac{m_2}{m_1}}$ times as much of the lighter isotope as in the initial substance, and through repeated 'ideal distillations' of the fractions thus obtained it is possible to carry the partial separation further. When 2700 ml of mercury were distilled, the residual fractions were shown to have the values to be expected from the considerations outlined above, as seen in Fig. 1.

Furthermore, the results of density measurements were corroborated by measurements of the atomic weight of the light and heavy mercury fractious obtained. Brønsted's work was thus the first in which the separation of isotopes on a preparative scale was unambiguously obtained, the claim of separation being based not only on a deviation of the density of a separated sample from that of a normal one, which might be due to the presence of minute impurities, but on a coincidene between the experimental values and those calculated from the kinetic theory of evaporation.

After the method of ideal distillation was successfully used by Brønsted and assoc.[4] for the separation of the isotopes of chlorine, concentrated solu-

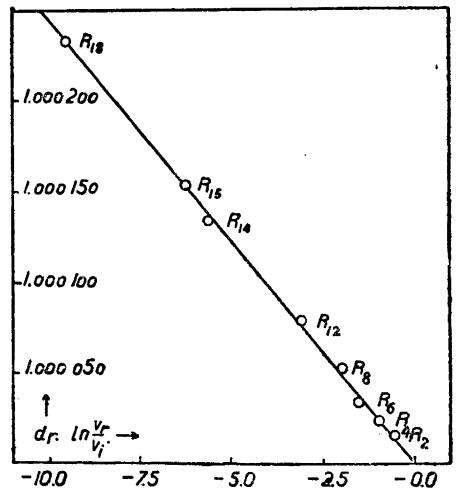

Fig. 1. Separation of mercury isotopes. — Density of mercury plotted as ordinate, the abscissa representing the natural logarithm of the ratio of the remainder and the initial volume.

tions of hydrochloric acid being distilled, attempts were made to carry out a partial separation of the isotopes of potassium. The investigation of the radioactivity of the fractions obtained was expected to elucidate whether the natural radioactivity of potassium is due to the main isotope K 39 or to a minor constituent of the mixed element potassium.

SEPARATION OF THE ISOTOPES OF POTASSIUM

In their work on the separation of the isotopes of potassium the present author and Lögstrup [5] distilled about 1 kg of metallic potassium in a pyrex bulb, the bottom of which was heated, while its top was cooled with solid carbon dioxide. The distillation was carried out as calculated from Knudsen's formula

$$p = \frac{G\sqrt{\frac{T}{M}}}{43.7 \times 10^{-6}}$$

where G is the number of grams distilled per second and M is the molecular weight of the potassium vapour, at a temperature of 160° C. The mean free path of potassium atoms was thus shorter than the distance between the warm and the cold surface ($<$ 1 cm). When the potassium residue was reduced to a few ml, the distillation was stopped and the residue shunted to another bulb inserted in the evacuated system. The distillation of the main potassium

fraction was then repeated. The combined residues consisted of 25 g of 'heavy' potassium. This 'heavy' potassium was converted into chloride, purified, and its radioactivity and atomic weight determined.

Potassium contains 93.4 % of K 39 and 6.6 % of K 40. Let us assume that the atomic weight determination indicates that the 'heavy' potassium has a K 41 content increased by 10 %. If K were responsible for the radioactivity of potassium, the activity of the 'heavy' sample would have increased by 10 % as well. If, however, K 39 is the radioactive isotope, the 'heavy' sample should show an activity decreased by 0.71 %. A third possibility is that the isotope K 40 (not yet discovered at the time when the described investigations were started) is responsible for the radioactivity of potassium. In this case, if the atomic weight determination indicates an increase by 10 % in the K 41 content of the sample, the increase in radioactivity will amount to 5 % only.

The atomic weight determination carried out by Hönigschmid and Goubeau [6] indicated an increase by 4.8 % in the K 41 content of the 'heavy' sample; radioactive determinations carried out first by using the Hoffmann electrometer indicated a difference of 4.2 % in the radioactivity of the samples and, using the Geiger-Müller tube in extended measurements, a value of 4.43 ± 0.5 % was found (Hevesy [7]). From these figures it had to be concluded that the radioactivity of potassium is due to its K 41 content.

In view of the fact that the value obtained by Baxter for the atomic weight of *normal* potassium was appreciably lower (39.096) than the value found by Hönigschmid and Goubeau (39.104), Professor Baxter kindly offered to compare the atomic weight of the 'heavy' sample with that of normal potassium. He (Baxter [8]) found a very much larger increase in the K 41 content of the heavy sample, namely 10.6 %, than did Hönigschmid and Goubeau [6]. In view of the much greater difference between the increase in the concentration of the isotope 41 in the heavy sample than in the increase of its radioactivity, Baxter's figure leads to the conclusion that the radioactivity of potassium is due to a lighter isotope than K 41 and a heavier isotope than K 39, thus that K 40 is responsible for the radioactivity of potassium. In view of the striking difference between the results obtained by the two greatest authorities in the field of atomic weight determination, it was imperative to attempt another approach.

SEARCH FOR CALCIUM 41

From the number of β-particles emitted by 1 g potassium it follows that 1 g potassium produced since the earth-crust solidified about 10^{-4} g calcium. Should the radioactivity of potassium be due to potassium 41, the decay of

this isotope in old minerals would produce detectable amounts of calcium 41. This reasoning induced the separation of calcium of apatite from Bamble, Norway (Hevesy [9]). This apatite of very considerable age has a potassium content of 6.5 % and a calcium content of less than 0.05 %. A decay of minute traces of the potassium present during geological times thus should have produced calcium 41 in sufficient amounts to be discernable in the mass spectrum of calcium isolated from biotite. Aston could not find any indication of the presence of calcium 41 secured in our sample from Bamble apatite. This result suggests that either K 41 is not radioactive or Ca 41 has a short life-time.

ARTIFICIAL PRODUCTION OF K 42

Amaldi and assoc. [14] observed, when bombarding potassium with neutrons, the formation of an artificially radioactive potassium isotope decaying with a period of about 1/2 day. When, later, the same isotope was obtained by bombardment of scandium according to the reaction

$$^{45}_{21}\mathrm{Sc} + ^{1}_{0}\mathrm{n} = ^{42}_{19}\mathrm{K} + ^{4}_{2}\alpha$$

the mass-number of the radioactive potassium isotope could be determined to be 42 (Hevesy [10]).

The fact that the bombardment of potassium 41 with neutrons leads to the formation of potassium 42 made it quite probable that the ex-terrestric bombardment of potassium 39 with neutrons leads to the formation of potassium 40 and that the radioactivity of potassium is due to this isotope (Hevesy [11]).

RESULT OF MASS-SPECTROSCOPIC INVESTIGATIONS

At the time when the above described partial separation of the potassium isotopes was carried out the sensitivity of the mass-spectroscopic method did not suffice to replace the atomic weight measurement of the fractions obtained by mass-spectroscopic determinations. Great progress was, however, achieved in recent years in the field of mass-spectroscopy and during the war Paul and Pahl [12] compared the K 39: K 40 ratio of the 'heavy' fraction obtaiend by Brönsted's method with that of normal potassium and found an increase of 8.7 % in the K 41 content of the heavy sample. From this figure and from that of the change in the K 39 content of the heavy fraction (— 0.62 %) the extent of concentration of the K 40 content of the sample was inter-

polated. The value obtained for the increase in the K 40 content 4.0 ± 0.5 % compares well with that obtained for the increase in the radioactivity of the sample (4.3 %), showing thus K 40, and this isotope alone, to be responsible for the natural radioactivity of potassium.

Brønsted's method thus permitted the determination of the mass-number of the isotope responsible for the natural radioactivity of potassium.

That potassium 40 is radioactive was also shown by Smythe and Hemmendinger [13] who prepared minute amounts of K 40 by means of the mass-spectrograph and showed this, and this isotope only, to be radioactive.

SUMMARY

The results obtained in the application of Brønsted's method to the determination of the isotope responsible for the natural radioactivity of potassium are reviewed.

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J. N. BRØNSTED MEMORIAL ISSUE

The Works of J. N. Brønsted

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J. N. BRØNSTED MEMORIAL ISSUE

J. N. Brønsted

Scientific Distinctions

Member of the Royal Danish Academy of Science, 1914.
Ramsay Silver medal, 1922.
Lectures at University College, London, 1923.
Visiting professor at Yale University, U. S. A., 1926.
Lectures at Columbia University and other universities in U. S. A., 1926/27.
H. C. Ørsted Medal, 1928.
Honorary member of The American Academy of Arts and Sciences, Boston, Mass. U. S. A., 1929.
Member of The Royal Physiographic Society, Lund, Sweden, 1934.
Honorary member of the Chemical Society, London, 1935.
Fellow of the Royal Society of Arts, London, 1936.
Member of the Norwegian Academy of Science, Oslo, 1937.
Member of the Danish Academy of Technical Sciences, 1937.
Member of the Commitee on Science and its Social Relations, 1937.
Member of the Royal Academy of Science, Stockholm, 1939.
Honorary Life Member of the New York Academy of Sciences, 1946.
Foreign Associate of the National Academy of Science of U. S. A., 1947.
Honorary Doctor of Science, University of London, 1947.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1279–1292

The Structure of Liquids. I

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The development of the X-ray diffraction methods for the investigation of the structure of matter exhibits certain peculiarities which are rather striking. The marked spots and sharp and well defined lines of the diagrams obtained from crystalline substances attracted, quite naturally, the interest of the scientists. The immediate result of this was the foundation of X-ray crystallography, which, in its turn, has furnished a vast amount of information concerning the constitution and properties of solid crystalline substances.

As early as 1915, Debye [1] and Ehrenfest [2] independently postulated that theoretically X-ray diagrams from non-crystalline substances should also possess a characteristic pattern from which certain conclusions concerning the distribution of the electrons in the substances in question could be drawn. However, little attention was paid to these results, and the possibility it offered of investigating the structure of liquids and other non-crystalline substances was not considered in the first decades.

The reason for this was certainly not lack of interest in the structure of liquids. It was probably due to the fact that the diffraction pattern from crystals was far more conspicuous to the naked eye than that produced by liquids. For this reason the interpretation of the latter requires a more advanced experimental technique.

The first attempt to determine the structure of a liquid by a Fourier-synthesis from experimental X-ray intensity data was made in 1930 by Debye and Menke [3]. Their investigation of liquid mercury represents, in fact, the first step towards a practical method for the determination of such structures. The small deviations between the results of Debye and Menke and later investigators in this field are quite negligible.

The application of Fourier-synthesis to X-ray data from liquids was originally proposed by Zernicke and Prins [4]. Their method, in a somewhat modified form, has been employed by Warren and Gingrich [5] and co-workers in their

determinations of atomic distribution functions for a great number of liquid elements and other liquids. Different authors have also reported the determination of atomic distribution functions for a series of more complicated substances by this method.

A structural interpretation of the atomic distribution functions has also been attempted in several cases, but with limited success, and Gingrich writes in a survey of the diffraction of X-rays by liquid elements [6]: 'These atomic distribution curves represent the time-averaged atomic environment about any given atom within the liquid, but this environment is neither permanent, as in a crystal, nor random, as in a gas, and *hence no simpler description of liquid structures can now be given to supply the same information.*'

A comparison of the atomic distribution functions for groups of similar substances seems to indicate that this statement is correct. On the other hand, such a conclusion seems rather strange from a physical point of view, especially as regards the structures of molten metals and liquid gases.

In recent years certain types of systematic errors which may enter into the Fourier-synthesis of the atomic distribution functions for liquids have been reported [7], and such errors may well account for the difficulty of a structural interpretation of the distribution functions. Any discussion of these problems must be based on theoretical expressions for the intensity of the coherent and incoherent scattering of X-rays by a liquid. It will therefore be necessary to give some basic details of the scattering theory for monochromatic X-rays.

II. THE SCATTERING OF X-RAYS BY A LIQUID

The intensity of the radiation scattered coherently by a system of electrons, when a parallel beam of X-rays of wavelength λ is falling on the system, may be expressed by the formula [8]:

$$I(s) = \frac{\varepsilon^4 I_0}{\mu^2 c^4 R^2} \cdot \frac{1 + \cos^2 2\Theta}{2} \int_0^\infty \sigma(r) \frac{\sin sr}{sr} \, dr \qquad (1)$$

$I(s)$ is the intensity of the radiation scattered coherently in a given direction, I_0 the intensity of the primary beam, ε and μ the charge and mass of an electron, c the velocity of light, R the distance from the center of the electron system to the point where the intensity is observed (this distance must be great compared with the extent of the system), 2Θ the angle between the incident beam and the direction in which the intensity is observed, s is equal to $\frac{4\pi \sin \Theta}{\lambda}$, r the distances occurring in the electron system, and $\sigma(r)\,dr$ is the

probability of finding electrons in the system at a mutual distance between r and $r + dr$.

Applying the Fourier integral theorem we may write:

$$\sigma(r) = r \frac{4R^2\mu^2c^4}{\pi\varepsilon^4 I_0} \cdot \int_0^\infty \frac{sI(s)}{1 + \cos^2 2\Theta} \sin sr \, dr \tag{2}$$

The function $\sigma(r)$ should, according to its definition, give maxima for r-values corresponding to distances between greater densities of electrons in the system in question.

For an electron system consisting of two atoms with spherical electron distribution at a fixed mutual distance, the $\sigma(r)$-function possesses only one maximum, and this maximum is found at an r-value which is somewhat greater than the distance between the nuclei. This shift of the maximum with relation to the internuclear distance increases with decreasing distance. Its numerical value will, however, in practice never exceed 0.005 Å and may therefore, in the case of liquids, for all practical purposes be neglected. It has been proved that the positions of the maxima in the function $\frac{\sigma(r)}{r}$ correspond exactly to the internuclear distances [9]. This function has, however, the disadvantage that the magnitude of maxima corresponding to the same two atoms is proportional to $\frac{1}{r}$ and accordingly decreases rapidly with increasing r-values.

The distribution function $\sigma(r)$ may be divided into terms in different ways. The electron system in question may, for instance, consist of atoms which are bound together to form molecules, and the molecules, in their turn, form still larger aggregates. The distribution function $\sigma(r)$ for such a system must contain, first of all, the sum of the distribution functions for all its component atoms $\sigma_{at}(r)$. Secondly it must contain the sum of the distribution functions for all the molecules, the distance between electron densities in the same atom being left out in the calculation of the molecular distribution functions $\sigma_m(r)$.

The distribution function for the whole system may thus be written:

$$\sigma(r) = \sum_{at} \sigma_{at}(r) + \sum_m \sigma_m(r) + \sigma_i(r) \tag{3}$$

Where the term $\sigma_i(r)$ results from all the inter molecular distances, *i. e.* distances between electron densities belonging to different molecules.

Introducing (3) in formula (1), leads to:

$$I(s) = \frac{\varepsilon^4 I_0}{\mu^2 c^4 R^2} \frac{1+\cos^2 2\Theta}{2} \left[\sum_{at} \int_0^\infty \sigma_{at}(r) \frac{\sin sr}{sr} dr + \right.$$
$$\left. \sum_m \int_0^\infty \sigma_m(r) \frac{\sin sr}{sr} dr + \int_0^\infty \sigma_i(r) \frac{\sin sr}{sr} dr \right] \quad (4)$$

The term:

$$\frac{\varepsilon^4 I_0}{\mu^2 c^4 R^2} \cdot \frac{1+\cos^2 2\Theta}{2} \int_0^\infty \sigma_{at}(r) \frac{\sin sr}{sr} dr \quad (5)$$

represents the intensity of the coherent scattering of one special atom or ion and may be replaced by the expression:

$$\frac{\varepsilon^4 I_0}{\mu^2 c^4 R^2} \cdot \frac{1+\cos^2 2\Theta}{2} f_{at}^2 \quad (6)$$

where f_{at} is the atomic scattering factor for the special atom or ion in question [10].

If the electron distribution of the system is altered during the exposure time or if more than one system contributes to the scattering, then the intensity of the total coherent scattering is represented by the weighted mean of the scattering corresponding to the individual electron distributions in the system. In that case the intensity may be expressed as:

$$I(s) = \frac{\varepsilon^4 I_0}{\mu^2 c^4 R^2} \cdot \frac{1+\cos^2 2\Theta}{2} \int_0^\infty \sum_i a_i \sigma_i(r) \frac{\sin sr}{sr} dr \quad (7)$$

where the summation must be taken over all the systems or electron distributions. a_i is a factor equal to the probability of each individual distribution and $\sigma_i(r)$ is the electron distribution function corresponding to this special electron distribution.

Formula (4) gives the intensity of the coherent scattering in a convenient form for our purpose. It should be noted, however, that certain structural problems may be simplified by splitting up the distribution function $\sigma(r)$ in a different way.

The intensity which we are able to determine experimentally from X-ray diagrams is the total scattering; that is the sum of the coherent and the incoherent scattering.

The intensity of the incoherent scattering may be given by the expression [11]:

$$I_{\text{inc}} = \frac{\varepsilon^4 I_0}{\mu^2 c^4 R^2} \cdot \frac{1+\cos^2 2\Theta}{2} \cdot \frac{1}{\left(1+\frac{h(1-\cos 2\Theta)}{\mu c \lambda}\right)^3} \cdot \sum_{at} S_{at}(s) \qquad (8)$$

The theoretical basis for the determination of the function $S_{at}(s)$ has been given by Heisenberg [12], and it is possible to determine the numerical values of this function for different atoms from a table given by Bewilouga [13].

$\frac{1}{\left(1+\frac{h(1-\cos 2\Theta)}{\mu c \lambda}\right)^3}$ represents the Breit-Dirac correction factor where h is Planck's constant. The summation in the last term in formula (8) is to be taken over all the atoms in the system.

The total intensity of the scattering may now be written:

$$I_{\text{total}}(s) = I(s) + I_{\text{inc}} =$$

$$= \frac{\varepsilon^4 I_0}{\mu^2 c^4 R^2} \cdot \frac{1+\cos^2 2\Theta}{2} \left[\sum_m \int_0^\infty \sigma_m(r) \frac{\sin sr}{sr} dr + \int_0^\infty \sigma_l(r) \frac{\sin sr}{sr} dr \right.$$

$$\left. + \sum_{at}\left(f_{at}^2 + \frac{1}{\left(1+\frac{h(1-\cos 2\Theta)}{\mu c \lambda}\right)^3} \cdot S_{at}(s)\right)\right] \qquad (9)$$

The last two terms in (9), representing the total scattering from all the isolated atoms in the system, can easily be calculated, as both f_{at} and $S_{at}(s)$ are tabulated for most types of atoms.

III. THE EXPERIMENTAL PROCEDURE

The X-ray diagrams for liquids may be obtained by an experimental technique which is similar to the powder method used in X-ray crystallography. The cameras and monochromators used by the different investigators for the study of liquids are of various types. In the following a brief description of the equipment used at the University of Oslo shall be given.

Between the X-ray tube, which was of the Müller Metallix type, and the camera, a monochromator was inserted a considerable distance into the camera. The distance between the focal spot of the tube and the sample in

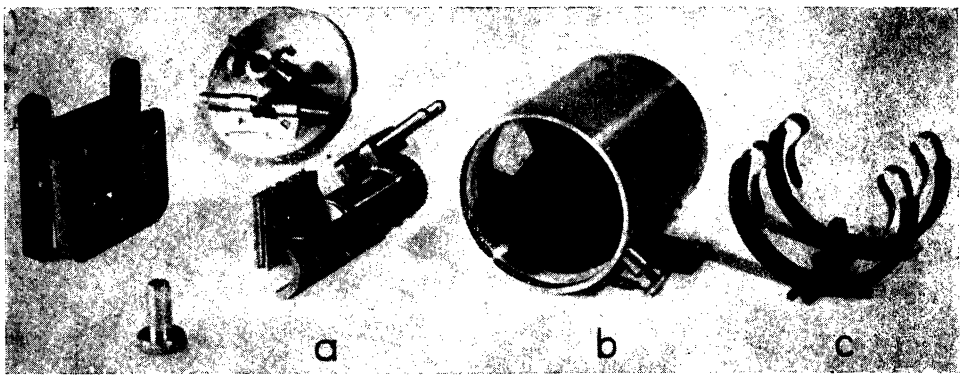

Fig. 1. Camera and monochromator.

the camera was only 8 cm. The different parts of the camera and the monochromator are illustrated in Fig. 1. The X-ray beam passes through three slits in the monochromator a, one between the tube and the reflecting crystal and two between the crystal and the sample. The monochromatic beam was the 200 reflection from a rocksalt crystal. The monochromator could be adjusted for any wavelength between 2 Å and 0.5 Å. The diameter of the cylindrical film was 57.3 mm and the film was held against the inner wall of the camera b by a spring system c.

The specimen was contained in a glass or rubber tube, the walls of which had a thickness of 0.02—0.01 mm. The diameter of the specimen ranged from 1 to 0.3 mm, depending on the absorption in the sample. The influence of the glass tube on the scattering could, in most cases, be neglected, but for substances which absorb X-rays strongly, effects due to scattering from the glass tube must be taken into consideration. This is also the case when rubber tubes are used.

The density D of the blackening of the film was determined by a Zeiss recording microphotometer, and the photometer reading, M, being transformed to density scale by the logarithmic formula:

$$D = -\log M + \log M_0 \tag{10}$$

where M_0 is the photometer reading for an unexposed spot on the film.

For small values of D, the connection between the total intensity of the scattering, $I_{exp}(s)$, and D may be given by:

$$I_{exp}(s) = c_1 D + c_2 \tag{11}$$

where c_1 and c_2 are constants. This formula is, however, only an approximation and is valid only for a rather narrow range in $I_{exp}(s)$.

$$I_a < I_{exp}(s) < I_b \tag{12}$$

For $I_{exp}(s) < I_a$ formula (11) gives too large values of $I_{exp}(s)$, and for $I_{exp}(s) > I_b$ the values are too small. Thegeneral shape of the experimental intensity curve of the liquids allows, however, an adjustment of the exposure time of the film to the correct range in D. The experimental intensity curve shows a main fall with incrasing s, and the magnitude of its maxima and minima decreases rapidly with increasing s. If, therefore, a diagram of short exposure-time gives too high values of the intensity for greater values of s compared with a diagram of longer exposure-time, then the diagram of short exposure-time is underexposed. If, on the other hand, a diagram of long exposure-time gives too small values of the intensity for smaller values of s compared with a diagram of shorter exposure-time, then the diagram of long exposure-time is overexposed. It is thus easy to pick out the diagrams of correct range in density D from a series of diagrams with different exposure-times. This procedure is, without doubt, laborious and requires the preparation of a considerable number of diagrams and photometer-records, of which a relatively large percentage must be rejected.

In order to increase the interval in s for which reliable observations of the intensity are available and also to check the observed intensity, sets of diagrams are always taken with both monochromatic CuK_α- and MoK_α-radiation. The best diagrams are then picked out from each set and used as the basis for the further treatment.

The reflection of the X-rays from the rock-salt crystal introduces a certain polarization to the monochromatic radiation, and the polarization factor, $\frac{1+\cos^2 2\Theta}{2}$, in formula (9) must be replaced by a factor

$$P(s) = \frac{1 + k\cos^2 2\,\Theta}{2} \tag{13}$$

where $k = 0.937$ for MoK_α- and $k = 0.723$ for CuK_α-radiation [14].

It should be remembered, however, that the radiation reflected in a given direction from a fixed crystal is not necessarily monochromatic. Braggs formula for the reflection from a crystal-lattice may be written:

$$2d \sin \Theta = n \lambda_0 \tag{14}$$

where n is a positive integer and d the distance between the reflecting planes in the crystal. In our case, the rock-salt crystal in the monochromator was set to reflect the desired wave-length λ_0 by the plane (200) through the slit-system. This setting of the crystal not only permits the passage of the radiation with wave-length λ_0 through the slits, but also all the wave-lengths λ_n which satisfy the condition:

$$\lambda_n = \frac{\lambda_0}{n} \tag{15}$$

where n again may be any positive integral number. This means that the wave-length λ_0 and all its 'overtones' can pass through the slits. The intensity of the 'overtones' must therefore be suppressed as much as possible relative to the intensity of the desired wave-length. This may be accomplished by running the X-ray tube at the correct tension.

Powder diagrams of substances, the lattices of which are well known, may be used for the control of the radiation. Lines originating from the 'overtones' may easily be spotted on the diagrams, as their position would correspond to superstructures in the lattices in question. However, we have never been able to observe such lines.

The absorption of the radiation in the cylindrical sample is compensated for by introducing the factor $A(s)$, which may be determined from tables published by Blake [15].

The intensity of the scattered radiation which is observed experimentally, $I_{exp}(s)$ may be written according to formula (9) and (13):

$$I_{exp}(s) = \frac{\varepsilon^4 I_0}{\mu^2 c^4 R^2} P(s) \cdot A(s) \left[\sum_m \int_0^\infty \sigma_m(r) \frac{\sin sr}{sr} dr \right.$$

$$\left. + \int_0^\infty \sigma_l(r) \frac{\sin sr}{sr} dr + \sum_{at} \left(f_{at}^2 + \frac{1}{\left(1 + \frac{h(1-\cos 2\Theta)}{\mu c \lambda}\right)^3} S_{at}(s) \right) \right] \tag{16}$$

From formula (16) an expression for the terms:

$$\sum_m \int_0^\infty \sigma_m(r) \frac{\sin sr}{sr} dr + \int_0^\infty \sigma_l(r) \frac{\sin sr}{sr} dr = I_{m+l}(s) \tag{17}$$

may be deduced:

$$I_{m+l}(s) = I_{\text{exp}}(s)\frac{\mu^2 c^4 R^2}{I_0 \varepsilon^4 P(s) A(s)} - \sum_{at}\left(f_{at}^2 + \frac{1}{\left(1 + \frac{h(1-\cos 2\Theta)}{\mu c \lambda}\right)^3} S_{at}(s)\right) \quad (18)$$

From formula (17) it is seen that the value of $I_{m+l}(s)$ must decrease rapidly with increasing a. This again means that the two terms in formula (18) must approach each other rapidly with increasing s. The second term:

$$B(s) = \sum_{at}\left(f_{at}^2 + \frac{1}{\left(1 + \frac{h(1-\cos 2\Theta)}{\mu c \lambda}\right)^3} S_{at}(s)\right) \quad (19)$$

may be calculated when the number of the different types of atoms or ions contained in the sample is known. Introducing (11), the first term in formula (18) may be written:

$$E(s) = \frac{c_1}{I_0} D(s) C(s) + \frac{c_2}{I_0} C(s) \quad (20)$$

where $D(s)$ represents the blackening of the film and $C(s)$ is given by:

$$C(s) = \frac{\mu^2 c^4 R^2}{\varepsilon^4 P(s) A(s)} \quad (21)$$

and may be determined when the experimental conditions are known. The two constants, $\frac{c_1}{I_0}$ and $\frac{c_2}{I_0}$, are given such values by a trial and error method that the function $E(s)$ coincides as close as possible to the function $B(s)$ for greater values of s. This operation is easiest to effect in the case of the experimental blackening obtained by the MoK_α-radiation. The problem is somewhat more complicated in the case of CuK_α radiation. In practice therefore the MoK_α-curve is first adjusted to the function $B(s)$ and then the CuK_α-curve is brought to coincidence with both the $B(s)$ and the adjusted MoK_α-curve.

An investigation of the errors which may be introduced by this adjustment has led to the conclusion that if the adjustment is carried out with care, the errors are negligibly small. In the next part of this paper, this type of error and a method for its removal will be treated in greater detail.

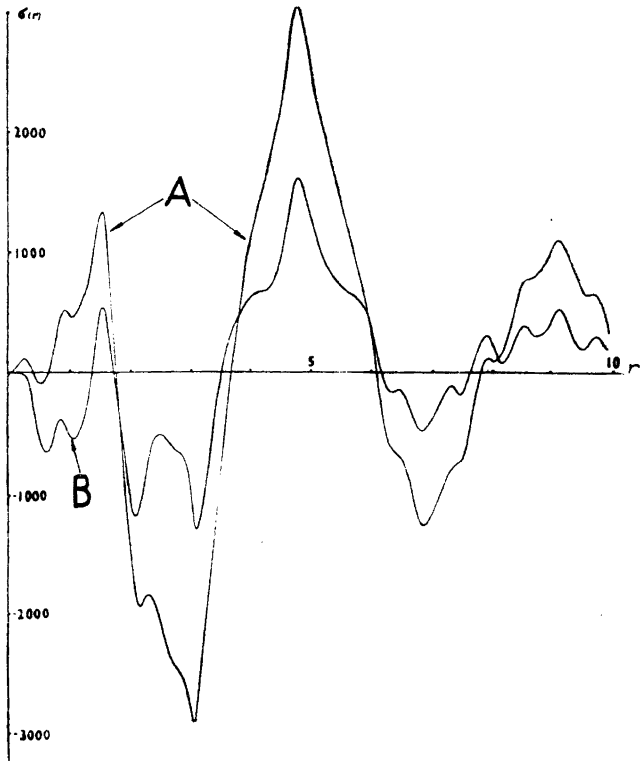

Fig. 2. Two electronic distribution curves for an aqueous solution of phosphoric acid. Curve A and B correspond to the intensity curves A and B in Fig. 3.

IV. A DISCUSSION OF CERTAIN SYSTEMATIC ERRORS

Both during the experimental procedure and as a result of the more theoretical treatment of the intensity curves, errors of different kinds may enter into the results of the Fourier synthesis. Some of these errors, like the influence of the exposuretime on the form of the intensity curve, has already been mentioned. Others, as for instance the influence of the film type and the developing process on the intensity, are of a more general character, and have therefore been treated thoroughly in publications on X-ray crystallography.

There are, however, some systematic errors which may ruin a Fourier synthesis of the distribution function for a non-crystalline material.

The first type may originate from two different sources. It may be introduced by an incorrect adjustment of the term $E(s)$, (20) to the function $B(s)$, (19), and this in turn may be due to an incorrect exposure-time. The

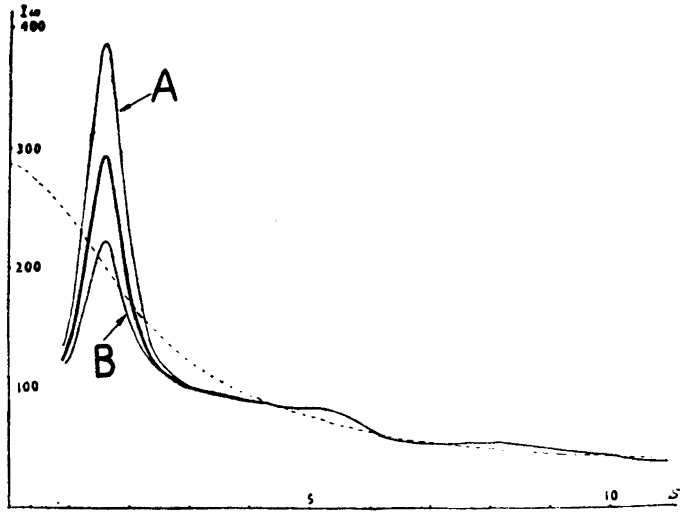

Fig. 3. Intensity curves visualizing the effect of various adjustments.

effect may also be caused by errors in the absorption factor, $A(s)$. The second of these two sources is certainly the more common.

The effect on the function $I_{m+i}(s)$ is the addition or subtraction of a term which is equal to nought for s equal to zero, reaches its greatest value for s equal to 1—2 $Å^{-1}$, where the intensity curve generally has its greatest peak, and decreases uniformly to zero with increasing s. The Fourier transformation of such a wedge-shaped term gives a function which has its greatest maxima and minima at smaller r-values, generally at 0.3—1.0 Å. In this region neither $\sigma_m(r)$ nor $\sigma_i(r)$ can have any maximum or minimum.

The influence of an incorrect adjustment of the MoK_α- and the CuK_α-intensity curves has been demonstrated in a work published in 1944 [7], where overexposed MoK_α- and underexposed CuK_α-diagrams from aqueous phosphoric acid are used as an example. In Fig. 2 two distribution curves for an aqueous solution, containing 86 % phosphoric acid, are given, and the corresponding intensity curves are shown in Fig. 3. These two intensity curves are the results of two adjustments which, as will be seen, differ greatly from each other. It should be noted that the position of the maxima is not shifted in the direction of the r-axis by the various adjustments. Their magnitude, or their position in the direction perpendicular to the r-axis, shows, however, a great variation from one case to the other.

Debye and Menke, in their pioneer work on the structure of liquid mercury [3], determined a probability function, $W(r)$, which is not identical with the

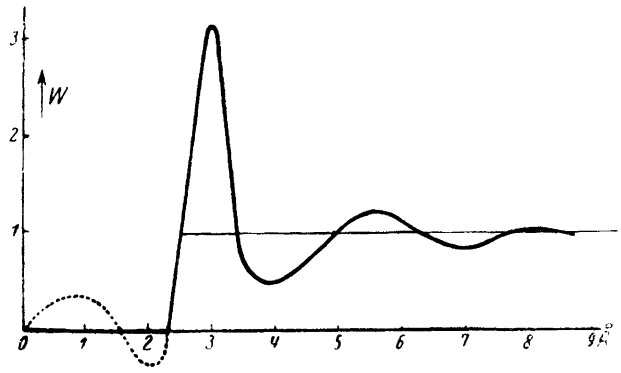

Fig. 4. Debye and Menkes probability function for liquid mercury.

distribution function, $\sigma(r)$. It is, however, derived from the intensity of the coherent scattering by a Fourier-transformation, which differs only slightly from the one by which the function $\sigma(r)$ is determined. The functions $W(r)$ and $\sigma(r)$ for the same substance have therefore many common properties and are subject to the same types of errors. In Debye and Menke's probability function for liquid mercury, a spurious maximum is observed at about 0.6 Å, and ascribed by the authors to errors in the intensity function for greater values of s. This spurious maximum may, however, be explained as due to the addition of such a wedge-shaped term to the intensity function. That the maxima observed at 5.5 and 8.5 Å in the $W(r)$ function may also arise from the same wedge-shaped term is not inconsistent with this explanation [7].

The occurrence of spurious maxima and minima at small values of r in the distribution function indicates very strongly the presence of an erroneous wedge-shaped term in the function $I_{m+I}(s)$, and this again in general may be traced back to an incorrect adjustment of the experimental intensity curve or an error in the absorption factor $A(s)$. The inner part of the $\sigma(r)$ curve should therefore be examined very carefully, and subsidiary maxima and minima in this part of the curve should always be taken into account.

The error in the absorption factor, which, as stated above, is the most common source of the wedge-shaped term, may be due to certain theoretical and experimental causes. Of these the most probable is perhaps the fact that the distribution of the intensity in the cross-section of the beam from the monochromator generally fails to meet the requirements of the theory. In the theoretical treatment it is assumed that the specimen is irradiated by a parallel beam of X-rays with constant intensity throughout its whole cross-section. A beam reflected from a plane crystal does not fulfill this require-

ment, as the intensity in a reflection from a crystal always decreases towards the edges.

The Fourier transformation in (2) requires that the intensity of the scattering be known from $s = 0$ to $s = \infty$ The experimental intensity curve is, however, only known between the limits $s = a$ to $s = b$. The value of a and b are, in general, of the magnitude $a \approx 1$ Å^{-1} and $b \approx 12$ Å^{-1}. When the upper limit of integration in (2) is changed from infinity to b, certain errors are introduced in the distribution functions. This error is of the same type as the diffraction effect met with in the Fourier synthesis of crystal lattices. It has been demonstrated by Bragg and West [16] that its influence may be reduced when the intensity is multiplied by a function e^{-ks^2}. Unfortunately this procedure also reduces the resolving power of the method. The reason for this is simply that the function e^{-ks^2} decreases rapidly with increasing s. Accordingly, it reduces the influence of the intensity on the distribution curve with increasing s, and this means a reduction of the resolving power. Warren, Gingrich and others [5,17], on the other hand, multiply the intensity curve by a factor $\frac{1}{f^2}$, which increases rapidly with increasing s. The resolving power of the method is in this way increased considerably, but the increase in the diffraction error caused by the factor $\frac{1}{f^2}$ has proved to be a great disadvantage, and may, as will be shown by a few examples, lead to serious difficulties in the interpretation of the experimental distribution curves.

The errors introduced in the distribution functions, when the lower limit of integration is changed from nought to a is not easily dealt with. Some estimate of its magnitude may be obtained by drawing two curves connecting the inner part of the observed intensity curve and the $I(s)$ axis. The two curves should be drawn in such a manner that one of them represents a probable upper limit of the intensity curve in this region, and the other represents the corresponding lower limit. The integration in the Fourier transformation is then carried out, using both the upper and the lower of the two curves. In this way two distribution curves are obtained, and the difference between them should represent a fairly good estimate of the magnitude of this error. For greater interatomic distances this procedure may prove less expedient, but for distances up to about 10 Å the method may be used safely.

In the following section some experimental results will be given, and, at the same time, some examples of the different kinds of errors discussed above will be shown. A more detailed discussion of the diffraction error, and its influence on the different types of distribution functions, is given by Viervoll [9].

SUMMARY

The theory of the scattering of monochromatic X-rays by a liquid is given and some details of the experimental method used at the University of Oslo for determination of electronic distribution functions are described. Some of the more common systematical errors are also discussed.

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Received July 14, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1293 – 1308

The Structure of Liquids. II

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V. THE EXPERIMENTAL RESULTS

The structural interpretation of the experimentally determined distribution curves seems, in the case of liquid elements, nearly always to lead to insuperable difficulties [1]. For more complicated structures, as for instance water and aqueous solutions of inorganic acids, bases and salts, such an interpretation has proved to be possible [2]. This situation must indeed be regarded as quite unusual, and should certainly not increase our confidence in the structures which have been reported for the more complicated substances. If, therefore, we are to accomplish anything in this field, it is obvious that the first problem which must be attacked is the structural interpretation of the distribution curves for the simplest possible liquids, namely the liquid noble gases and the molten metals.

The reason why a structural interpretation of these substances has not yet been possible may be due either to the complexity of the actual structures of even the simplest liquids, or to the fact that systematic errors of some sort have entered into the experimental distribution curves. The first reason is regarded as the only one of any importance by the great majority of the investigators, and the second has, as far as can be ascertained, only been considered by Campbell and Hildebrand [3]. In a discussion of some peculiar peaks which were observed in a great many of the experimental distribution curves for liquid elements, they state: 'One might be tempted to regard these subsidiary peaks as illusory, since they are so small as to suggest experimental errors, and they do not correspond to any expected geometrical structure. However, their occurence again and again with different liquids and independent investigators make them appear real', and thus the second possibility is rejected by Campbell and Hildebrand also. The solution of the problem is, however, as will be seen later, to be found precisely in this direction.

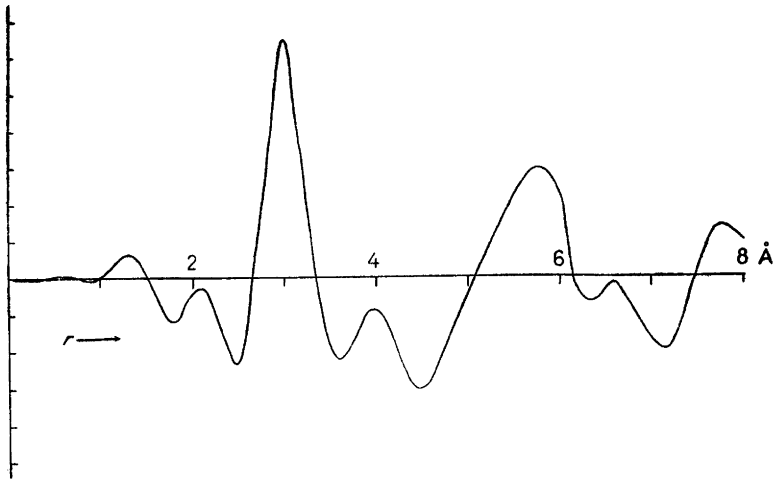

Fig. 1. Campbell and Hildebrand's atomic distribution curve for liquid mercury at — 38° C.

VI. MOLTEN METALS AND LIQUID NOBLE GASES

Atomic distribution curves have been published for a considerable number of liquid noble gases and molten metals. Many of these distribution curves possess subsidiary peaks of the type mentioned above. For nine of these substances: lithium, argon, mercury, thallium, lead, indium, tin, bismuth and germanium, a complete recalculation of the distribution curves has been accomplished. The results for mercury at — 38° C and tin at 280° C will be discussed as examples.

The reasons the distribution curve of mercury, determined by Campbell and Hildebrand [3], was chosen are that the subsidiary peaks in this case are very marked, the diagrams are taken at a temperature which is only a few degrees above the meltingpoint of mercury, and that mercury melts at a comparatively low temperature and therefore should be expected to possess an extraordinarily high degree of order in the liquid state. Moreover the experimental distribution curve of mercury at — 38° C, as determined by Campbell and Hildebrand, offers a good example of the influence of an error in the absorption or in the adjustment.

The probability function $W(r)$ of tin at 280° C, as determined by Hendus [4], possesses three large maxima. Superimposed on the second and the third of these, is a ripple of constant period equal to about 0.95 Å. Such a ripple is very often met with in experimental distribution curves, and for this reason tin was used as the second example.

If the term $4\,\pi\,r^2\,\varrho_0$, representing the distribution function of a constant average density of atoms equal to ϱ_0 [5], is subtracted from the atomic distribution curve for liquid mercury at — 38° C, determined by Campbell and Hildebrand, then the curve given in Fig. 1 is obtained. The part of this curve $r = 0$ to $r = 2.5$ Å is not given in the original paper, and has been determined from the intensity data published. The larger peaks of this distribution curve are observed at 3 and 5.7 Å. Smaller peaks are found at 0.6, 1.3, 2.1, 3.9, and 6.5 Å. The large peak at 3 Å corresponds very closely to the interatomic distances in the crystal lattice of solid mercury. The maximum at 2.1 Å and especially the comparatively large one at 1.3 Å are very difficult to explain as due to interatomic distances between mercury atoms. An interatomic distance of 1.3 Å, which would correspond to an atomic radius of the mercury atoms equal to 0.65 Å, is quite incompatible with the values derived from crystal lattices. The maxima at 0.6, 1.3, and 2.1 Å indicate, therefore, very strongly the presence of some error in the distribution curve. The fact that the small maxima at 2.1 and 3.9 Å are located symmetrically round the large peak at 3 Å makes the assumption that the atomic distribution curve is influenced by a diffraction error quite likely — the large maximum at 3 Å being real and the two subsidiary peaks at 2.1 and 3.9 Å, corresponding to the first maximum of the diffraction, ripples on both sides. From the observed distance 0.9 Å between the central peak and the first maximum in the ripple, an upper limit of integration equal to 8.6 $Å^{-1}$ is computed — a result which is in the best accordance with the actual upper limit of integration equal to 9 $Å^{-1}$.

An electronic distribution curve was then determined from Campbell and Hildebrand's intensity data. The result is given in Fig. 2. The lower limit of integration for all curves was by an approximation to nought made equal, and the upper limits, which are different for the different curves, are indicated on the right of the respective curves. The maximum at 1.3 Å is already seen when the interval of integration is $s = 0$ to $s = 4.3$ $Å^{-1}$, and this maximum is only slightly affected when the upper limit of integration is shifted even to $s = 9$ $Å^{-1}$. This proves that the maximum at 1.3 Å must be due namely to an error in the inner part of the intensity curve between $s = 0$ and $s = 4.3$ $Å^{-1}$.

The subsidiary peaks at 2.1 and 3.9 $Å^{-1}$ are considerably reduced in the electronic distribution curve, corresponding to an upper limit of integration equal to 9 $Å^{-1}$. The first one has nearly vanished, but the second is somewhat more persistent. This is exactly what would happen if the subsidiary peaks were actually caused by a diffraction effect. It should also be noted that when the upper limit of integration is $s = 7.3$ $Å^{-1}$, the two subsidiary maxima are moved to a distance of about 1.1 Å from the central peak.

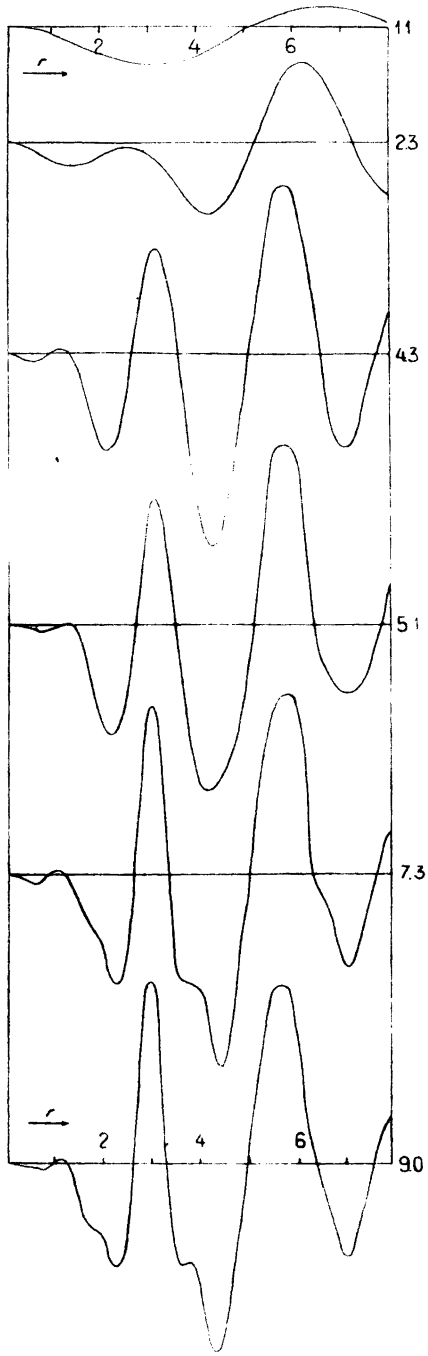

Fig. 2. The electronic distribution curves for liquid mercury at — 38° C determined from Campbell and Hildebrand's data.

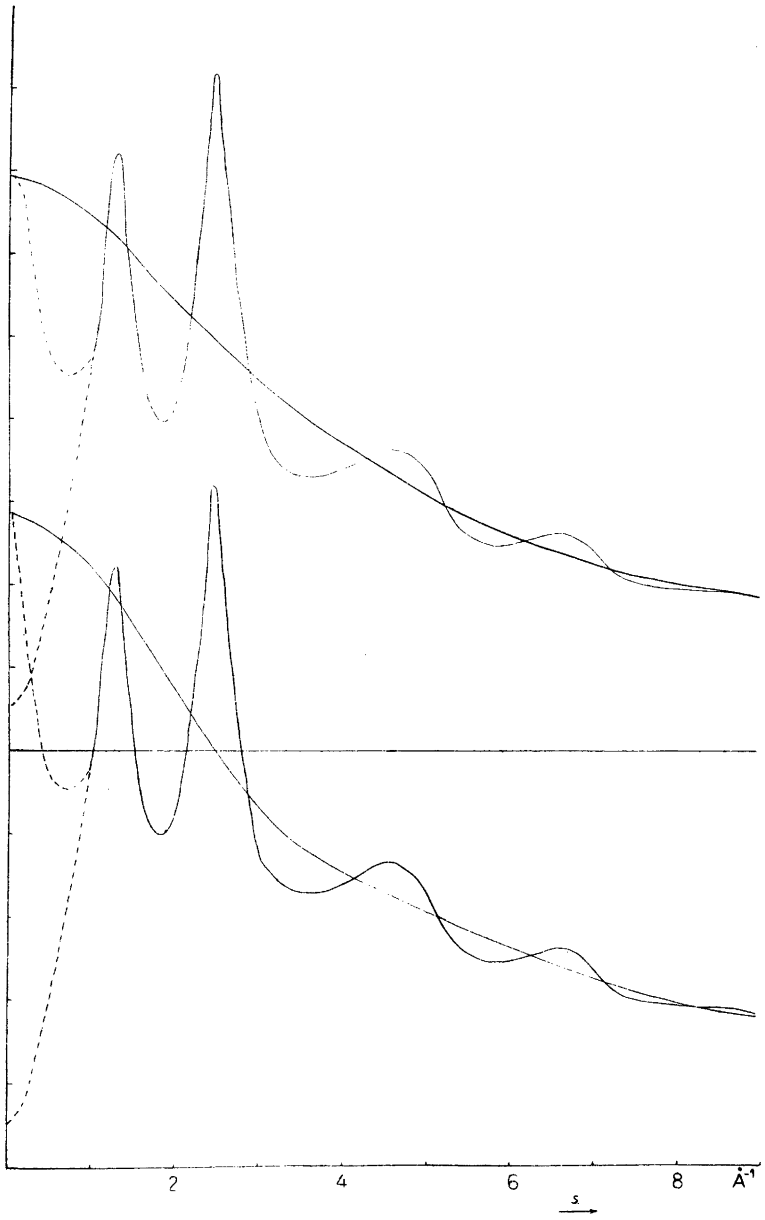

Fig. 3. The upper curve represents Campbell and Hildebrand's intensity curve for liquid mercury at — 38° C, the lower one the corresponding curve with corrected atomic scattering, B(s).

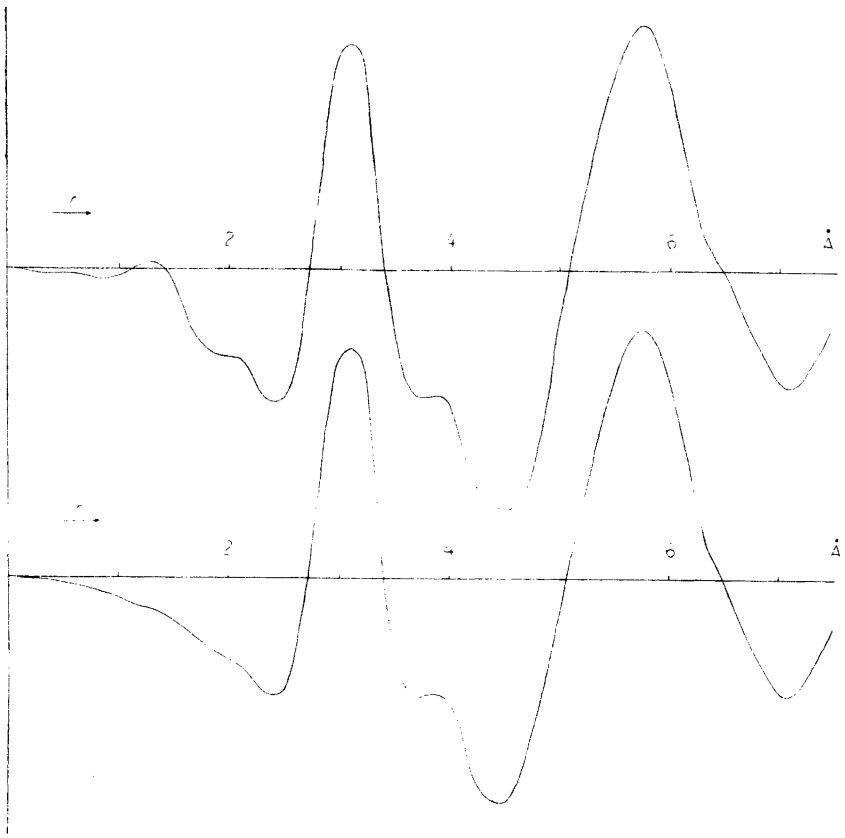

Fig. 4. Electronic distribution curves determined from the intensity curves in Fig. 3.

From this distance, an upper limit of integration, equal to 7.0 $Å^{-1}$, is again computed. The maximum which was observed at 6.5 Å in Campbell and Hildebrand's atomic distribution curve has vanished leaving only a flattening of the distribution curve in this region. The subsidiary peaks observed at 2.1, 3.9, and 6.5 Å are therefore undoubtedly due to a diffraction error of the ordinary type.

The peak at 1.3 Å, which is also found in the electronic distribution curve, can hardly be caused by a diffraction effect. An error in the adjustment of the intensity curve or in the absorption factor $A(s)$ would, as stated in part I, give rise to such maxima or minima.

Since the linear absorption coefficient, μ, of mercury is large, an error of this type and of a magnitude sufficiently great to explain the occurence of the peak at 1.3 Å may easily enter into the intensity curve.

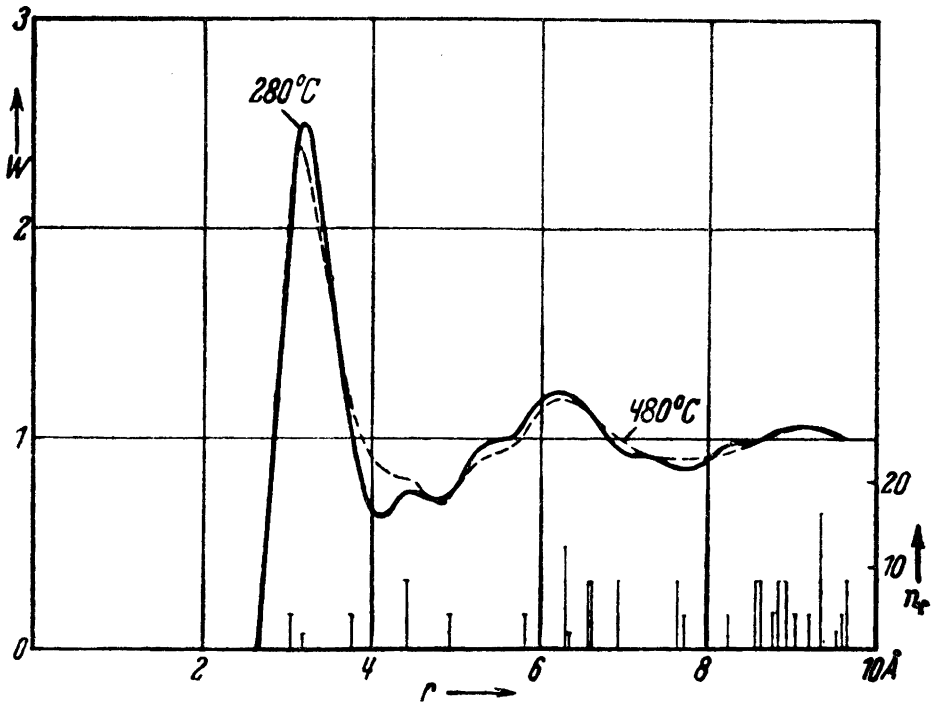

Fig. 5. Probability function, $W(r)$ for liquid tin determined by Hendus.

The effect of an error in $A(s)$ on the intensity curve should also increase rapidly with decreasing s, and as will be seen from Fig. 2, this is exactly what happens in our case. It is difficult to correct for such an error using the data given by Campbell and Hildebrand. The best we can do is to introduce a correction in the total atomic scattering curve $B(s)$ and then make a new adjustment of the corrected atomic scattering curve to the observed intensity curve. In this way it is possible to find how a variation in the absorption factor alters the intensity curve. In order to determine the magnitude of the error in $B(s)$, a trial and error method must be applied. For each new value of $B(s)$ a part of the distribution curve stretching to both sides from $r = 1.3$ Å must be computed, and the value of the function $B(s)$, which causes the subsidiary peak to vanish, is chosen for the final determination of the total distribution curve.

The upper curve in Fig. 3 is the intensity curve given by Campbell and Hildebrand. The lower one represents the same intensity curve with the corrected total atomic scattering curve $B(s)$. Fig. 4 gives the corresponding

Table 1.

Position of maxima observed	Difference in Å	Computed position
4.45		4.25
	0.95	
5.4		5.25
	0.9	
6.3		6.25
	1.0	
7.3		7.25
	0.9	
8.2		8.25
	1.0	
9.2		9.25
Mean difference	0.95 Å	

electronic distribution curves. The only difference between these two distribution curves is that the maximum at 1.3 Å has vanished completely and the small maximum at 2.1 Å has been reduced still further. This result does not prove with absolute certainty that an error in the absorption factor $A(s)$ really is incorporated in the original intensity curve, but it makes such an assumption quite likely.

The structure of liquid tin has been studied by Gamertsfelder [6] and Hendus [7]. The probability function $W(r)$ determined by Hendus (Fig. 5) for liquid tin at 280° C has its first and greatest maximum at 3.20 Å. The inner part of the function from $r = 0$ to $r = 2.8$ Å is not given by Hendus, and it is therefore difficult to judge whether the curve is influenced by some of the errors discussed above or not. From $r \approx 4$ to $r \approx 10$ Å the curve shows six smaller maxima which are superimposed on two broad maxima at about 6.2 and 9.2 Å. The position of the six small peaks are given in Table 1.

The six small maxima are, as will be seen from the table, approximately equidistant. An error in a narrow interval of the intensity curve always introduces a periodic term in the Fourier transformation, and thus gives rise to a sequence of small periodic maxima of equal amplitude. If the distance between these maxima is known, the position of the interval in the intensity curve from which the periodic maxima originate may easily be computed. In our case the period of the small peaks is approx. 0.95 Å, and the interval on the intensity curve from which they may originate would be found at approx. $s = \frac{2\pi}{0.95} \approx 2\pi$ Å^{-1} or $\frac{\sin\Theta}{\lambda} \approx 0.5$ (Fig. 6). In this part of the intensity

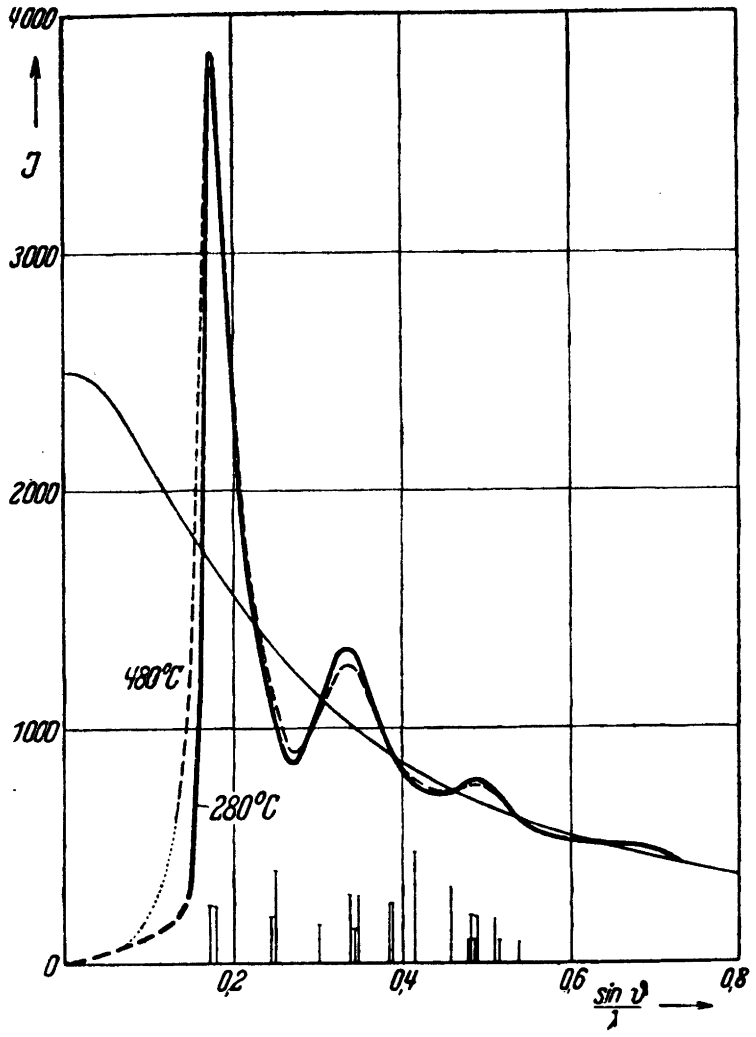

Fig. 6. The intensity curve from which W(r) in Fig. 5 was determined.

curve a maximum is found. If the magnitude of this maximum is too great, this would introduce a periodic term having maxima at the positions given in the third column in Table 1. The deviation between the observed and the computed position of the maxima at 4.45 and 5.4 Å may be due to the influence of a diffraction ripple from the great peak at 3.20 Å. The fact that the small maxima in the probability function $W(r)$ may be caused by the maximum in the intensity curve at about $s \approx 2\pi \text{Å}^{-1}$, does not prove that the

small maxima are spurious. When, however, the method of computation used by Hendus is considered, such an assumption becomes quite likely. This method of computation has much in common with a method used by Warren, Gingrich and others [5], and the distribution curves which are obtained by this procedure correspond to the distribution of the atoms, not to the electronic distribution in the system.

According to Warren and Gingrich, the atomic distribution function $4\pi r^2\sigma^1(r)$, representing the difference between the actual distribution of the atoms in the sample and the distribution in a liquid with constant atomic density equal to the mean atomic density in the sample, is given by the expression:

$$4\pi r^2 \sigma^1(r) = \frac{2r}{N}\int_0^\infty \frac{s(I_{m+l}(s))}{f^2} \sin sr\, dr \tag{22}$$

The corresponding electronic density function may be deduced from formula (2) and (17).

$$\sum_m \sigma_m(r) + \sigma_l(r) = Cr\int_0^\infty s\, I_{m+l}(s) \sin sr\, ds \tag{23}$$

where C is a constant.

The difference between the integrals in (22) and (23) is that in (22) a factor $\frac{1}{f^2}$ is introduced, where f is the atomic scattering factor. The factor $\frac{1}{f^2}$ must therefore always increase rapidly with increasing s. In Fig. 7 the function $\frac{1}{f^2}$ for tin is given.

The connection between the function $I_{m+l}(s)$ and the experimentally observed intensity I_{exp} is given by (18). From this formula it will be seen that I_{exp} is multiplied by a factor

$$\frac{\mu^2 c^4 R^2}{I_0 e^4} \cdot \frac{1}{P(s)} \cdot \frac{1}{A(s)} \tag{24}$$

This factor is again, to a certain degree, dependent on the form and dimensions of the sample, the type of monochromator used, and other similar technical details of the experimental procedure. The factor is also greatly influenced by the absorption coefficient of the substances in question. In general, the best results are obtained when the value of this factor multiplied by s is as constant as possible for the whole interval in s for which intensities

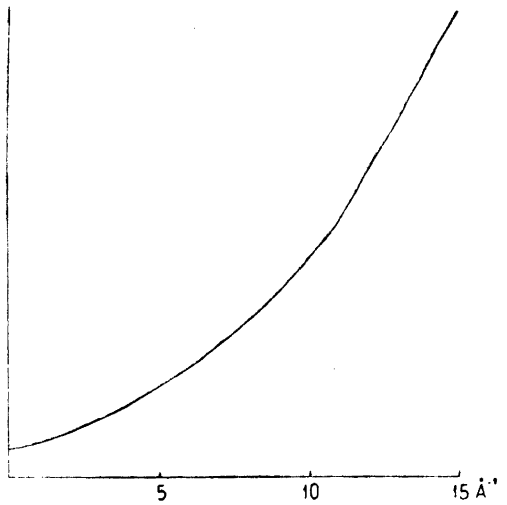

Fig. 7. The function $\frac{1}{f^2}$ *for tin.*

are observed. The reason for this is simply that equal weight must be attached to all parts of the experimentally observed intensity curve. If therefore, as in (22), $I_{m+l}(s)$ is multiplied by a factor $\frac{1}{f^2}$, very great importance is assigned to the experimentally observed intensity for greater values of s, and the observations corresponding to smaller values of s are of little significance to the distribution curve. Under such circumstances an accidental error in the outer part of the experimental intensity curve may show up very clearly in the atomic distribution curve, whereas an error of equal magnitude in the inner part of the intensity curve does not count at all. This is, in fact, a great defect in this method, as the spurious maxima generated by an accidental error in the intensity corresponding to greater values of s are always sharp and well defined. The maxima caused by an error in the inner part of the intensity curve are necessarily of a considerable width, and for this reason are not so easily mistaken for marked interatomic distances. Both types of errors may, however, influence the magnitude of the maxima and minima in the distribution curve.

In the case of liquid tin at 280° C, an electronic distribution curve was then computed, making use of the intensity data published by Hendus. The result is given in Fig. 8. This curve shows maxima at 3.28, 6.21 and 9.15 Å. The six small periodic maxima observed in the probability function $W(r)$ have totally vanished. A slight undulation of the electronic distribution curve in the interval $r = 0$ to $r = 2.5$ Å, having maxima at about 1.5 and 2.3 Å,

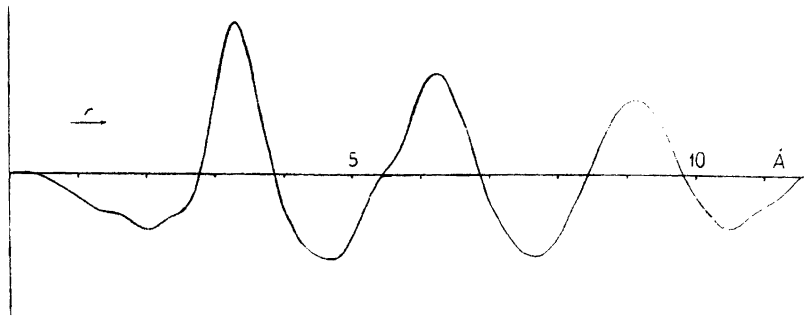

Fig. 8. Electronic distribution curves for tin.

seems to indicate a somewhat exaggerated value of the maximum in the region $s \approx 2\pi\text{Å}^{-1}$ of the intensity curve.

The small periodic maxima in the function $W(r)$ of Hendus, may therefore, in all probability, be ascribed to a slight occasional error in the intensity curve at about $s = 2\pi\text{Å}^{-1}$, and this rather negligible error is, owing to the special method of computation, made to interfere with the whole probability function.

Further typical examples of this error will be given in a subsequent part of this report, and for the sake of simplicity an error of this type will be termed general ripple.

A recomputation of the electronic distribution curves has, as mentioned above, been effected in nine cases. In all of these, the originally published atomic distribution curves have possessed marked subsidiary peaks or other individual peculiarities. In all nine cases it has been possible to show that the peculiarities of the distribution curves may be ascribed to diffraction ripples, general ripples, or to errors in the adjustment of the $B(s)$ function or in the absorption factor $A(s)$.

For liquid lithium the recomputation was based on intensity data given by Gamertsfelder [6], for argon at 91.8° K and 84.4° K the intensities were determined by Eisenstein and Gingrich [7]. A comparison of the atomic and electronic distribution curves, shows very strongly that the subsidiary peaks in these cases also are spurious, being due to diffraction ripples. The peak at 4.1 Å in the atomic distribution curve of lithium, at 4.5 Å in liquid argon at 91.8° K and at 5.1 Å in argon at 84.4° K are not found at all in the corresponding electronic distribution curves. Similar subsidiary peaks are also reported in the experimental distribution curves for some other liquid noble gases and molten metals; for instance in liquid sodium at 100° C [8] and in cadmium at 350° C [6]. In the case of sodium, the distribution curve has its greatest maximum

Table 2.

Substance		Position of second peak in diameters	Position of third peak in diameters	Position of first peak in Å
Li	200° C	1.80		3.24
Na	100° C	1.84		3.83
Na	400° C	1.87		3.90
Al	700° C	1.86		2.96
Ar	84.4° K	1.98		3.79
Ar	91.8° K	1.95		3.80
Ar	126.7° K	1.80		3.90
Ar	144.1° K	1.85	2.65	3.90
K	70° C	1.83	2.67	4.64
K	395° C	1.87	2.55	4.76
Zn	460° C	1.84	3.00	2.94
Ga	18° C	1.98	2.61	2.83
In	390° C	1.97		3.30
In	160° C	1.93		3.36
Sn	280° C	1.89	2.77	3.28
Sn	390° C	1.93		3.36
Hg	−38° C	1.90		3.00
Hg	0° C	1.9[illegible]		3.00
Hg	50° C	2.00		3.00
Hg	100° C	2.00		3.00
Hg	150° C	2.00		3.00
Hg	200° C	2.02		3.05
	Mean	1.91	2.70	

at about 5 Å at a distance of 1.17 Å from the greater peak. This corresponds to an upper limit of integration $s'_{max} = 6.7$ Å^{-1}, if the subsidiary peak is due to a diffraction effect, which agrees well with the actual limit of integration $s = 6.28$ Å^{-1}. For cadmium $s'_{max} = 8.6$ Å^{-1} and $s_{max} = 7.54$ Å^{-1}.

The agreement between the value s'_{max} computed from the distance between the first large peak and its subsidiary maximum and the actual value s_{max} is, as will be seen, on the whole remarkably good. Only in the case of cadmium is the deviation relatively large. An error of 0.13 Å in the distance between the two peaks would, however, account for this deviation, and it is indeed difficult to localize the small subsidiary maxima with greater accuracy from the curves in the publications.

From the above discussion it can be seen that there is good reason to believe that the subsidiary peaks observed in the atomic distribution curves of liquid noble gases and molten metals are spurious.

When the small extra maxima are removed, all these distribution curves become astonishing similar and extremely simple. Only two or three maxima are left, and the ratio of their mutual distances is approximately the same, as will be seen from Table 2. In this table the position of the maxima along the r-axis are given, while the distance from the origin to the first and most marked peak in each curve is taken as unity. This coordinate may be termed diameters, by which is meant atomic diameters.

The interpretation of an atomic distribution curve possessing only two or three maxima ought to be simple. There is, however, a special type of error which may give rise to maxima in the same positions as the second and third peak in the experimental curves. It has been demonstrated [9] that if the first and greatest maximum of the experimental intensity curve is too great, smaller spurious maxima may occur at 0.26, 1.83 and 2.67 diameters, provided that the distribution curve possesses a maximum at 1 diameter. This special error must always give a maximum at about 0.26 diameters, and the magnitude of the maxima at about 1.8 and 2.6 diameters should always be small in comparison with the principal peak at 1 diameter. Unfortunately the inner part of the distribution curves is omitted in most of the publications. For sodium at 100° C and 400° C, potassium at 70° C and 395° C and for tin at 250° C and 390° C a sufficient number of points are given on the inner part of the distribution curves and in these six cases no maximum is observed at about 0.26 diameters. The final electronic distribution curve for mercury at — 38° C based on Campbell and Hildebrand's intensity data possesses no maximum in the critical interval. To this must be added the fact that the maximum which is observed at about 1.9 diameters for most of the substances is approximately of the same magnitude as the principal peak at 1 diameter and is not small in comparison with that maximum. It is obvious therefore that the error in question is not the cause of the maxima at about 1.9 and 2.7 diameters. These maxima can in any case be influenced by such an effect only to a slight degree.

The main source of the two maxima at approx. 1.9 and 2.7 diameters must, therefore, be found in some characteristic properties of the order of the atoms in the liquids. It follows therefore that it should be possible to construct a structure of similar atoms which would give a distribution function with maxima in the correct positions. As a first approximation, liquid noble gases and molten metals may be regarded as systems of hard spheres of the same size.

The formation of irregular packings of hard spheres of equal size has been given an extensive theoretical treatment by Kirkwood and Boggs [10]. The distribution curves for the centers of the spheres which Kirkwood and Boggs have determined on a wholly theoretical basis resemble the experimentally de-

Table 3.

Position of maxima in diameters.

First	Second	Third	
1	1.91	2.70	Mean of observations from Table 2.
1	1.71	2.44	Kirkwood and Boggs $\alpha = 0.72$, $\beta = 5.90$

termined distribution curves for liquid noble gases and molten metals in a striking manner. The relative position of the maxima is, however, not in the best accordance with the experimental results.

In Table 3 the relative position of the maxima according to Kirkwood and Boggs are compared with the mean values from Table 2.

The positions of the maxima in the theoretical curve depend, to a certain degree, on some parameters which are introduced by Kirkwood and Boggs. The deviation between the experimental and theoretical relative position of the maxima may perhaps be eliminated by a variation in these parameters. The deviation may, however, also to some degree originate from uncertainty as to the location of the correct position of the maxima in the experimental distribution curves.

The results of Kirkwood and Boggs have subsequently been confirmed by Born and Green [11] in a more general theory of the liquids. A comparison between this theory and the experimental distribution curves for more complicated liquids will be given in the subsequent parts of the present paper.

SUMMARY

Using liquid mercury at — 38° C and liquid tin at 280° C as examples, the effects of some types of systematical errors on the distribution curves of liquids are demonstrated. The subsidiary peaks observed in the distribution curves of a series of liquids are proved to be spurious. It is shown that when the influence of the systematical errors is removed the electronic distribution curves for molten metals and liquid noble gases are very simple and show great similarity.

The work on this publication was started in Cambridge in England in 1946, and I want to express my sincere thanks to Professor Sir W. Lawrence Bragg for his encourage-

ment and for the great interest he took in these problems. My thanks are also due to The British Council who made that stay in England possible.

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Received July 14, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1309–1317

A Simple Method for the Measurement of Turbidity

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If, during transmission measurements of turbid suspensions with a photoelectric colorimeter, the distance between the sample holder and the photocell is increased, the amount of light received by the photocell decreases. This is due to the fact that the amount of scattered light which strikes the photocell, diminishes with the solid angle within which it is seen from the suspension. Dognon [1, 2] discussed the error caused hereby in colorimetric measurements and possible ways of improving the determinations. Later the same author [3–5] showed that the relative change in apparent absorption of light, when the photocell is moved from the sample holder to a position infinitely distant from it, should be independent of the concentration of the particles. He also discussed at length the possibility of using experimentally obtained values to get information about the size and shape of the particles.

This paper will describe how transmission measurements with different distances between photocell and suspension can give the relation between turbidity and concentration. Any of the commercially available photoelectric colorimeters which has a large compartment for the sample holders can be used without alterations. The influence of the color of the liquid is eliminated and it is not necessary to utilize a standard at each measurement. Correspondence with generally accepted standards for turbidity, such as the silica scale, can be established once and for all as long as the same instrument is used. The method has been in use in our laboratory for routine turbidimetric measurements on viscose for some time and has been found reliable and rapid.

THEORY

A beam of parallel light enters a suspension in a plane parallel sample holder at right angles. The light is received by a photocell on the other side.

The following notations will be used:

I_0 = intensity of light entering the suspension
I_t = intensity of transmitted (undeviated) light
I_s = total intensity of scattered light
aI_s = fraction of scattered light, striking the photocell
I_a = intensity of absorbed light
x = distance between suspension and photocell
d = length of light path through suspension
T_x = intensity of light received by the photocell when the distance from the suspension is x

According to the definitions given above, we have

$$I_0 = I_t + I_s + I_a \tag{1}$$

$$\text{and } T_x = I_t + aI_s \tag{2}$$

where a is a function of x and the experimental arrangements, as well as of the form and size of the scattering particles. Reflections at the glass walls of the sample holder are neglected.

It is assumed that Lambert's law is valid, *i.e.*

$$I_t = I_0 \, e^{-\varkappa d} \tag{3}$$

($\varkappa$ = extinction coefficient)

Different cases will be considered.

First case: Dilute suspension with a measurable amount of directly transmitted light; no light absorption.

If measurements are made at two different distances x_1 and x_2 ($x_2 > x_1$), we get the formula:

$$\frac{T_{x_2}}{T_{x_1}} = \frac{a_2(1 - e^{-\varkappa d}) + e^{-\varkappa d}}{a_1(1 - e^{-\varkappa d}) + e^{-\varkappa d}} \tag{4}$$

As only dilute suspensions are considered, we may substitute $e^{-\varkappa d} = 1 - \varkappa d$. This gives

$$\frac{T_{x_2}}{T_{x_1}} = \frac{1 - \varkappa d\,(1 - a_2)}{1 - \varkappa d\,(1 - a_1)} \tag{5}$$

and as a special case:

$$y = \frac{T_\infty}{T_{x_1}} = \frac{1 - \varkappa d}{1 - \varkappa d\,(1 - a_1)} \tag{6}$$

If Beer's law is valid, $\varkappa$ can be substituted by kc (k = constant, c = concentration). The equations thus obtained are referred to as (5a) and (6a).

Because of the identity $T_\infty = I_t$, (1) can also be written

$$\frac{I_s}{I_0} = 1 - y\frac{T_{x_1}}{I_0} \tag{7}$$

As long as (6) or (6a) is valid, we also have

$$I_s/I_0 = \varkappa d = kcd = \frac{1-y}{1-y(1-a_1)} \tag{8}$$

By definition also

$$a_1 = \frac{1-y}{I_0/T_{x_1} - y} \tag{9}$$

By derivation of (6a) an expression is obtained which, when the concentration approaches zero, becomes simply

$$\lim_{c=0} \mathrm{d}y/\mathrm{d}c = -kda_1 \tag{10}$$

[the corresponding expression derived from (5a) is: $-kd(a_1 - a_2)$]

With the aid of the equations derived, the method can now be discussed in detail. It can be seen from (10) that the sensitivity of the method increases with the difference $(a_1 - a_2)$. The difference in distance $(x_2 - x_1)$ should consequently be as great as possible. It is not possible, however to diminish the distance x_1 between photocell and suspension below a certain value. The photocell can, on the other hand, without difficulty be placed so far from the suspension that the values for $x_2 = \infty$ (*i. e.* $a_2 = 0$) can be found by extrapolation. The discussion will, for the sake of simplicity, be limited to the case where the photocell is moved from a position as near the suspension as possible, to a position infinitely distant from it.

The value of $y = T_\infty/T_{x_1}$ is obtained through extrapolation from the experiments, as is described below. The relationship between y and c, as seen from (6a), is not linear. It is advantageous to measure T_{x_1}/I_0. at the same time. For that purpose the sample holder is first filled with the liquid medium and then with the suspension. The amount of light lost through reflections in the glass walls of the sample holder is thus compensated for.

Regardless of the validity of the Lambert-Beer law, the two experimentally obtained quantities allow determination of α_1 and I_s/I_0, *i.e.* the percentage of scattered light. The latter quantity is independent of the experimental arrangements. For very dilute suspensions, α_1 can also be calculated from (10) after a separate determination of kd. If different suspensions are studied with the same apparatus and the values of α_1 compared, some conclusions as to form and size of scattering particles might be drawn. The possibilities in this respect have been discussed by Dognon in his papers cited above.

Second case: Dilute suspension in a light-absorbing medium.

It is assumed that the absorption follows the laws of Lambert and Beer; the intensity of the directly transmitted light is thus:

$$I_t = I_0 \, e^{-(\varkappa + k'c')d} \tag{11}$$

(k' = extinction coefficient and c' = concentration of light absorbing substance). Formula (4), however, assumes the same form as before, which means that the light absorption is without influence on the turbidity measurement. The result is easily understandable if we think of the sample holder as divided into two compartments; one filled with the light absorbing matter, the other with the scattering substance.

Formulas (5), (6), and (10) remain unchanged. Instead of (7) we get

$$\frac{I_s + I_a}{I_0} = 1 - y\frac{T_{x_1}}{I_0} \tag{12}$$

I_a/I_0 is determined separately from a sample of the colored solution without scattering particles. It is then a simple matter to calculate $I_s/(I_0 - I_a)$. Likewise

$$\alpha_1 = \frac{1 - y}{I_0/T_{x_1}\left(1 - \frac{I_a}{I_0}\right) - y} \tag{13}$$

Third case: Concentrated suspension. The directly transmitted intensity I_t practically = 0.

In this case the suspension itself acts as source of light for the photocell. Changes in concentration of the scattering particles mean only a change in the intensity of the light source. The ratio y is therefore independent of the concentration. It is necessary either to dilute the suspension or to use sample holders with a shorter light path.

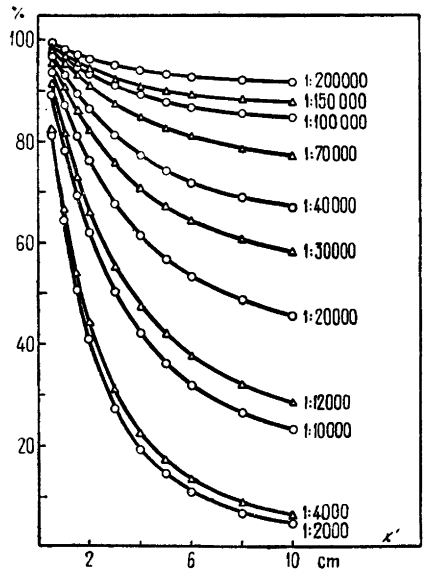

Fig. 1. Measurements on latex. Decrease in illumination of photocell when sample holder is moved away from it.

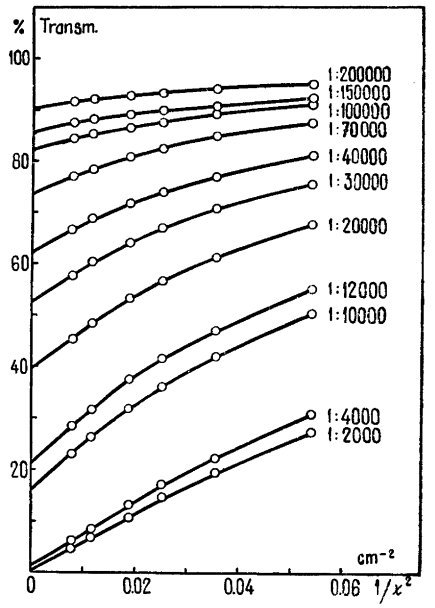

Fig. 2. Measurements on latex. Extrapolation of transmission measurements.

EXPERIMENTS

The measurements described were all made with a Lumetron Photoelectric Colorimeter, Model 402 E (from Photovolt Corp., New York, USA). No changes were made on the instrument. A special feature of this colorimeter is the large compartment for the sample holders, which makes it possible to move a 2 cm sample holder to a maximum distance of 13 cm from the photocell. The light beam is somewhat divergent because of the extension of the light source.

The sample holder was first put in position 'zero', that is as near the photocell as possible, and the instrument balanced to read 100 %. The illumination was then determined at different distances from the photocell. Fig. 1 shows a set of curves obtained with dilute rubber latex solutions. It is obvious that due to the extension of the 'light source' in the sample holder, the illumination of the photocell from the suspension does not vary linearly with $1/x^2$. Nevertheless, plotting the transmission values against $1/x^2$ greatly facilitates the extrapolation necessary to find values of y (Fig. 2).

From the extrapolated values, the concentration dependence of a number of quantities have been calculated and are shown in Fig. 3. It is difficult to

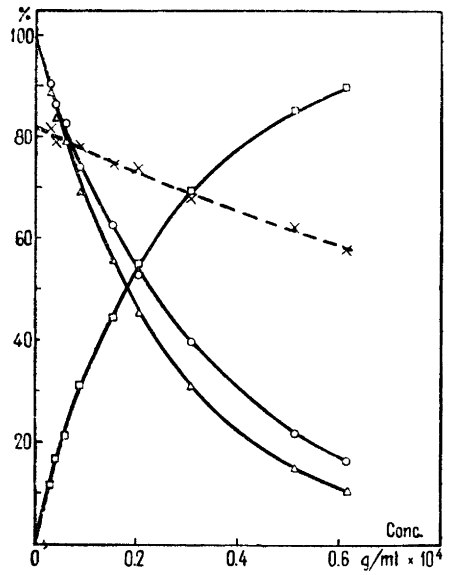

Fig. 3. Measurements on latex. Concentration dependence of different quantities calculated from measurements in Figs. 1—2.

○ T_∞/T_{x_1}
□ I_s/I_0
△ T_∞/I_0
× α_1

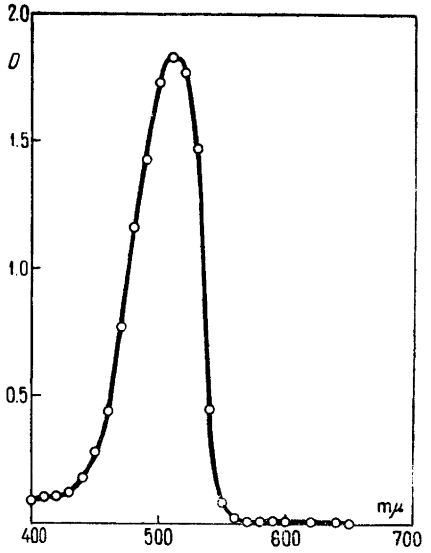

Fig. 4. Absorption curve of eosin.

calculate the value which α_1 should have if the scattering were symmetrical, but it would necessarily always be less than 50 %. The obtained values of around 80 % therefore mean, that there is a pronounced forward scattering, which decreases slightly with increasing concentration. Calculation of α_1 at infinite dilution according to (10) gives 80 %, in good agreement with the extrapolated value in the diagram. I_s/I_0 varies linearly with concentration only for rather dilute suspensions.

Experiments were made in which eosin was added to dilute solutions of latex in order to test the conclusion drawn above that the color of the liquid medium has no influence on the turbidity measurement. The absorption curve of eosin (Fig. 4) shows that this substance is well suited to experiments of this kind. It is possible, by changing the light filter, to vary the light absorption from zero to high values.

Measurements were made at two dilutions and with three filters: red, green, and blue (Table 1). Values of y, T_{x_1}/I_0, and I_a/I_0 were determined. The

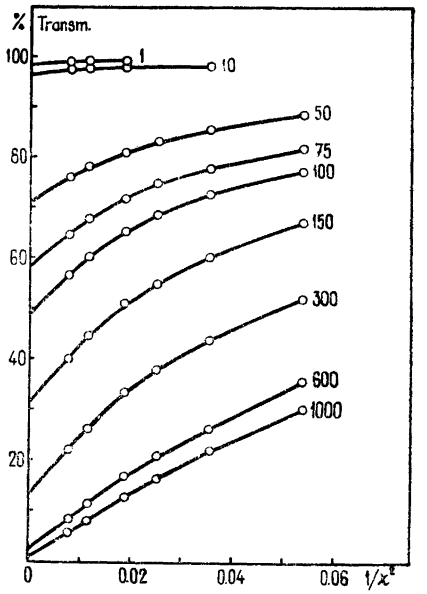

Fig. 5. Measurements on turbidity standard. Extrapolation of transmission measurements.

last column of the table shows that the percentage of scattered light is unaffected by the light absorption even at wave-lengths where the light absorption amounts to as much as 80 % of the incident light. This result proves, that the turbidity determinations are independent of the color of the suspension.

The relationship between the latex turbidities already described and the standard silica scale [6] was established by means of determinations on diluted samples of a turbidity standard no. 1 000 for water analysis (Fig. 5). The result is shown in Fig. 6. It can be seen that the turbidity of a sample to be investigated should preferably be greater than 20 p.p.m. For lower values the method is too insensitive.

If the sample holder were filled with water, the value of y differed from unity by only a few tenths of one percent, *i.e.* not more than the uncertainty of the instrument. Scattering and reflections in the glass walls therefore do not disturb the measurements.

For routine measurements the extrapolation is often unnecessary. It is sufficient to measure T_x at two positions as far apart as the instrument allows.

DETAILS OF THE MEASUREMENTS

The rubber latex ('T-Revertex') had a solids content of 61.6 % (after drying for 3 hours at 105° C). It was diluted with ammonia (approx. 5 %).

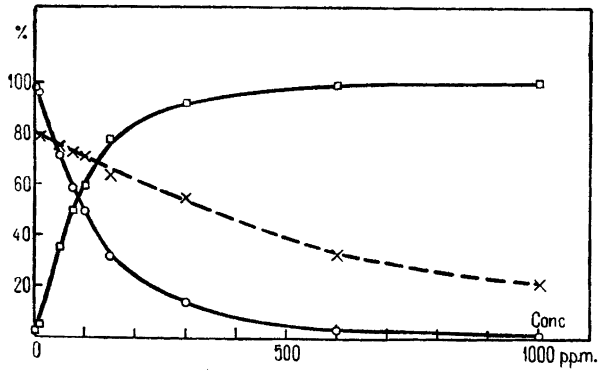

Fig. 6. Measurements on turbidity standard. Concentration dependence of different quantities.

$\circ\ T_\infty/T_{x_1}$
$\square\ I_s/I_0$
$\times\ a_1$

The turbidity standard (from Hartman-Leddon Co, Philadelphia, USA) was prepared from specially treated fullers' earth and standardized with a Jackson candle turbidimeter according to the specifications of the American Public Health Association [6]. The undiluted suspension had a turbidity corresponding to 1000 p. p. m. Dilutions were made with distilled water.

In all experiments with suspensions the light path through the suspension was 2 cm long.

Figs. 1—3. Measurements were made with filter M 550, the transmission of which lies between 530 and 570 mμ. The length x' in Fig. 1 is simply the distance the sample holder had been moved from the position closest to the photocell. By x in Fig. 2 is meant the distance from the middle of the suspension to the photocell. In this case $x = x' + 1.3$

Table 1. Comparison between turbidity measurements on latex with and without eosin. The letter 'e' in the first column indicates measurements with eosin.

Dilution of latex	Filter	T_∞/T_{x_1} %	T_{x_1}/I_0 %	$(I_s + I_a)/I_0$ %	I_a/I_0 %	$I_s/(I_0 - I_a)$ %
20 000	M 620	47.7	80.7	61.5	0	61.5
e		47.5	80.9	61.6	0.2	61.5
	M 550	40.2	77.8	68.7	0	68.7
e		41.2	42.3	82.6	47.5	66.9
	M 465	31.5	73.2	76.9	0	76.9
e		32.6	14.3	95.3	80.0	76.5
100 000	M 620	86.2	95.7	17.5	0	17.5
e		86.0	95.5	17.9	0.2	17.7
	M 550	82.9	95.5	20.8	0	20.8
e		82.5	50.0	58.7	47.5	21.3
	M 465	78.0	93.8	26.8	0	26.8
e		75.6	19.5	85.3	80.0	26.5

cm. In fact, as far as the extrapolation is concerned it is without importance from what point in the suspension the distance x is calculated, although the assumption made is the most natural.

The figures to the right of the curves indicate the dilution of the original latex. The concentration is expressed in g solids content per ml suspension.

Fig. 4. The absorption curve of eosin was measured with a Beckman Spectrophotometer, model DU, against distilled water. Concentration 0.005 % eosin in 5 % NH_3. Light path of sample holder 1.00 cm. Optical density $D = -\log^{10} I_t/I_0$.

Figs. 5—6. Same filter as in Figs. 1—3. The concentration is expressed in parts per million of silica. In order to avoid sedimentation of the particles, it was necessary to shake the suspension immediately before every measurement. Dilution with a more viscous liquid, *e. g.* glycol or glycerine, would probably have been better.

Table 1. Filters M 620 and M 465 transmit light in the intervals 610—640 mμ and 435—480 mμ resp. Concentration of eosin 0.005 %.

SUMMARY

A simple method for turbidity measurements with a commercially available photoelectric colorimeter is described. The change in apparent transmission with increasing distance between photocell and suspension is measured. The influence of color is eliminated, as is the necessary of utilizing a standard sample at each measurement. A theory has been worked out and tested by experiments with rubber latex and silica suspension. The method can be used for turbidities equal to and higher than 20 parts per million (calculated according to the silica standard scale).

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Received September 7, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1318–1325

A Potentiometric Study of the Complex Compounds between Silver and Benzoate Ions

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The object of this work was a quantitative determination of the complex compounds, formed by dissolving silver perchlorate and sodium benzoate in water and adding sodium perchlorate to a constant ionic strength. In one series an ionic strength of 0.2 C and in another 1 C was chosen.* Due to the careful work on the solubility of silver benzoate, carried out by Larsson and Adell [1], the activity coefficients are well known even in rather concentrated salt solutions, so an attempt has also been made to calculate the thermodynamic equilibrium constant of the formation of the simplest complex, *i. e.* the silver benzoate molecule itself.

EXPERIMENTAL DETAILS

Potentiometric measurements were carried out in a thermostat at 25.00° C, in the same manner as previously described by the author [2]. The potentiometer was from Leeds & Northrup, type K_1.

The silver perchlorate was prepared by dissolving newly precipitated and carefully washed silver oxide in perchloric acid, *p. a.* The surplus of silver oxide was filtered away from the resulting neutral (pH≈6) silver perchlorate solution, which was then analyzed by gravimetric determination of the silver ions as silver chloride. Due to the high solubility of silver perchlorate in water, no purification by recrystallization was attempted. The analyzed stock solution of silver perchlorate was about 3 C, and all the solutions of silver perchlorate were prepared from this stock by dilution.

The sodium perchlorate, *purum*, was purified by recrystallization until it became free from chloride. The recrystallization was carried out at such a high temperature (>60° C) that a nonaqueous salt resulted. From this product stock solutions were prepared.

* C stands for gram formula weight per liter.

Stock solutions of sodium benzoate were obtained by careful neutralization of a known amount of recrystallized benzoic acid, *p. a.*, with carbonate free sodium hydroxide solution. Attempts to recrystallize sodium benzoate directly seemed to indicate that it was very difficult to obtain a chloride free product in this way.

Mercury electrodes in 1 C or 0.2 C solutions of sodium perchlorate, saturated with mercurous sulphate, were used as reference electrodes.

Brown's [3] silver-silver chloride electrodes were used to measure the silver ion concentrations. These electrodes functioned well also in benzoate solutions. The reproducibility of the electromotive forces decreased with increasing benzoate concentrations, but that was probably due to the increasing liquid junction potentials of the cells. When junction solutions were used, 1 or 0.2 C $NaClO_4$ respectively, the emf of a cell could be reproduced with an accuracy of 0.3—0.4 mV at the highest benzoate concentrations, but with less deviations with decreasing amounts of benzoate in the solution.

MEASUREMENTS AND CALCULATIONS OF THE EQUILIBRIUM CONSTANTS

The measurements at an ionic strength 1 C are shown in Table 1. The solutions in question were produced by adding portions of a 1 C sodium benzoate solution from a burette to an exactly known volume of an initial solution, containing 0.000950 C $AgClO_4$ + 0.999 C $NaClO_4$ or 0.00295 C $AgClO_4$ + 0.997 C $NaClO_4$ in the electrode vessel. After every portion added from the burette, the emf of the cell was measured. In Table 2 the measurements at the ionic strength 0.2 C are to be found. Here the 'titration' was performed in the same way with a 0.2 C solution of sodium benzoate in the burette and an initial solution in the electrode vessel of 0.00299 C $AgClO_4$ + 0.197 C $NaClO_4$. The emf E in the tables is the difference between the emf of the initial cell free from benzoate, and the emf of the same cell after the addition of benzoate.

c_{Ag} and c_B represents the total concentration of silver and benzoate respectively, while $[Ag^+]$ and $[B^-]$ is the concentration of free silver and benzoate ions. $[Ag^+]$ is calculated from Nernst's formula, which for our purpose may be written:

$$E_{corr} = 59.16 \log \frac{c^0_{Ag}}{[Ag^+]} \qquad \text{I}$$

where c^0_{Ag} is the silver concentration of the 'benzoate free' initial solution and E_{corr} the already mentioned emf E, corrected for the liquid junction potential according to Henderson (see *e. g.* Bjerrum and Unmack [4]). For the calculation of this correction the ionic mobility at infinite dilution has been used, *viz.* $u_{Na} = 50.1$, $v_{ClO_4} = 68$ and $v_B = 32.5$, *i. e.* the values in the tables of Landolt-Börnstein. In consequence of the great difference between the mobilities of benzoate and perchlorate ions, this correction becomes exceptionally great and probably rather uncertain. By putting in a suitable salt bridge between

Table 1. Titration with 1 C sodium benzoate. Ionic strength 1 C.

c_{Ag} mC	c_B mC	E mV	E_{corr} mV	$[Ag^+]$ mC	$[B^-]$ mC	$F_1(B^-)$ C^{-1}
0.950	0	0	0	0.950	0	× *
0.941	9.70	1.2	1.1	0.910	9.67	3.4
0.932	19.22	2.3	2.1	0.875	19.16	3.4
0.914	37.9	4.4	4.1	0.809	37.8	3.44
0.897	55.9	6.4	6.0	0.752	55.7	3.46
0.864	90.4	10.3	9.6	0.654	90.2	3.56
0.849	106.7	12.1	11.3	0.612	106.4	3.64
0.820	137.3	15.4	14.3	0.545	137.0	3.68
0.792	166.1	18.7	17.4	0.483	165.8	3.86
0.732	229.9	25.5	23.7	0.378	229.5	4.20
0.679	284.9	31.5	29.2	0.306	284.5	4.28
0.595	374	41.1	38.1	0.215	373	4.74
0.529	443	48.9	45.3	0.163	442	5.08
0.476	499	54.9	50.8	0.132	498	5.17
2.95	0	0	0	2.95	0	⊙ *
2.93	9.70	1.2	1.1	2.83	9.60	3.3
2.89	19.22	2.3	2.1	2.72	19.1	3.3
2.84	37.9	4.6	4.3	2.50	37.5	3.6
2.79	55.9	6.7	6.3	2.31	55.4	3.7
2.68	90.4	10.6	9.9	2.01	89.7	3.7
2.64	106.7	12.3	11.5	1.89	105.9	3.75
2.54	137.3	15.5	14.4	1.69	136.4	3.7
2.46	166.1	18.7	17.4	1.50	165.1	3.9
2.27	229.9	25.3	23.5	1.18	228.7	4.0
2.11	284.9	31.0	28.7	0.967	283.7	4.2
1.847	374	40.5	37.5	0.685	373	4.5
1.642	443	48.1	44.5	0.522	442	4.85
1.474	499	54.2	50.1	0.420	498	5.0
1.180	600	66.2	61.3	0.272	599	5.6

the two half-elements of the cells, the liquid junction potential might have been diminished, but at the same time it would have been quite impossible to estimate the necessary correction.

The liquid junction potential was calculated for the boundaries

$$1\ C\ NaClO_4 \left| \begin{matrix} x\ C\ NaB \\ (1-x)\ C\ NaClO_4 \end{matrix} \right. \text{(Table 1) and } 0.2\ C\ NaClO_4 \left| \begin{matrix} x\ C\ NaB \\ (0.2-x)\ C\ NaClO_4 \end{matrix} \right. \text{(Table 2)}$$

* These signs are used to distinguish $F_1(B^-)$-values, connected with different c_{Ag} (*cf.* Fig. 1).

Table 2. Titration with 0.2 C sodium benzoate. Ionic strength 0.2 C.

c_{Ag} mC	c_B mC	E mV	E_{corr} mV	$[Ag^+]$ mC	$[B^-]$ mC	$F_1(B^-)$ C^{-1}
2.99	0	0	0	2.99	0	●
2.96	1.94	0.5	0.4	2.94	1.90	3.6
2.93	3.84	1.2	1.0	2.88	3.77	4.5
2.88	7.58	2.2	2.0	2.78	7.47	4.8
2.82	11.18	3.0	2.6	2.70	11.03	4.0
2.72	18.08	4.9	4.2	2.54	17.87	4.0
2.67	21.34	5.8	4.9	2.47	21.08	3.8
2.58	27.5	7.6	6.5	2.32	27.2	4.1
2.49	33.2	9.2	7.9	2.20	32.8	4.0
2.30	46.0	12.8	11.0	1.95	45.5	4.0
2.14	57.0	16.3	14.0	1.73	56.5	4.2
1.872	74.8	22.3	19.2	1.42	74.2	4.3
1.664	88.6	27.1	26.2	1.20	88.0	4.4
1.498	99.8	31.3	27.2	1.04	99.2	4.4

for some round values of the benzoate concentration x. The correction was then drawn in a diagram as a function of $x = c_B$. In such a diagram, the correction could be interpolated for the c_B values of the tables. The influence upon the liquid junction potential by the small amounts of silver in the solutions has thus been neglected. The correction is largest at the ionic strength 0.2 C, where a greater percentage of perchlorate is exchanged for benzoate. One advantage of working at a high ion concentration, *e. g.* 3 C, is that there are less liquid junction potentials; but, on the other hand, the calculations of the activity coefficients turn out to be more difficult at increasing concentrations. The curves, describing the variation of the activity coefficients, regarded as functions of the ionic concentration, are perhaps also steeper at a concentration of 3 C, as the activity coefficients often have a minimum about 0.5 or 1 C, where it thus ought to be easiest to maintain constant activity conditions.

The benzoate ion concentration, $[B^-]$, has been calculated by the methods previously described [2]. The calculation is very simple in the present case, since c_{Ag} is small and the complex compounds are weak, and therefore $[B^-]$ is nearly equal to c_B. The little correction that must be done, is limited by $(c_{Ag} - [Ag^+])$ and $2(c_{Ag} - [Ag^+])$, and as $(c_{Ag} - [Ag^+])$ is so small compared to c_B, it is of very little importance if c_B is corrected with $(c_{Ag} - [Ag^+])$ or twice this quantity when calculating $[B^-]$.

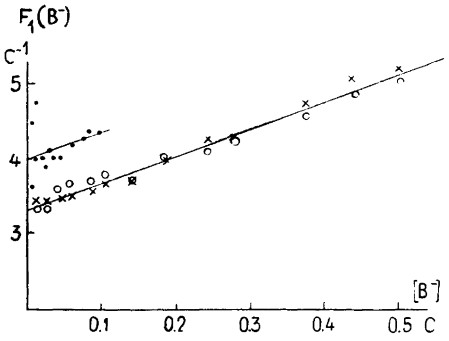

Fig. 1. $F_1\,(B^-)$ *in the ion media 0.2 and 1 C* $NaClO_4$.

The points: $F_1\,(B^-) = \dfrac{c_{Ag} - [Ag^+]}{[Ag^+] \cdot [B^-]}$

The lower line (1 C): $F_1\,(B^-) = 3.3 + 3.6\,[B^-]$. *The upper line (0.2 C) is drawn parallel to the lower one, as* β_2 *is perhaps relatively independent of the ionic strength.*

$F_1(B^-)$, in the tables, is the function previously used by the author to calculate the complexity constants β_n [2]. At the ionic strength 1 C, $F_1(B^-)$ can be described by a straight line within the limits of unavoidable errors of measurements (*q. v.* Fig. 1). The diagram gives $\beta_1 = 3.3 \pm 0.1$ C^{-1} and $\beta_2 = 3.6 \pm 0.5$ C^{-2} as values of the complexity constants of AgB and AgB_2^- respectively. The attainable range of measurements is too small to let us conclude with certainty that $F_1(B^-)$ does not deviate from a straight line. Such a deviation would mean that complex ions with more than two benzoate ligands should exist in the solutions. At the ionic strength 0.2 C the attainable range of c_B is still less, and therefore not even the slope of the line $F_1(B^-)$ can be settled. Thus only $\beta_1 = 4.0 \pm 0.2$ C^{-1} is calculated from the measurements in Table 2.

THE THERMODYNAMIC EQUILIBRIUM CONSTANT β_1^0

The calculated values of β_1 are concentration constants, valid only in the corresponding ionic conditions, *i. e.* 0.2 and 1 C sodium perchlorate solutions. Thus

$$\beta_1 = \frac{[AgB]}{[Ag^+]\,[B^-]} \qquad \text{II}$$

The real equilibrium constant β_1^0 is defined by $\beta_1^0 = \dfrac{(AgB)}{(Ag^+)\,(B^-)}$, III

if, as usual, (s) means the activity and [s] the concentration of a substance s. β_1^0 can be calculated, if the activity coefficients, f_s, are known in the ionic media used, as

$$\beta_1^0 = \frac{f_{AgB}}{f_{Ag} \cdot f_B} \cdot \frac{[AgB]}{[Ag^+]\,[B^-]} = \frac{f_{AgB}}{f_{Ag} \cdot f_B} \cdot \beta_1 \qquad \text{IV}$$

The mean activity coefficient of silver benzoate, at 18° C $f = \sqrt{f_{Ag} \cdot f_B}$, has been determined by Larsson and Adell [1] in several salt solutions. At an ionic concentration of 0.2 C these authors found -log $f = 0.14$, *i. e.* $f^2 = 0.52$, determined in solutions of sodium-, potassium- and barium nitrate with insignificant deviations. At the ionic concentration 1 C they found -log f = 0.21, *i. e.* $f^2 = 0.38$, determined in sodium and potassium nitrate. The authors, it is true, assumed the silver benzoate to be completely dissociated, but their method of extrapolation might partly compensate the rather insignificant error that was caused by the small amounts of undissociated silver benzoate, which existed in their solutions notwithstanding the small concentrations of benzoate and silver ions.

The calculated mean activity coefficients thus seem to be rather independent of the nature of the ionic conditions and therefore ought to be valid in solutions of sodium perchlorate. The fact that Larsson and Adell apparently found very differing coefficients in acetate solutions, can now be accounted for by the formation of complex compounds between silver and acetate ions, taking place to a great extent in the concentrated acetate solutions also examined by the authors [Cf. 2]. As activity coefficients are known to vary only slowly with changing temperature, the coefficients, determined by Larsson and Adell at 18°, can also be used at a temperature of 25° in the following approximate calculations.

The activity coefficient, f_{AgB}, of the uncharged compound AgB is not known, but its value is probably nearly equal to 1. Larsson [5] determined the activity coefficient of the undissociated molecule HB of benzoic acid in several salt solutions and found that this coefficient was very little influenced even by great concentrations of sodium perchlorate, contrasting to other salt media investigated.

Thus if $f_{AgB} = 1$, and $f_{Ag} \cdot f_B = f^2$ is taken from [1], β_1^0 can be calculated from equation IV as follows. From the measurements at the ionic strength 0.2 C: $\beta_1^0 = 4.0/0.52 = 7.7 \pm 1$, and at 1 C: $\beta_1^0 = 3.3/0.38 = 8.7 \pm 0.5$, or as a mean $\beta_1^0 = 8.2 \pm 1$ C^{-1}. The accordance of the two calculated values of β_1^0 is as good as can be expected, especially in view of the uncertainty of the liquid junction potential, which is most pronounced at an ionic strength of 0.2 C. The value of this potential, calculated according to Henderson *(l. c.)*, is perhaps to be regarded more as an approximate measure of the possible error of the measured emfs than as a quantitatively reliable correction. By calculating β_1 at the ionic strength 0.2, without any correction for the diffusion potential, we arrive at a value of 4.6 instead of 4.0.

Kolthoff and Bosch [6] have determined the silver ion activity by potentiometric measurements in solutions, saturated with silver benzoate. They

draw only the qualitative conclusion that silver benzoate is not completely dissociated. From their measurements, however, an approximate value of β_1^0 can be computed. By dissolving silver benzoate in destilled water at 25°, the authors determine the solubility $c_{Ag} = 0.01162$ C and the silver ion activity $(Ag^+) = 0.009462$ C. The latter value was assigned an uncertainty of 2 %, arising from an assumed fault of 0.5 mV in the emf measurements. From these figures the authors calculated the apparent activity coefficient of the silver ion to be $0.009462/0.01162 = 0.813$. This value is so much less than the theoretical value, calculated according to the limit law of Debye and Hückel, *i. e.* $-\log f_{Ag} = 0.5 \sqrt{0.01162}$, giving $f_{Ag} = 0.883$, that the authors concluded that the silver benzoate was incompletely dissociated. From the cited values, we can calculate β_1^0, assuming that the real activity coefficients can be calculated from the limit law of Debye and Hückel and that AgB is the only complex compound in such a diluted solution ($[AgB_2^-]$ ought to be less than 1 % of [AgB], if the values of β_1 and β_2 of the present paper are approximately correct). Then the following equations are valid:

$$\begin{cases} [Ag^+] \cdot f_{Ag} = 0.009462 \\ -\log f_{Ag} = 0.5 \sqrt{[Ag^+]} \end{cases}$$

an equation system, giving $[Ag^+] = 0.01066$ and $f_{Ag} = 0.887$. Thus the concentration of undissociated silver benzoate $[AgB] = c_{Ag} - [Ag^+] = 0.00096$, and $\beta_1^0 = [AgB]/(Ag^+)^2 = 10.7 \pm 2.5$ C^{-1}, where the proposed uncertainty corresponds to an uncertainty of 2 % in (Ag^+). The value, calculated from the measurements of Kolthoff and Bosch, thus coincides within the limits of experimental error with β_1^0 from the present paper.

SUMMARY

1. By 'potentiometric titrations' of silver perchlorate in 1 C $NaClO_4$ with 1 C sodium benzcate (NaB) the existence of the complex compounds AgB and AgB_2^- was proven and their complexity constants determined to $\beta_1 = 3.3 \pm 0.1$ C^{-1} and $\beta_2 = 3.6 \pm 0.3$ C^{-2} (concentration constants) at 25° C.

2. Complexes containing more than two ligands, *e. g.* AgB_3^{2-}, could not be discovered. It cannot, however, be absolutely denied that such compounds might exist in small concentrations in the solutions examined, and that they thus might be dominant at sufficiently high concentrations of benzoate ions.

3. Neither did binuclear compounds of the type Ag_2B^+, Ag_2B_2, $Ag_2B_3^-$ *etc.* exist in detectable concentrations, as is seen from the fact that the function

$F_1(B^-)$ in Table 1 or Fig. 1 is independent of c_{Ag} [Cf. 2]. As some of the solutions investigated are supersaturated with silver benzoate, such polynuclear compounds could never be formed in aqueous solutions in detectable concentrations, except possibly in those solutions made by dissolving silver benzoate in concentrated solutions of a very soluble silver salt.

4. In 0.2 C $NaClO_4$ β_1 has been determined to be 4.0 ± 0.2 C^{-1}.

5. By the aid of the mean activity coefficient of silver benzoate, found in the literature, the thermodynamic equilibrium constant β_1^0 has been calculated to be 8.2 ± 1 C^{-1}.

Finally I wish to express my gratitude to Prof. Erik Larsson, the head of the Institution of Organic Chemistry of Chalmers' Institute of Technology, who made the present work possible by placing the precious potentiometer of his institution at my disposal. I also wish to thank Mrs. Barbro Aggeryd, who has carried out a great part of the laboratory work for this paper.

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Received September 9, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1326–1328

The Structure of DL- and D-Leucine

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It would appear possible by a comparison of the crystal structures of optically active compounds with those of the corresponding racemic forms, to obtain some information about the rôle played by the D- and L-molecules in the latter *e. g.* whether they are distributed at random in solid solution or whether distinct pairs of DL-molecules do exist. For this reason and also in order to see how α-amino acid molecules with relatively large branched carbon chains may be packed together, we have examined the crystal structure of DL-, L- and D-leucine by x-rays. Although we have not been able to determine the complete structure, some results have been obtained which may be of interest.

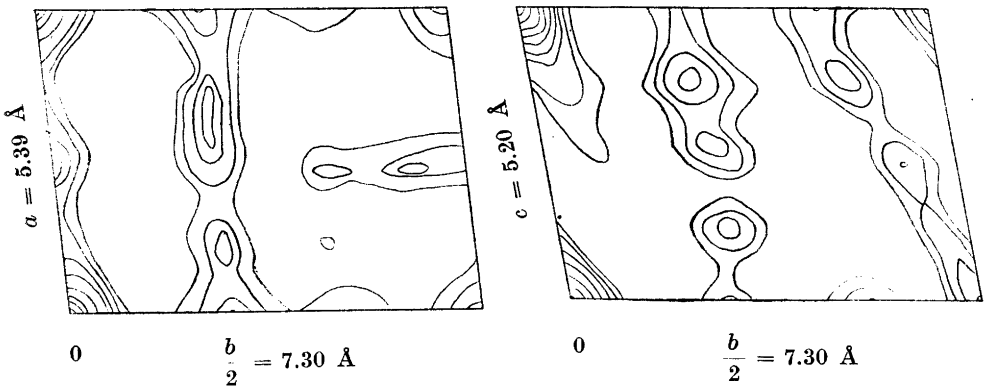
Oscillation- and Weissenberg photographs were taken of several crystals about different axes with CoKα and CuKα radiation, a Buerger-Weissenberg camera of 57.3 mm diameter being used. The density of the crystals was determined by the flotation method in mixtures of xylene and ethylene chloride.

DL-Leucine

Crystals of a preparation from Schering-Kahlbaum were used for the experiments. They were thin tablets with angles of approx. 70 and 110° between the adjacent sides. The extinction directions were respectively parallel and perpendicular to the one of these which was chosen as a-axis. The refractive indices parallel and perpendicular to the a-axis were measured in natural light by embedding the crystals in mixtures of paraffin and 1-bromonaphthalene. Axial dispersion could be observed.

$$\alpha' \sim n_{\parallel a} : 1.525 \qquad \gamma' \sim n_{\perp a} : 1.552$$

The crystals were triclinic with a density of 1.18 g/cm^3. The unit cell dimensions were: $a = 5.39$ Å, $b = 14.6$ Å, $c = 5.18$ Å and the angles between the axes: $\alpha = 103°$; $\beta = 111.5°$, $\gamma = 96°$. There are two molecules in the unit



Figs. 1 and 2. The Patterson-function of DL-*leucine parallel-projected on the ab- and bc-planes.*

cell. Systematic absences of reflections were not observed, and, as the crystals turned out to be piezoelectric according to a test performed by Mr. E. Blomgren, Uppsala, the space group is P 1.

Patterson projections were calculated on the b^* c^* and a^* b^* planes from the visually estimated intensities, but too many maxima appeared and made an interpretation very difficult. Strong maxima, however, corresponding to the distances 2.75 and 2.90 Å at about right angles to each other were present. They probably are the O_I—(H)—O_{II} and N_IH_2—(H)—O_{II} distances, while another set of maxima from 3.60—4.00 Å corresponds to the C_IH_3—$C_{II}H_3$-distances. The Patterson projections indicate a layer structure with the molecules nearly parallel to the b-axis.

An attempt was made to calculate the electron projection on the b^*-axis from the first eleven orders of the ($0k0$)-reflections. The signs were obtained by calculations on models based on previous investigations of amino acids [1, 2]. From the one-dimensional electron projection, mean values of the y-parameters of the atoms in the two molecules could be obtained, and by using Robertson and Woodward's empirical atomic scattering factors [3], the ($0k0$)-intensities were calculated. Smaller displacements of the atoms were tried. The y-parameters giving best agreement between calculated and observed intensities were:

$C'H_3$: $\pm$ 0.030, $C''H_3$: $\pm$ 0.110, CH: $\pm$ 0.115, CH_2: $\pm$ 0.215, CH: $\pm$ 0.285, C: $\pm$ 0.380, NH_2: $\pm$ 0.220, O': $\pm$ 0.390, O'': $\pm$ 0.430. — And the relative intensities were:

(0k0)	010	020	030	040	050	060	070	080	090	0100	0110
Calculated intensity	72	6	1	46	78	79	1	7	5	19	4
Observed »	v.s	w	v.w	s	v.s	s	—	—	w-m	w-m	w

From the optical anisotropy one would expect the COO-groups to be approximately perpendicular to the a-axis.

A calculation of the two-dimensional electron projections was attempted, again neglecting the lack of a centre of symmetry. The intensities calculated from the atomic parameters obtained in this way, however, did not agree very well with the experimental ones.

The crystals from Schering-Kahlbaum were exceedingly twinned on the (010)-plane. This was deduced from the fact that all reflections except the (0k0)-ones were doubled in such a way that upon constructing the reciprocal lattice, two sets of points resulted, one being obtained from the other by a rotation of 180° about the b^*-axis. A less pronounced twinning about the a-axis, also visible in the reciprocal lattice, sometimes makes the crystals look like irregular hexagons. These twin-formations may be easily understood if one pictures the structure as consisting of double layers of molecules stacked together with the methyl groups from neighbouring layers towards each other at distances of 3.6—4.0 Å, while the polar groups, COO^- and NH_3^+, make strong bonds of length 2.75—2.90 Å between and in the two single layers. — This also explains why the crystals do not wet, since, clearly, the CH_3-groups must stick out.

D-Leucine

Dr. S. E. Darmon, Colloid Science Lab., Cambridge, kindly supplied me with some very good crystals. They too were tablets, but with a shape different from the DL-leucine.

The crystals were orthorhombic in agreement with previous optical investigations [4,5]. Cell dimensions were: $a = 5.36$ Å. $b = 14.7$ Å, and $c = 9.65$ Å, with the axes chosen in accordance with those of DL-leucine. From the density of 1.17 g/cm^3, we get four molecules per unit cell. (A reference book [5] gives the density as 1.29 g/cm^3 but this figure leads to the improbable value $z = 4.5$). From systematic absences of reflections, the space group may be P 2_122_1, but P 2_122 and P 222 cannot be excluded. It is interesting to note that the a- and b-axes are nearly identical in DL- and L-leucine and the twinning of the DL-leucine crystals makes them simulate the macroscopic symmetry of the D-leucine.. Furthermore, the (0k0)-reflections from the two kinds of

crystals have very similar intensities and, in fact, the electron projections on the b*-axis calculated from these show maxima for practically the same values of y. Hence the D-leucine crystals are also built up of double layers in a similar way to DL-leucine.

Some single crystals of a L-leucine preparation from S. A. Hoffmann-La Roche and Co. were also examined. They, however, gave x-ray-diagrams identical with those of DL-leucine except that reflections due to twin-formation did not appear on the equator diagram and that some extra, relatively weak, reflections accompanied the (0k0)-reflections showing the same variation in intensity as these. These diagrams show that crystals of DL-leucine have been present in the L-leucine preparation and that there are built in (0k0)-layers of orthorhombic L-leucine in them. This is in accordance with the powder photographs of DL- and L-leucine: Nearly all lines on the latter may be indexed on the basis of the orthorhombic unit cell, but some lines, which correspond to the strongest lines on the powder photograph of DL-leucine, still remain. Fractional recrystallization of L-leucine gave, as less soluble produet, orthorhombic single crystals with x-ray diagrams similar to those of D-leucine. — The reason that other extra (hk0)-reflections are not easily observed may be that they are weaker and very nearly coincide with the somewhat drawn out (hk0)-reflections from DL-leucine.

In order to ascertain if the triclinic DL-leucine was a true racemic compound, the solubilities were determined.

10 g	aqueous	solution	saturated	with	DL-leucine	contain	0.104 g	leucine
10 »	»	»	»	»	L- »	»	0.231 »	»
10 »	»	»	»	»	L and DL »	»	0.259 »	»

The triclinic DL-leucine thus appears to be a true racemic compound with one D- and one L-molecule in the unit cell, arranged in such a way that no centre of symmetry results. This is not in accordance with the assumption, based on previous investigations, that one must be able to bring the molecules of the two antipodes in a true racemic compound to coincide by means of a symmetry operation [6]. It is tempting to regard the twin-formation as a substitute for the desired symmetry operation.

As characteristic features of this amino acid in the solid state may be mentioned the low symmetry of the DL-form, the layer structure along the b-axis, and the ease with which 'mistakes' are introduced into the sequence of the (0k0)-layers.

SUMMARY

X-ray investigation shows that crystals of DL-leucine are triclinic with unit cell dimensions $a = 5.39$ Å, $b = 14.6$ Å, $c = 5.18$ Å, $\alpha = 103°$, $\beta = 111.5°$, $\gamma = 96°$ and 2 molecules per unit cell while those of D-leucine are orthorhombic with $a = 5.36$ Å, $b = 14.7$ Å, $c = 9.65$ Å, and 4 molecules per unit cell. Although it has not been possible to determine the structure completely it is inferred from two-dimensional Patterson- and one-dimensional electron-projections that the crystals in both cases are built up of double-layers consisting of two layers of molecules with the methyl groups pointing outwards. Different kinds of disorder structure have been observed and may be explained by the forces between adjacent double-layers being weak. The symmetry of the DL-form is lower than expected.

I am indebted to the University in Copenhagen for a grant, to Dr. S. E. Darmon, Cambridge, for supplying me with crystals and to Mr. E. Blomberg, Uppsala, for performing the tests for piezoelectricity. I also wish to express my cordial thanks to Professor N. Bjerrum and Dr. A. Tovborg Jensen for much help and many valuable discussions.

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Received September 12, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1331 – 1342

Enzymatic Breakdown of Polymetaphosphate

IV. The Activation and the Inhibition of the Enzyme

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It has been known for several years that some phosphatases are activated by ions of bivalent metals. In 1927 Erdtman [1, 2] found that phosphatase from kidney was activated by magnesium ions. Quantitative measurements on the activation of this enzyme by Mg^{++} ions were carried out by Jenner and Kay [3] some years later, and in 1940 Bamann and Heumüller [4] published an investigation on the action of some bivalent cations on the alkaline phosphatase from liver using $Na_4P_2O_7$, $Na_3(PO_3)_3$, $Na_5P_3O_{10}$ and glycerophosphate as substrate. However, the activating effect of bivalent cations is not restricted to the enzymes from liver and kidney; the polymetaphosphate degrading enzymes from moulds and yeasts are also largely influenced. This paper will deal with the action of metal ions on these enzymes and with some experiments performed with compounds reacting specifically towards certain essential groups of enzymes.

The enzymes used in the experiments were extracted from *Aspergillus niger* and *Saccharomyces cerevisiae* (baker's yeast). For details of the enzyme preparation see Ingelman and Malmgren [5, 6]. In these experiments a high molecular potassium metaphosphate $(KPO_3)_n$ — designated as K 15 — was used as substrate, the molecular weight of which was more than one million. The polymetaphosphate is prepared by heating primary potassium orthophosphate.

$$n\,KH_2PO_4 \longrightarrow (KPO_3)_n + n\,H_2O$$

This polymetaphosphate is insoluble in water but soluble, for instance, in sodium salt solutions of suitable concentration. For particulars concerning the synthesis and properties of these substances see [7, 8]. 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:

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

is used, where η_{sp} = specific viscosity and t = time. For a more detailed discussion of this method of calculation, see Ingelman and Malmgren [7].

INFLUENCE OF CATIONS ON THE ENZYME ACTIVITY

The enzymes in question are activated by some metal ions and inhibited by others. However, the measurements are complicated by the properties of the substrate, which is an electrolyte with colloidal properties due to the anion. The viscosity of a colloid of this type is not only dependent on the concentration of the substance but on the concentration and nature of the salts of low molecular weight which are also present. The effect of these salts is to cause a diminution of the solvation and of the charge on the anion; both of which factors contribute largely to the viscosity. One might expect the charge effect of the anion to be depressed in the presence of cations — the decrease being greater the higher the valency of the cations added. However, on reduction of the charge probably also the shape of the substrate is changed [8]. (A more detailed investigation on the physico-chemical properties of the substrate will shortly be published in *Acta Chem. Scand.*) Fig. 1 shows the variation of the intrinsic viscosity $(\eta_{sp}/c)_{c\to 0}$ as a function of the atomic quotient: bivalent metal/phosphorus (Me:P) for some metals. The measurements have been performed in acetate buffer of ionic strength 0.3 and pH 5.3.

As can be seen from Fig. 1 the values of the intrinsic viscosity do not differ very much from that of a metal-free solution as long as the quotient Me:P is not too great. Most bivalent metals from complexes of great stability with the polymetaphosphate and only a minor part of the metal is present in a free ionic state. In fact, the concentration of the Me^{++} *ions* in the solutions is very small. However, if the quotient Me:P exceeds a certain value the Me-polymetaphosphate is precipitated. For most metals, *e.g.* Mg and Ca a

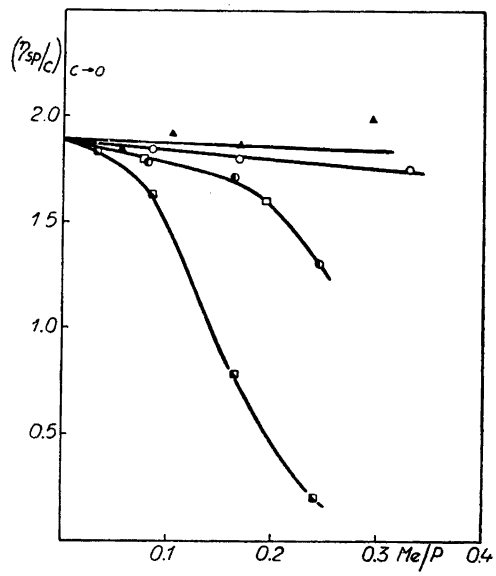

Fig. 1. $(\eta_{sp}/c)_{c\to 0}$ *as a function of Me/P for some metals* ▲ = *Zn*; ○ = *Mg*; □ = *Mn*; ◐ = *Ca*; ◹ = *Pb*

permanent precipitate is formed when Me:P $\sim$ $^1/_3$*. This value seems to vary a little depending on the metal added. The equilibrium:

bivalent metal ion + polymetaphosphate $\rightleftharpoons$ complex anion

is lagerly displaced to the right. (It should be noted that the monovalent silver ion forms complexes with the polymetaphosphate.)

The experiments have been performed as follows. The activity of the enzyme is determined when using a solution of K 15 of the following composition (50 mg K 15 + 10 ml buffer + 2 ml 0.15 *M* $NaNO_3$) as substrate. To 5 ml of this solution 1 ml of the enzyme solution is added and the deter-

* A rough estimation of the order of magnitude of the Me^{++} concentration may be made from the knowledge of the solubility products of some slightly soluble salts of the metal in question. Thus using the solubility products of the oxalates, the following values were found with respect to the substrate solutions

$$C_{Zn}^{++} < 10^{-7};\ 10^{-7} < C_{Ca}^{++} < 10^{-6};\ \text{at pH } 5.4$$

A review of the literature dealing with complex-formation between alkaline earths and heavy metals and a sodium polymetaphosphate called Graham's salt (in principle not differing very much from the substrate of this work though being of lower molecular weight) is given by Karbe and Jander [9].

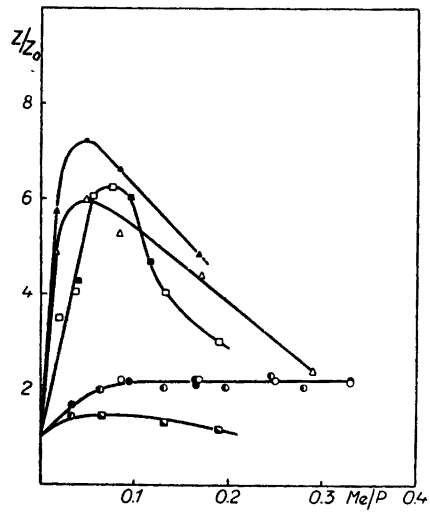

Fig. 2. z/z_0 as a function of Me/P for some metals (A. niger; pH = 5.4).
▲ = *Zn (serie I)*; ● = *Mg (serie I)*
△ = *Zn (serie II)*; ○ = *Mg (serie II)*
■ = *Mn (serie I)*; ◐ = *Ca*; ◹ = *Pb*;
□ = *Mn (serie II)*.

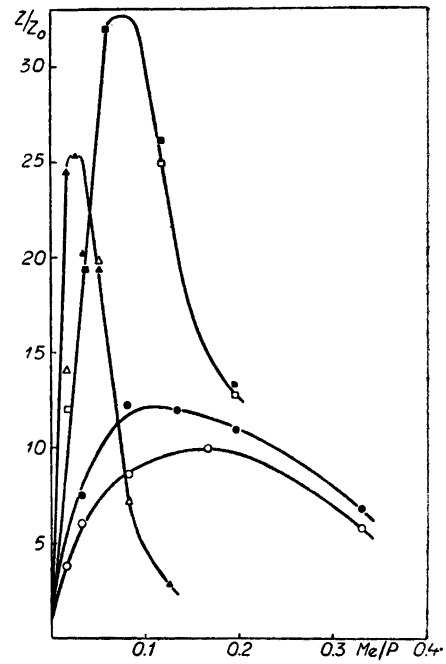

Fig. 3. z/z_0 as a function of Me/P for some metals (A. niger; pH = 6.6).
▲ = *Zn (serie I)* ■ = *Mn (serie I)*
△ = *Zn (serie II)* □ = *Mn (serie II)*
● = *Mg (serie I)*
○ = *Mg (serie II)*

mined activity is designated as z_0. Then the substrate solution is changed by substituting part of the sodium nitrate with 0.15 *M* $Me(NO_3)_2$ its composition being 50 mg K 15 + 10 ml buffer + *a* ml 0.15 *M* $Me(NO_3)_2$ + (2 — *a*) ml 0.15 *M* $NaNO_3$ (*a* changing from $\sim$ 0.05 — 1.00) and the enzyme activity is determined as usual. (The measurements of the activation of a certain metal were completed in a few hours to ensure that the z_0-value remained unchanged.) The influence of the following metals has been investigated Mg, Ca, Zn, Mn, Fe, Ba, Pb, Hg and the monovalent metals Tl and Ag.

Aspergillus niger. The enzyme from *A. niger* is completely inhibited by Ag^+ and Hg^{++}-ions * but activated by ions of the other metals mentioned above. In Figs. 2 and 3 the z/z_0-values, *i. e.* the activation, have been plotted as a

* The mecury-organic compound 'merthiolat' does not inhibit the enzyme.

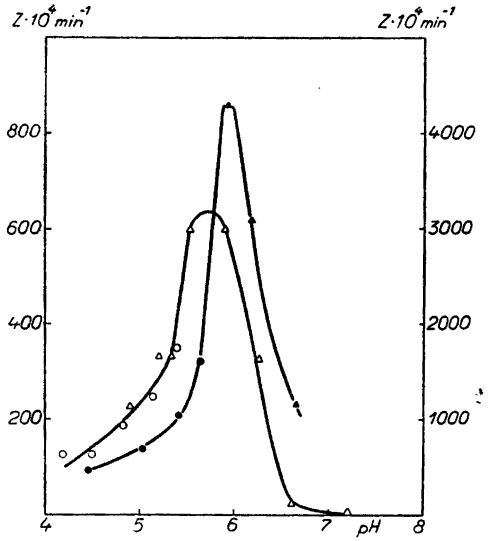

Fig. 4. z-values as a function of pH. Ordinata to the left: no activator; ○ = acetate buffer; △ = phosphate buffer. Ordinata to the right: activation by Mn^{++} ions; ● = acetate buffer; ▲ = phosphate buffer.

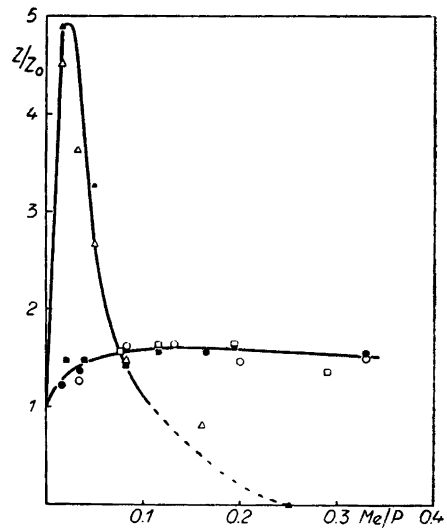

Fig. 5. z/z_0 as a function of Me/P for some metals (S. cerevisiae; pH = 6.6).
▲ = Zn (serie I); ■ = Mn (serie I);
△ = Zn (serie II); □ = Mn (serie II);
● = Mg (serie I);
○ = Mg (serie II).

function of the atomic quotient Me/P for some metals of biochemical importance, *viz.* Mg, Ca, Mn, Zn and Pb; the last mentioned metal being an activator instead of an inhibitor which was not expected. The medium used was acetate buffer of ionic strength 0.3 and pH 5.4 (Fig. 2), pH = 6.3 (Fig. 3).

The fact that zinc is a better activator than magnesium is concordant with the observation by Pett and Wynne[10] who state that zinc replaces magnesium as activator of phosphatases from bacteria. Under similar conditions the activation by zinc ions, for example, on the enzymes from *A. niger* and *Saccharomyces cerevisiae* is by no means identical; a fact justifying a suggestion that these enzymes are two different chemical entities. This is supported by the observed difference between the pH-optima[6].

The activating effect of a given metal ion seems to increase when the pH is changed towards the alkaline side, all other factors such as substrate and enzyme concentration, ionic strength, Me/P ratio etc. being constant. Thus, in the presence of a good activator *i.e.* Mn^{++} the pH-optimum is displaced a little towards the alkaline side. This is illustrated by Fig. 4 showing

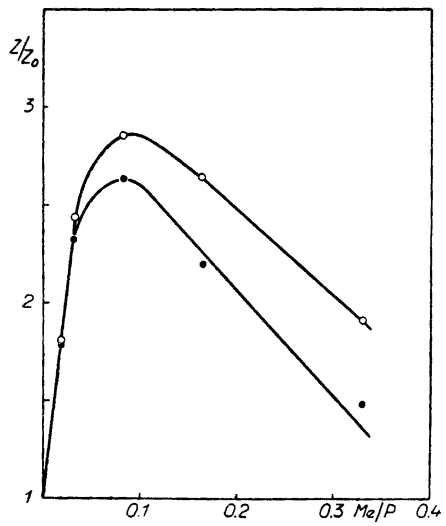

Fig. 6. z/z_0 as a function of Mg/P; (S. cerevisiae; pH = 7.3).
● = *serie I*
○ = *serie II*

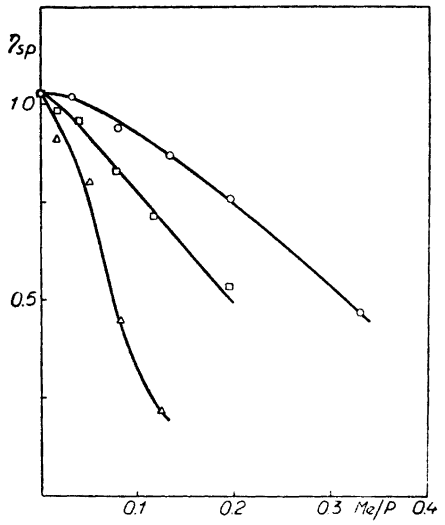

Fig. 7. η_{sp} as a function of Me/P for some metals. 0.42 % solutions in acetate buffer of pH = 5.4.
○ = *Mg*; □ = *Mn*; △ = *Zn*.

the pH-optima of *A. niger* enzyme in the presence and absence of Mn^{++} ions. The quotient Mn/P is 1 : 10.

Saccharomyces cerevisiae. The enzyme from *S. cerevisiae* behaves qualitatively like that from *A. niger* though under similar conditions (the same pH, the same ionic strength etc.) the activation of the *S. cerevisiae* enzyme seems to be smaller, see Figs. 5 and 6.

As seen from the figures the curves mostly show maxima. In some cases, however, the z/z_0-values are almost constant after a certain Me/P-ratio is exceeded. These Me/P-values are rather low, and at these low Me-concentrations probably the shape of the substrate molecule does not differ very much from that of Me-free polymetaphosphate. In Fig. 7 the $(\eta_{sp})_{t=0}$ values corresponding to the curves shown in Fig. 3 are plotted as a function of the Me/P-values. As long as the ratio Me/P does not exceed $\sim$ 0.05—0.10 the changes of the substrate are small if not negligible in comparison with the increase in the z/z_0-values.

The results of the activation experiments are given in Table 1.

Table 1. Data from activating experiments.

	pH	metal	z/z_0 (maximum value)
A. niger	5.4	Zn	6—7
		Mn	6
		Ca	2.2
		Mg	2.2
		Pb	1.5
	6.6	Mn	32
		Zn	25
		Mg	10—12
S. cerevisiae	6.6	Zn	5
		Mn	1.6
		Mg	1.6
	7.3	Mg	2.7

INFLUENCE OF ANIONS ON THE ENZYME ACTIVITY

Fluorine ions. According to Bauer [11] the activity of the pyrophosphatase of yeast (bottom yeast) is inhibited by NaF. However, the activity of the *A. niger* and *S. cerevisiae* enzyme is only slightly inhibited by NaF. See Table 2.

Cyanide-ions. Some enzymes, for instance part of the respiratory ones, are known to be inhibited by cyanide ions. With these polymetaphosphatases the inhibiting effect is rather small in the case of the enzyme from *A. niger*, but is almost complete with that from *S. cerevisiae*. See Table 2. The ionic strength of the buffers used in these experiments is 0.3. The buffer solutions were made 0.005 *M* with respect to NaCN, NaF and $NaNO_3$ (blank solution). Substrate concentration 0.5 %. 1 ml enzyme solution/5 ml substrate solution.

Table 2. Influence of fluorine and cyanide ions on the enzyme activity.

	Substance added to the buffer	z/z_0 Expt. I	z/z_0 Expt. II
A. niger	$NaNO_3$ (blank) $z = z_0 =$ 360 (I), 459 (II)		
acetate buffer	NaF	0.98	0.99
pH = 5.4	NaCN	0.96	0.99
S. cerevisiae	$NaNO_3$ (blank) $z = z_0 =$ 77 (I), 80 (II)		
phosphate buffer	NaF	0.94	0.96
pH = 7.3	NaCN	0.08	0.04

INFLUENCE OF SOME SPECIFIC REAGENTS ON THE ENZYME ACTIVITY

Iodoacetic acid is an inhibitor of enzymes containing the sulphydryl radical -SH as essential group, but has no appreciable effect on the polymetaphosphate degrading enzymes in question.

Arsenite recognised as an inhibitor of some oxidizing enzymes has no influence on the enzyme from *A. niger*, but reduces the activity of the *S. cerevisiae* enzyme to about 30—40 %.

The bile acids generally inhibit the activity of phosphatases according to Uraki[12]. The effect of taurocholic acid on the polymetaphosphatases is, however, rather small. Owing to the difficulty in obtaining satisfactory solutions of the taurocholic acid under these experimental conditions the reproducibility is rather poor.

Formaldehyde in small concentration does not disturb the function of the polymetaphosphate degrading enzymes.

The substrate solution used in these experiments was 0.5 % with respect to K 15 in acetate- and phosphate buffers of ionic strength 0.3. As usual 1 ml of the enzyme solution was added to 5 ml of the substrate solution. The results of the measurements are collected in Table 3.

Table 3. Influence of some compounds on the enzyme activity.

	'Inhibitor' added to 5 ml substrate solution	z/z_0 Expt. I	Expt. II
	none (blank) $z = z_0 = 275$ (I), 427 (II)		
A. niger	5 mg CH_2ICOOH	0.99	0.93
acetate buffer	5 mg Na_3AsO_3	1.15	1.14
pH = 5.4	5 mg taurocholic acid	0.95	1.25
	0.1 ml 35 % formaldehyde	0.98	0.93
	none (blank) $z = z_0 = 83$ (I), 112 (II)		
S. cerevisiae	5 mg CH_2ICOOH	1.04	0.97
phosphate buffer	5 mg Na_3AsO_3	0.39	0.27
pH = 7.3	5 mg taurocholic acid	1.25	0.86
	0.1 ml 35 % formaldehyde	1.10	1.13

DISCUSSION

The mechanism of activating is not definitely known. E. Bauer [9] who worked on pyrophosphatase from yeast assumes the formation of a 'magnesium-bridge' between the substrate and the enzyme. Janner and Kay [3] report in their investigation on phosphatase from kidney that they have found a maximum activation by Mg^{++} ions at a certain concentration of those ions. Their curves resemble in principal those of Fig. 3 in this paper. They ascribe the decrease of the activity to the formation of a substrate-magnesium-enzyme complex with two magnesium atoms, which is formed when the amount of magnesium is greater than that required for the formation of the complex containing one atom of magnesium. However, one must not forget that there is another competitive reaction, *viz.* the complex formation between the Me^{++} ions and the polymetaphosphate. Perhaps the differences of activation of the metal ions in question may be due to differences in the equilibrium positions of these complex formations. Another possibility is that at a certain Me:P ratio the shape and charge or the substrate molecule is most suited to enzymatic breakdown without the Me-atom acting as a bridge between substrate and enzyme.

In a previous work [7] the sedimentation constant of the enzyme from *A. niger* has been determined as 3.2 Svedberg units, using a separation cell and calculating the *s*-value from the analysis of the activity of the original solution and that of the upper part of the cell after the run. The sedimentation constant of the substrate is $\sim$ 26 S in 0.1 % solution in acetate buffer of ionic strength 0.3. The sedimentation constant is largely dependent on the species and concentration of the low molecular salts present in the solution. However, if a complex substrate-enzyme is formed one may expect the sedimentation constant of the enzyme to increase in the presence of its high molecular weight substrate.

In order to decide whether such a complex is formed or not the following experiments were carried out. To 10 ml of a buffered polymetaphosphate solution (0.1 % with respect to K 15) was added 2 ml of an enzyme solution of high activity and the mixture was then immediately run in a separation cell, in the ultracentrifuge. During the run part of the mixture was kept at about the same temperature as that of the rotor chamber and after the run the activities were determined as described above. The data and the results of these runs are collected in Table 4.

As seen from the table there is an increase of the *s*-values at the pH-value neighbouring that of optimum activity. The *s*-values at more acid or alkaline pH-values show no appreciable deviation from 3.2. The *s*-values never amount

Table 4. Sedimentation constant of the enzyme in the presence of its high molecular weight substrate.

Activator	pH	s
none	4.3	4.1
	5.4	4.7
	6.6	6.4
	6.9	4.8
	7.3	3.7
	8.0	3.6
0.15 ml 0.07 *M* $Zn(NO_3)_2$/10 ml substrate solution	4.3	3.6
	6.6	5.3

to that of the substrate because of the breakdown of the polymetaphosphate during the centrifugation. In the presence of activating metal ions the breakdown of the substrate results in a lower s-value than in the absence of the same activator. However, the increase of the sedimentation constant in the experiments described above is not absolutely valid proof of a specific substrate-enzyme combination. It is well known, for instance by the works by Perlmann,[13] Hermann and Perlmann[14] and Briggs[15], that metaphosphoric acid forms compounds with proteins. However, if some essential groups of the enzyme protein are blocked by silver one may expect the enzyme to sediment with normal velocity, *i.e.* $s = 3.2$, even at the pH optimum and in the presence of the high molecular weight substrate, if these essential groups form the substrate-enzyme linkage. Now it is possible to inactivate the enzyme by addition of a silver salt and then revive the activity by removing the silver from the protein by KCN, which does not appreciably decrease the original enzyme activity.

To 5 ml of a 0.1 % K 15 solution in acetate buffer of pH = 5.4, 0.001 *M* with respect to $AgNO_3$, 2 ml of an enzyme solution *(A. niger)* was added. Part of the solution was run in the separation cell in the ultracentrifuge, and after the run the blank solution and centrifuged solution (upper cell) were made 0.015 *M* with respect to KCN. The activities were then measured as usual. Hitherto only three experiments have been carried out and the results $s = 6.6$, 7.9 and 7.3 respectively, seems to indicate that other groups than those blocked by silver atoms are involved in the formation of a substrate-enzyme complex. These investigations are beeing continued and will be extended to systems of an uncharged, non dissociating substrate.

ELECTRODIALYSIS

One could possibly assume that the activating action of some metal ions is due to the fact that the metal atoms play the role of a prosthetic group or part of such a group. However, if this assumption were true one might expect these metal atoms to be rather easily removable by electrodialysis, thus causing the enzymes to lose their activity.

The experiments were carried out in a cell of about 200 ml capacity; current $\sim$ 30 m A; current density $\sim$ 0.3 m A/cm^2, time 8 hours. Part of the enzyme solution was retained as a blank. During the experiment, the blank solution was stored in a test tube in the outer liquid of the electrodialysis cell in order to keep the blank solution at approx. the same temperature as the electrodialysed solution. The activity of both solutions was measured after the electrodialysis.

A. niger. The activity of an enzyme solution (in diluted acetate buffer) was decreased from $z = 865$ (blank solution) to 345 by the electrodialysis. (The activity measurements were carried out on 0.5 % K 15 solutions in acetate buffer of pH = 5.4.) However, if the substrate solution was made 0.01 *M* with respect to $Mg(NO_3)_2$ the activity was increased 2.21 times. Under similar conditions the enzyme blank was activated 2.26 times. Hence it is more likely that the activity decrease is due to denaturation, for instance at the membranes, than to removing of a prosthetic group.

S. cerevisiae. A solution of *S. cerevisiae* enzyme (in diluted phosphate buffer) lost all its activity after electrodialysis under the same experimental conditions as described above for *A. niger*. The activity could not be restored afterwards by addition of zinc or magnesium salt, and it appears that the *S. cerevisiae* enzyme is more easily denatured than that from *A. niger*.

SUMMARY

The enzymatic breakdown of a high molecular weight polymetaphosphate has been studied in the presence of some metal ions under various conditions. Among the metals investigated Zn and Mn are the best activators for the enzymes used (*A. niger* and *S. cerevisiae*); Ag and Hg completely inhibit the enzyme activity.

The formation of a substrate-enzyme complex has been shown by means of the ultracentrifuge using a separation cell. It is not likely that all the essential groups of the enzyme are involved in this complex formation.

The author wishes to express his sincere thanks to Prof. The Svedberg and Prof. A. Tiselius for the privilege of carrying out this work in their laboratories. He is also in-

debted to Dr. B. Ingelman for stimulating and valuable discussions and to Miss A. Karlsson for technical assistance. The work has been supported financially by *Statens Naturvetenskapliga Forskningsråd.*

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Received September 16, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1343–1349

The Effect of Some Antibiotic Substances on the Germination of the Conidia of *Polyporus annosus* Fr.

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The spruce (*Picea abies* Karst.) is subject to many diseases. One of the most important of these is root rot, caused by the root rot fungus, *Polyporus annosus* Fr. The losses to our forestry due to this fungus amounts to several million kronor a year. A throrough study of its biology and biochemistry is accordingly a matter of great urgency, and Professor Erdtman and the author have therefore initiated an investigation which it is hoped will, among other things, contribute to our knowledge of the effects on the fungus of antibiotic agents occurring in the soil.

Before entering upon that question, a brief review of the biology and manner of dispersion of the infecting fungus will be necessary. The principal host of the fungus is the spruce, and it is attacked in most parts of the country. Scots pine (*Pinus silvestris* L), is as a rule resistant to the disease, but in the southernmost counties this tree is also attacked. In the spruce the fungus is generally a saprophyte, and the disease is chronic. The mycelium produces a central rot in the heartwood, spreading slowly to the sapwood. In the pine, on the other hand, the disease is of acute character. In this tree the fungus appears as a parasite, kills the cambium and the outer sapwood, and causes the standing tree to dry very quickly. It does not, however, attack the heartwood.

The means available for the spreading of the fungus are its mycelium, its basidiospores, and conidia which are formed asexually on the mycelium. The infection enters the spruce through wounded or dead roots. It is not yet quite clear how the mycelium reaches the root system, at least two possibilities are conceivable. One is that an affected root establishes contact with an injured, but not yet attacked root of another root system. Such root contacts, actually, have been demonstrated on many occasions, and infection has been experimentally produced in the same manner [1–4].

It is also conceivable that the mycelium may spread through the soil from infected roots to the roots of unaffected trees, an opinion which is held by several authors [5–7]. This, however, is not easily demonstrated, as the root rot mycelium cannot be cultivated by spreading out a soil sample on malt agar in a Petri dish. In such experiments numerous mould fungi, bacteria, *etc.* will grow out instead, smothering any relatively slow growing

annosus mycelium that may be present. Nor can *annosus* mycelium be induced to grow in unsterilized samples of humus or litter in a flask under laboratory conditions. However, it will grow readily if the material is sterilized. This is usually taken to mean that other micro-organisms in the samples actively prevent the *annosus* mycelium from growing [8, 9].

A close study of the distribution of root rot in Sweden shows that this disease is not evenly spread over the whole country. In some districts spruce is very severely attacked, *e. g.* in Silurian districts and on calcareous soil in general. In the northern spruce district of Sweden, which is situated on primary rock, hardly any root rot is found. Other rot fungi occur there instead [3].

Root rot may be very unevenly distributed even in small, restricted areas. In the Tönnersjöheden Experimental Park in Halland it is, for instance, common in spruce forest planted on the old moors, while the beautiful spruce stands growing on what used to be beechforest ground are practically free from root rot.

The composition of the microflora differs in different soils. In soil where root rot occurs sparingly, or not at all, antibiotic fungi and bacteria counteracting the *annosus* mycelium may be richly represented. This is difficult to demonstrate by analysing the microflora of the soil, a procedure which is, in any event, very time-consuming. A beginning has been made by Björkman, who has isolated from some soils fungi of antagonistic effects on *P. annosus* [7]. Other investigations indicate that a good many soil fungi — mainly moulds, but also bacteria — are able to check the growth of *annosus* mycelium on malt agar and on nutritive solutions [10, 11].

In the present investigation we have examined the effects of a number of antibiotic agents on the germination of *annosus* conidia. These conidia, which form on the mycelium, can easily be obtained in sufficient quantities for laboratory examinations, and are accordingly suitable for investigating the inhibiting effects of antibiotic agents on germination. The biology of germination of the conidia has been more closely examined in another connexion [11]. Here we will only note that the best germination was obtained in a 0.5 % solution of malt extract. Between 50 and 60 % of the conidia germinated in this substratum after being kept one day in a moist chamber at 22° C. The

Table 1. Inhibiting effect of various fungicides (in p. p. m.) on the germination of annosus conidia.

Fungicides	Per cent germination				
	0	0—5	5—20	20—50	50—60
Corrosive sublimate	20	10	5	1	0.05
Copper sulfate	100	50		20	10
Phenol	10 000	5 000	2 000	1 000	100
Pentachlorophenol-Na	1 000	500	200		100

Table 2. Inhibiting effect of various fungal antibiotics (in p. p. m.) on the germination of annosus conidia.

Antibiotics	Per cent germination				
	0	0—5	5—20	20—50	50—60
Patulin	50	25	10	5	2
Enniatin		500	50	25	5
Griseofulvin				500	10
Penicillin	1 000	500	250	25	5
Spinulosin	250		100	50	10
Streptomycin	1 000	500	100	25	5

antibiotic agents were therefore dissolved in 0.5 % malt, and the same solution was used for all dilutions, and control tests. Its pH was about 5.0.

Germination was first examined in some common fungicides (Table 1). Of these, sublimate had strongest effect. It stopped germination completely at 20 p. p. m. and did not allow the conidia to germinate as well as in the control tests until at 0.05 p. p. m. Phenol had but little effect, and a relatively high concentration was required to inhibit germination of the spores even in sodium pentachlorophenolate.

Some agents antibiotic to fungi have been examined in Table 2. Of these, patulin was the most effective. Spinulosin also proved fairly effective. Enniatin, which is isolated from some species of *Fusarium* and is very toxic to tubercle bacteria [12, 13], had no great effect on *annosus* conidia. The same applies to both penicillin and streptomycin. Penicillin was also examined at pH 3.6 and streptomycin at pH 7.2, but their antibiotic effect was no greater at these pH-values.

Brian and his collaborators [14, 15] isolated from *Penicillium Janczewskii* a substance called griseofulvin. At low concentrations (1.0—0.2 p. p. m.) this substance produced peculiar curls and ramifications in the germ tubes of *Botrytis allii*. It had no similar effect on the germ tubes of *annosus* spores, nor was the germination of these appreciably inhibited. More than 30 % of the conidia germinated with normal germ tubes even at 500 p. p. m.

The effect of most antibiotic agents is bacteriostatic or fungistatic, and only a small number of them are bactericides or fungicides. Comparatively little is known of how they affect the bacteria and fungi, but presumably they interfere at one vital stage or another of their metabolism. Waksman [16] has pointed out many possible ways in which this might be done. It has for instance been shown that the pH of the solvent greatly influences the efficacy [17, 18]. As several of these agents are either acids or bases, the influence

Table 3. Inhibiting effect of various fungal antibiotics (in p. p. m.) on the germination of annosus conidia.

Antibiotics	pH	Per cent germination				
		0	0—5	5—20	20—50	50—60
Alternaria acid	3.6	1000	500	200	50	25
	5.0	1000	500	200	100	25
Citrinin	3.6	25	10	5	2	1
	5.0	250		100	50	10
Gladiolic acid	3.6	10	5	0.05	0.005	0.0005
	5.0	50	20		10	0.2
Gliotoxin	3.6	5	2	0.5	0.25	0.1
	5.0	25		10	5	2
Glutinosin	3.6	5	2	0.2	0.05	0.01
	5.0	5	2	0.05	0.02	0.01
Mycophenolic acid	3.6	1	0.2	0.02	0.005	0.0005
	5.0	500	250	100	10	5
Penicillic acid	3.6	250	100	50	2	0.1
	5.0	500	100	50	25	10
Puberulic acid	3.6	10	2	0.05	0.02	0.005
	5.0	250		100	50	25
Puberulonic acid	3.6	100	50	25	2	0.1
	5.0	250		100	25	5
'Red pigment'	3.6	5	2	0.5	0.2	1
	5.0	5	2	0.5	0.2	1
Stipitatic acid	3.6	100	50	25	5	2
	5.0	250	100		50	25
Viridin	3.6	5	2	1	0.1	0.02
	5.0	0.2	0.2	0.1	0.02	0.005

of pH is probably connected with the degree of dissociation of the antibiotic substances.

The effect of the pH of the solvent on some antibiotic agents produced by fungi is quite distinct (Table 3). In an acid solution (0.5 % malt extract + 0.001 *M* citric acid, pH = 3.6) a lower concentration was usually, *e. g.* in the case of citrinin, gladiolic acid, puberulic acid, *etc.*, required to inhibit the germination of conidia than in malt extract with a pH of about 5.0. In mycophenolic acid the difference was very large. Germination was completely inhibited by 1 p. p. m. at pH 3.6, while 500 p. p. m. were required in malt extract.

Some of these antibiotic agents are exceedingly effective on the *annosus* conidia. This statement applies to the 'Red pigment' — produced from *Penicillium nigricans-Janczewskii* [15] — gliotoxin, and viridin. The two last-

Table 4. Inhibiting effect of various bacterial antibiotics (in p. p. m.) on the germination of annosus conidia.

Antibiotics	pH	Per cent germination				
		0	0—5	5—20	20—50	50—60
Gramicidin	3.6			200	100	25
	5.0			200	100	25
Gramicidin S	3.6	100	50	10	2	1
	5.0	100	50	10	2	1
Pyocyaninchloride	3.6	200			100	25
	5.0	500			200	100
Tyrocidinchloride	3.6	100	50	25	5	2
	5.0	100	50	25	5	2

named substances, which are produced by *Trichoderma viride*, are of particular interest in this connexion. This fungus is very common in the soil, and may possibly counteract the *annosus* mycelium. Weindling, who among others has been examining the antibiotic properties of *Trichoderma*, found that this fungus was most active against *Rhizoctonia solani* in acid soils [19]. Rishbeth [4] points out that the *annosus* mycelium is possibly counteracted in the soil by *Trichoderma viride*, which in some parts of Great Britain is more common in acid than in alkaline soils.

Some bacteria-produced antibiotic agents have also been examined (Table 4). Gramicidin and tyrocidine are two closely related high-molecular polypeptides obtained from *Bac. brevis* and other soil bacteria. Of the two gramicidin preparations, the Russian 'Gramicidin S' was appreciably more effective than the British one. Tyrocidine chloride was also rather active, while pyocyanin, the blue pigment of *Pseudomonas aeruginosa*, had little effect.

These experiments show that the *annosus* conidia are sometimes inhibited by very small quantities of antibiotic substances. Since its mycelium will, as a rule, hardly tolerate them in appreciable concentrations [20], the spreading of the fungus in the soil might possibly be prevented — or at least made more difficult — by the presence in the soil of suitable organisms producing antibiotic agents. The mycelium would then be dispersed mainly via the root systems, in which moulds and bacteria cannot penetrate as easily as a fungus mycelium with cellulose-decomposing properties [3, 4, 9]. It must be borne in mind, however, that conditions are much more complicated in nature than in pure laboratory experiments. There are many other organisms in the soil that are not affected by these substances, but are perhaps instead able to break them down. At least some of these antibiotic agents are not very stable, and

Table 5. Inhibiting effect of various heartwood constituents (in p. p. m.) on the germination of annosus conidia.

Heartwood substances	Per cent germination				
	0	0—5	5—20	20—50	50—60
Pinosylvin	50	25	10	2	0.5
Pinosylvin monomethyl ether	50	25	5	1	0.5
Dihydropinosylvin *	100		50	25	5
Conidendrin				500 ***	100
Pinoresinol **			500 ***	100	50

* Not found in nature.

** Constituent of pine and spruce gum resin.

*** Saturated solution.

are easily inactivated in the presence of organic substances, *e. g.* sugars and proteins, at unfavourable pH-values, *etc.*

Assuming, however, that the *annosus* mycelium has successfully reached a root, despite all dangers in the soil, it must still penetrate into the host plant. Pine heartwood contains the strongly fungicide pinosylvin phenols [20, 21], which has a strong inhibiting effect on the germination of *annosus* conidia (Table 5). Spruce heartwood, on the other hand, contains hardly any fungicide substances. Saturated solutions of conidendrin or sulphite liquors lactone have only an insignificantly inhibiting effect on the germination of *annosus* conidia, and so has pinoresinol, a constituent of spruce gum resin, *etc.* (Table 5).

The dead heartwood cannot provide any active, physiological protection against penetrating organisms, as do for example the rustresisting cereals, constituents of whose living protoplasm can actively counteract the growth of rust fungi. Once the mycelium has penetrated into the heartwood of spruce, the host is defenceless against the root rot fungus. To be resistant to root rot, spruce heartwood would have to contain toxic substances able to prevent the growth of the fungal mycelium, and — as we have just said — none such has been found there.

Conditions are different in the pine. The pinosylvin phenols have a strongly inhibiting effect not only on the germination of the *annosus* conidia but also on its mycelium [20]. In malt agar their inhibiting limit is at about 0.02 %, or 200 p. p. m., of pinosylvin. The root rot mycelium has obviously no chance of penetrating into the heartwood of pine, since the pinosylvin content is as a rule largest (1—2 %) in its peripheral parts [22, 23]. Nor has any rot due to *P. annosus* been observed in the heartwood of pine trees; in this tree the root rot mycelium apparently never grows except in the sapwood.

SUMMARY

The effects of antibiotic agents from fungi and bacteria on the germination of the conidia of the root rot fungus, *Polyporus annosus* Fr., have been investigated. Some of these agents, *e. g.* gladiolic acid, mycophenolic acid, and viridin, are very effective against the conidia of *P. annosus*, surpassing sublimate in this respect. In most cases the effect is greater at pH 3.6 than at pH 5.0. Germination is also effectively inhibited by pinosylvin. No substance possessing similar properties has, however, been found in spruce wood.

I am indebted to *Fonden för Skoglig Forskning* for financial support. The laboratory work has been carried out by Miss Sonja Andersson and Mrs. Silvia Jögi. The antibiotics have been kindly put at my disposal by several scientists. I wish to thank especially professor H. Raistrick, London, Dr. P. W. Brian, Butterwick Research Laboratories, England, Dr. R. L. M. Synge, Edinburgh, professor Pl. A. Plattner, Zürich, civilingenjör B. Sandberg, Kärnbolaget AB, Stockholm, and professor H. Erdtman, Royal Institute of Technology, Stockholm.

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Received October 21, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1350 – 1354

Action of Strong Acids on Acetylated Glycosides

IV. Investigation of Disaccharide Models

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In a previous paper in this series [1], it was reported that the velocity of transglycosidation of alkyl glucosides decreases with increasing + I activity (electron attraction) of the alkyl group. In the disaccharides, gentiobiose and cellobiose, there are one and two oxygen atoms respectively on the carbon atoms in β-position to the glycosidic linkage, which will attract electrons and thus lower the reactivity. In order to investigate the possibility of transforming disaccharides, a number of acetylated glucosides of halogen and oxygen substituted alcohols have been investigated, using the methods outlined in the previous paper. Thus the velocity constants given in Table 1 are relative, the velocity constant for the transformation of β-glucose pentaacetate to the equilibrium mixture taken as unity.

Table 1. Transglycosidation and acetolysis of some substituted alkyl glucosides.

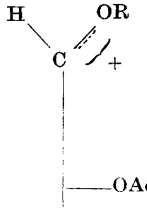
β-Glucoside tetraacetate	$k_{\text{transglyc.}}$	$\alpha_{\max}$	$k_{\text{acetolysis}}$	α_∞
$-O\cdot CH_2\cdot CH_2\cdot CH_3$	15	+ 5.13°	0.08	+ 2.71°
$-O\cdot CH_2\cdot CH_2\cdot CH_2OH$	2.5	4.66	0.06	2.95
$-O\cdot CH_2\cdot CH_3$	15	5.20	0.07	3.53
$-O\cdot CH_2\cdot CH_2\cdot OOC\cdot CH_3$	0.85	3.99	0.025	2.55
$-O\cdot CH_2\cdot CH_2\cdot Cl$	0.40	3.99	0.03	2.40
$-O\cdot CH(CH_3)_2$	50	5.50	0.08	1.25
$-O\cdot CH(CH_2Cl)_2$	$\simeq$ 0.02	1.71	–	1.55

In all runs 0.500 g of glucoside were dissolved in 20 ml of a solution of sulfuric acid in acetic anhydride-acetic acid (10 : 3). The rotation was measured at 20.0° in 2 dm tubes. As the velocity constants differed by several powers of ten, the concentration of sulfuric acid was varied between 0.1 C and 1.5 C in the different runs in order to get a convenient velocity of the reaction studied. The final rotation for glucose pentaacetate in the parallel runs was + 4.90°.

When a hydroxyl group is introduced at the γ-carbon atom in propyl β-glucoside tetraacetate, the velocity of the transglycosidation decreases from 15 to 2.5. The hydroxyl group becomes acetylated, but this does not seem to complicate the kinetics. The velocity of the acetolysis also decreases, but to a much smaller extent.

An electron attracting group in the β-position has a still greater effect. This is demonstrated with the ethyl, β-acetoxyethyl and β-chloroethyl glucosides.

The 1,3-dichloropropyl(2-) β-glucoside tetraacetate reacts extremely slowly compared to the unsubstituted isopropyl glucoside. The velocity of transglycosidation is of the same magnitude as that of acetolysis, which is indicated by the low maximum. Consequently, no exact values can be calculated. The transglycosidation seems to be about 2500 times slower than that of the unsubstituted glucoside.

The final rotations of the substituted alkyl glucosides do not differ appreciably from those of the unsubstituted ones. This is rather unexpected. The low final rotation indicates a high percentage of glucose heptaacetate, but the yield of heptaacetate from the unsubstituted glucosides, however, increases with the velocity of transglycosidation. If this were also true for the unsubstituted glucosides, one would get very little glucose heptaacetate and consequently much higher values for the final rotation than those obtained. These results are difficult to interpret. According to the theory outlined in the preceeding paper, transglycosidation and acetolysis have the same intermediate step — the cation  . If this theroy is correct, one

must assume that 'acetylation and acetolysis' of this cation, leading to glucose heptaacetate, is much more rapid for the substituted glucosides than for the unsubstituted ones.

From the above results, one may conclude that disaccharides of the gentiobiose type, analogous to the chloroethyl β-glucoside and the acetoxyethyl β-glucoside, could be transformed into the α-derivates. (Helferich and Werner [2] have synthesized chloroethyl α-glucoside from the β-glucoside with titanium tetrachloride as a catalyst.) The cellobiose type seems to be more difficult to transform. The reaction with a strong acid always involves some decomposition, and the transglycosidation would be so slow that most of the substance would probably be destroyed before any appreciable amounts of the α-glucoside were formed.

EXPERIMENTAL

The experimental conditions were the same as in the preceding paper. The syntheses of the glucosides investigated, with the exception of 1,3-dichloropropyl (2-) β-glucoside tetraacetate, are described in an earlier communication [3].

1,3-Dichloropropyl (2-) glucoside tetraacetate

A solution of acetobromoglucose (8.22 g) and mercuric acetate (3.03 g), in a mixture of absolute benzene (40 ml) and glycerol α,γ-dichlorohydrin (20 g), was boiled on the steam bath for 15 minutes. After cooling, it was washed several times with water, dried

Typical runs

Table 2. Transglycosidation of chloroethyl β-glucoside tetraacetate.

β-Glucose pentaacetate and chloroethyl β-glucoside tetraacetate, 0.500 g of each, dissolved in 20 ml of 0.36 C sulfuric acid in acetic anhydride-acetic acid, 10 : 3. t = 20° C. Rotations determined in 2 dm tubes. (The table gives only a part of the observed values.)

Time min	β-Glucose pentaacetate		Chloroethyl β-glucoside tetraacetate	
	α_D	k	α_D	k
0	+ 0.37°		− 0.98°	
20	2.27	0.0120	− 0.06	0.0044
40	3.36	0.0119	+ 0.68	0.0044
60	4.02	0.0121	1.36	0.0046
80	4.42	0.0126	1.87	0.0046
100	4.60	0.0124	2.31	0.0046
226	4.86		3.60	0.0047
313			3.85	
370			3.99	
1440			3.64	
2880			3.25	
	Mean value	0.0121	Mean value	0.0046

Table 3. Acetolysis of acetoxyethyl β-glucoside tetraacetate. β-Glucose pentaacetate and acetoxyethyl β-glucoside tetraacetate, 0.500 g of each, dissolved in 20 ml of 1.65 C sulfuric acid in acetic anhydride-acetic acid, 10 : 3. t = 20° C. Rota- tions determined in 2 dm tubes.

Time min	β-Glucose pentaacetate		Acetoxyethyl β-glucoside tetraacetate	
	α_D	k	α_D	k
0	+ 0.37°		− 1.04°	
10	4.53	0.092	+ 1.70	
20	5.00		2.81	
50			3.62	
60			3.64	
70			3.60 *	
80			3.56	0.0017
90			3.50	0.0022
100			3.46	0.0021
110			3.41	0.0022
120			3.35	0.0024
1374			2.55	
	Mean value	0.097	Mean value	0.0024

Table 4. Transglycosidation and acetolysis of 1,3-dichloropropyl (2-) β-glucoside tetra- acetate. β-Glucose pentaacetate and 1,3-dichloropropyl (2-) β-glucoside tetraacetate, 0.500 g of each, dissolved in 20 ml of 1.65 C sulfuric acid in acetic anhydride-acetic acid, 10 : 3. t = 20° C. Rotations determined in 2 dm tubes. (The table gives only a part of the observed values.)

Time min	β-Glucose pentaacetate		1,3-Dichloropropyl (2-) β-glucoside tetraacetate
	α_D	k	α_D
0	0.37°		− 0.68°
10	3.05	0.038	− 0.65
25	4.50		− 0.62
50	4.91		− 0.47
100	4.99		− 0.26
200			+ 0.08
1340			1.40
1570			1.50
2800			1.70
4205			1.71
7000			1.57
8500			1.55
	Mean value	0.039	

* Chosen as initial value.

over calcium chloride and concentrated under reduced pressure. The oily residue was recrystallized from ethanol-water, 1 : 1. After three recrystallizations, the melting point was constant at 122—123° (uncorr.). Yield 1.3 g (14 %). $[\alpha]_D^{20} - 10.8°$ in chloroform ($c = 2.5$). The melting point is identical and the yield, although very low, is somewhat better than that obtained by Coles, Dodds and Bergeim [4], who synthesized the same substance by the method of Koenigs and Knorr. They did not determine the optical rotation.

SUMMARY

The transglycosidation and acetolysis of some halogen and oxygen substituted alkyl glucosides have been investigated in acetic anhydride-acetic acid solution. The substituents reduce the reactivity of the glucoside considerably. With one substituent on the β-carbon atom of the agluconic group (the gentiobiose type), transglycosidation is more rapid than acetolysis and the α-glucoside accumulates in the reaction mixture. With substituents on two β-carbon atoms (the cellobiose type), both transglycosidation and acetolysis are very slow, and no appreciate accumulation of α-glucoside can be observed.

1,3-Dichloropropyl (2-) β-glucoside tetraacetate has been synthesized by the mercuric acetate method.

The author wishes to thank *Statens Naturvetenskapliga Forskningsråd* for financial support and Mr. L. Asp for skilful assistance.

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Received October 7, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1355–1357

Action of Strong Acids on Acetylated Glycosides

V.* Synthesis of β-Isomaltose Octaacetate

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From the investigations on halogen and oxygen substituted alkyl glucosides reported in Part IV of this series [1], the possibility of transglycosidation of certain disaccharides has been elucidated. There should be a great difference between disaccharides of the gentiobiose type and those of the cellobiose type. In the former there is only one carbon atom with an electron attracting substituent in β-position in the agluconic part of the molecule, while in the latter there are two. These electron attracting groups reduce the velocity of transglycosidation considerably, and consequently the glycosidic bond in cellobiose is very stable towards reagents that normally catalyze transglycosidation. The glycosidic bond in gentiobiose should be much more sensitive. This is indicated by the experiments of Pascu [2], who treated gentiobiose octaacetate with titanium tetrachloride in order to obtain gentiobiose chloride heptaacetate. He obtained a product which had a higher specific rotation and a lower melting point than that reported by other authors for the substance. Pascu's value for the optical rotation was also not in agreement with that calculated by the application of Hudson's rules of *iso*-rotation. One must therefore conclude that his product contained considerable amounts of isomaltose chloride heptaacetate.

The difference between gentiobiose and cellobiose is further demonstrated by the following experiment. α-Gentiobiose octaacetate and α-cellobiose octaacetate were dissolved in 2 C sulfuric acid in acetic anhydride-acetic acid, 10 : 3, and the optical rotation was measured at appropriate intervals. The reaction is very complex, involving α/β-transformation of the acetates, transglycosidation, acetolysis, and finally α/β-transformation of the glucose penta-

* A preliminary communication on this subject has been published in *Nature* **164** (1949) 706.

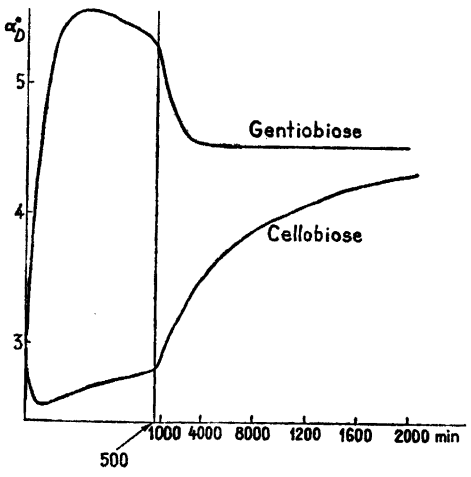

Fig. 1. Transglycosidation and acetolysis of the octaacetates Hof α-gentiobiose and α-cellobiose.

acetate formed by the acetolysis. The results are given in Fig. 1. The results may be interpreted by assuming that the gentiobiose derivative is transformed rather quickly, but that no transglycosidation can be observed with the cellobiose derivative.

Finally, the octaacetate of isomaltose, 6-α-D-glucopyranosyl-D-glucose, has been prepared from the gentiobiose derivative by transglycosidation. Crystalline derivatives of isomaltose have been prepared recently by hydrolysis of dextran [3] and by hydrolysis of starch [4], but the present paper reports the first total synthesis of this sugar and also one of the first syntheses of a disaccharide with an α-glycosidic bond. The substance was prepared in the following manner. Gentiobiose octaacetate (α- or β-) was treated with a large excess of titanium tetrachloride in absolute chloroform. The resulting mixture of isomaltose and gentiobiose chloride heptaacetate was treated with mercuric acetate in acetic acid. By this reaction the β-octaacetates were obtained, and they were then separated by recrystallization.

EXPERIMENTAL

α-Gentiobiose octaacetate

α-Gentiobiose octaacetate was prepared from amygdalin heptaacetate by hydrogenolysis, according to Bergmann and Freudenberg [5], but the method was improved to give better yields. Amygdalin heptaacetate (10 g) was dissolved in hot acetic acid (60 ml) and palladium black (1 g) was added. This mixture was shaken mechanically in an atmosphere of hydrogen and the temperature maintained at 40° by illumination with an infrared lamp. In about 15 minutes the optimum amount of hydrogen (740 ml) had been consumed. The palladium was removed by filtration and the solution was concentrated to a thick sirup under reduced pressure. Instead of isolating the gentiobiose heptaacetate formed, the sirup was dissolved in a solution of anhydrous zinc chloride (6 g) in acetic anhydride (60 ml) and kept at room temperature for 24 hours. The solution was then poured into ice water (1000 ml) and the precipitate was collected and recrystallized from methanol (75 ml). Two recrystallizations yielded the pure substance. M. p. 186—186.5°. $[\alpha]_D^{20}$ + 51°*. The average yield of several runs was 4.4 ± 0.4 g.

β-Gentiobiose octaacetate was prepared according to Reynolds and Evans [6].

The treatment of gentiobiose and cellobiose octaacetate with sulfuric acid was made

* All melting points uncorrected. All rotations in chloroform, $c = 2$.

by the method described in Part IV[7] of this series. The only difference was that the concentration of cellobiose octaacetate was much smaller than that of the other glucosides, owing to its low solubility. In order to obtain greater accuracy, the rotation was measured in a 4 dm tube.

β-Isomaltose octaacetate

Gentiobiose octaacetate (5 g, α- and β- are equally suitable) was dissolved in absolute chloroform (70 ml) and mixed with a solution of titanium tetrachloride (6 g) in the same solvent (70 ml). A yellow precipitate was formed immediately. The mixture was boiled on a water bath (65—70°) for five hours. (When the mixture was heated on a steam bath, some decomposition occurred and the yield was smaller.) After cooling, the mixture was poured into ice water (500 ml). The precipitate dissolved and the chloroform phase became almost colorless. It was separated, washed with water, dried over calcium chloride, and concentrated to a sirup under reduced pressure. This sirup, consisting of the chloride heptaacetates of isomaltose and gentiobiose, was dissolved in a solution of mercuric acetate (4 g) in acetic acid (40 ml). By this treatment the chloride heptaacetates were transformed into the β-octaacetates. (In a control experiment, glucose chloride tetraacetate was transformed into the β-pentaacetate in a quantitative yield by this method.) After two hours the solution was poured into water (500 ml) and extracted with chloroform (2 × 50 ml). The chloroform was washed with dilute sodium carbonate and water, dried over calcium chloride, and concentrated under reduced pressure. The residue was recrystallized from ethanol (25 ml). β-Gentiobiose octaacetate (1.0 g) of m. p. 188—189° separated. The mother liquor was concentrated under reduced pressure and the residue dissolved in methanol (7 ml) and seeded with pure β-isomaltose octaacetate *. The substance crystallized very slowly, complete separation requiring about one week in the refrigerator. Yield 1.85 g (46 %). M. p. 129—130°. $[\alpha]_D^{20}$ + 95°. Two further recrystallizations from methanol yielded the pure substance. M. p. 142—143°. $[\alpha]_D^{20}$ + 98°. The melting point was not depressed when mixed with the β-isomaltose octaacetate prepared by Wolfrom, Georges and Miller [3].

SUMMARY

β-Isomaltose octaacetate has been prepared from gentiobiose octaacetate in a series of reactions, involving a transglycosidation.

The author wishes to thank *Statens Naturvetenskapliga Forskningsråd* for financial support, and Mr. L. Asp for skilfulassistance.

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Received October 31, 1949.

* A sample of crystalline β-isomaltose octaacetate was kindly supplied by Professor Wolfrom.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1358–1374

Aromatic Keto- and Hydroxy-polyethers as Lignin Models. II *

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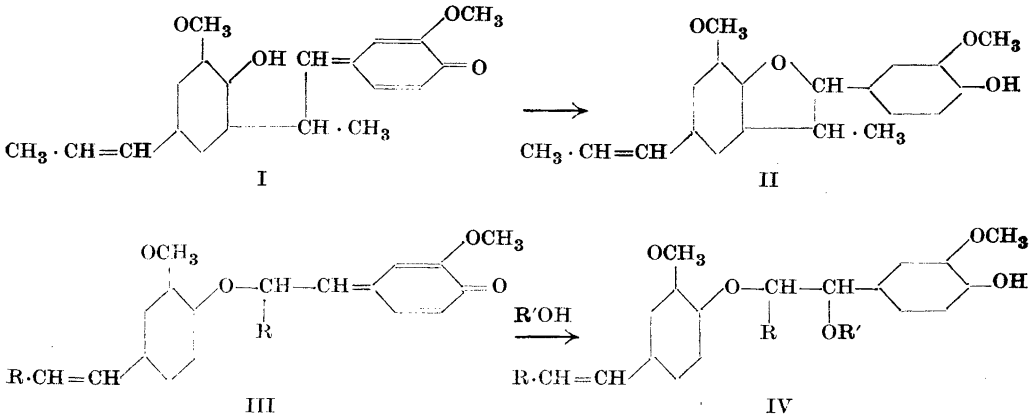
Studies on the analytical composition, physical properties, and degradation of lignins and lignin derivatives conclusively demonstrate the fundamental correctness of the view that lignin is related to phenyl propane which was already advanced by P. Klason more than fifty years ago.

Much interest is now devoted to the mechanism of lignin formation, the nature of the lignin precursors, and the reactive groups of the lignins which are responsible for their facile sulphonation with sulphites and their reactions with several other reagents.

A reaction of great interest in connection with lignin formation is the dehydrogenation of *iso*eugenol with phenol oxidases or ferric chloride to 'dehydrodi-*iso*eugenol'. The investigation of this compound revealed that it is not a diphenyl derivative, as previously supposed, but a coumarane derivative [1]. The coupling takes place between two molecules of *iso*eugenol in such a way that the carbon atom in the ortho position to the hydroxyl group in one molecule is linked to the β-carbon atom in the side chain of another molecule (II), probably via the intermediate quinone I.

Another type of coupling should be theoretically possible, namely, the formation of ethers of structure IV. The introduction of the benzyl alcoholic hydroxyl group involves the addition of the elements of water (R′ = H) in the same way as the formation of dehydrodi-*iso*eugenol from the postulated quinonoid intermediate I. Such a reaction would be of very great interest in relation to the chemistry of lignin, and during the last fifteen years several attempts have been made in these laboratories to effect the dehydrogenation of *iso*eugenol and similar substances to compounds of this type. The products,

* Part I. Preliminary communication. *Acta Chem. Scand.* 2 (1948) 535.



however, invariably were ill-defined and amorphous, and similar to those obtained by Freudenberg and his collaborators [2].

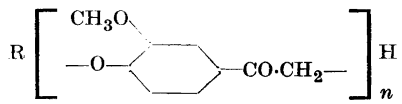
Since such coupling reactions could easily be imagined to proceed further with the formation of high molecular products containing only very few free phenolic hydroxyl groups, it was suggested in 1933 that lignin may owe its formation in Nature to the dehydrogenation of progenitors of coniferyl type [1]. This theory now appears to be widely accepted [3] and quite recently Freudenberg reported the dehydrogenation of coniferyl alcohol to products which resemble 'Brauns native lignin', a lignin-like material that occurs in most woods [4]. In many respects, however, these products differ from the main portion of lignin.

Elements of type IV contain hydroxyl groups of the benzyl alcoholic type which, according to Holmberg [5], may be responsible for the sulphonation of lignin. In these laboratories Lindgren recently showed that such alcohols are excellent lignin models provided that the nuclei are substituted as in lignin [6].

Pending advance in our continued experiments on the dehydrogenation of C_6—C = CH—R compounds, we have decided to synthesize substances of type IV by orthodox methods.

We have chosen to start with acetoguaiacone, but experiments with propioguaiacones are also in progress (B. Lindgren).

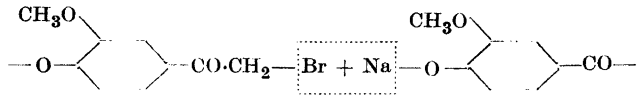
A series of compounds of general type V have been prepared.



$n = 2-4$ R = CH_3 or H

V

The syntheses were carried out according to the following scheme:



In the synthesis of the members of the 'phenol series' (R = H) the hydroxyl group had to be protected by acetylation or, preferably, benzoylation. The condensations were generally carried out in anhydrous methyl ethyl ketone in the presence of potassium carbonate, but sometimes in absolute alcohol, using the sodium salt of the phenol.

In the latter case, it was advantageous to use an excess of the phenol in order to prevent deacylation and other complications. Good yields of the phenolic ethers (V, R = H) were obtained by deacylation with piperidine in alcohol.

Ultraviolet absorption spectra.
(Extinctions per C_6C_2-unit.)

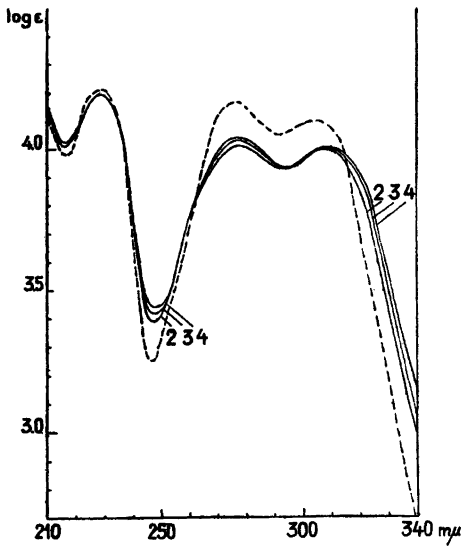


Fig. 1. *2 : V, n = 2, R = H;*
3 : V, n = 3, R = H;
4 : V, n = 4, R = H;
dashed line: acetoguaiacone;
all in absolute alcohol.

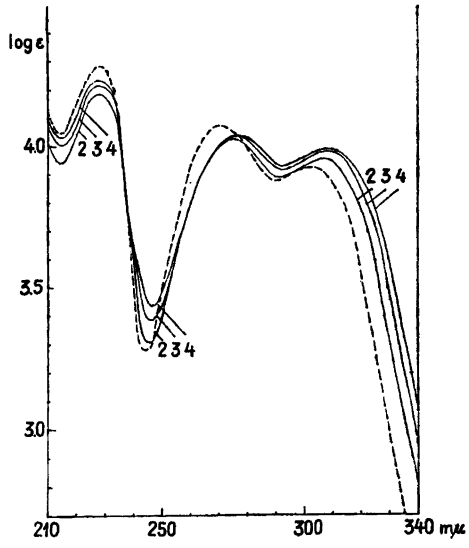


Fig. 2. *2 : V, n = 2, R = CH_3;*
3 : V, n = 3, R = CH_3;
4 : V, n = 4, R = CH_3;
dashed line: acetoveratrone;
all in absolute alcohol.

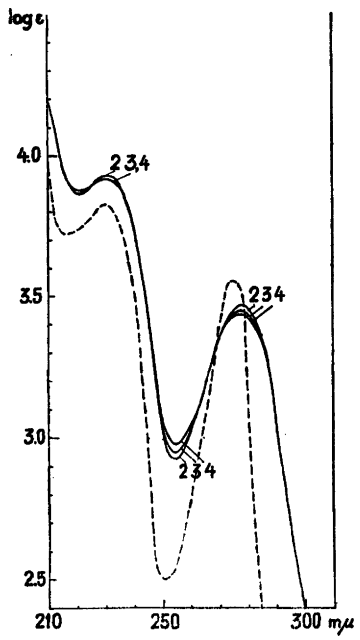


Fig. 3. *2 : VI, n = 2;*
3 : VI, n = 3;
4 : VI, n = 4;
*dashed line: vanillyl alcohol *;*
all in absolute alcohol.

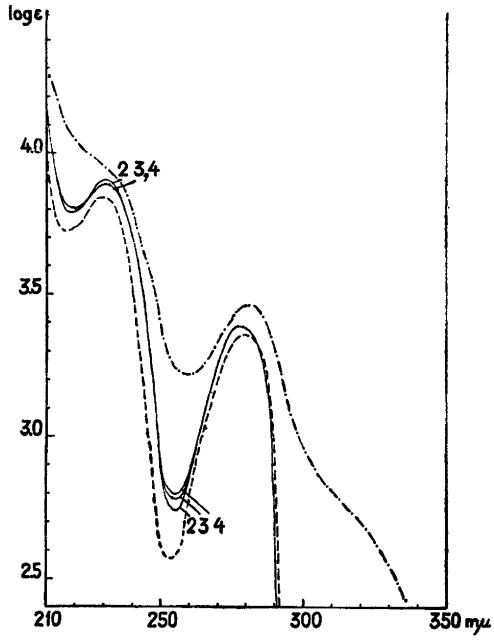
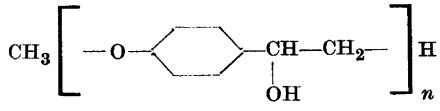


Fig. 4. *The sulphonic acids of*
2 : VI, n = 2;
3 : VI, n = 3;
4 : VI, n = 4;
*– – – – vanillyl alcohol *;*
–.–.–. lignin;
all in water.

The various ketonic ethers (V, R = CH_3) were reduced to the corresponding alcohols (VI).

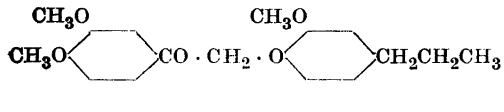


$n = 2—4$

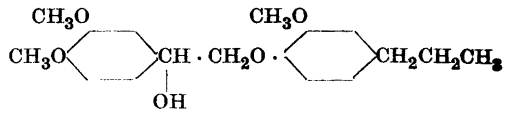
VI

The reduction was accomplished by the Meerwein-Ponndorff method, and the reaction was first tested in the case of 3,4-dimethoxyphenacyl coerulignol ether (VII). This was easily converted into the dihydroderivative VIII.

* These spectra have kindly been put at our disposal by Dr. B. Lindgren.



VII



VIII

Both compounds are crystalline solids. The reduction of the compounds V (n = 2—4 R = CH_3) must lead to a mixture of diastereoisomerides, and the products obtained from these ketones were amorphous but showed no ketonic properties. The compounds V (R = $COCH_3$ or CO—C_6H_5), as expected, could not be properly reduced by this method. Complications occurred owing to deacylation and formation of products with an abnormally high alkoxyl content.

Spectrochemically, the ketones V (R = H or CH_3) as well as the secondary alcohols VI (R = CH_3) offered no surprises (Fig. 1—4). Only small but regular changes were observed with increasing molecular weight as was to be expected from the results of several similar instances already studied.

All alcohols VI and the alcohol VIII were subjected to sulphite cooks under varying conditions. In all cases the hydroxyl groups were replaced by sulphonic acid groups, and the sulphonic acids from VI were all amorphous and yielded no crystalline derivatives. The barium salt of the sulphonic acid from VIII, possessing only one asymmetric carbon atom, yielded a crystalline salt with S-l-naphthylmethylthiuronium chloride.

The fundamental studies of Hägglund, on the sulphonation of lignin during the sulphite delignification, clearly indicate the presence of two different groups in lignin characterized by different reactivity to sulphites [7].

One group ('A') reacts readily with sulphite at any pH (even in alkaline solution). This yields the low sulphonated, solid, insoluble, lignin sulphonic acids of Hägglund. It is believed that these groups are benzyl alcoholic hydroxyl groups, as assumed by Holmberg, and belong to elements of type IV[8].

In Hägglund's laboratories one of the present authors showed that certain reactive phenols condense easily with the groups A in lignin to form high-molecular insoluble products [9]. This reaction takes place only under acid conditions. It was therefore of interest to investigate the influence of phenols (resorcinol) on the sulphonation of our lignin models (VI), which are all substituted benzyl alcohols.

Table 1 summarises the results of these experiments.

The yields of sulphonic acids formed under various conditions were determined by isolation of the barium salts according to methods described earlier [10]

and also indirectly by determining the sum of undissolved solid organic material and products which could be extracted from the 'waste liquors' with chloroform. At low pH's the insoluble materials just mentioned were hard, resinous condensation products.

Table 1. Sulphonation of lignin models under various conditions.

Substance (1.0 g was always employed)	Time h	Per cent yield of sulphonic acid (The total amount of 'sulphur dioxide' was always 6.0 % and the amount of 'sodium hydroxide' present in the cooking acids as well as the pH' s (at room temperature) are indicated below. Reaction temperature 135°. Amount of cooking acid 65 ml.)				
		1.4 % NaOH pH = 1.1-1.2	2.8 % NaOH pH = 1.7-1.8	4.0 % NaOH pH = 3.5	7.5 % NaOH pH = 6.5	11.0 % NaOH pH = 11.5
VI. $n = 2$	1.0	70	70			
»	3.5	100	90	70	30	
»	19.0					15
VI. $n = 2+$ 1.0 g resorcinol	3.5	18		50		
VI. $n = 3$	1.0	25	35			
»	3.5	50	80			
VI. $n = 4$	3.5	20	35			
VIII.	1.0		5	10		
»	3.5	10	15			
»	19.0	60				

As seen from this table the rate of sulphonation decreases with increasing chain length. This conforms with the results of Lindgren [6], who found that the 'monomolecular' lignin models, vanillyl and veratryl alcohol, are sulphonated more rapidly than lignin. The decreasing solubility of the higher molecular compounds in sulphite cooking acid reduces the rate of sulphonation. The 'trimolecular' lignin model VI ($n = 3$) is more easily sulphonated at pH 1.8 than at pH 1.1. This may be due to the fact that condensation and sulphonation occur simultaneously, the rates of both reactions depending on the acidity as well as the chain length. The condensation of the 'dimolecular' lignin model VI ($n = 2$) with resorcinol is far greater at pH 1.1 than at pH 3.5. This is in agreement with the assumption that the group 'A' in lignin is of a benzyl alcohol type. Substance VIII reacts much more slowly than the compounds VI ($n = 2$—4). This may be explained by its low solubility in sulphite cooking acid due to the hydrophobic character of the coerulignol part of the molecule.

The sulphonic acids obtained were precipitable as S-l-naphthylmethylthiuronium salts but not with the reagents ordinarily employed for the precipitation of lignin sulphonic acids. This conforms with the known difficulty to precipitate the highly sulphonated low molecular lignin sulphonic acids. Such acids, however, may be precipitated with the above-mentioned reagent.

The group B in lignin which is hydrolyzed and sulphonated in the second step of the sulphite delignification is nonreactive with phenols. It has already been assumed that this group constitutes a cycloacetal group [10]. It is presumably similar to dehydrodi-*iso*eugenol, but has been assumed to possess an hydroxyl group instead of the hydrogen atom at carbon atom 2 in the coumarane ring. It could be imagined to be formed by dehydrogenation of a glucosidic derivative of α-hydroxy coniferyl alcohol. Acetal linkages in lignin have already been assumed by Holmberg [11]. Further experiments on the synthesis of suitable model substances of type B are in progress.

The degradation of the various ketones, alcohols and sulphonic acids described in this paper with nitrobenzene and alkali will be the subject of forthcoming communications which will also contain degradation experiments with dehydrodi-*iso*eugenol and compounds similar to V but also containing dehydrodi-*iso*eugenol components.

EXPERIMENTAL

Acetoveratrone

30 g of acetoguaiacone and 60 ml of dimethyl sulphate were dissolved in 300 ml of alcohol. 60 ml of sodium hydroxide (40 g per 100 ml) were slowly added, in order to keep the temperature of the mixture below 50° C. After refluxing for one hour and cooling, the solution was poured into 750 ml of water. The resulting mixture was extracted with ether, the ethereal solution washed with water, dried over sodium sulphate and evaporated. A readily crystallizing pale yellow oil was obtained.

Yield 24.9 g (78 %). M. p. 50—51°. (M. p. of the pure product 52—53° [12].)

The product may be further purified by distillation under reduced pressure, but in this case the crude product was used directly for further syntheses.

ω-Bromo-acetoveratrone

The compound was prepared mainly according to Mannich and Hahn [13], but the product was recovered in a more careful manner in order to avoid discoloration and to increase the yield.

20 g of acetoveratrone were dissolved in 100 ml of chloroform. 17.7 g of bromine in 50 ml of chloroform were slowly added at room temperature. At the beginning, a dark red solution was obtained. When still more bromine was added, however, the colour disappeared and hydrogen bromide was formed. After all bromine had been added, the solution was more or less reddish. On shaking the latter with bicarbonate solution, the

red colour disappeared. The solution was washed with water and dried over sodium sulphate. The chloroform was evaporated under reduced pressure. In order to avoid excessive discoloration of the product, 200 ml of light petroleum (b. p. 40—60°) were added in several portions during the distillation. On cooling, the resulting light brown oil crystallized. The product was recrystallized from a small amount of methanol. Yield 24 g (84 %). M. p. 80—81°.

Acetoguaiacyl-acetoguaiacone methyl ether[14] (V, $n = 2$, $R = CH_3$)

20 g of ω-bromo-acetoveratrone and 10 g of acetoguaiacone were dissolved in 300 ml of absolute alcohol in a flask provided with a reflux condenser and an inlet for nitrogen. The mixture was heated to boiling on the steam bath and nitrogen was passed in, in order to remove all the air from the flask. Then 14.5 g of the sodium salt of acetoguaiacone were added. The reddish yellow mixture was boiled until all solids dissolved and then another five minutes. (About 15 minutes total.) On cooling, small needle shaped crystals separated. The crystals were collected by filtration and the filtrate concentrated to about 75 ml, yielding more crystals on cooling. Yield 24 g (83 %). The product was recrystallized twice from alcohol. M. p. 138—139°.

$C_{19}H_{20}O_6$	Calc.	C	66.3	H	5.86	OCH_3	27.0
	Found	»	66.1	»	5.90	»	27.0

The first batches of this product had a m. p. of 120.5—121°. This substance was shown to be a dimorphous modification of the above-mentioned substance of m. p. 138—139°. By dissolving in hot alcohol and seeding the cooled solution with the substance of m. p. 138—139°, a product crystallized identical with the latter.

The compound (0.5 g) was boiled for three hours with semi-carbazide hydrochloride (0.5 g) and potassium acetate (0.5 g) in alcohol. A white crystalline *semi-carbazone* was obtained. Recrystallization from dilute acetic acid yielded crystals of m. p. 235—236° (decomp.).

$C_{21}H_{26}O_6N_6$ Calc. OCH_3 20.3 Found OCH_3 20.5

ω-Bromo-acetoguaiacyl-acetoguaiacone methyl ether

15 g of acetoguaiacyl-acetoguaiacone methyl ether were dissolved in 100 ml of chloroform. 7.2 g of bromine in 50 ml of chloroform were slowly added. The reaction was started by irradiation with ultraviolet light and gentle warming. After all bromine was added, the slightly red mixture was extracted with bicarbonate solution, washed with water, and dried over sodium sulphate. The chloroform was evaporated under reduced pressure. The resulting oil crystallized on cooling. Yield 12.2 g (66 %). M. p. 133—138°. Repeated recrystallization from ethyl acetate yielded small crystal needles of m. p. 148.5—149.5°.

$C_{19}H_{19}O_6Br$	Calc.	Br	18.9	OCH_3	22.0
	Found	»	18.9	»	22.2

Di-(acetoguaiacyl)-acetoguaiacone methyl ether (V, $n = 3$, R = CH_3)

a) 5 g of ω-bromo-acetoguaiacyl-acetoguaiacone methyl ether and 1.75 g of acetoguaiacone were dissolved in 150 ml of boiling absolute alcohol in a nitrogen atmosphere. Then 2.37 g of the sodium salt of acetoguaiacone were added, and the mixture was treated as described above. On cooling, a yellowish powder separated. Yield 6 g (80 %). M. p. 140—140.5°. The product was recrystallized twice from dioxane. A white crystalline substance containing dioxane was obtained. This product was dissolved in a small amount of chloroform. To the boiling solution a large amount of alcohol was added. The mixture was boiled in order to evaporate the chloroform and then filtered. On cooling small, colourless crystals separated. M. p. 145—146°.

$C_{28}H_{28}O_9$	Calc.	C	66.1	H	5.56	OCH_3	24.4	Mol. wt.	508	
	Found	»	65.8	»	5.57	»	24.6	» »	511	(Rast)

b) 1.5 g of acetoguaiacyl-acetoguaiacone (see p. 1368) and 1.18 g of ω-bromo-acetoveratrone were dissolved in 50 ml of absolute methyl ethyl ketone. 2 g of anhydrous potassium carbonate were added. After boiling under anhydrous conditions for 45 minutes, the mixture was filtered. On cooling, a yellowish powder (1.0 g) separated from the filtrate. The filter cake was dissolved in dilute sulphuric acid, yielding another 0.8 g of the product. After recrystallization according to a) a colourless product was obtained. M. p. 144—145°. Mixed m. p. with the product of a), 143—145.5°.

A *semi-carbazone* was obtained as described above. M. p. 241—242° (decomp.).

$C_{31}H_{37}O_9N_9$ Calc. OCH_3 18.3 Found OCH_3 18.6

Tri-(acetoguaiacyl)-acetoguaiacone methyl ether (V, $n = 4$, R = CH_3)

2.75 g of acetoguaiacyl-acetoguaiacone, 2.24 g of ω-bromo-acetoguaiacyl-acetoguaiacone methyl ether and 0.36 g of sodium ethoxide in 120 ml of absolute alcohol were treated as described above. A precipitate was formed even in the boiling solution, and the amount increased on cooling. The yellowish powder (3.9 g, 70 %) was collected by filtration and recrystallized three times from dioxane, yielding a colourless product containing dioxane. On repeated recrystallizations from methyl ethyl ketone a microcrystalline product of m. p. 145—147° was obtained.

$C_{37}H_{36}O_{12}$	Calc.	C	66.1	H	5.40	OCH_3	23.1	Mol. wt.	672	
	Found	»	65.9	»	5.79	»	23.2	» »	702	(Rast)

A crystalline semi-carbazone could not be obtained from this compound.

ω-Bromo-acetyl-acetoguaiacone

10 g of acetyl-acetoguaiacone (prepared according to Finnemore [15]) were dissolved in 75 ml of chloroform. 7.7 g of bromine in 35 ml of chloroform were slowly added. The reaction was started by adding a drop of concentrated hydrochloric acid and irradiation

with ultraviolet light. The chloroform solution was treated as described above. Evaporation of the chloroform yielded a readily crystallizing oil. On recrystallization from alcohol, needle shaped crystals of m. p. 88—88.5° were obtained. Yield 8.7 g (67 %).

$C_{11}H_{11}O_4Br$	Calc.	C	46.0	H	3.86	OCH_3	10.8
	Found	»	46.0	»	3.86	»	10.8

Acetoguaiacyl-acetoguaiacone acetate
(V, $n = 2$, R = $COCH_3$)

5 g of ω-bromo-acetyl-acetoguaiacone and 3.3 g of the sodium salt of aceto-guaiacone were refluxed in 200 ml of absolute alcohol for 15 minutes. After cooling, the solution was poured into ether, yielding a white precipitate, consisting partly of sodium bromide. By dissolving in a hot mixture of benzene and petroleum (b. p. 120—140°) and filtering, the product was separated from the sodium bromide. On cooling, white crystals separated. Recrystallization from alcohol yielded 0.7 g (13 %) of needle shaped crystals. M. p. 126—127°.

$C_{20}H_{20}O_7$	Calc.	OCH_3	16.7	Found		OCH_3	16.7

Benzoyl-acetoguaiacone

To 10 g of acetoguaiacone in 20 ml of anhydrous pyridine, 7.0 ml of benzoyl chloride were added in small portions. The temperature of the mixture increased, and finally a crystal slurry was formed. After about half an hour 100 ml of water were added, yielding a white, crystalline product. The product was collected by filtration, dried and dissolved in a small amount of benzene. Petroleum (b. p. 120—140°) was added until the solution just turned cloudy. Long thin colourless needles of m. p. 108—109° [12] slowly separated. Yield 14.5 g (90 %).

ω-Bromo-benzoyl-acetoguaiacone

10 g of benzoyl-acetoguaiacone were brominated as described above. Yield 11.5 g (88 %). Recrystallization from alcohol yielded long, colourless needles of m. p. 105—106°.

$C_{16}H_{13}O_4Br$	Calc.	Br	22.9	Found		Br	22.7

Acetoguaiacyl-acetoguaiacone benzoate
(V, $n = 2$, R = C_6H_5CO)

10 g of ω-bromo-benzoyl-acetoguaiacone and 4.75 g of acetoguaiacone were dissolved in 100 ml of anhydrous methyl ethyl ketone, containing 15 g of anhydrous potassium carbonate, and boiled under anhydrous conditions for 20 minutes. The mixture was poured into water and acidified. The oily layer was separated and the water phase extracted with ether. The oil and the ethereal solution were combined, dried over sodium sulphate and evaporated. On cooling, the resulting oil crystallized. Yield 8.8 g (70 %).

M. p. 110—132°. The product was recrystallized three times from methanol. Colourless crystal needles of m. p. 138—139°.

$C_{25}H_{22}O_7$	Calc.	C	69.1	H	5.11
	Found	»	68.9	»	5.13

The compound was also prepared as described above, in absolute alcohol. A more discoloured product was obtained in a lower yield (55 %).

ω-Bromo-acetoguaiacyl-acetoguaiacone benzoate

To 5 g of acetoguaiacyl-acetoguaiacone benzoate in 100 ml of chloroform, 1.85 g of bromine in 35 ml of chloroform were slowly added. The reaction was very difficult to start, and only after adding a drop of concentrated hydrochloric acid, irradiation with ultraviolet light and heating to boiling, did the colour of the bromine disappear. The reaction continued comparatively slowly. After being treated in the usual way, the chloroform solution was filtered through a column of aluminium oxide. The resulting colourless solution was evaporated, yielding a slightly yellow oil. On treating with methanol the oil crystallized after cooling in the refrigerator. Yield 4.4 g (73 %).

The compound was very difficult to purify and even after repeated recrystallizations from different solvents the bromine content was found to be somewhat too low. M. p. 137—138°.

$C_{25}H_{21}O_7Br$ Calc. Br 15.6 Found Br 15.1

Acetoguaiacyl-acetoguaiacone (V, $n = 2$, R = H)

a) 0.5 g of acetoguaiacyl-acetoguaiacone acetate were refluxed in a nitrogen atmosphere with 0.2 g of potassium hydroxide in 50 ml of alcohol for ten minutes. The solution was poured into water and extracted with ether. The water phase was acidified and extracted with chloroform. The chloroform solution was washed with water, dried over sodium sulphate and filtered through a column of aluminium oxide. On evaporation there remained a yellowish, readily crystallizing oil. Yield 0.30 g (67 %). Recrystallization from methanol yielded crystal needles of m. p. 141—143°.

b) 5 g of acetoguaiacyl-acetoguaiacone benzoate were hydrolyzed by refluxing with 2 g of piperidine in 100 ml of alcohol for 20 minutes. The solution was poured into water, acidified and extracted with chloroform. The chloroform solution was treated in the above-mentioned way, yielding 5.2 g of an oil. On treatment with ether the oil crystallized, yielding 3.1 g (90 %) of crystals. M. p. 136—139°. (From the ether solution 1.8 g (91 %) of benzoyl-piperidine, m. p. 42—43° were obtained.) On recrystallization, needles of m. p. 140—142° were obtained. Mixed melting point with the product of a) 140—142°.

$C_{18}H_{18}O_6$	Calc.	C	65.5	H	5.50	OCH_3	18.8
	Found	»	65.6	»	5.62	»	18.9

The compound was soluble in alkali and gave a blue green colour with ferric chloride.

0.2 g were dissolved in acetone and an excess of diazomethane in ether was added. Evolution of gas was observed. After standing in the refrigerator for 24 hours the solu-

tion was evaporated, yielding a solid residue (0.2 g). Recrystallization from alcohol yielded thin needles of m. p. 119—120°. Mixed m. p. with acetoguaiacyl-acetoguaiacone methyl ether 119—120°.

(These experiments were carried out with one of the first batches of the product, before the dimorphous modification of m. p. 138—139° had been obtained.)

The *semi-carbazone* of the compound was prepared as described above. M. p. 180—182° (from alcohol).

$C_{20}H_{24}O_6N_6$ Calc. OCH_3 14.0 Found OCH_3 13.9

Di-(acetoguaiacyl)-acetoguaiacone benzoate (V, $n = 3$, R = C_6H_5CO)

a) To 1.5 g of acetoguaiacyl-acetoguaiacone and 1.6 g of ω-bromo-benzoyl-acetoguaiacone in 50 ml of anhydrous methyl ethyl ketone, 2 g of anhydrous potassium carbonate were added. The reaction was accomplished as described above. 1.8 g (68 %) of a yellowish product was obtained. The substance was recrystallized twice from ethyl acetate. Thin needles of m. p. 169—171°.

b) 7.75 g of ω-bromo-acetoguaiacyl-acetoguaiacone benzoate and 2.25 g of acetoguaiacone were reacted with 2.85 g of the sodium salt of acetoguaiacone in 250 ml of absolute alcohol in the manner described above. After evaporating the solution to 75 ml, 4.5 g (50 %) of a yellowish powder separated on cooling. On recrystallization according to a), thin needles, m. p. 170.5—171.5°, were obtained. Mixed m. p. with the product, prepared according to a), 168—171°.

$C_{34}H_{30}O_{10}$ Calc. OCH_3 15.6 Found OCH_3 15.7

Di-(acetoguaiacyl)-acetoguaiacone (V, $n = 3$, R = H)

2 g of di-(acetoguaiacyl)-acetoguaiacone benzoate were hydrolyzed with 0.6 g of piperidin in 50 ml of alcohol by the procedure described above. 1.0 g (60 %) of an oil was obtained. The oil crystallized on treatment with methanol. The substance was recrystallized from dioxane, followed by recrystallization from methyl ethyl ketone. On further recrystallizations from methanol, thin needles of m. p. 184—186° were obtained.

$C_{27}H_{26}O_9$ Calc. OCH_3 18.8 Found OCH_3 18.8

To 0.05 g of the compound in acetone an excess of diazomethane in ether was added. On standing in the refrigerator for 24 hours, colourless crystals of m. p. 144—145° (0.05 g) separated. Mixed m. p. with di-(acetoguaiacyl)-acetoguaiacone methyl ether 144—145°.

The *semi-carbazone* was prepared as before. M. p. 198—199° (from a large amount of alcohol).

$C_{30}H_{35}O_9N_9$ Calc. OCH_3 14.9 Found OCH_3 14.0

Tri-(acetoguaiacyl)-acetoguaiacone benzoate
(V, $n = 4$, R = C_6H_5CO)

a) 1.1 g of di-(acetoguaiacyl)-acetoguaiacone, 0.49 g of ω-bromo-benzoyl-acetoguaiacone, and 0.098 g of sodium ethoxide in 110 ml of absolute alcohol were treated as described above. On cooling, 0.5 g (45 %) of a yellowish product separated. After boiling with acetone to remove impurities, the product was recrystallized twice from methyl ethyl ketone. Flat crystals were obtained of m. p. 181—182° (decomp.).

b) 1.5 g of acetoguaiacyl-acetoguaiacone, 1.47 g of ω-bromo-acetoguaiacyl-acetoguaiacone benzoate, and 3 g of anhydrous potassium carbonate in 50 ml of absolute methyl ethyl ketone were treated as described above. 1.8 g (85 %) of a yellowish product were obtained. The product was treated according to a). M. p. 182—183°. Mixed m. p. with the substance of a) 181—183°.

$C_{43}H_{38}O_{13}$ Calc. OCH_3 16.3 Found OCH_3 16.2

Tri-(acetoguaiacyl)-acetoguaiacone
(V, $n = 4$, R = H)

1.2 g of tri-(acetoguaiacyl)-acetoguaiacone benzoate were hydrolyzed with 0.5 g of piperidine in 100 ml of alcohol as described above. 0.7 g (70 %) of an oil, crystallizing on treatment with methanol, resulted. After three recrystallizations from methyl ethyl ketone, colourless small crystals of m. p. 201—202° (decomp.) were obtained.

$C_{36}H_{34}O_{12}$ Calc. OCH_3 18.8 Found OCH_3 18.9

0.05 g of the compound in methyl ethyl ketone were methylated with diazomethane as described above. After 24 hours in the refrigerator, small crystals of m. p. 145—146° separated. Mixed m. p. with tri-(acetoguaiacyl)-acetoguaiacone methyl ether 145—147°.

The *semi-carbazone* was prepared as described above and recrystallized from a mixture of alcohol and acetic acid. M. p. 212—214° (decomp.).

$C_{40}H_{46}O_{12}N_{12}$ Calc. OCH_3 14.0 Found OCH_3 14.3

3,4-Dimethoxyphenacyl coerulignol ether (VII)

5 g of ω-bromo-acetoveratrone, 4.5 g coerulignol, and 1.3 g of sodium ethoxide in 150 ml of absolute alcohol were treated as described above. The mixture was poured into 350 ml of water and extracted with ether. The ethereal extract was washed with 0.2-n sodium hydroxide and water and dried over sodium sulphate. After evaporation, an oil was obtained. The oil could be distilled at a pressure of 1 mm of mercury, yielding 5.0 g (75 %) of an almost colourless oil, crystallizing on treatment with methanol. Recrystallization from methanol yielded crystals of m. p. 73—74°.

$C_{20}H_{24}O_5$	Calc.	C	69.7	H	7.03
	Found	»	69.7	»	7.04

REDUCTION OF THE KETO GROUPS[16]

Dihydro-3,4-dimethoxyphenacyl coerulignol ether (VIII)

6.88 g of 3,4-dimethoxyphenacyl coerulignol ether and 4.0 g of aluminium isopropoxide were boiled in 25 ml of anhydrous isopropyl alcohol until no more acetone was formed. The product was hydrolyzed with dilute hydrochloric acid, yielding a yellow oil. The water phase was extracted with ether. The ethereal solution was combined with the oily layer, washed with water, and dried over sodium sulphate. The solution was filtered through a column of aluminium oxide and evaporated, yielding a light yellow crystallizing oil. Yield 5.3 g (75 %). M. p. 57—59°. On repeated recrystallization from ether, flat crystals of m. p. 62—63° were obtained. Mixed m. p. with the unreduced product (m. p. 73—74°) 45—52°. The analyses showed that the substance contained one mole of water of crystallization.

$C_{20}H_{26}O_5 + H_2O$	Calc.	C	65.9	H	7.76	OCH_3	25.5
	Found	»	66.3	»	7.78	»	25.6

On fusing the substance *in vacuo* and recrystallization from anhydrous ether, crystals of m. p. 65—66° were obtained.

$C_{20}H_{26}O_5$	Calc.	OCH_3	26.9	Found	OCH_3 27.0

The compound did not form a precipitate with 2,4-dinitrophenyl-hydrazine.

Tetrahydro-acetoguaiacyl-acetoguaiacone methyl ether (VI, $n = 2$)

6.4 g of acetoguaiacyl-acetoguaiacone methyl ether and 16.0 g of aluminium isopropoxide in 60 ml of absolute isopropyl alcohol were treated as described above, extracting with chloroform instead of ether. On drying *in vacuo*, the resulting oil turned into a yellowish, amorphous powder. Yield 4.5 g (70 %). No precipitate with 2,4-dinitrophenyl-hydrazine.

$C_{19}H_{24}O_6$	Calc.	OCH_3	26.7	Found	OCH_3 26.3

When the substance was heated in a capillary tube, softening commenced at about 50°. The 'melt' was very viscous but became transparent at about 60°. 'Collaps' took place at about 100°.

Hexahydro-di-(acetoguaiacyl)-acetoguaiacone methyl ether (VI, $n = 3$)

5.6 g of di-(acetoguaiacyl)-acetoguaiacone methyl ether and 12 g of aluminium isopropoxide in 60 ml of absolute isopropyl alcohol were treated as above. An almost white, amorphous powder was obtained. Yield 4.4 g (74 %). No precipitate with 2,4-dinitrophenyl-hydrazine.

$C_{28}H_{34}O_9$	Calc.	OCH_3	24.1	Found	OCH_3 24.7

Octahydro-tri-(acetoguaiacyl)-acetoguaiacone methyl ether (VI, $n = 4$)

2.9 g of tri-(acetoguaiacyl)-acetoguaiacone methyl ether and 5 g of aluminium isopropoxide in 35 ml of absolute isopropyl alcohol were treated as described above. 2.66 g (90 %) of an amorphous powder were obtained. No precipitate with 2,4-dinitrophenylhydrazine.

$C_{37}H_{44}O_{12}$ Calc. OCH_3 22.8 Found OCH_3 22.8

When the substance was heated in a capillary tube, softening commenced at about 70°. The 'melt' was very viscous but became transparent at about 100°. 'Collaps' took place at about 130°.

Sulphite cookings

The ketonic compounds (V) were treated in the following manner:

0.5—1 g of the substance and 100 ml of sulphite cooking acid (5—6 % SO_2 and 1.4 % NaOH) were sealed in a pyrex glass tube. The tube was heated in an oven at 135° for 20 hours.

After cooling, all the tubes contained undissolved residues, consisting of unchanged starting material. Since the recovery of the latter amounted to 88—100 %, no attempts were made to work up the aqueous solutions.

The compounds also were heated with a cooking acid, containing 9—10 % SO_2 and 0.7 % NaOH as described above. The compounds were slightly decomposed, but more than 80 % could be recovered.

The above mentioned alcohols (VI and VIII) were cooked in autoclaves, rotating in an oil bath under conditions specified in table 1.

The 'waste liquors' were treated in the following way:

After removing the excess sulphur dioxide by passing in carbon dioxide or nitrogen, the solution was extracted with chloroform in order to dissolve decomposition products. The cations of the solution were then exchanged for hydrogen ions in a column of amberlite. SO_2 was removed and an excess of a slurry of barium carbonate in water was added. After standing over night, the mixture was heated to boiling and filtered hot. The filtrate was evaporated to dryness. The residue was dissolved in a small amount of water and filtered. The resulting solution was precipitated with alcohol and ether, yielding the barium salt of the sulphonic acid as a white precipitate of small particle size. The latter was centrifuged off and washed with alcohol and ether. For purification the precipitation was repeated several times.

The barium salts obtained were dissolved in water and precipitated with an aqueous solution of S-l-naphthylmethylthiuronium chloride. The resulting products were isolated and analyzed in only two cases.

Sulphonic acid from VIII.

Barium salt:

$(C_{20}H_{25}O_7S)_2Ba$	Calc.	Ba	14.4	OCH_3	19.5
	Found	»	14.7	»	19.3

Naphthylmethylthiuronium salt:
Recrystallized from water. M. p. 148—150°.

$C_{32}H_{38}O_7N_2S_2$	Calc.	S	10.2	OCH_3	14.9
	Found	»	10.3	»	15.1

Sulphonic acid from VI, n = 2.
Barium salt:

$C_{19}H_{22}O_{10}S_2Ba$	Calc.	Ba	22.5	OCH_3	15.2
	Found	»	22.6	»	15.1

Naphthylmethylthiuronium salt:

$C_{43}H_{48}O_{10}N_4S_4$	Calc.	S	14.1		
	Found	»	13.8		

Sulphonic acid from VI, n = 3.
Barium salt:

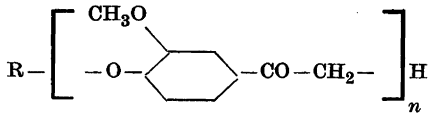
$(C_{28}H_{31}O_{15}S_3)_2Ba_3$	Calc.	Ba	22.7	OCH_3	13.6
	Found	»	22.5	»	13.4

Sulphonic acid from VI, n = 4.
Barium salt:

$C_{37}H_{40}O_{20}S_4Ba_2$	Calc.	Ba	22.8	OCH_3	12.8
	Found	»	23.0	»	12.8

SUMMARY

A series of ethers of ω-hydroxy-acetoguaiacone of the general formula



have been prepared and reduced to the corresponding secondary alcohols.

The sulphonation of the compounds with sulphite cooking acid has been studied.

The authors wish to acknowledge their indebtedness to *Statens Tekniska Forskningsråd* for financial support.

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Received September 29, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1375–1380

Constituents of Pine Heartwood

XV.* The Heartwood of *Pinus excelsa* Wall.

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Pinus excelsa, 'Bhutan Pine', is a *Haploxylon* pine growing on the slopes of the Himalayas in northern India. The botanists [1] consider it to belong to the group *Strobi*, containing, among other pines, *P. strobus* and *P. monticola*. The heartwood constituents of these latter two pines have already been investigated (see Parts V[2] and XIV[3]). The present investigation shows that the botanical relationship between *P. excelsa* and the above-mentioned pines is clearly demonstrated by the heartwood constituents.

The heartwood was extracted with ether and acetone as described before [4]. The ether extract (14 % of the heartwood) deposited large crystals after some days. Just as in the case of *P. strobus* and *P. monticola*, even the ether extract had to be investigated. The 0.2 % sodium hydroxide fraction contained small amounts of chrysin and pinobanksin, and the 4 % sodium hydroxide fraction contained tectochrysin and a considerable amount of pinosylvin monomethyl ether (about 40 % of the total yield of this substance).

The acetone extract was fractionated in the usual manner. The water-soluble portion contained pinitol and *l*-arabinose, which were separated by precipitation of the sugar as its *p*-bromophenylhydrazone. The sodium carbonate fraction gave a good yield of pinobanksin, precipitated as its sodium salt. Some chrysin was co-precipitated with the pinobanksin, and more chrysin was found in the filtrate. (Pure chrysin gives no precipitate with sodium carbonate solution.) Pinocembrin could be isolated from the 0.2 % sodium hydroxide fraction. The 4 % sodium hydroxide fraction contained a small quantity of tectochrysin (precipitated as its sodium salt) along with much pinosylvin monomethyl ether.

* XIV. *Acta Chem. Scand.* 3 (1949) 1147.

The yields obtained from 6.5 kg of air-dry heartwood are tabulated below. Only 392 g of the ether extract (total weight 917 g) were investigated, and from those values the theoretical yields for the entire ether extract have been calculated.

Substance	Found in acetone extract	Found in 392 g of ether extract	Calc. for the entire ether extract	Total yield
'Membrane substances'	8.6 g	—	—	8.6 g (0.13 %)
Pinitol + *l*-arabinose	7.4 g *	—	—	7.4 g (0.11 »)
Chrysin	2.4 g	1.0 g	2.3 g	4.7 g (0.07 »)
Tectochrysin	0.4 g	1.8 g	4.2 g	4.6 g (0.07 »)
Pinobanksin	7.5 g	0.4 g	0.9 g	8.4 g (0.13 »)
Pinocembrin	0.9 g	—	—	0.9 g (0.01 »)
Pinosylvin monomethyl ether	53 g	16 g	38 g	91 g (1.4 »)
Neutral fraction	5.1 g	12 g	28 g	33 g (0.5 »)

The specimen of *P. excelsa* investigated here gave very high yields of phenolic substances. The content of pinosylvin monomethyl ether was of the same magnitude as in the best specimens of *P. sylvestris* hitherto investigated. *P. excelsa* resembles other *Haploxylon* pines in its content of pinitol, chrysin, and tectochrysin, but differs from them in its large content of pinobanksin. This substance has been isolated from several *Diploxylon* pines, but of *Haploxylon* pines, only *P. strobus* yielded a very small quantity of it [2]. Strobopinin, a characteristic heartwood constituent of *P. strobus*, *P. monticola*, and *P. Lambertiana*, could not be found in *P. excelsa*. A comparatively large fraction of the heartwood could be extracted with ether (14 %), which indicates that the wood was rich in resins. The ether also extracted large amounts of phenols, although the wood had a comparatively large content of 'membrane substances'. *P. strobus* behaves like *P. excelsa* in this respect [2], but from *P. monticola* only a small part of the phenols can be extracted by ether [3].

To sum up, the heartwood of *P. excelsa* contains the substances which seem to be characteristic of *Haploxylon* pines (pinitol, chrysin and tectochrysin). It also, however, has some features in common with many *Diploxylon* pines (high yields of pinobanksin, pinosylvin monomethyl ether and ether extract).

* The whole mixture was not separated. Only 1.0 g of pinitol and 0.2 g of *l*-arabinose were isolated in a pure state.

EXPERIMENTAL

The wood used for the investigation came from Wood Technologist Forest Research Institute, Dehra Dun, India. The heartwood gave a strong dark red colour when stained with diazotised benzidine solution. The air-dried, fine-ground heartwood (6.5 kg) was extracted with ether for 24 hours and then with acetone for 48 hours. The extracted wood then gave a somewhat weaker colour reaction with the benzidine reagent, but further extraction did not weaken this colour reaction any more. (Acetone for seven days and benzene, ethanol and ethyl acetate for 24 hours were tried.) It is evident that part of the colour obtained with diazotised benzidine is due to insoluble products in the wood.

The ether extract (917 g) was a dark brown syrup, which deposited large crystals in a few days. 36.7 g of the extract were treated with 200 ml of light petroleum, and the yellowish brown sticky residue was extracted with boiling water (2 × 250 ml). The aqueous extracts were cooled and shaken with ether. The ether solution was dried over anhydrous sodium sulphate and concentrated, leaving 0.57 g of a colourless oil, which deposited small crystals. Since the ether extract seemed to contain comparatively large amounts of phenols, it was necessary to investigate a larger portion of it.

Investigation of the ether extract

Part of the ether extract (392 g) was treated with light petroleum (1 l). The solution was separated from the residue by decantation and the solvent evaporated, leaving 193 g of a pale yellow oil, which deposited large crystals (probably resin acids). It was not investigated any further. The residue was dissolved in ether (700 ml) and the ether solution was shaken with saturated sodium bicarbonate, saturated sodium carbonate, 0.2 % sodium hydroxide (200 + 150 ml of each) and, finally, with 4 % sodium hydroxide solution (6 × 150 ml). The three first fractions were called EB, EC and EH_1. A yellow crystalline precipitate was formed in the 4 % sodium hydroxide solution. It was separated by filtration (EH_{21}). Filtrate = EH_{22}. Each fraction (except EH_{21}) was acidified and extracted with ether. The ether solutions were dried over anhydrous sodium sulphate and concentrated. The remaining 'neutral fraction' was a reddish-brown oil with a strong fluorescence. Yield, 12.0 g.

EB and *EC* yielded small amounts of a brown sticky product, which did not crystallise.

EH_1 yielded a large quantity of a brown oil (about 100 g). After a few days it began to deposit a small amount of crystals. The mixture was treated with ether and the crystals removed by filtration. They were brownish-yellow and melted gradually at 250—257°*. After vacuum-sublimation and recrystallisation from ethanol, pale yellow crystals (m. p. 272—274°) were obtained. Yield, 1.0 g. A small part of this substance was acetylated with acetic anhydride and pyridine. The acetate, when recrystallised from ethanol, formed long, thin, colourless needles melting at 193—195°. The mixed m. p. with chrysin diacetate was 193—195°.

The ether filtrate was concentrated to an oil again and extracted with boiling water several times. The water extracts were cooled, forming milky suspensions which were extracted with ether. The ether solutions were combined and dried over anhydrous sodium sulphate. On evaporation they yielded a crystalline residue which was recrystal-

* All melting points uncorrected.

lised twice from 50 % acetic acid and once from toluene. Thick colourless needles (0.4 g), m. p. 175—176°, were obtained. They gave no m. p. depression when mixed with pinobanksin. The insoluble residue from the water extraction was a brown sticky product which did not crystallise.

EH_{21}: The crystalline sodium salt was acidified with dilute sulphuric acid, and the liberated phenol was washed and dried, sublimated in a vacuum, and recrystallised twice from chloroform-light petroleum. Yield, 1.8 g of yellow crystals, m. p. 163—164°. The acetate, prepared by heating the substance with acetic anhydride and pyridine for half an hour on a water bath, melted at 154—155° after one recrystallisation from ethanol, and gave no m. p. depression with tectochrysin acetate.

EH_{22} was a brown oil which crystallised readily. It was distilled in a vacuum and the crystallised distillate recrystallised three times from 50 % acetic acid and once from chloroform, yielding 16.3 g of an almost colourless crystalline product, melting at 119—121°. A mixture with pinosylvin monomethyl ether melted at the same temperature.

Investigation of the acetone extract

The acetone extract was concentrated on the steam bath. The residue consisted of a brown resinous product and an aqueous solution (= W). They were separated by decantation and the resinous product treated with ether to precipitate 'membrane substances'. The ether filtrate (1 l) was shaken with 300 ml of water which was combined with W, and the ether solution then divided into fractions in the same way as described for the ether extract. The fractions are called B, C, H_1 and H_2, respectively. The 'membrane substances' were stirred with cold water, the suspension filtered and the filtrate added to W. The 'membrane substances' were then dried, yielding 8.6 g of a light brown powder.

The 'neutral fraction', which was left in ether solution after the alkali extractions, yielded 5.1 g of a brown turpentine-smelling oil on evaporation. It showed a strong fluorescence in ultra-violet light.

W was shaken with a little ether which was combined with the main ether solution (before the fractionation). The water solution was then evaporated to dryness, by vacuum distillation, and the remaining brown syrup dissolved in boiling ethanol. When the ethanol solution was evaporated to a small volume and cooled, it deposited a colourless crystalline precipitate which was separated by filtration. Further evaporation of the mother liquor yielded an additional amount of crystalline products. The combined precipitates (7.4 g) melted gradually between 140 and 165°, reduced Fehling's solution, and gave a pentose colour reaction with phloroglucinol-hydrochloric acid. Further recrystallisation yielded no pure products. Pinitol could easily be isolated after precipitation of the sugar as its phenylosazone. The excess of phenylhydrazine was removed by benzaldehyde, and the excess of benzaldehyde extracted with ether. The remaining water solution was concentrated to a syrup, from which pinitol was obtained after recrystallisation from ethanol. 2.1 g of the mixture thus yielded 1.0 g of pinitol. Its m. p. was 181—184° but could be raised to 184—186° by further recrystallisation. $[\alpha]_D^{20}$ + 64.5° ± 0.5° (water, c = 3.2). A small amount of *l*-arabinose (0.2 g) was isolated by precipitation as *p*-bromophenylhydrazone. The free sugar was liberated from it by treatment with benzaldehyde as described for *P. monticola* [3]. The sugar melted at 156—158° and gave no m. p. depression with *l*-arabinose. $[\alpha]_D^{20}$ + 101° ± 1° (equilibrium rotation in water, c = 2.3).

B was acidified and extracted with ether. On evaporation of the solvent, the ether solution yielded a brown amorphous product (2 g). This was not further investigated.

C: The sodium carbonate extract deposited a pale yellow crystalline precipitate (C_1), which was separated by filtration. The filtrate was extracted with ether (ether solution = C_2) and then acidified. A sticky brown precipitate was formed. It was taken up in ether, the ether solution dried over anhydrous sodium sulphate and then concentrated, leaving a brown oil which slowly crystallised (= C_3).

C_1 was stirred with dilute sulphuric acid, yielding a brown sticky solid, which was extracted with ether. The insoluble residue was separated, dried in the air and extracted with ether in a Soxhlet apparatus for 48 hours. Most of it dissolved, leaving a dark brown insoluble residue (6 g). The ether extracts were combined, dried over anhydrous sodium sulphate and concentrated to a small volume. A yellow precipitate was formed and separated by filtration. It melted at 265—273° and consisted of crude chrysin. The ether solution was shaken with a saturated sodium carbonate solution to precipitate the pinobanksin sodium salt again. The precipitate was collected, treated with dilute sulphuric acid, combined with crude pinobanksin from C_3 (see below) and then recrystallised several times from 50 % acetic acid and from toluene. Finally, 7.5 g of pinobanksin, m. p. 176—177°, were obtained. $[\alpha]_D^{20}$ + 14° ± 1° (methanol, c = 4.5). The m. p. was not depressed on admixture of pinobanksin from *P. Banksiana.*

C_2 was concentrated to a small volume. A pale yellow precipitate was formed and collected. It melted at 255—259° and was combined with the chrysin found in C_1. The crude chrysin was purified by vacuum sublimation and recrystallisation from ethanol, yielding 2.4 g of pure chrysin, m. p. 275—276°. Its acetate melted at 193—195° and gave no m. p. depression when mixed with chrysin diacetate.

C_3 was treated with ether, and the brownish insoluble crystals were collected. They were dissolved in ether, and the solution filtered through aluminium oxide, which adsorbed most of the coloured impurities. The filtrate was evaporated, yielding a crystalline residue, which was recrystallised from toluene. A small amount of crude pinobanksin (m. p. 168—172°) was obtained and combined with the corresponding product from C^I.

H_1 was acidified and taken up in ether. The ether solution was dried over anhydrous sodium sulphate. On concentration, it yielded a reddish-brown oil which deposited a small quantity of crystals. It was treated with methanol and ether and the crystals collected. They were purified by recrystallisation from 50 % acetic acid and vacuum sublimation, yielding a small amount of crude chrysin (m. p. 270—275°) which was added to the corresponding fraction from C_1 and C_2.

After removal of the chrysin, the oil was left standing for some days. Additional crystals were formed and collected after treatment with ether. This product (m. p. 190—192°) was recrystallised twice from 50 % acetic acid. Colourless needles, m. p. 193—194°, were obtained. Yield, 0.9 g. $[\alpha]_D^{20}$ −54.0° ± 0.5° (methanol, c = 3.7). The substance melted at the same temperature when mixed with an equal amount of pinocembrin.

H_2: The 4 % sodium hydroxide extract deposited a yellow crystalline precipitate (H_{21}), which was separated by filtration and treated with dilute sulphuric acid to liberate the phenol. It was sublimated in a vacuum and recrystallised from methanol and from chloroform-light petroleum. Yellow crystals (0.4 g), m. p. 165—166°, were obtained. The m. p. was not depressed on admixture of tectochrysin from the ether extract.

The sodium hydroxide solution was acidified and extracted with ether. The ether solution was dried over anhydrous sodium sulphate and the solvent evaporated. The

brownish residue crystallised readily. The crystals were dissolved in ether, the solution decolourised by filtration through aluminium oxide and evaporated. The crystalline residue was recrystallised twice from 50 % acetic acid, yielding 53 g of a pale flesh-coloured crystalline substance. M. p. 118—119°. The m. p. was not depressed on admixture of pinosylvin monomethyl ether.

SUMMARY

The heartwood of *Pinus excelsa* Wall. has been investigated. Pinitol, *l*-arabinose, chrysin, tectochrysin, pinobanksin, pinocembrin, and pinosylvin monomethyl ether were isolated from it.

The author is indebted to Dr. K. A. Chowdhury, Dehra Dun, India, for supplying the wood, and to Mrs. B. Strömgren and Mr. A. Misiorny for skilful experimental assistance. The investigation was facilitated by a grant from *Fonden för Skoglig Forskning*.

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Received October 1, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1381–1384

Constituents of Pine Heartwood

XVI.* The Heartwood of *Pinus virginiana* Mill.

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Pinus virginiana, 'Scrub Pine', growing in the southeastern part of the United States of America, is a pine belonging to the group *Insignes* of the section *Diploxylon* [1]. This group contains, among other species, *P. Banksiana*, *P. contorta* and *P. radiata*, the heartwood constituents of which have been previously investigated [2-4].

The heartwood was extracted with ether and acetone in the usual way (see Part IX[5]). The ether extract (11 % of the heartwood) crystallised partly. Treatment with light petroleum separated it into a soluble and an insoluble fraction. The insoluble fraction was found to contain pinobanksin. No other crystalline products could be isolated from it. The water-soluble fraction of the acetone extract contained comparatively large quantities of *l*-arabinose, and the sodium carbonate fraction yielded pinobanksin (3—4 times the quantity found in the ether extract). Pinocembrin was found in the 0.2 % sodium hydroxide fraction, and also, in a small quantity, in the 4 % sodium hydroxide fraction. This fraction consisted mainly of resinous products, from which 60 mg of pinosylvin monomethyl ether could be isolated after a very tedious process of separation.

The total yields from 6.3 kg of air-dried heartwood were:

'Membrane substances'	7.5 g	(0.12	%)
l-Arabinose	8.3 g	(0.13	»)
Pinobanksin	15.2 g	(0.24	») **
Pinocembrin	5.6 g	(0.09	»)
Pinosylvin monomethyl ether	0.06 g	(0.001	»)
Neutral fraction of acetone extract	14.9 g	(0.24	»)
» » » ether »	3.8 g	(0.06	»)

* XV. *Acta Chem. Scand.* 3 (1949) 1375.

** 11.4 g of pinobanksin were obtained from the acetone extract. Only a small part (about 13 %) of the ether extract was investigated. The calculated yield of pinobanksin from the entire ether extract was 3.8 g.

Just as its relatives *P. Banksiana*, *P. radiata* and *P. contorta*, *P. virginiana* also contains pinobanksin (probably 3,5,7-trihydroxyflavanone). The yield from the specimen used for the present extraction was better than from any pine previously investigated. The very low content of pinosylvin monomethyl ether is remarkable. Due to the lack of additional wood samples, it is impossible to say if this anomaly is characteristic of the whole species, or if the specimen investigated here just happened to be extraordinarily poor in pinosylvin monomethyl ether.

EXPERIMENTAL

The wood used for the investigation came from a tree grown at Lee Experimental Forest, Buckingham, Virginia, U.S.A. The heartwood gave a rather weak red colour when stained with diazotised benzidine solution.

6.3 kg of air-dried heartwood were extracted with ether for 24 hours and then with acetone for 48 hours. The ether extract (686 g) was a brown syrup which partly crystallised. 283 g of it were treated with light petroleum (1.5 l), leaving an insoluble yellowish precipitate which was collected, washed, and dried. Yield 22.2 g. It melted gradually at 70—100° under decomposition. Its solution in acetone gave no precipitate with cyclohexylamine, but the light petroleum solution gave a thick precipitate, indicating that it contained resin acids.

Part of the insoluble fraction (7 g) was extracted with boiling water, and the extract cooled and shaken with ether. The ether was evaporated, yielding a semi-crystalline residue, from which pure pinobanksin (0.4 g) was obtained after one recrystallisation from 50 % acetic acid and two from toluene. The m. p. of the substance was 176—177°.* A somewhat better yield of pinobanksin (0.5 g) was obtained from another 7 g portion of the insoluble fraction, which was dissolved in ether and divided into fractions by shaking with sodium bicarbonate, sodium carbonate, 0.2 % sodium hydroxide and 4 % sodium hydroxide solutions in the usual way. The sodium carbonate fraction was acidified and the resulting brown precipitate extracted with ether. The ether solution was concentrated and the residue recrystallised from 50 % acetic acid and from toluene to yield pure pinobanksin. The 0.2 % sodium hydroxide fraction yielded a brown oil which did not crystallise, and the 4 % sodium hydroxide fraction a very small quantity of a brown sticky product. The remaining 'neutral fraction' was concentrated to a brown turpentine-smelling syrup (0.5 g).

The acetone extract was concentrated to a brown resinous product and a small volume of water solution. The water phase (= W) was separated by decantation, and the resin treated with 400 ml of ether to precipitate 'membrane substances'. They were separated, dried in the air and stirred with cold water (100 ml). The suspension was then filtered, and the filtrate combined with W. The 'membrane substances' were air-dried, yielding 7.5 g of a light brown powder.

The ether solution was first shaken with water (100 ml), which was combined with W. It was then divided into fractions by successive shaking with saturated sodium bicarbonate (3 × 250 ml), saturated sodium carbonate (3 × 500 ml), 0.2 % sodium

* All melting points uncorrected.

hydroxide (2 × 200 ml), and 4 % sodium hydroxide (2 × 200 ml) solutions. The fractions are referred to as B, C, H_1, and H_2, respectively. The remaining ether solution, containing neutral products, was concentrated to a yellowish-brown oil of low viscosity (14.9 g), which had a turpentine-like odour and showed a strong fluorescence in ultraviolet light.

W was concentrated by vacuum distillation, yielding a brown syrup, which was dissolved in boiling ethanol. The extract was concentrated and cooled, yielding a colourless crystalline precipitate, which was separated and recrystallised twice from ethanol. Yield, 8.3 g of *l*-arabinose, m. p. 159—161°. $[a]_D^{20}$ + 106° ± 1° (equilibrium rotation in water, c = 3.0). The sugar gave a crystalline precipitate with *p*-bromophenylhydrazine in acetic acid solution. No additional crystalline products could be obtained from the filtrate after this precipitation.

B was acidified and extracted with ether. The ether solution was concentrated to a yellowish sticky product (12 g) which did not crystallise.

C: The sodium carbonate solution deposited a precipitate, consisting of yellow crystals and a brown resinous, semi-solid product. The entire precipitate (C_1) was separated from the solution, which was then extracted with ether. Ether extract = C_2. The carbonate solution was then acidified and extracted with ether again. This ether solution yielded a yellow solid after evaporation of the ether. A small quantity of colourless crystals (0.7 g) could be separated from it. They melted at 133—140° and gave a yellow precipitate with saturated sodium carbonate, indicating the presence of pinobanksin. This fraction was not investigated further.

C_1 was acidified and extracted with ether. The brown insoluble residue was dried in the air and extracted with ether again in a Soxhlet apparatus for about 90 hours, leaving 7.0 g of a brown insoluble powder. Further extraction with ether was of no effect. The combined ether extracts were dried over anhydrous sodium sulphate and the ether evaporated. The residue was a yellow crystalline product which was recrystallised from toluene, dissolved in ether, and decolourised by filtration through aluminium oxide. The filtrate was evaporated to dryness and the crystals recrystallised from toluene once again, yielding pale yellow crystals melting at 174—175°. After one recrystallisation from methanol-water, the m. p. was raised to 175—177° and was not depressed on admixture of pinobanksin from *P. Banksiana*. $[a]_D^{20}$ + 13.5° ± 0.5° (methanol, c = 3.6). Yield, 11.4 g. The toluene mother liquors were concentrated by vacuum distillation and the residue recrystallised from 50 % acetic acid, yielding crude pinocembrin (2.5 g), m. p. 191—193°, which was combined with the corresponding fraction from H_1.

C_2 was dried over anhydrous sodium sulphate and concentrated, yielding a yellow crystalline residue, which was recrystallised from 50 % acetic acid. A small quantity of crude pinocembrin was obtained. It was combined with H_1.

H_1: The 0.2 % sodium hydroxide extract was acidified and extracted with ether. The ether solution was dried over anhydrous sodium sulphate and the solvent evaporated. The pale yellow crystalline residue was recrystallised from 50 % acetic acid, yielding pale yellow crystals, m. p. 190—192°. This substance was combined with the crude pinocembrin from C and H_2, dissolved in ether, and decolourised by filtration through aluminium oxide. The filtrate was evaporated to dryness and the residue recrystallised twice from 50 % acetic acid. Yield, 5.6 g of pinocembrin, m. p. 194—195°. $[a]_D^{20}$ − 54° ± 1° (methanol, c = 2.1).

H_2: The alkaline solution was acidified and extracted with ether. The ether extract was dried and concentrated to a yellowish-brown syrup, which showed some tendency to crystallise. After treatment with a little ether, an insoluble precipitate could be separated. It melted at 187—189° and was combined with the crude pinocembrin from H_1. The ether filtrate was concentrated to a syrup again, which did not crystallise in a week. It was then distilled in a vacuum, yielding a reddish-brown syrupy distillate. To remove the last traces of pinocembrin, this distillate was dissolved in ether and shaken with 0.1 % sodium hydroxide solution. The ether solution was then dried and filtered through aluminium oxide, which adsorbed most of its colour. After concentration, the ether filtrate deposited a small amount of a crystalline substance, m. p. 112—119°. After recrystallisation from chloroform-light petroleum, colourless crystals (60 mg), m. p. 118—120°, were obtained. A mixture with pinosylvin monomethyl ether melted at the same temperature. The remaining yellow syrup did not deposit any more crystals.

SUMMARY

The heartwood of *Pinus virginiana* Mill. has been investigated. *l*-Arabinose, pinobanksin (probably 3,5,7-trihydroxyflavanone), pinocembrin (5,7-dihydroxyflavanone) and a very small quantity of pinosylvin monomethyl ether were isolated from it.

The author is indebted to Mrs. B. Strömgren for skilful assistance with the experimental work and to Dr. I. T. Haigh, Southeastern Forest Experimental Station, Asheville, North Carolina, U.S.A., for supplying the wood. The investigation was facilitated by a grant from *Fonden för Skoglig Forskning.*

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Received October 12, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1385 – 1399

Displacement Equilibria and Catalysis on Thiosulphates, Xanthates and Dithiocarbamates of Divalent Sulphur, Selenium and Tellurium

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Pentathionate and hexathionate, in reactions with nucleophilic reagents, behave [1] as thiosulphates of divalent sulphur, S^{++} and S_2^{++}. In aqueous solutions, as well as in the solid state, they have a tendency to liberate sulphur, forming tetrathionate [2–11]:

$$S_2(S_2O_3)_2^{--} = S + S(S_2O_3)_2^{--} \quad (1)$$

$$S(S_2O_3)_2^{--} = S + (S_2O_3)_2^{--} \quad (2)$$

These changes, in solutions, are strongly catalyzed by thiosulphate [1, 4, 7, 10, 11] and by hydroxyl ions [1–11].

In this article, some analogous catalytic reactions are described. Thus, monoselenopentathionate [12], a thiosulphate of divalent selenium, tends to decompose into selenium and tetrathionate:

$$Se(S_2O_3)_2^{--} = Se + (S_2O_3)_2^{--} \quad (3)$$

In this case also, thiosulphate [12, 13] and hydroxyl ions act as catalysts. Likewise, monotelluropentathionate [14], a thiosulphate of divalent tellurium, liberates tellurium, to give tetrathionate:

$$Te(S_2O_3)_2^{--} = Te + (S_2O_3)_2^{--} \quad (4)$$

Here, iodide ions, as well as thiosulphate and hydroxyl ions, serve as catalysts.

Thiocarbonyl derivatives of divalent sulphur and selenium undergo the same type of change. In these cases, disulphides are formed, instead of tetrathionate, together with sulphur or selenium. *E. g.*:

$$Se(S_2COR)_2 = Se + (S_2COR)_2 \quad (5)$$

$$Se(S_2CNR_2)_2 = Se + (S_2CNR_2)_2 \quad (6)$$

These reactions are strongly catalyzed by xanthate and by dithiocarbamate ions.

Xanthates and dithiocarbamates of divalent tellurium are more stable than the corresponding sulphur and selenium compounds. With the tellurium compounds, displacement equilibria of the following type may be demonstrated:

$$Te(S_2CNR_2)_2 + 2\ R'_2NCS_2^- \rightleftharpoons Te(S_2CNR'_2)_2 + 2\ R_2NCS_2^- \quad (7)$$

Under special conditions, the selenium compounds react in the same way.

The purpose of this article is, chiefly, to demonstrate the symmetry with respect to catalytic phenomena which is displayed within the class of compounds named in the title. The mechanism of the catalysis is a subject of uncertainty. The experiments are, though, in harmony with a hypothesis, advanced earlier [1], that the catalysis is due, in some way, to ionic displacement equilibria of the type represented by Eq. (7).

XANTHATES AND DITHIOCARBAMATES

Russell [15] reported that selenious acid reacts with sodium dithiocarbamates to give dithiocarbamates of tetravalent selenium. He stated that the product 'frequently, if not usually' appeared as an equimolar mixture of the divalent selenium dithiocarbamate and the corresponding *bis*(thiocarbamyl) disulphide. In the case of the ethyl compounds, the two components could be separated by treatment of the product with cold benzene, giving a selenium *bis*(diethyldithiocarbamate) of m. p. 110° C.

It was shown recently [12] that monoselenopentathionate reacts with sodium diethyldithiocarbamate to give selenium *bis*(diethyldithiocarbamate), m. p. 116° C, in quantitative yield. Likewise [14], monotelluropentathionate reacts with sodium diethyldithiocarbamate and potassium ethylxanthate to give tellurium *bis*(diethyldithiocarbamate) and di(ethylxanthate), respectively.

In this work, xanthates of divalent selenium are described for the first time, together with two new dithiocarbamates, and two new xanthates and dithiocarbamates of divalent tellurium. They were prepared from monoselenopentathionate and monotelluropentathionate, the reactions being, in the case of the xanthates:

$$Se(S_2O_3)_2^{--} + 2\ ROCS_2^- = Se(S_2COR)_2 + 2\ S_2O_3^{--} \quad (8)$$

$$Te(S_2O_3)_2^{--} + 2\ ROCS_2^- = Te(S_2COR)_2 + 2\ S_2O_3^{--} \quad (9)$$

with similar equations for the dithiocarbamates. With a slight excess of the thiocarbonyl salt, the reactions are complete in a few minutes:

To 0.01 mole of sodium or potassium monoselenopentathionate or monotelluropentathionate dissolved in 100 ml of water were added, with stirring, 0.025 mole of sodium or potassium xanthate or dithiocarbamate dissolved in the same amount of water. After a few minutes stirring the product had coagulated, leaving the liquid clear. It was filtered off, washed with water and with methanol, and dried *in vacuo* over sulphuric acid. The selenium ethyl- and *iso*-propylxanthates separated out as oils, which were extracted from the aqueous layer by means of ether. The ether extracts were dried over anhydrous sodium sulphate, and the ether subsequently distilled off.

Table 1. Xanthates and dithiocarbamates of divalent selenium and tellurium.

Compound	M. p., °C (uncorr.)	% Se or Te	
		Calc.	Found
$Se(S_2COCH_3)_2$	106[a]	26.93	26.91
$Se(S_2COC_2H_5)_2$	Oil	24.57	24.41
$Se(S_2COCH(CH_3)_2)_2$	Oil	22.60	22.53
$Se(S_2CN(CH_3)_2)_2$	182—3[b]	24.72	24.57
$Se(S_2CN(C_2H_5)_2)_2$	116[c]	21.03	21.01
$Se(S_2CNC_5H_{10})_2$	175—6[d]	19.76	19.70
$Te(S_2COCH_3)_2$	89[e]	37.31	37.33
$Te(S_2COC_2H_5)_2$	94[f]	34.48	34.35
$Te(S_2COCH(CH_3)_2)_2$	87[g]	32.05	32.12
$Te(S_2CN(CH_3)_2)_2$	>250[h]	34.67	34.74
$Te(S_2CN(C_2H_5)_2)_2$	164[i]	30.07	29.89
$Te(S_2CNC_5H_{10})_2$	>250[h]	28.46	28.31

a Recrystallized from ethylacetate (5 g dissolved in 75 ml at 60°). Plates or prisms.

b Recrystallized from chloroform (1 g dissolved in about 300 ml at boiling temperature). Microcrystalline powder.

c Recrystallized from benzene (5 g dissolved in 80 ml at 60—70°). Plates or leaves.

d Recrystallized from chloroform (5 g dissolved in about 250 ml at boiling temperature). Tiny plates.

e Recrystallized by dissolving 5 g in 20 ml of warm benzene, and adding 50 ml of warm methanol. Flat needles.

f Recrystallized by dissolving 5 g in 10 ml of warm benzene, and adding 25 ml of warm ethanol. Long needles.

g Recrystallized by dissolving 5 g in 30 ml of warm ether, and adding 100 ml of methanol. Needles.

h Too insoluble in ordinary organic solvents to be conveniently recrystallized.

i Recrystallized by dissolving 5 g in 15 ml of warm carbon disulphide, and adding 25 ml of ether.

In Table 1 the three compounds prepared previously [12, 14] are included for comparison. For analysis, the selenium compounds were oxidized by means of nitric acid-sulphuric acid, the excess of nitric acid destroyed by means of urea, and the selenious acid determined volumetrically by means of the Norris and Fay method. The tellurium compounds were oxidized by means of nitric acid, and the excess of nitric acid was removed by heating with several portions of concentrated hydrochloric acid. The tellurous acid was reduced by means of hypophosphorous acid, and the tellurium weighed.

The selenium compounds are greenish yellow, while the tellurium compounds are red. In the solid state, the dithiocarbamates are quite stable, whereas the xanthates may liberate selenium or tellurium after a few days.

The compounds are insoluble in and unaffected by water.

The selenium *bis*(dimethyldithiocarbamate) was proved to be identical with a compound obtained from a commercial sample * of selenium dimethyldithiocarbamate, prepared from selenious acid and sodium dimethyldithiocarbamate.

The tellurium compounds readily exchange their thiocarbonyl groups in reactions with dithiocarbamate ions.

Tellurium *bis*(dimethyldithiocarbamate) and di(piperidyldithiocarbamate) have a very low solubility in ordinary organic solvents. If an excess of sodium dimethyl- or piperidyldithiocarbamate, dissolved in methanol or ethanol, is added to solutions of tellurium *bis*(diethyldithiocarbamate) or a tellurium di(xanthate) in ethylacetate or chloroform, a displacement takes place, and tellurium *bis*(dimethyldithiocarbamate) or tellurium di(piperidyldithiocarbamate) separates out. The reactions of tellurium di(ethylxanthate) and *bis*-(diethyldithiocarbamate) were studied in some detail. They may be formulated as follows:

$$Te(S_2COC_2H_5)_2 + 2\ R_2NCS_2^- = Te(S_2CNR_2)_2 + 2\ C_2H_5OCS_2^- \qquad (10)$$

$$Te(S_2CN(C_2H_5)_2)_2 + 2\ R_2NCS_2^- = Te(S_2CNR_2)_2 + 2\ (C_2H_5)_2NCS_2^- \qquad (11)$$

where R_2N— is dimethylamino or piperidyl.

With tellurium di(ethylxanthate) the reactions are quantitative even in the presence of potassium ethylxanthate. In the case of tellurium *bis*(diethyldithiocarbamate) the yields depend upon the excess of sodium dimethyl- or piperidyldithiocarbamate employed. Also, in presence of sodium diethyldithiocarbamate the yields become considerably lower. This shows that an equilibrium is established which is not displaced completely to the right, in

* Sharples Chemicals Inc. The *bis*(dimethylthiocarbamyl) disulphide, present in equimolar proportions, was extracted by means of cold chloroform. The author is indebted to the Airco Export Corp., New York, for samples of Sharples Chemicals Inc.'s sodium and selenium dimethyldithiocarbamate and diethyldithiocarbamate.

spite of the high solubility difference between the tellurium compounds on the left and the right side. Furthermore, it follows that the nucleophilic reactivity of the ethylxanthate ion is markedly lower than that of the dithiocarbamate ions. This difference in nucleophilic reactivity is in accordance with the relative inductive effects of alkoxy and dialkylamino groups, the latter groups having a higher electron releasing power than the former.

No catalytic decompositions were observed in experiments with the tellurium compounds. In the case of the selenium derivatives, a marked catalysis by xanthate and dithiocarbamate ions occurs.

0.01 *M* solutions of selenium di(methylxanthate) and *bis*(diethyldithiocarbamate) in chloroform are stable for over a week. If an equal amount of 0.01 *M* sodium or potassium xanthate or dithiocarbamate in ethanol is added to these yellowish green solutions, red selenium is liberated within one half to four minutes. In the case of selenium *bis*(diethyldithiocarbamate) the second product was identified as *bis*(diethylthiocarbamyl) disulphide, and it may, by analogy, be assumed that di(methylxanthyl) disulphide is formed in the case of selenium di(methylxanthate):

$$Se(S_2COCH_3)_2 = Se + (S_2COCH_3)_2 \tag{12}$$

$$Se(S_2CN(C_2H_5)_2)_2 = Se + (S_2CN(C_2H_5)_2)_2 \tag{13}$$

The xanthate liberates selenium more rapidly and completely than the dithiocarbamate. With the xanthate, dithiocarbamate ions are slightly stronger catalysts than are xanthate ions, whereas with the dithiocarbamate, the reverse is the case.

If selenium di(methylxanthate), dissolved in chloroform, is added rapidly to cold methanol solutions of an excess of sodium dimethyl- or piperidyldithiocarbamate, a displacement takes place just as in the case of the tellurium compounds:

$$Se(S_2COCH_3)_2 + 2\ R_2NCS_2^- = Se(S_2CNR_2)_2 + 2\ CH_3OCS_2^- \tag{14}$$

where R_2N— is dimethylamino or piperidyl. The selenium dithiocarbamates separate out so rapidly that only a negligible amount of catalysis has time to take place.

Thiocarbonyl derivatives of divalent sulphur, S^{++} and S_2^{++}, are subject to the same type of catalysis as the xanthates and dithiocarbamates of divalent selenium.

Bloch and Bergmann [16] found that monosulphur and disulphur di(thiobenzoate) readily liberate sulphur, giving dibenzoyl disulphide. The disulphur compound reacted in two stages, first forming the monosulphur compound:

$$S_2(S(O)CC_6H_5)_2 = S + S(S(O)CC_6H_5)_2 \quad (15)$$

$$S(S(O)CC_6H_5)_2 = S + (S(O)CC_6H_5)_2 \quad (16)$$

These reactions are catalyzed [16] by dimethylaniline and by potassium thiobenzoate.

In this work, the corresponding reactions of monosulphur and disulphur di(ethylxanthate) have been studied. These compounds [17, 18], like the sulphur thiobenzoates [16], were first prepared from the sulphur chlorides and the respective potassium thiocarbonyl salts. They are also formed, in quantitative yields, from aqueous solutions of potassium pentathionate and hexathionate [1]:

$$S(S_2O_3)_2^{--} + 2\ C_2H_5OCS_2^- = S(S_2COC_2H_5)_2 + 2\ S_2O_3^{--} \quad (17)$$

$$S_2(S_2O_3)_2^{--} + 2\ C_2H_5OCS_2^- = S_2(S_2COC_2H_5)_2 + 2\ S_2O_3^{--} \quad (18)$$

with similar equations for the thiobenzoates. These reactions are analogous to the reactions of monoselenopentathionate and monotelluropentathionate with thiocarbonyl salts.

Monosulphur and disulphur di(ethylxanthate) are faintly yellowish-green oils, very slightly soluble in ethanol, but miscible with ethylacetate in all proportions. In substance and in ethylacetate solutions they seem to be quite stable. In the presence of potassium ethylxanthate, sulphur is rapidly liberated:

$$S_2(S_2COC_2H_5)_2 = S + S(S_2COC_2H_5)_2 \quad (19)$$

$$S(S_2COC_2H_5)_2 = S + (S_2COC_2H_5)_2 \quad (20)$$

Other alkali xanthates, and dithiocarbamates, also catalyze these reactions.

Experimental

25 millimoles of tellurium di(ethylxanthate) or *bis*(diethyldithiocarbamate) dissolved in 20 ml of chloroform were employed in each experiment. To such solutions were added sodium dimethyl- or piperidyldithiocarbamate dissolved in 15 ml of methanol. Addition of 15 ml of methanol alone did not cause any precipitation. The products were filtered off, washed with ether, methanol, and ether, dried *in vacuo* over sulphuric acid, and weighed.

Tellurium di(ethylxanthate). (1) 25 % excess (6.25 millimole) of sodium dimethyldithiocarbamate. Yield 0.9281 g = 100.9 %. (2) As (1) but in presence of 6.25 millimole of potassium ethylxanthate. Yield 0.9255 g = 100.6 %. (3) 25 % excess of sodium piperidyldithiocarbamate. Yield 1.1210 g = 100.1 %. (4) As (3) but in presence of 6.25 millimole of potassium ethylxanthate. Yield 1.1182 g = 99.8 %.

For the products from (1) and (2) were found 34.66 % and 34.78 % Te, respectively; calc. for $Te(S_2CN(CH_3)_2)_2$: 34.67 %. For those from (3) and (4) were found 28.30 % and 28.42 % Te; calc. for $Te(S_2CNC_5H_{10})_2$: 28.46 %.

Tellurium bis(*diethyldithiocarbamate*). (5) 100 % excess (10 millimole) of sodium dimethyldithiocarbamate. Yield 0.8756 g = 95.2 %. (6) 25 % excess (6.25 millimole) of sodium dimethyldithiocarbamate. Yield 0.5853 g = 63.6 %. (7) As (6) but in presence of 6.25 millimole of sodium diethyldithiocarbamate. Yield 0.0352 g = 3.8 %. (8) 100 % excess of sodium piperidyldithiocarbamate. Yield 1.1038 g = 98.4 %. (9) 25 % excess of sodium piperidyldithiocarbamate. Yield 0.9464 g = 84.4 %. (10) As (9) but in presence of 6.25 millimole of sodium diethyldithiocarbamate. Yield 0.7344 g = 65.6 %.

For the products from (5) and (6) were found 34.80 % and 34.68 % Te, respectively. For those from (8) and (10) were found 28.51 % and 28.57 % Te, respectively.

No tellurium was liberated in any of the experiments.

Selenium compounds

Catalysis. The following solutions were employed: (a) 0.01 *M* selenium di(methylxanthate) in chloroform, (b) 0.01 *M* selenium *bis*(diethyldithiocarbamate) in chloroform, (c) 0.01 *M* solutions of potassium methyl-, ethyl- and *iso*-propylxanthate, in ethanol, (d) 0.01 *M* solutions of sodium dimethyl-, diethyl- and piperidyldithiocarbamate, in ethanol. The experiments were made at 20° C. Time from mixing till appearance of red selenium:

5 ml each of (a) and (c): 45 seconds
5 ml each of (a) and (d): 30 seconds
5 ml each of (b) and (c): 2 minutes
5 ml each of (b) and (d): 4 minutes
5 ml of (a) and 2 ml of (c): 4 minutes
5 ml of (a) and 2 ml of (d): 2 minutes
5 ml of (b) and 10 ml of (c): 45 seconds
5 ml of (b) and 10 ml of (d): 1 ½ minutes

To 5 millimoles of (1) selenium di(methylxanthate) and (2) selenium *bis*(diethyldithiocarbamate) dissolved in 20 ml of chloroform were added 5 millimoles of potassium methylxanthate and sodium diethyldithiocarbamate, respectively, dissolved in 10 ml of methanol. Selenium immediately separated out. After one hour's standing the selenium was filtered off, dried, and weighed: (1) 0.3862 g = 97.8 %. (2) 0.2783 g = 70.5 %.

To 5 millimoles of selenium *bis*(diethyldithiocarbamate) in 20 ml hot ethanol (it did not dissolve completely) were added 5 millimoles of solid sodium diethyldithiocarbamate. The selenium compound dissolved, and selenium separated out. The mixture was filtered while hot: 0.3375 g Se = 85.5 %. The filtrate was cooled to room temperature, and 0.27 g of greenish yellow crystals separated out. They consisted mainly of unchanged selenium *bis*(diethyldithiocarbamate). On addition of 50 ml of water, 1.09 g of faintly greenish crystals were obtained, melting at 69—71° C and containing only a trace of selenium. They were recrystallized from 10 ml of ethanol: Yield 0.70 g of faintly greenish crystals, m. p. 72° C, not depressed in mixture with a sample of *bis*(diethylthiocarbamyl) disulphide obtained from other sources.

Displacements. 2.5 millimoles of selenium di(methylxanthate), dissolved in 15 ml of chloroform, were cooled in ice water and added rapidly to 100 % excess (10 millimoles) of (1) sodium dimethyldithiocarbamate and (2) sodium piperidyldithiocarbamate dissolved in 15 ml of methanol and cooled in ice water (the beakers containing the selenium compound were rinsed with 5 ml of chloroform). Greenish yellow crystals immediately separated out, which after about 30 seconds began to acquire a very faint reddish colour. They were filtered off as rapidly as possible, and washed with methanol and ether: (1) 0.7761 g = 97.2 %. They melted at 179—80° C and were found to contain 24.69 % Se; calc. for $Se(S_2CN(CH_3)_2)_2$: 24.72 %. (2) 0.9482 g = 95.0 %. They melted at 170—1° C and were found to contain 19.77 % Se; calc. for $Se(S_2CNC_5H_{10})_2$: 19.70 %.

Sulphur compounds

To 5 millimoles of (1) monosulphur di(ethylxanthate) and (2) disulphur di(ethylxanthate), dissolved in 10 ml of ethylacetate, were added 5 millimoles of potassium ethylxanthate in 10 ml of ethanol. Sulphur immediately separated out. It was filtered off, washed with methanol, dried, and weighed: (1) 0.1037 g = 64.7 %. After 24 hours, more sulphur had crystallized from the filtrate: Total 0.1598 g = 99.3 %. (2) 0.2281 g, corresponding to 1.42 at. S per mole of disulphur di(ethylxanthate). After 24 hours: Total 0.3223 g, corresponding to 2.01 at. S per mole.

THIOSULPHATES: PENTATHIONATE, HEXATHIONATE, MONOSELENOPENTATHIONATE, AND MONOTELLUROPENTATHIONATE

The properties of these ions as thiosulphates of divalent electropositive sulphur, selenium and tellurium follow from the reactions of pentathionate and hexathionate with piperidine and hydroxide and with ethylxanthate and thiobenzoate ions[1], and of monoselenopentathionate and monotelluropentathionate with hydroxide and with xanthate and dithiocarbamate ions [12, 14]. In these reactions, the two thiosulphate groups are rapidly and quantitatively displaced by the respective nucleophilic reagents. Thiosulphate itself reacts with sulphur compounds containing groups of a lower nucleophilic reactivity, displacing these groups and forming pentathionate and hexathionate[1].

In presence of small amounts of alkalies, pentathionate and hexathionate rapidly decompose into sulphur and tetrathionate, as mentioned on p. 1385. Hexathionate reacts in two stages, first forming pentathionate [10].

Beside hydroxyl ions, thiosulphate ions catalyze these reactions. The catalytic effect of thiosulphate on pentathionate was first studied by Foerster and Hornig [4] and Kurtenacker and Kaufmann [7], and recently by Goehring, Helbing and Appel [11]. An analogous effect on hexathionate was first reported by Kurtenacker, Mutschin and Stastny [10].

It is shown in this work that thiosulphate and hydroxyl ions have a marked catalytic effect on the corresponding reactions (3) and (4) of monoselenopentathionate and monotelluropentathionate.

That hydroxyl ions act as catalysts, may be concluded from the fact that hydrochloric acid greatly stabilizes the aqueous solutions of these compounds. In the case of monotelluropentathionate, potassium iodide also acts as a catalyst.

The relative stabilities of pentathionate, monoselenopentathionate and monotelluropentathionate in 0.064 *M* neutral aqueous solutions at 25° C are as follows.

Monoselenopentathionate solutions remain unchanged for 10 hours, monotelluropentathionate solutions for 3 hours. Once the monoselenopentathionate solutions have started to decompose, the process proceeds faster than in the case of monotelluropentathionate. According to Goehring, Helbing and Appel [11], pentathionate solutions are turbid after 6 hours.

The catalytic effect of thiosulphate increases in the order monotelluropentathionate, pentathionate, and monoselenopentathionate. The catalysis of iodide on monotelluropentathionate is less pronounced than that of thiosulphate.

The reactions are subject to a marked positive salt effect, as demonstrated in this work in the case of monoselenopentathionate and monotelluropentathionate. It seems likely that the salt effect on solutions to which no catalyst, such as thiosulphate or iodide, has been added, is an effect on a hydroxide catalysis. Such an effect on pentathionate was reported earlier [19].

The positive salt effect indicates that the catalysis is due to reactions between ions with similar charge, which, obviously, is negative.

The presence of tetrathionate seems to have no effect on the stability of monoselenopentathionate and monotelluropentathionate solutions, although it is said to decrease the stability of pentathionate solutions [20].

The reactions are of the autocatalytic type. The only possible autocatalysts are, beside thiosulphate, selenium and tellurium. Colloidal sulphur has a strong catalytic effect on the corresponding reactions of pentathionate and hexathionate [10]. These facts indicate that the release of sulphur, selenium and tellurium is facilitated if the atoms can be deposited directly on a colloidal particle of the same element. This is perhaps part of a nucleus formation effect. Also, the heats of combustion of potassium tetrathionate and pentathionate show [21] that (in the solid state) the change of pentathionate into tetrathionate and atomic sulphur is endothermic, whereas the change into tetrathionate and monoclinic S_8 is exothermic.

Experimental

Measurements were made in a thermostat at 25° C with 0.064 *M* solutions of sodium monoselenopentathionate and monotelluropentathionate, in 50 ml volumetric flasks. This concentration was chosen in order to allow a comparison with the results of Goehring,

Helbing and Appel[11] on potassium pentathionate. For analysis, 5 ml samples were pipetted out, and the selenium or tellurium was filtered off. In the filtrates, the monoselenopentathionate was titrated with bromate as described earlier[12], and the selenious acid determined by means of the Norris and Fay method. In each case, the relative decreases in the bromate and the Norris and Fay titer values corresponded to one mole of tetrathionate formed per mole of monoselenopentathionate consumed. The monotelluropentathionate was titrated directly with iodine[14], and the tetrathionate subsequently determined by means of the sulphite method. The cyanide method [22, 1] was also used sometimes, to check on a possible rearrangement of the tetrathionate.

No acidity developed in the solutions during the experiments. In the case of monoselenopentathionate, filtered samples were tested with iodine: No thiosulphate was formed, or where it had been added as a catalyst, the amount did not change.

0.064 *M* sodium monoselenopentathionate

(1) In water. Selenium appeared after 10—11 hours. After 24 hours: 4 % decrease. (2) In 2 *M* sodium chloride. Selenium appeared after 2 hours. After 4 hours: 3.1 % decrease; 6 hours: 11.6 %; 8 hours: 20.1 %. (3) In 2 *M* sodium chloride and 0.0013 *M* or 0.064 *M* potassium tetrathionate. As (2). (4) In 2 *M* sodium chloride and 0.06 *N* hydrochloric acid. No change after 12 hours. After 24 hours: 1.8 % decrease. (5) In 0.07 *M* sodium thiosulphate. Selenium appeared after 1 minute. After 15 minutes: 74.8 % decrease; 30 minutes: 87.5 %; 1 hour: 91.8 %; 2 hours: 93.0 %. (6) In 0.01 *M* sodium thiosulphate. Selenium appeared after 10 minutes. After 30 minutes: 20.8 % decrease; 1 hour: 39.3 %; 2 hours: 68.3 %; 3 hours: 79.8 %. (7) In 0.01 *M* sodium thiosulphate and 2 *M* sodium chloride. Selenium appeared after 3 minutes. After 15 minutes: 59.8 % decrease; 30 minutes: 84.8 %; 1 hour: 95.9 %.

0.064 *M* sodium monotelluropentathionate

(8) In water. Tellurium appeared after 3 hours. After 24 hours: 2.1 % decrease. (9) In 2 *M* sodium chloride. Tellurium appeared after 20 minutes. After 4 hours: 0.6 % decrease; 6 hours: 0.9 %; 24 hours: 4.2 %. (10) In 2 *M* sodium chloride and 0.0013 *M* or 0.064 *M* potassium tetrathionate. As (9). (11) In 2 *M* sodium chloride and 0.06 *N* hydrochloric acid. No change after 3 hours. After 9 hours: 0.5 % decrease; 24 hours: 2 %. (12) In 0.07 *M* sodium thiosulphate. Tellurium appeared after 6 minutes. After 30 minutes: 1.7 % decrease; 1 hour: 3.8 %; 2 hours: 7.6 %; 3 hours: 11.6 %; 5 hours: 21.6 %. (13) In 0.07 *M* sodium thiosulphate and 2 *M* sodium chloride. Tellurium appeared after 2 minutes. After 30 minutes: 4.0 % decrease; 2 hour: 10.5 %; 3 hours: 52.3 %. (14) In 0.04 *M* sodium thiosulphate and 2 *M* sodium chloride. Tellurium appeared after 3 minutes. After 30 minutes: 2.5 % decrease; 1 hour: 5.1 %; 2 hours: 15.2 %; 3 hours: 27.4 %. (15) In 0.07 *M* potassium iodide. After 30 minutes: 0.7 % decrease, 1 hour: 1.5 %; 2 hours: 2.3 %; 3 hours: 3.8 %, 16 hours: 18.9 %. (16) In 0.07 *M* potassium iodide and 2 *M* sodium chloride. Tellurium appeared after 1 minute. After 30 minutes: 6.5 % decrease, 1 hour: 10.3 %, 2 hours: 17.4 %, 3 hours: 23.3 %, 4 hours: 28.2 %. (17) In 0.14 *M* potassium iodide. Tellurium appeared after 2 minutes. After 30 minutes: 3.4 % decrease, 1 hour: 5.9 %, 2 hours: 9.2 %, 3 hours: 12.6 %.

The amount of tetrathionate present in the titrated solutions, as determined by means of the sulphite and the cyanide methods, remained constant, except in the experiments with thiosulphate as a catalyst. In Expt. (12), after 5 hours, the amount of tetrathionate had changed as from 0.099 *M* to 0.0949 *M* and that of pentathionate as from zero to 0.0019 *M*. In Expt. (13), after 3 hours, from 0.099 *M* to 0.0923 *M* and from zero to 0.0041 *M*, respectively, and in Expt. (14), after 3 hours, from 0.099 *M* to 0.0962 *M* and from zero to 0.0017 *M*, respectively. These changes correspond roughly to a thiosulphate-catalyzed rearrangement of some of the tetrathionate, formed from the monotelluropentathionate, into trithionate and pentathionate.

The experiments (5) and (12) may be compared with the following experiment of Goehring, Helbing and Appel[11]: 0.0643 *M* potassium pentathionate, in 0.072 *M* sodium thiosulphate, at 25° C. After 15 minutes: 44.4 % decrease; 1 hour: 51.1 %; 2 ½ hours: 64.5 %; 5 hours: 68.9 %.

Acetate and monohydrogenphosphate ions also bring about an increased instability of monotelluropentathionate. These phenomena have not been investigated in any detail.

THE NATURE OF THE CATALYSIS

Reference to the structure of the reacting compounds is pertinent to the succeeding discussion of the mechanism of the catalytic reactions.

There is some controversy in the literature as to whether the pentathionate and hexathionate ions and the organic tri- and tetrasulphides are built up of unbranched, zigzag sulphur chains, or of branched (coordinated) structures. The more recent evidence definitely favours the unbranched chain formulae. A chemical proof of an unbranched structure for polythionic compounds of the pentathionic type (combinations of two thio anions with S^{++}) is as follows[1]:

In reactions with nucleophilic reagents, such as piperidine and thiocarbonyl anions, these compounds eliminate their thio groups as anions, the reagents, having a higher nucleophilic reactivity, taking their place and becoming linked to S^{++} (*cf.* p. 1392). These ionic displacement reactions show that in each case a divalent sulphur atom forms a bridge between the thio sulphur atoms of two thio groups[1].

The polythionic compounds of the hexathionic type (combinations of two thio anions with S_2^{++}) react in an analogous way with piperidine and thiocarbonyl anions, and thus, in these cases a divalent disulphur group forms a bridge between the thio sulphur atoms of two thio groups[1]. Here, the disulphur group, as far as the ionic displacement reactions are concerned, may have a branched structure ($\rangle S \rightarrow S$ or $\rangle S = S$) or an unbranched chain structure ($-S-S-$). However, in compounds of divalent sulphur the sulphur atom has in no case been proved able to add sulphur to form bonds of the branched type.

Likewise, structure investigations by physical methods are in favour of unbranched sulphur chains. Particularly, the comprehensive studies by Koch[23] of the ultraviolet absorption spectra of organic disulphides and polysulphides, provide strong evidence on this point.

The chemical reactions of polythionic compounds and analogous sulphur compounds are, predominantly, with nucleophilic reagents. These reactions may be explained in a straight-forward and consistent way on the basis of the electrophilic reactivity of sulphur and formulae with unbranched sulphur chains[1, 24].

As for the thiosulphates, xanthates and dithiocarbamates of divalent selenium and tellurium, the ionic displacement reactions demonstrated earlier [12, 14] and in this work prove that these compounds, also, are built up of unbranched chains.

The catalytic effect of thiosulphate on pentathionate was attributed by Kurtenacker and Kaufmann [7] to the existence of an equilibrium:

$$S_2O_3^{--} \rightleftharpoons S + SO_3^{--} \tag{21}$$

Through the tendency of thiosulphate to liberate sulphur, the corresponding tendency of pentathionate was assumed to be increased.

However, the equilibrium (21) does hardly exist [1]. Thus, an exchange:

$$S_2O_3^{--} + S^*O_3^{--} \rightleftharpoons SS^*O_3^{--} + SO_3^{--} \tag{22}$$

does not take place at ordinary temperatures [25].

Goehring, Helbing and Appel [11] formulated the thiosulphate catalysis on pentathionate as due to an equilibrium:

$$S_5O_6^{--} + S_2O_3^{--} \rightleftharpoons S_6O_6^{--} + SO_3^{--} \tag{23}$$

followed by

$$S_6O_6^{--} = S + S_5O_6^{--} \tag{24}$$

$$S_5O_6^{--} + SO_3^{--} \rightleftharpoons S_4O_6^{--} + S_2O_3^{--} \tag{25}$$

Hexathionate *is* more unstable than is pentathionate; however, the difference in stability is hardly large enough to account for the marked catalysis on pentathionate as being due to the formation of a relatively small amount of hexathionate. There is a thiosulphate catalysis on hexathionate also, and the acceptance of Goehring, Helbing and Appel's theory would thus merely shift the problem of thiosulphate catalysis from pentathionate to hexathionate.

A different mechanism, which has to be considered, is the following. The thiosulphate catalysis on pentathionate, hexathionate, monoselenopentathionate and monotelluropentathionate may be due to a nucleophilic attack by thiosulphate on the thio sulphur atom of one of the two thiosulphate groups of the compounds, with a simultaneous or subsequent release of the other of the thiosulphate groups. Such a displacement would give rise to branched (coordinated) structures, and these would, according to the structural evidence, be unstable and liberate the coordinated sulphur, selenium or tellurium. One might also imagine the thiosulphate group and the sulphur, selenium or tellurium being released simultaneously. A similar picture would apply in

the case of the xanthate and dithiocarbamate catalysis on the xanthates and dithiocarbamates of sulphur, selenium and tellurium, *i. e.*, a nucleophilic attack, not on the central sulphur, selenium or tellurium atoms, but on one of the sulphur atoms linked thereto.

There are, though, two points which argue against such a mechanism. First, it accounts only for the catalysis by thio anions, not for the catalysis by hydroxide or iodide. The catalytic phenomena seem so closely related that a common mechanism must be thought to be responsible. Next, the fact that iodide acts as a catalyst on monotelluropentathionate, but not on pentathionate and monoselenopentathionate, indicates that an attack on tellurium is involved, not on one of the sulphur atoms linked to it.

A theory, which avoids these difficulties, was put forward [1] in 1945 to account for the hydroxide and thiosulphate catalysis on pentathionate and hexathionate. The theory is in accordance with the experiments on selenium and tellurium compounds recorded in this work, and is outlined on the remaining pages.

Large amounts of hydroxyl ions rapidly and completely displace the two thiosulphate groups of pentathionate, hexathionate, monoselenopentathionate and monotelluropentathionate [1, 11, 14]. It seems likely that smaller amounts, such as those which effect the catalytic decompositions, react to displace only one of the thiosulphate groups, like small amounts of sodium diethyldithiocarbamate do in reactions with pentathionate and hexathionate [1]. Accordingly, it appears as if the catalysis takes place as a consequence of ionic displacement equilibria such as:

$$Se(S_2O_3)_2^{--} + OH^- \rightleftharpoons Se\begin{matrix}\diagup OH \\ \\ \diagdown S_2O_3^-\end{matrix} + S_2O_3^{--} \qquad (26)$$

and similar equations with S, S_2 or Te instead of Se.

In the same way, a nucleophilic attack by iodide on the tellurium of monotelluropentathionate leads to the following displacement equilibrium:

$$Te(S_2O_3)_2^{--} + I^- \rightleftharpoons Te\begin{matrix}\diagup I \\ \\ \diagdown S_2O_3^-\end{matrix} + S_2O_3^{--} \qquad (27)$$

Furthermore, in view of the properties of pentathionate, hexathionate, monoselenopentathionate and monotelluropentathionate as thiosulphates of divalent sulphur, selenium and tellurium, it seems likely that in the presence of thiosulphate, ionic displacement equilibria are established, of the type:

$$Se(S_2O_3)_2^{--} + S_2^*O_3^{--} \rightleftharpoons Se\begin{matrix}\diagup S_2^*O_3^- \\ \\ \diagdown S_2O_3^-\end{matrix} + S_2O_3^{--} \qquad (28)$$

and likewise with S, S_2 or Te instead of Se. Since catalysis occurs, these equilibria are, possibly, the cause of the catalysis. Analogous considerations apply to the xanthate and dithiocarbamate catalysis on xanthates and dithiocarbamates of divalent sulphur and selenium. The experimental evidence for displacements are the reactions represented by Eqs. (7), (10), (11) and (14). Thus, it appears as if the catalysis is connected with the occurrence of ionic displacements such as:

$$Se(S_2COR)_2 + ROCS_2^{*-} \rightleftharpoons Se\begin{matrix}\diagup S_2^*COR \\ \\ \diagdown S_2COR\end{matrix} + ROCS_2^- \qquad (29)$$

$$Se(S_2CNR_2)_2 + R_2NCS_2^{*-} \rightleftharpoons Se\begin{matrix}\diagup S_2^*CNR_2 \\ \\ \diagdown S_2CNR_2\end{matrix} + R_2NCS_2^- \qquad (30)$$

Especially the experiments made with the selenium compounds, where an excess of sodium dithiocarbamate brings about displacements, while smaller amounts cause decompositions, suggest that there is a link between the displacements and the catalysis.

If the equilibria (26)—(30) are responsible for the catalysis, there is still the problem of why the molecules thereby become unstable. A possibility is the following. The reactions (26)—(30) proceed through a transition state, the energy of which is higher than that of the molecules in the initial state. The molecules are labile even in the initial state, and the increase in energy associated with the passing of the transition state serves to decrease the thermodynamic stability and accelerate the decompositions.

SUMMARY

The decompositions of monoselenopentathionate and monotelluropentathionate into tetrathionate, and selenium and tellurium, respectively, are catalyzed by thiosulphate and hydroxide, the last change also by iodide. There is a positive salt effect on these decompositions.

Some new xanthates and dithiocarbamates of divalent selenium and tellurium are described. The decompositions of the selenium compounds into

the disulphides, and selenium, are catalyzed by xanthate and dithiocarbamate ions. Monosulphur and disulphur di(ethylxanthate) are subject to the same type of catalysis.

Tellurium di(ethylxanthate) and *bis*(diethyldithiocarbamate) react with sodium dimethyl- and piperidyldithiocarbamate to give tellurium *bis*(dimethyldithiocarbamate) and di(piperidyldithiocarbamate). Under special conditions, the selenium compounds react in the same way.

Dithiocarbamate ions have a higher nucleophilic reactivity than xanthate ions, in displacements on tellurium.

The cause of the catalytic decompositions is discussed.

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Received October 7, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1400–1404

The Effect of Complex Formation between Cupric and Sulphate Ions on the Equilibrium of Cupric Trihydroxysulphate in Mixed Aqueous Solutions of Cupric and Potassium Sulphate

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In a recent paper by Näsänen and Tamminen [1], the solubility product of cupric trihydroxysulphate ($Cu(OH)_{1.5}(SO_4)_{0.25}$) was determined in potassium sulphate solutions as a function of the ionic strength. This solubility product

$$[Cu^{++}]\ (OH^-)^{1.5}\ [SO_4^{=}]^{0.25} \qquad (1)$$

where $[Cu^{++}]$ and $[SO_4^{=}]$ are molarities and (OH^-) hydroxyl ion activity, was calculated by means of the stoichiometric concentrations and the measured pH. In these calculations the complex formation between cupric and sulphate ions could not be taken into account because the complexity constants had not been determined before. Furthermore opinions diverged [2] regarding, whether or not cupric sulphate, is completely dissociated. The effect of potassium sulphate additions was, however, so great that complex formation seemed to be possible. Therefore a spectrophotometric investigation on the complex formation between cupric and sulphate ions was later carried out by the present author [3]. In this study the first complexity constant

$$k_1 = 1/K_1 = [CuSO_4] / [Cu^{++}]\ [SO_4^{=}] \qquad (2)$$

was determined in solutions of some alkali salts. The results seemed to show that practically no higher complexes between cupric and sulphate ions exist. On the basis of these results, the effect of complex formation may be estimated.

For the determination of the solubility product, cupric sulphate, sodium hydroxide, and potassium sulphate were mixed. The total copper concentration (c_{Cu}) was relatively low ($c_{Cu} < 0.02$), and the stoichiometric concentration of sodium hydroxide (c_B) less than 1.5 c_{Cu} because cupric trihydroxysulphate is not stable when $c_B > 1.5\ c_{Cu}$. When equilibrium was reached, the pH of the solution was measured. The concentrations of cupric and sulphate ions were calculated by means of the equations

$$[Cu^{++}] = c_{Cu} - x - [CuSO_4] \tag{3}$$

and

$$[SO_4^{=}] = c + c_{Cu} - 0.25\,x - [CuSO_4] \tag{4}$$

where c is the molarity of the added potassium sulphate and x the decrease in concentration of cupric ion owing to precipitation. The electro-negativity equation gives

$$x = 0.667\ (c_B + [H^+] - [OH^-]) \tag{5}$$

When $c_B < 1.5\ c_{Cu}$, the hydroxyl ion concentration in this equation is negligible, and also, as a rule, the hydrogen ion concentration. From equations (2), (3), and (4) it follows that

$$[CuSO_4] = \frac{[Cu^{++}]_0\,[SO_4^{=}]_0}{[Cu^{++}]_0 + [SO_4^{=}]_0 + K_1 - [CuSO_4]} \tag{6}$$

where

$$[Cu^{++}]_0 = c_{Cu} - x \tag{7}$$

$$[SO_4^{=}]_0 = c + c_{Cu} - 0.25\ x \tag{8}$$

The dissociation constant of cupric sulphate was calculated with the aid of the relation

$$pK_1 = 2.099 - \frac{4.05\ \sqrt{I}}{1 + 1.762\ \sqrt{I}} + 0.155\ I \tag{9}$$

obtained earlier [3].

The results are recorded in Table 1. The relation between solubility product and ionic strength may be represented by means of the equation

$$pS = pS_0 - \frac{2.53\ \sqrt{I}}{1 + \alpha\ \sqrt{I}} + BI \tag{10}$$

For the parameters, the values

$$pS_0 = 17.133,\ \alpha = 1.495 \text{ and } B = 0$$

Table 1. Solubility product of cupric trihydroxysulphate in potassium sulphate solutions at 25° C.

I	$c_{Cu} \cdot 10^2$	$c_B \cdot 10^2$	c	pH	pK_1	pS
0.122	1.01	0.465	—	4.99	1.695	16.903
0.117	1.01	0.555	—	5.11	1.709	16.896
0.112	1.00	0.645	—	5.38	1.722	16.855
0.191	2.04	0.374	—	4.60	1.527	16.840
0.183	2.01	0.574	—	4.70	1.544	16.786
0.177	2.00	0.733	—	4.75	1.558	16.803
0.169	1.97	0.924	—	4.84	1.575	16.810
0.159	1.95	1.178	—	5.04	1.600	16.809
0.151	1.93	1.344	—	5.36	1.620	16.790
0.332	2.02	0.555	0.0255	4.80	1.268	16.573
0.322	1.98	0.906	0.0250	4.97	1.283	16.542
0.315	1.95	1.116	0.0246	5.16	1.294	16.549
0.310	1.93	1.327	0.0244	5.41	1.302	16.575
0.636	1.98	0.906	0.125	5.09	0.949	16.340
0.628	1.95	1.161	0.123	5.28	0.952	16.342
0.624	1.93	1.327	0.122	5.55	0.957	16.333
0.894	2.01	0.574	0.255	5.09	0.817	16.149
0.881	1.97	0.924	0.249	5.21	0.822	16.190
0.874	1.95	1.178	0.246	5.43	0.824	16.157
0.869	1.93	1.340	0.244	5.73	0.828	16.164
1.19	2.01	0.574	0.458	5.21	0.763	16.044
1.17	1.97	0.924	0.449	5.34	0.765	16.067
1.16	1.95	1.178	0.443	5.55	0.764	16.046
1.16	1.93	1.344	0.438	5.85	0.764	16.051

were obtained by the method of least squares. Previously, when the complex formation was not taken into account, a value of 17.115 was obtained for pS_0. The difference is thus of the order of magnitude of experimental errors. The parameter α is of a reasonable magnitude. In Fig. 1, where pS is plotted against $\sqrt{I}$, the two lower curves refer to these measurements in potassium sulphate solutions. The solid line represents the results when complex formation is taken into consideration and the dotted line when it is neglected. The effect of complex formation is thus considerable and, as expected, is greater the greater the concentration of sulphate ion.

In the paper by Näsänen and Tamminen [1], mentioned above, it was shown that the solubility of cupric hydroxyperchlorate was considerably greater than that of cupric trihydroxysulphate. Therefore in a mixed solution of cupric

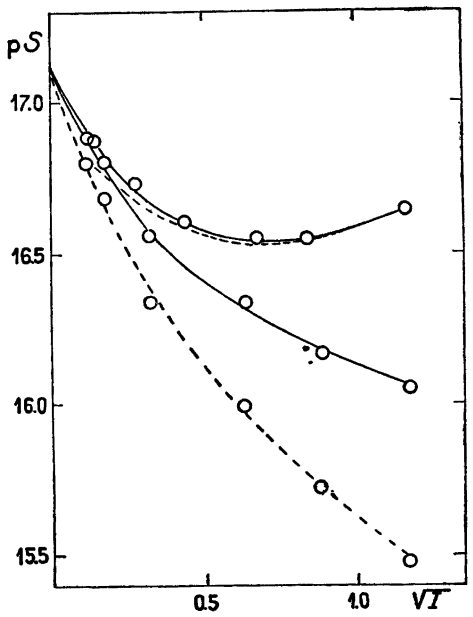

Fig. 1. Solubility product of cupric trihydroxysulphate in potassium sulphate and sodium perchlorate solutions as a function of ionic strength.

sulphate and sodium perchlorate, trihydroxysulphate is precipitated and not hydroxyperchlorate. In this way it was possible to determine the solubility product of cupric trihydroxysulphate in sodium perchlorate solutions. The results of these measurements are given in Table 2. Also in this case, the equations derived above were used in the calculations, but the dissociation constant of cupric sulphate was calculated from the equation

$$\mathrm{p}K_1 = 2.099 - \frac{4.05\sqrt{I}}{1 + 1.527\sqrt{I}} + 0.161\ I \qquad (11)$$

The results may be represented by equation (10). For the parameters the values

$$\mathrm{p}S_0 = 17.133,\ a = 2.077 \text{ and } B = 0.278$$

were obtained. In Fig. 1 the two upper curves represent these results. In this case the effect of complex formation is slight, especially at higher ionic strengths.

As is seen in Fig. 1, the solubility product in sodium perchlorate solution differs considerably from that in a potassium sulphate solution with the same ionic strength. The activity coefficients, at least the activity coefficient of the

Table 2. Solubility product of cupric trihydroxysulphate in sodium perchlorate solutions at 25° C.

$\sqrt{I}$	$c_{Cu} \cdot 10^2$	$c_{SO_4} \cdot 10^2$	pH	pK_1	pS
0.139	1.04	0.216	4.81	1.637	16.873
0.271	1.03	0.454	5.05	1.335	16.731
0.433	1.02	0.677	5.50	1.073	16.609
0.671	1.03	0.456	5.22	0.829	16.541
0.836	1.04	0.225	4.98	0.724	16.555
1.160	1.04	0.216	4.91	0.622	16.649

cupric ion, are thus considerably greater in sodium perchlorate solutions than in potassium sulphate solutions. In this connection it may be emphasized that the above values for the solubility products and complexity constants were obtained at relatively high ionic strengths; in the former case $\sqrt{I} > 0.1$ and in the latter $\sqrt{I} > 0.2$. The equations (9), (10), and (11) are therefore valid at these ionic strengths. It is quite possible that at still lower ionic strengths, the results would agree with the expectation of the extended Debye theory, as developed by Gronwall, La Mer and Sandved. If so, the true thermodynamic constants would be somewhat smaller than the above values, which were extrapolated with the aid of the Debye-Hückel equation.

SUMMARY

The significance of complex formation in the equilibrium between cupric trihydroxysulphate and solutions of potassium sulphate or sodium perchlorate was investigated. In potassium sulphate solutions the effect was considerable, but in sodium perchlorate solutions it was very slight. The apparent and true solubility products were determined in both cases as a function of ionic strength. The true solubility product may be represented by means of the Debye-Hückel equation. The parameters were of reasonable magnitudes.

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Received October 12, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1405 – 1417

Investigations on Dextranase

I. On the Occurence and the Assay of Dextranase

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Dextran is built up of glucose residues, which are presumed [1] to be connected mainly by α-1,6 linkages. The same type of linkage also occurs in starch but only at the points of ramification. We began this investigation in order to find some enzyme, capable of hydrolyzing α-1,6 linkages without simultaneous action on α-1,4 linkages.

Enzymic hydrolysis of α-1,6 linkages has already been reported. Thus Myrbäck and Ahlborg [2] have shown that the limit dextrins of starch, especially the simplest one — isomaltose — can be broken down by amylase preparations, although the splitting proceeds very slowly. Attempts at breaking down dextran by means of amylase and phosphorylase preparations have been performed without success by Swanson [3]. Colin and Belval [4] showed that various amylases and digestive juice of a snail do not act on dextran. Analogous experiments have been carried out by Drake [5], who hydrolyzed by means of a commercial enzyme preparation, Luizym, both lichenin with β-1,4 linkages and pustulin with β-1,6 linkages between the glucose residues.

After the completion of the experiments in this investigation, the results of which have previously been communicated [6], Ingelman [7] reported the occurence of a dextran splitting enzyme in *Cellvibrio fulva*, whereas he found preparations of other bacteria and of various moulds to be without any action.

It may also be mentioned here that phosphorylases are known, which act on α-1,6 linkages [8–10].

We have tried in vain to split dextran with enzyme solutions prepared from green malt and from Takadiastase, Chlarase, Luizym and other commercial enzyme preparations.

THE PRODUCTION OF DEXTRANASE

The adaptive formation of dextranase and amylase

It was known previously that some bacteria and moulds, when grown on nutritient solutions containing certain substances, are capable of forming enzymes that can break down these substances. We examined about 30 different moulds, most of which we isolated ourselves, as to their tendency to grow on nutritient solutions containing dextran as the single source of carbohydrate. We hoped thereby to find some species which can utilize this polysaccharide by forming a dextran splitting enzyme and presumed that only the moulds growing vigorously on these media produce dextranase in large quantities.

The media used in these experiments had the following comparatively simple composition (a variation of Pringsheim and Aronovsky's [11] nutritient solution): 20 g dextran, 20 g agar, 2 g ammonium sulphate, 0.5 g potassium dihydrogen phosphate, 0.1 g magnesium sulphate and 1 liter of tap water.

After inoculation the various cultures were left for about one week at 20° C, whereupon the growth of the various moulds was examined. We found thereby that the following moulds grew strongly: *Penicillium funiculosum* Thom, *Penicillium lilacinum* Thom, and *Verticillium coccorum* (Petch) Westerdijk.

These moulds were cultivated in fluid nutritient solutions containing dextran, in the manner described later on. From the moulds thus obtained we prepared extracts, which proved to contain dextranase. They did not, however, contain amylase or saccharase.

In some cultivations, starch was substituted for dextran. The extracts preparad from moulds grown in such solutions contained on the contrary amylase but no dextranase.

These experiments indicate that amylase and dextranase are formed only adaptively.

The production of mould spores

We needed a good supply of spores for the cultivation of our moulds, and this was accomplished by growing the moulds in Petri dishes on a medium of the following composition (*cf.* the medium used by Moyer and Coghill [12]):

Distilled water	1000 g	KH_2PO_4	0.06 g
Agar	20 g	$MgSO_4 \cdot 7H_2O$	0.05 g
Dextran	10 g	$ZnSO_4 \cdot 7H_2O$	0.02 g
Glycerol	8 g	$MnSO_4 \cdot 4H_2O$	0.01 g
Pepton	5 g	KNa tartrate	0.005 g
NaCl	4 g	$Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$	0.005 g
		$CuSO_4 \cdot 5H_2O$	0.004 g

This solution was sterilized, cooled to 40° C, mixed with mould spores and poured into sterilized Petri dishes and left at room temperature. The layer was about 3—4 mm. A vigorous production of spores was obtained in 5 days.

Surface cultivation of moulds

First we made some attempts at surface cultivation of moulds in 500 ml Erlenmeyer flasks containing 200 ml of sterilized nutritient solution of the following composition:

Tap water	1000 ml
Dextran	20 g
$(NH_4)_2SO_4$	2 g
KH_2PO_4	0.5 g
$MgSO_4 \cdot 7H_2O$	0.1 g

The pH of the solution was 4—5.

The flasks were inoculated with spores of *Penicillium funiculosum* and *Penicillium lilacinum*, respectiviely, and were left at room temperature for 10 days. The surface was covered with moulds, from which we prepared enzyme solutions as described later on.

As surface cultivation soon proved to give less yield and was more laborious than submerged cultivation, we passed over entirely to the latter method.

Submerged cultivation of moulds

We made several experiments in submerged cultivation of moulds to produce dextranase, and as a result of our experience we recommend the following procedure.

The nutritient solution has this composition (*cf.* the media used by Moyer and Coghill [13] and Jarvis and Johnson [14]):

Distilled water	1000 g		
Dextran (low molecular)	20 g	$MgSO_4 \cdot 7H_2O$	0.25 g
		$FeSO_4 \cdot 7H_2O$	0.10 g
$NaNO_3$	1.6 g	$ZnSO_4 \cdot 7H_2O$	0.044 g
$(NH_4)_2SO_4$	1.0 g	$MnSO_4 \cdot 4H_2O$	0.020 g
KH_2PO_4	0.50 g	$CuSO_4 \cdot 5H_2O$	0.005 g

The pH of the medium should be between 3.5 and 5.

500 ml of nutritient solution is poured into 1000 ml Erlenmeyer flasks, which are plugged with cotton, and sterilized. The medium is inoculated with a few milliliters of a 0.1 % sterile soap solution containing an abundance of spores. The flasks are shaken for 10 days at 25° C, whereupon the moulds are worked up in the way described later on in this article.

Alterations in the pH of submerged cultures

pH changes usually take place in nutritient solutions for submerged cultures when the moulds grow. We have found that this alteration depends on the proportions of the amounts of nitrate ion and ammonium ion in the solution. If the solution contains ammonium sulphate but no sodium nitrate, the mould is entirely left to the ammonium ion as its only source of nitrogen, and the solution will become more and more acid, as the mould consumes ammonium ion. If, on the other hand, the solution contains sodium nitrate but no ammonium sulphate, the pH of the solution will rise as the mould uses up nitrate ion.

We have tried to stabilize the pH of solutions containing ammonium sulphate as the only supply of nitrogen by addition of calcium carbonate. The pH of these media were rather high in comparison with the pH optimum for the stability [6] of our dextranase. The activity of the enzyme solutions from these cultivations was always very low. Other methods for stabilizing the pH had therefore to be substituted.

Since the pH of the nutritient solution will rise if it contains sodium nitrate and will decrease if it contains ammonium sulphate, we made some experiments to find a solution with suitable proportions of sodium nitrate and ammonium sulphate so that the pH alterations of the medium are as small as possible. A somewhat similar procedure has been employed previously by Jarvis and Johnson [14].

Five flasks with 500 ml of nutritient solution were prepared in the way recommended with the exception of the amounts of sodium nitrate and ammonium sulphate, the proportions of which were varied as shown in Table 1. The pH of the solutions was adjusted to about 4.5. After sterilization the flasks were inoculated with the same amount of a spore suspension of *Penicillium funiculosum* and shaken for 9 days at 25° C. The pH of the medium and the total yield of dextranase were then assayed.

The results, which are given in Table 1, indicate that the pH alterations are least if about 55 % of the nitrogen is administered as sodium nitrate and 45 % as ammonium sulphate and that the enzyme yield decreases if the pH of the medium is raised or lowered considerably from 4.

The red pigment of *Penicillium funiculosum*

We also noticed in these experiments with submerged cultures of *Penicillium funiculosum* in solutions with various proportions of nitrate ion and ammonium ion that the moulds were almost colourless in solutions free of

Table 1. Submerged cultivation of Penicillium funiculosum in media containing various mole percent of ammonium ion and nitrate ion.

Meq $NaNO_3$ per litre	Meq $(NH_4)_2SO_4$ per litre	pH of the media		Dextranase	
		after adjustment	after 9 days	yield μA	activity $\mu A/g$ dry weight
0.0352	0.0000	4.60	7.2	980	1070
0.0264	0.0088	4.68	7.6	282	272
0.0176	0.0176	4.65	4.1	2660	2660
0.0088	0.0264	4.67	2.0	102	102
0.0000	0.0352	4.63	2.0	116	102

nitrate and that in presence of nitrate they assumed a red colour, the intensity of which intensified as the proportions of nitrate ion to ammonium ion increased. The red pigment funiculosin in *P. funiculosum* has already been investigated by Igarasi [15].

The extraction of dextranase from the moulds

The moulds from surface cultures were collected on a filter, washed with water and cut into small pieces. The moulds from submerged cultures were centrifuged off and washed with water.

The moulds were then ground with sand, toluene and a small amount of acetate buffer (pH = 5.0). The mixture thus obtained was left for one day at room temperature for autolysis, whereupon it was extracted twice with dilute acetate buffer (pH = 5.0). The extract was centrifuged and the supernatant, the crude dextranase solution, stored under toluene. We have kept such a solution in a refrigerator for 8 months without any appreciable loss of activity.

The nutritient solutions had no detectable dextranase activity.

The power of the various moulds to produce dextranase

In addition to the qualitative estimation of the tendency of various moulds to form dextranase, we have made hitherto only a few quantitative experiments on the power of the moulds to produce the enzyme. We got the best yields of dextranase from two strains of *Penicillium funiculosum*, and hence cultivated mainly this mould. For the activity determinations the reader is referred to the next chapter of this article.

In one submerged cultivation of *P. funiculosum* in 250 ml of nutritient solution for 9 days we got a total dextranase yield of 3140 μA. Calculated on dry weight basis the activity of the preparation was 3150 μA/g. The activities of our dextranase preparations from *P. funiculosum* were usually between 2000 and 6000 μA/g.

A dextranase solution prepared from a surface culture of *Penicillium lilacinum* had an activity of 55 μA/g dry weight.

Submerged cultivation of *Penicillium lilacinum* and *Verticillium coccorum* was carried out in 400 ml nutritient solutions for 10 days at 25° C and pH 4.5—5.5. The dextranase yield was 150 μA and 120 μA respectively.

THE ASSAY OF DEXTRANASE

The dextranase activity has been assayed both viscosimetrically and by iodimetrical determination of the liberated reducing sugars. We have used mainly the viscosimetric method as it is the more sensitive one.

The viscosimetric assay of dextranase

The viscosimetric assays were performed by a method previously described by one of us [16–20].

a. Procedure

For substrate we used dextran produced from sucrose by the bacterium *Leuconostoc mesenteroides* (Cienkowski) van Tieghem by the usual method [1]. 1.5 to 3 % solutions of dextran were prepared in the following way. High molecular dextran was dissolved in boiling distilled water, whereupon the solution was poured into a Pasteur flask and sterilized at about 125° C. Such solutions could be kept for several weeks. The dextran concentration of the solutions was assayed from the dry weight, whereby about 25 g dextran solution was evaporated in an Erlenmeyer flask in an electric oven at 105° C.

Buffer solutions for the activity determinations were prepared by mixing 0.3 *M* KH_2PO_4 and 0.3 *M* Na_2HPO_4 in suitable proportions.

2 ml of enzyme solution, diluted to a suitable strength, were mixed with 1 ml buffer solution, and 20 ml of dextran solution were added. When not otherwise stated, the assay was performed at pH 5.9, where our dextranase has its optimum activity, and at 30° C. The assay was otherwise carried out in the manner described previously by one of us [19,20] for viscosimetric assay of amylase.

b. Theoretical

If all linkages in a polymeric homologous series are broken with equa ease, the following relation [16] is valid between the enzyme activity A, the substrate concentration c_s, the specific viscosity η_{sp} and the time t:

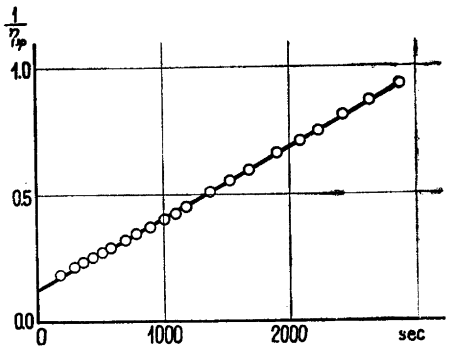


Fig. 1. Viscosity measurements at the enzymic break down of dextran.

$$A = c_s^2 \frac{\mathrm{d}\frac{1}{\eta_{sp}}}{\mathrm{d}t} \tag{1}$$

A condition is, however, that Staudinger and Heuer's [21] equation can be applied to the substance in question

$$\eta_{sp} = K_m c_{gm} M \tag{2}$$

where

K_m = the viscosity molecular weight constant,
c_{gm} = the concentration in primary moles per litre, and
M = the molecular weight.

A similar formula has also been suggested [22] (η_r = the relative viscosity)

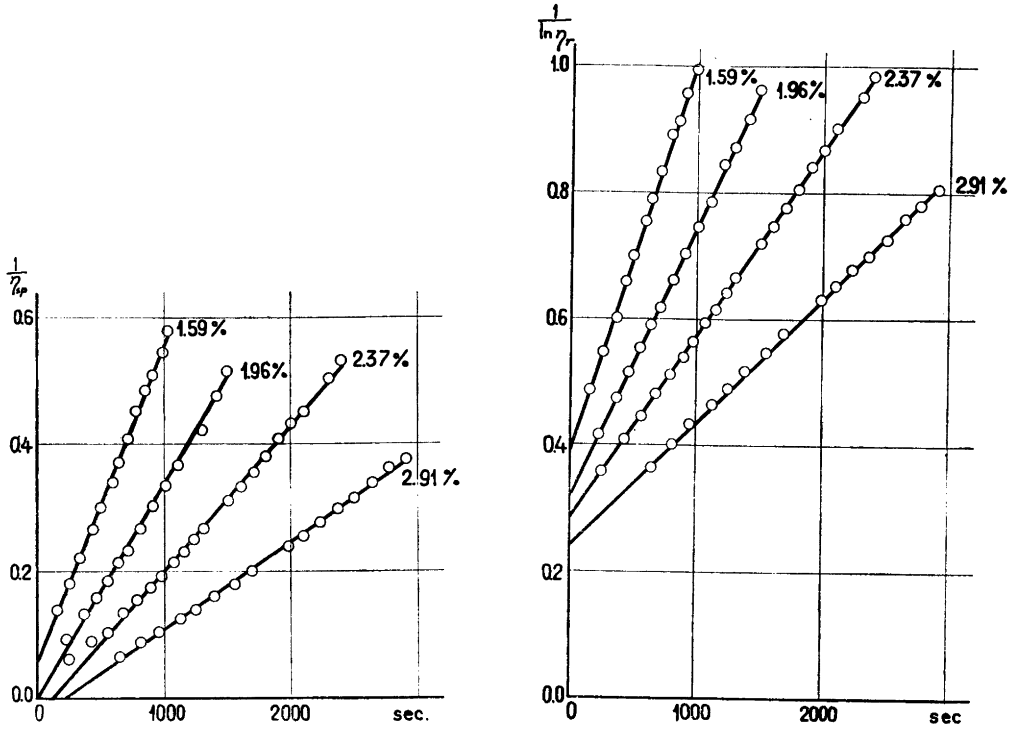
$$A = c_s^2 \frac{\mathrm{d}\frac{1}{\ln \eta_r}}{\mathrm{d}t} \tag{3}$$

This formula was derived from the modified Arrhenius-Staudinger formula [23–25]

$$\ln \eta_r = K_m c_{gm} M \tag{4}$$

c. The applicability of the formulas

We have investigated whether formula (1) can be used for the determination of dextranase activity by performing some experiments with various enzyme and substrate concentrations.



Figs. 2 and 3. Activity measurements of a dextranase solution, using dextran of various concentrations.

If the reciprocal value of the specific viscosity is plotted against time, the points will lie on a straight line within a considerable range, as shown in Figs. 1 and 2. The decrease in the viscosity could not be followed accurately any longer than shown in Fig. 1 as the flow time of the solvent in the Oswald viscosimeter used was about 10 seconds. At the beginning of the break down, when the solution still has a high viscosity, the points lie, however, above the straight line, making formula (1) somewhat less applicable for dextranase assays.

Formula (3) has also been employed in activity calculations, and in Fig. 3, which corresponds to Fig. 2, the reciprocals of the natural logarithms of the relative viscosities are plotted against time. The points lie here on a straight line, within the errors in the measurements. From this we conclude that formula (3) is preferable to formula (1) when activity determinations are carried out at high viscosities. If measurements at moderate viscosities only are taken into consideration, formula (1) can be used. For a theoretical dis-

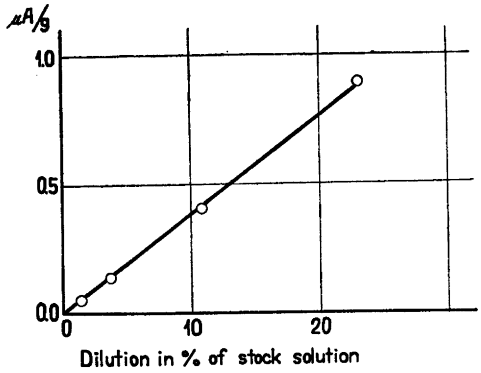


Fig. 4. Activity measurements of dextranase solutions of various concentrations.

cussion on the applicability of formulas (1) and (3) and related formulas, the reader is referred to an article by one of us [22] already quoted.

We have assayed the activity of a dextranase solution using dextran solutions of various concentrations. The results, which are given in Figs. 2 and 3 and Table 2, indicate that equations (1) and (3) are valid at various substrate concentrations.

Table 2. Activity assay of a dextranase solution, using dextran of various concentrations.

c_s	Activity in μA/ml	
	using (1)	using (3)
2.91	1.47	1.43
2.37	1.53	1.43
1.96	1.53	1.43
1.59	1.48	1.36

We have also measured the activity of various dilute solutions prepared from a stock enzyme solution. We see from the results given in Fig. 4 that the prepared concentration of the enzyme and the assayed activity calculated by the formulas are proportional.

From the applicability of the viscosimetric formulas we conclude that all or almost all of the 1,6 linkages are broken with equal ease and that the dextranase does not break down dextran from the ends of the molecules.

d. On an attempt to generalize the formulas

An attempt to generalize equation (1) has been made by Ingelman and Malmgren [26] and by Ingelman [7], who give the following formula, characterized by the exponent n, to which the concentration of the substrate is raised:

$$A = c_s^n \frac{\mathrm{d}\frac{1}{\eta_{sp}}}{\mathrm{d}t} \tag{5}$$

This equation demands (*cf.* the deduction of equation (1) from (2) [16]) the following expression for the specific viscosity

$$\eta_{sp} = K_m c_{gm}^{n-1} M \tag{6}$$

It seems improbable that the specific viscosity should not even in very dilute solutions be proportional to the concentration of the solute, as in Einstein's [27] formula, which can be written

$$\eta_{sp} = \varkappa \varphi \tag{7}$$

where $\varkappa$ = a constant depending on the shape of the particles and φ = the ratio between the total volume of the dispersed substance and the volume of the 'solution'.

In all ordinary formulas for the viscosity of polymeric homologous substances, proportionality between the specific viscosity (or the logarithm of the relative viscosity) and the solute concentration is presumed [28–30].

Using formula (5) we can, however, by determining the exponent n, easily prove whether the formulas are applicable or not. We can foresee deviations from the theoretically expected value $n = 2$, either if an ionic factor influences the viscosity of the substrate [18,31] or if the viscosity of the substrate does not follow the formulas used by Staudinger but for example Mark's [32] formula. Deviations can also be expected if the measurements have been performed at so high viscosity that equation (3) should have been used or at so low a degree of polymerization of the substrate that the presumptions on which the deduction of the formulas were founded are no longer valid.

These presumptions imply that the ratio between molecules of various size in the reaction mixture is what it would have been if they had all originated from one single giant molecule by hydrolysis in which all linkages are broken with equal ease. Thus the reaction mixture will contain molecules of all sizes. This indicates that, if for some reason the substrate is partly hydrolyzed before the enzymic break down, fractionation ought usually to be avoided.

In viscosimetric enzyme assays, experimental conditions should be aimed at such that a possible ionic factor is restrained and the polymerization degree of the substrate so high that the viscosimetric formulas can be applied.

Finally, deviations can be expected if the affinity of the enzyme to the substrate is not very high. Then the enzyme assay gives the activity of the enzyme at the particular substrate concentration in question.

The assay of dextranase by measuring its power to liberate reducing sugars

The power of dextranase to break down dextran into reducing sugars can be estimated in a manner similar to that in which the corresponding amylase assays are performed.

We make up the reaction mixture so that 100 ml solution contain 1 g dextran (a suitable amount of about 2 % dextran solution is used, the percentage dry weight of which has been previously determined), 5 ml phosphate buffer pH 5.9 (0.7 ml 0.3 *M* Na_2HPO_4 + 4.3 ml 0.3 *M* KH_2PO_4) and 5 ml enzyme solution. Dextran solution, buffer solution and the water necessary are first mixed in a flask which is placed in a thermostat at 30° C. After the temperature has equilized, the enzyme solution, which has also been warmed to 30° C, is added. At suitable intervals samples of 5 ml are withdrawn and their content of reducing sugar determined by the modification of Linderstrøm-Lang and Holter's [33] method, given by Blom and Rosted [34] (the concentrations of the stock solutions are changed to suit 5 ml samples, but the reaction mixture is the same).

A sample of 5 ml is pipetted into 5 ml of a 0.2 *N* iodine solution. 20 ml bicarbonate buffer solution is added. The mixture is shaken and left at room temperature for 30 min. Then 5 ml 4 *N* sulphuric acid is added and the iodine remaining titrated with 0.02 *N* sodium thiosulphate solution and starch as indicator.

Bicarbonate buffer solution: 21.1 g Na_2CO_3 and 4.2 g $NaHCO_3$ are dissolved in distilled water and made up to 1 liter.

The volume of thiosulphate solution used for the various samples is plotted against the time when the samples were taken. A straight line can be fitted to some points, since the liberation of reducing sugars is proportional to time in the beginning of the enzymic hydrolysis. The inclination of the line gives the liberation of reducing sugars.

Since the products of the enzymic hydrolysis of dextran, at least at the beginning, are not monosaccharides or oligosaccharides but polysaccharides of various sizes, we consider it more correct to express the dextranase amount

in milliequivalents of reducing sugars per minute than in, for example, mg glucose per minute or mg isomaltose per minute.

A conversion factor for dextranase assay

The activity of a dextranase solution was determined viscosimetrically. Its power of liberating reducing sugars was also determined. From these preliminary experiments we found that a dextranase solution, whose activity is 1 μA/ml liberates 0.00032 milliequivalents reducing sugars/min · ml.

SUMMARY

In cultivating moulds of the species *Penicillium lilacinum* Thom, *Penicillium funiculosum* Thom and *Verticillium coccorum* (Petch) Westerdijk, we found a new enzyme capable of hydrolyzing dextran. This enzyme will appear only if the nutritient solution contains dextran, i addition to nutritient salts. If *Penicillium funiculosum* is cultivated in nutritient solutions containing dextran as the only carbohydrate, it does not form appreciable amounts of amylase or saccharase. However, if it is cultivated in media containing starch as the only carbohydrate, it contains amylase but no dextranase.

Submerged cultures gave better yields and were less laborious than surface cultures. In submerged cultures the pH of the solution may change and with it the yield of dextranase. The optimum acidity of the medium is about pH = 4. The pH alterations are restrained if the mole percent of ammonium ion and nitrate ion in the nutritient solution are about 45 and 55 % respectively.

If *Penicillium funiculosum* is cultivated in a medium, containing no nitrate, it will appear almost colourless. In the presence of nitrate ion it forms a red pigment, funicusolin, the colour intensity of which intensifies as the mole percent of the nitrate ion increases.

The activity of dextranase solutions can be determined by viscosimetric assay and by assay of its power of liberating reducing sugars. The following conversion factor is tentatively given: 1 μA = 0.00032 milliequivalents reducing sugars/minute.

It is demonstrated viscosimetrically that dextranase attacks all or almost all 1,6 linkages of the dextran molecule with equal ease and does not break down the molecule from its ends.

We wish to express our gratitude to Prof. Karl Myrbäck for his incouraging interest in this investigation and for the opportunity we have had to carry out the experiments in his laboratories. Thanks are also due to Prof. Erik Björkman of Skogshögskolan, Stockholm, for some moulds, among which we mention *Penicillium funiculosum*, to Prof.

Johanna Westerdijk at whose institute, Centralbureau voor Schimmelcultures, Baarn, Holland, the moulds were identified, and to Mrs William Cameron who revised the English text.

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Received October 13, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1418–1429

The Iodine-Azide Reaction

II. The Catalytic Effect of Carbon Disulphide

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In connection with the senior author's investigations on the iodine-azide reaction, it was of obvious interest to examine the kinetics with carbon disulphide as catalyst.

The overall equation of the iodine-azide reaction is:

$$2\ N_3^- + I_2 = 3\ N_2 + 2\ I^- \qquad (1)$$

This reaction takes place only if a suitable catalyst is present. Substances which contain sulphide sulphur, in general, act as catalysts.

Browne and Hoel[1] were the first to ascertain that carbon disulphide also will catalyze the iodine-azide reaction. These authors have investigated the stoichiometry of this process and found excellent conformity with equation (1). Furthermore, it has been shown that if carbon disulphide vapour is bubbled through an aqueous solution of sodium or potassium azide, exactly one mole carbon disulphide per mole azide will be gradually absorbed with the formation of one mole of alkali azido-dithiocarbonate. If iodine solution is added to such a solution of azido-dithiocarbonate, a white precipitate of azido-carbon disulphide will be formed. The last mentioned compound together with sodium azide solution gives a brisk evolution of nitrogen, while at the same time reduction to azido-dithiocarbonate ions possibly takes place. On the basis of the above mentioned experiences, the following mechanism for the iodine-azide reaction with carbon disulphide as the catalyst was proposed:

$$\begin{aligned} N_3^- + CS_2 &= SCSN_3^- \\ 2\ SCSN_3^- + I_2 &= (SCSN_3)_2 + 2\ I^- \\ (SCSN_3)_2 + 2\ N_3^- &= 2\ SCSN_3^- + 3\ N_2 \end{aligned}$$

after which the azido-dithiocarbonate ions again react with iodine, and so on. Browne and Hoel emphasize that the azido-dithiocarbonate ions are the actual catalyst, and that the formation of this ion is irreversible, so that no carbon disulphide is regenerated after it has once become "fixed".

Feigl and Chargaff [2] — apparently without having noticed the work of Browne and Hoel — have also dealt with the catalytic action of carbon disulphide on the iodine azide reaction. Partly on the basis of various qualitative experiments and partly on Sommer's[3] observation that carbon disulphide and azide ions react with the formation of azido-dithiocarbonate ions, a reaction mechanism was proposed nearly identical to that of Browne and Hoel. The only difference was that Feigl and Chargaff assumed that carbon disulphide is regenerated when the reaction takes place. The investigations recorded in the present paper agree with the mechanism of Feigl and Chargaff, and the formation of azido-dithiocarbonate ions has been found to be the rate determining reaction step.

EXPERIMENTAL

N. Hofman-Bang [4] has shown that the rate of the iodine-azide reaction — catalyzed by tetrathionate ions — is proportional to the concentration of azide ions and tetrathionate ions, but independent of the concentration of iodine. Therefore it was possible to make kinetic experiments by adding a small amount of iodine solution together with some starch solution, to a solution of sodium azide and potassium tetrathionate. When the quantity of iodine was used up, the solution changed momentarily from a blue colour to colourless, and thus it was easy to measure the reaction time. It soon became evident that the rate of the carbon disulphide catalyzed iodine-azide reaction was also independent of the iodine concentration, for which reason an analogous experimental method could be applied.

Experimental procedure (for further details see ref. 4): Into a 500 ml Erlenmeyer flask was pipetted iodine in potassium iodide, sodium azide, sodium perchlorate, starch indicator, and water; and the flask was placed in a water thermostat, so that only the neck was above the water. After the elapse of 30 minutes (for sake of temperature adjustment) an aqueous solution (usually 10 ml) of carbon disulphide was sucked into a pipette. The solution was immediately allowed to run down in the Erlenmeyer flask, which at the same time — partly immersed in the thermostat water — was kept in a rotary motion with the left hand. When half of the carbon disulphide solution had run out of the pipette, a stopwatch was started. The last drop in the pipette was blown out, and the flask was kept rotating for an additional 10 sec. The instant the blue colour of the solution disappeared, a new portion of iodine was added from a pipette, and the time of reaction was determined again. The volume of iodine solution added was small — usually 0.1—0.5 ml — in relation to the total volume of the reacting solution (250 ml). In nearly all the experiments carried out five portions of iodine solution were added one after another. The addition of the first portion of iodine solution — especially if operating

with a small quantity of iodine — does not always yield such fine results as the following portions, because the experimental solution usually consumes a small amount of iodine itself.

As soon as this work was started, it became evident that it was necessary to find an easy method for the direct determination of the concentration of carbon disulphide in an aqueous solution. For that reason the well known iodometric determination of carbon disulphide was modified [5] so that titration with iodine could be used even if carbon disulphide was on hand in a dilute aqueous solution. The determinations of carbon disulphide were carried out, in all the present series of experiments, in the following way: immediately after taking out 10 ml carbon disulphide solution for the catalytic experiment, another 10 ml was removed and was allowed to run down into 20 ml 10 % alcoholic potash contained in a 300 ml Erlenmeyer flask with glass stopper. After replacing the stopper, the flask was shaken for a few seconds and left in darkness for 30 minutes. Thereafter the stopper was removed and washed with a small amount of water, one drop of phenolphthalein was added, and neutralization by dropwise addition of 60 % acetic acid took place. An excess of 3—4 drops was added. For the sake of imparting to the solution an adequate reaction one gram of calcium carbonate was added, and the flask shaken for about 15 seconds. After the addition of 1 ml of starch indicator, the solution was diluted with oxygen-free water to 150 ml and titrated with 0.01 *N* iodine. The iodine solution was added to an excess of about 0.5 ml. The excess was determined by back titration with 0.01 *N* thiosulphate.

Solutions used: The iodine solution was 0.100 *N* to iodine and 0.180 *M* to potassium iodide. In the experiments the iodine solution was added by means of a Krogh syringe pipette. The sodium azide solution was made 0.1000 *M* by weighing out pure sodium azide (analyzed according to [4]) and dissolving in a volumetric flask. The solutions of potassium iodide and of sodium perchlorate were also made 0.1000 *M* by weighing out the calculated amounts of the pure, dried salts.

EFFECT OF IODINE CONCENTRATION

From Table 1 it can be seen that the rate of reaction is, as a whole, independent of the iodine concentration. But the rate seems to be somewhat slower if the iodine concentration is very low (Expt. no. 6). This phenomenon is perhaps related to the fact that iodine is bound to starch and therefore does not react quite so readily as does free iodine. In all experiments 10 ml of 0.1 *M* potassium iodide were added in order to keep the iodide concentration constant during an experiment, as the reduction of iodine to iodide otherwise would cause a considerable percentile change. Furthermore, the colour change of the starch indicator will be more abrupt and sharp if the concentration of iodide ions is fairly large. The ionic strength was kept constant (0.02) in all experiments by the addition of varying amounts of 0.1 *M* sodium perchlorate. The concentrations of potassium and perchlorate ions were in no case so high that precipitation of potassium perchlorate took place. From t_3 (the time of

reaction for the third addition of iodine) was calculated the corresponding rate constant k_3, according to the second order rate expression:

$$k = \frac{2.303}{c \cdot t} \cdot \log \frac{a}{a - x} \qquad (2)$$

where c is the concentration of carbon disulphide, t is the time of reaction, a is the initial concentration of sodium azide, and $a - x$ is the concentration of sodium azide to the time t. Analogously was calculated, from the experimental times of reaction, the rate constants k_2, k_4 and k_5. From these constants together with k_3, the average k_a was taken, which is given in the table.

Table 1. Effect of iodine concentration on the reaction between sodium azide and iodine at 20° C.

Catalyst: Carbon disulphide. In all experiments — besides the solutions mentioned in the table — were added 1 ml 0.5 % starch indicator, 10 ml 0.1 *M* potassium iodide, and water, so that the total volume was 250 ml. The added amounts of iodine in potassium iodide, sodium azide, carbon disulphide and sodium perchlorate are given in ml 0.1 *N* solution. t_3 is the reaction time for the third addition of iodine, k_3 is the corresponding rate constant. k_a is the average of k_2, k_3, k_4 and k_5. Ionic strength in all experiments: 0.02.

Expt. no.	Iodine solution		Sodium azide	Carbon disulphide	Sodium perchlorate	t_3 min	k_3	k_a
	Iodine	Potassium iodide						
1	0.40	0.72	25	0.78	14.2	12.28	4.35	4.35
2	0.40	0.72	25	0.78	14.2	12.28	4.35	4.32
3	0.80	1.44	25	0.98	13.5	20.27	4.38	4.38
4	1.60	2.88	25	0.90	12.1	47.86	4.42	4.40
5	0.50	0.90	12.5	0.47	26.6	52.73	4.49	4.47
6	0.10	0.18	12.5	0.50	27.3	10.48	3.90	3.87

EFFECT OF CARBON DISULPHIDE CONCENTRATION

From Table 2 it can be seen that the rate of reaction is proportional to the concentration of carbon disulphide. From Expt. no. 1 to Expt. no. 8 the

Table 2. Effect of carbon disulphide concentration on the reaction between sodium azide and iodine at 20° C.

Catalyst: Carbon disulphide. In experiments 1, 2, 3 and 4 — besides the solutions mentioned in the table — were added 1 ml 0.5 % starch indicator, 10 ml 0.1 *M* potassium iodide, and water, so that the total volume was 250 ml. In experiments 5, 6, 7 and 8 were added the same solutions except that the 10 ml 0.1 *M* potassium iodide was replaced by 10 ml 0.1 *M* sodium perchlorate. The added amounts of iodine in potassium iodide, sodium azide, carbon disulphide, and sodium perchlorate are given in ml 0.1 *N* solution. t_3 is the reaction time for the third addition of iodine, k_3 is the corresponding rate constant. k_a is the avarage of k_2, k_3, k_4 and k_5. Ionic strength in all experiments: 0.02.

Expt. no.	Iodine solution		Sodium azide	Carbon disulphide	Sodium perchlorate	t_3 min	k_3	k_a
	Iodine	Potassium iodide						
1	0.40	0.72	25	1.44	14.2	6.42	4.51	4.46
2	»	»	»	0.73	»	12.57	4.54	4.52
3	»	»	»	0.37	»	25.11	4.49	4.47
4	»	»	»	0.19	»	48.75	4.50	4.46
5	0.10	0.18	1.25	1.46	38.6	39.00	4.40	4.38
6	»	»	»	2.79	»	21.47	4.18	4.19
7	»	»	»	5.64	»	10.48	4.23	4.23
8	»	»	»	10.80	»	5.60	4.14	4.05

relation between the concentrations of azide and carbon disulphide is changed by a factor of about 10^3, but nevertheless, the difference between the two corresponding rate constants is only approx. 10 %.

EFFECT OF SODIUM AZIDE CONCENTRATION

Table 3 shows that the rate of reaction is proportional to the concentration of sodium azide.

Table 3. Effect of sodium azide concentration on the reaction between sodium azide and iodine at 20° C.

Catalyst: Carbon disulphide. In all experiments — besides the solutions mentioned in the table — were added 1 ml 0.5 % starch indicator, 10 ml 0.1 *M* potassium iodide and water, so that the total volume was 250 ml. The added amounts of iodine in potassium iodide, sodium azide, carbon disulphide, and sodium perchlorate, are given in ml 0.1 *N* solution. t_3 is the reaction time for the third addition of iodine, k_3 is the corresponding rate constant. k_a is the average of k_2, k_3, k_4 and k_5. Ionic strength in all experiments: 0.02.

Expt. no.	Iodine solution		Sodium azide	Carbon disulphide	Sodium perchlorate	t_3 min	k_3	k_a
	Iodine	Potassium iodide						
1	0.50	0.90	6.25	0.477	32.70	123.07	4.26	4.25
2	»	»	12.50	0.488	26.60	53.28	4.28	4.25
3	»	»	12.50	0.497	26.60	52.32	4.27	4.25
4	»	»	25.00	0.504	14.10	24.60	4.24	4.20

EFFECT OF IONIC STRENGTH

Since the rate of the carbon disulphide catalyzed iodine-azide reaction is directly proportional to the concentration of sodium azide and to the concentration of carbon disulphide, it is reasonable to assume that the rate determining reaction step is a reaction between azide ions and carbon disulphide molecules

$$N_3^- + CS_2 \longrightarrow SCSN_3^- \qquad (3)$$

An investigation of the primary salt effect might support this assumption. According to Brønsted[6] the ratio between the rate constant at ionic strength 0.1 and the rate constant at ionic strength 0.02 should be about one, when an ion of one charge reacts with a neutral molecule. From Table 4 it is seen that the experimental ratio is 1.005, which agrees well with the assumption made as to the rate determining step.

Table 4. Effect of ionic strength on the reaction between sodium azide and iodine at 20° C.

Catalyst: Carbon disulphide. In the two experiments — besides the solutions mentioned in the table — were added 1 ml 0.5 % starch indicator, 10 ml 0.1 *M* potassium iodide, and water, so that the total volume was 250 ml. The added amounts of iodine in potassium iodide, sodium azide, carbon disulphide, and sodium perchlorate are given in ml 0.1 *N* solution. t_3 is the reaction time for the third addition of iodine, k_3 is the corresponding rate constant. k_a is the average of k_2, k_3, k_4 and k_5. Ionic strength in Expt. 1 is 0.02, and in Expt. 2 it is 0.1.

Expt. no.	Iodine solution		Sodium azide	Carbon disulphide	Sodium perchlorate	t_3 min	k_3	k_a
	Iodine	Potassium iodide						
1	0.50	0.90	12.5	1.58	26.60	15.73	4.47	4.41
2	»	»	»	1.36	226.60	18.22	4.48	4.43

EFFECT OF pH

According to Brønsted [6] a reaction between two un-charged molecules would show a very small primary salt effect, for which reason the reaction

$$HN_3 + CS_2 \longrightarrow HSCSN_3$$

would also agree with the salt effect found. Hydrazoic acid is an acid of about the same strength as acetic acid, *i. e.* the acid constant is approx. 10^{-5}. In a sodium azide solution, with pH > 7, the concentration of un-ionized hydrazoic acid will be proportional to the concentration of hydrogen ions, or, if the pH decreases by 1, the concentration of hydrazoic acid increases ten times. As can be seen from Table 5, a variation of pH does not change the rate constant. For this reason the rate determining step cannot be a reaction between hydrazoic acid and carbon disulphide.

A few experiments have been carried out with identical concentrations of sodium azide, carbon disulphide, and iodine. In addition, varying amounts of perchloric acid were added, so that the azide ions were partly converted into un-ionized hydrazoic acid. The rate of reaction gradually decreases as the conversion of the azide ions into hydrazoic acid proceeds, and when a small excess of perchloric acid (or another strong acid) is added, the rate of reaction becomes extremely small. Consequently we have good reasons to assume that only free azide ions — and not hydrazoic acid — take part in the reaction.

Table 5. Effect of pH on the rate of the reaction between sodium azide and iodine at 20° C.

Catalyst: Carbon disulphide. In the experiments — besides the solutions mentioned in the table — were added 1 ml 0.5 % starch indicator, 10 ml 0.1 *M* potassium iodide, 0.1 *M* boric acid, and 0.1 *N* sodium hydroxide, so that the total volume was 250 ml. The added amounts of iodine in potassium iodide, sodium azide, and carbon disulphide are given in ml 0.1 *N* solution. t_3 is the reaction time for the third addition of iodine, k_3 is the corresponding rate constant, k_a is the average of k_2, k_3, k_4 and k_5. The given values of pH were measured by means of a glass electrode.

Expt. no.	Iodine solution		Sodium azide	Carbon disulphide	pH	t_3 min	k_3	k_a
	Iodine	Potassium iodide						
1	0.50	0.90	12.5	1.55	7.80	15.93	4.50	4.44
2	»	»	»	1.54	7.80	15.98	4.52	4.52
3	»	»	»	1.53	8.40	16.10	4.50	4.50
4	»	»	»	1.53	8.80	16.00	4.55	4.46

ENERGY OF ACTIVATION

The energy of activation of the carbon disulphide catalyzed iodine-azide reaction was determined by experiments analogous with those previously described. The rate of reaction was determined in the temperature range 0.4 to 20° C. Due to the volatility of carbon disulphide from an aqueous solution, it was impossible to use temperatures higher than 20° C. But at 20° C or less, the loss of carbon disulphide during an experiment was negligible (this is also the reason why all the previous experiments were carried out at 20° C — and not 25° C). In Table 6 are given the average values of the rate constant, k_a. Using the method of least squares, the numerical values of H and A were calculated according to the equation:

$$\log k_a = H - \frac{A}{T}$$

where T is the absolute temperature. The result is

$$\log k_a = 15.537 - \frac{4364}{T}$$

From this equation values of k were calculated by substituting T with the experimental temperatures. These values are, together with the experimental ones, recorded in Table 6. The energy of activation is:

$$A \times 4.571 = 19\,950 \text{ kcal/mole}$$

The frequency exponent H, which has a value of 15.537 with 1 min as time unit, is — with 1 sec. as unit — 15.537—log 60 = 13.759.

Table 6. Energy of activation of the carbon disulphide catalyzed iodine-azide reaction.

In all experiments — besides the carbon disulphide solution — were added 1 ml starch indicator, 10 ml 0.1 M potassium iodide, 12.5 ml 0.1 M sodium azide, 0.50 ml 0.1 N iodine, which was 0.18 M with respect to potassium iodide, 26.60 ml 0.1 M sodium perchlorate, and water, so that the total volume was 250 ml. The added amounts of carbon disulphide are given in ml 0.1 M solution. t_3 is the reaction time for the third addition of iodine, k_3 is the corresponding rate constant; k_a is the average of k_2, k_3, k_4 and k_5. $k_{calc.}$ was calculated from the equation $\log k_a = H - \frac{A}{T}$, which is a straight line fitted to the experimental k_a and T values. Ionic strength in the experiments: 0.02.

Expt. no.	Temp. °C	Carbon disulphide	t_3 min	k_3	k_a	$k_{calc.}$
1	20	0.470	52.38	4.52	4.44	4.45
2	»	0.500	49.40	4.50	4.49	
3	15	0.925	51.03	2.35	2.33	2.45
4	»	0.940	48.37	2.44	2.39	
5	12.5	1.675	36.87	1.80	1.82	1.81
6	»	1.695	36.73	1.79	1.80	
7	10	1.530	52.65	1.38	1.40	1.33
8	»	1.546	52.67	1.37	1.38	
9	0.4	2.230	138.47	0.360	0.375	0.380
10	»	2.165	139.72	0.367	0.375	

IS CARBON DISULPHIDE OR AZIDO-DITHIOCARBONATE IONS THE ACTUAL CATALYST?

In order to ascertain whether or not the carbon disulphide that combines with azide ions in accordance with equation (3), is subsequently regenerated during the following stages of the iodine-azide reaction, Browne and Hoel [1]

have carried out an experiment as follows: 'A sample of pure, dry, solid potassium azido-dithiocarbonate, weighing 0.1974 g, was dissolved in 20 cc of a 20 % solution of potassium trinitride. A concentrated solution of iodine, in potassium iodide, was introduced, drop by drop, below the surface of the solution, while a slow current of air was continuously bubbled through the reacting mixture and then through an alcoholic solution of potassium hydroxide. The azido-salt used would correspond to a total weight of carbon disulphide amounting to 0.0955 g. No indication whatever of the presence of carbon disulphide in the gases from the flask was obtained when the alcoholic solution was tested in the usual way with acetic acid and copper sulfate, although the operation of bubbling air through the flask was continued for 20 minutes.'

From this experiment Browne and Hoel concluded that azido-dithiocarbonate is the actual catalyst, and that no carbon disulphide is regenerated after it has become 'fixed' as azido-dithiocarbonate. This assumption does not agree with our kinetic experiments. If this assumption were true, the rate of reaction in an experiment would increase until all carbon disulphide present had combined with azide ions. We always found the rate of reaction strictly proportional to the concentration of carbon disulphide, and the interaction between azide ions and carbon disulphide is known to be a slow process.

We have carried out two experiments as follows: Into a Friedrichs gas washing bottle were introduced 50 ml 0.1 *M* sodium azide, an amount of carbon disulphide solution corresponding to 2.00×10^{-4} mole carbon disulphide, 1 ml starch indicator, and water, to a total volume of 90 ml. This solution was allowed to stand for one hour, so that the carbon disulphide would be partly 'fixed' as azido-dithiocarbonate ions. After addition of a further 10 ml of water, a constant air current was bubbled through the solution for 10 minutes. The carbon disulphide vapour contained in the air current was absorbed in another Friedrichs bottle containing 30 ml 10 % alcoholic potash, and estimated — by iodine titration — to be 1.03×10^{-4} moles. Consequently, 0.97×10^{-4} moles of carbon disulphide had been converted into azido-dithiocarbonate ions. A parallel experiment was carried out differing only in that after 1 hour 10 ml 0.1 *N* iodine solution was added instead of 10 ml of water. The amount of carbon disulphide driven off by the air current was estimated to be 1.82×10^{-4} moles. From a control experiment it was found that only a negligible amount of iodine was volatilized during the aeration. These experiments show that carbon disulphide was regenerated when the iodine-azide reaction took place. The probable reason why Browne and Hoel found no regeneration is that they added iodine drop by drop so that only small amounts of carbon disulphide were liberated; and, due to the

extremely high concentration of sodium azide (20 %), the re-formation of azido-dithiocarbonate ions took place before the carbon disulphide had a chance to escape.

IODINE AZIDE, IN_3

As can be seen below, it is a possibility that iodine azide, IN_3, is an intermediate product in the iodine-azide reaction. If so, iodine azide would have to react vigorously with azide ions with the formation of free nitrogen. An aqueous solution of iodine azide was prepared according to Hantzsch [7]. All operations were carried out at + 5° C. The solution was mixed with a 5 % solution of sodium azide (at + 5° C). Only very slow liberation of a gas took place — possibly due to spontaneous decay of iodine azide.

DISCUSSION

From all the experiments mentioned it is evident that the rate of the carbon disulphide catalyzed iodine-azide reaction is proportional to the concentration of carbon disulphide, proportional to the concentration of free azide ions, and independent of the concentration of iodine. Therefore, the rate determining reaction step must be:

$$N_3^- + CS_2 \longrightarrow SCSN^-$$

The reverse of this reaction is without importance in this case, and the formation of azido-dithiocarbonate ions has been shown [1,3] to take place even if iodine is not present. Therefore it is reasonable to assume that it is also the *first* step in a sequence of reactions. Investigations by Browne, Smith, Gross and Brandes [8] on the conductivity of the azido-dithiocarbonate ion have shown that azido-dithiocarbonic acid is a moderately strong acid, and hence only azido-dithiocarbonate ions will be on hand in slightly basic solution.

As the reactions in which iodine takes part are instantaneous, it is impossible by simple kinetic investigations to elucidate them further. Since Browne and Hoel [1] have shown that iodine reacts immediately with azido-dithiocarbonate ions with formation of azido-carbon disulphide, which again reacts with sodium azide solution with the evolution of nitrogen, it is reasonable to assume a reaction mechanism rather similar to that of Browne and Hoel.

$$N_3^- + CS_2 \longrightarrow SCSN_3^-$$
$$2\ SCSN_3^- + I_2 \longrightarrow (SCSN_3)_2 + 2\ I^-$$
$$(SCSN_3)_2 + 2\ N_3^- \longrightarrow 2\ CS_2 + 2\ N_3^- + 3\ N_2$$

This mechanism differs from the one proposed by Browne and Hoel only in that carbon disulphide — and not only azido-dithiocarbonate ions — is regenerated.

Possible reaction steps — after the formation of azidodithiocarbonate ions — could also be:

$$SCSN_3^- + I_2 \longrightarrow CS_2 + IN_3 + I^-$$
$$IN_3 + N_3^- \longrightarrow I^- + 3\ N_2$$

but as iodine azide does not react — or at least only very slowly — with azide ions, this possibility must be excluded.

SUMMARY

Kinetic investigations have been carried out on the iodine-azide reaction catalyzed by carbon disulphide. The reaction is a second order reaction as to azide ions and carbon disulphide molecules. The rate of reaction was found to be independent of the concentration of iodine. The investigations agree with a reaction mechanism rather similar to that proposed by Browne and Hoel[1]. The difference is that carbon disulphide — and not azido-dithiocarbonate ions — must be considered as the actual catalyst. The relation between rate constant and absolute temperature has been found to be

$$\log_{10} k = 13.759 - \frac{4364}{T}$$

with seconds as the time unit, and k calculated in natural logarithms. The energy of activation is 19 950 kcal/mole.

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Received October 24, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1430–1436

A Study of Ammonium Permolybdates

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During the investigation of the structure of polymolybdates, which is now being carried out at this institute, it seemed possible to obtain further information on the paramolybdates by studying the perparamolybdates. A study of the ammonium perparamolybdates was therefore begun.

Werther [1] in 1861 noted the yellow colour obtained by the action of H_2O_2 on acid solutions of ammonium molybdate. The crystals of ammonium perparamolybdate were then investigated by Baerwald [2] in 1885. He dissolved ammonium paramolybdate in an excess of H_2O_2 and let the solution evaporate isothermally. From his primitive analyses he arrived at the formula $14NH_3 \cdot 18MoO_3 \cdot 3H_2O_2 \cdot 18H_2O$. The large lemon-yellow crystals were monoclinic with $a : b : c = 1.4727 : 1 : 1.0268$ and $\beta = 74°\ 32'$ ($180° - \beta = 105°\ 28'$). Péchard [3] used the same method at 100° C and undoubtedly obtained the same crystals, because Dufet [4] who measured them crystallographically found $a : b : c = 1.4682 : 1 : 1.0259$ and $\beta = 105°\ 41'\ 40''$. Péchard, however, described this compound as $(NH_4)_2O \cdot Mo_2O_7 \cdot 4H_2O$.

Moeller [5] claimed to have obtained a water-free compound $(NH_4)_2Mo_2O_8$ by the action of H_2O_2 on ammonium molybdate. His crystals were measured by Fock[6] who found $a : b : c = 0.4693 : 1 : 0.2956$ and $\beta = 112°\ 39'\ 30''$, so this compound was obviously not identical with that described by Baerwald and Péchard. The only analytical value, given by Moeller, is 80.89 % MoO_3, which is possibly in agreement with 81.52 % calculated for ammonium paramolybdate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. Since the crystals are bright yellow and described as easily cleaved along (010), we tried transforming the axial system given by Groth [13] for $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ in order to show an eventual identity with Moeller's crystals. It was possible to get $a : b : c = 0.460 : 1 : 0.294$ and $\beta = 114.0°$ in clear agreement with Fock's values. As is stated below Moeller obviously obtained a solid solution of small amounts of peroxide oxygen in $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$. (It is, however, interesting to note that the

habit of the crystals obtained by crystallization from an H_2O_2 solution is different from that obtained from an H_2O_2 free solution.)

The most critical analytical work in this field is surely that by Muthmann and Nagel [7], which appeared in 1898. They found two different salts. From concentrated H_2O_2 solutions, saturated with $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ they obtained at first orange coloured crystals and then from the mother liquor lemon yellow crystals of a quite different habit. The two compounds were given the formulae $3(NH_4)_2O \cdot 7MoO_4 \cdot 12H_2O$ and $3(NH_4)_2O \cdot 5MoO_3 \cdot 2MoO_4 \cdot 6H_2O$ respectively. The latter is certainly the salt formerly described by Baerwald and Péchard.

A very confusing contribution to the knowledge of the ammonium perparamolybdates was published in 1931 by Cagliotti [8]. He gives analytical results without stating the analytical methods used and X-ray crystallographic data without any statement about the sort of radiation. He claims to have prepared the following three compounds:

$$5(NH_4)_2O \cdot 12MoO_4 \cdot 18H_2O,\ 5(NH_4)_2O \cdot 9MoO_3 \cdot 3MoO_4 \cdot 10H_2O \text{ and } 5(NH_4)_2O \cdot 6MoO_3 \cdot 6MoO_4 \cdot 13H_2O.$$

(The latter was obtained from solutions of normal ammonium molybdate in 30 % H_2O_2.) In varying the ratio $MoO_3 : O_{active}$ in the solutions of paramolybdate in H_2O_2 between 1 and 6 he obtained crystals with active oxygen contents from 1.38 % to 1.90 %. He also studied the crystallization from the solution with the ratio $MoO_3 : O_{active} = 1$ at different temperatures. The results are here given in Table 1. The colour of these crystals changes from intense yellow to very bright yellow. In his X-ray investigation, Cagliotti studied ammonium perparamolybdates with oxygen contents of 0.99, 1.42 and 2.26 % and found great similarities between the powder photographs of paramolybdate and perparamolybdates.

Finally Rosenheim, Hakki and Krause [9] have accepted the two formulae $5(NH_4)_2O \cdot 12MoO_3 \cdot 12O \cdot 21H_2O$ and $5(NH_4)_2O \cdot 12MoO_3 \cdot 3\ O \cdot 12H_2O$ with-

Table 1. (From Cagliotti [8]).

	t °C	$(NH_4)_2O$	MoO_3	O	H_2O
1	30	11.58	77.47	1.97	8.98
2	40	12.00	77.49	1.80	8.71
3	50	12.00	77.45	1.84	8.71
4	60	11.98	77.60	1.75	8.67
5	70	11.62	77.38	1.94	9.06
6	80	11.21	78.44	0.99	8.36
7	90	11.30	81.02	0.36	6.32
8	96	11.42	80.82	0.30	6.46

out giving any analytical methods. In order to get better results, however, they prepared a guanidinium perparamolybdate for which they determined the following composition:

$(CN_3H_6)_2O$	26.01;	26.35 %	
MoO_3	65.20;	64.90;	65.02 %
O	1.64;	1.95;	1.51 %

These values may give weight ratios $MoO_3/(CN_3H_6)_2O$ between 65.20/26.01 = 2.51 and 64.90/26.35 = 2.46. The calculated values are for $12MoO_3/5(CN_3H_6)_2O$ = 2.54 and for $7MoO_3/3(CN_3H_6)_2O$ = 2.47. But Rosenheim, Hakki and Krause concluded that they had obtained the compound $5(CN_3H_6)_2O \cdot 12MoO_3 \cdot 3O \cdot 11H_2O$!

EXPERIMENTAL

We have investigated crystals obtained by isothermal evaporation from solutions of ammonium paramolybdate, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$*, in H_2O_2.

Analytical methods and checks

NH_3: Kjeldahl distillation and titration to colourless solution with bromcresol green + methyl red as indicator. In a solution containing MoO_3, H_2O_2 and a known amount of NH_3($MoO_3 : H_2O_2 : NH_3$ approximately as in the solutions analyzed during the investigation), we determined NH_3. Weighed amount: 0.04669 g. Observed amount: 0.04665 g.

MoO_3: Heating to constant weight in a furnace at 450° C. After one hour the constancy is good. The residue (MoO_3) always has a pale grey-green colour. The check was carried out as under NH_3 with a known amount of MoO_3. Weighed amount: 0.6166 g. Observed amount: 0.6158 g.

Active oxygen: Titration in acid solution with 0.1 *N* $KMnO_4$. Ferroine sulphate was used as indicator. To avoid catalytical decomposition of H_2O_2 we added $MnSO_4$ to the solution before the titration. The check was carried out as under NH_3 with a known amount of H_2O_2. Weighed amount: 0.00440 g. Observed amount: 0.00438 g.

RESULTS

1. $3(NH_4)_2O \cdot 7MoO_3 \cdot xO \cdot 4H_2O$ ($x<0.3$)

From dilute solutions we obtained paramolybdate crystals with up to 0.2 weight % *O* without any visible change in the powder photographs. It is

* By analyzing a commercial preparation labelled Ammonium molybdate, $(NH_4)_6Mo_7O_{24} \cdot \cdot 4H_2O$ (Kemikaliebolaget Kebo, Stockholm), this was found to have the formula of the dimolybdate $(NH_4)_2Mo_2O_7$, the existence of which has been doubted (*cf.* Gmelin [12]). By recristallization we obtained, however, the paramolybdate. The dimolybdate will be further investigated.

very strange, however, that even at these low oxygen contents the crystals may be lemon yellow. During his study of the crystallization at different temperatures, given in Table 1, Cagliotti surely has obtained this phase (nos. 7 and 8), quite as Moeller, as already has been pointed out in the historical review. We have not tried to determine the exact limit of the solid solution.

2. $3(NH_4)_2O \cdot 7MoO_3 \cdot (2-x)O \cdot 6H_2O \cdot (x<0.5)$

This compound was obtained by evaporation of solutions containing H_2O_2 in a mole ratio H_2O_2 : Mo larger than 0.75 and is surely identical with the compound having the least oxygen, obtained by Baerwald, Péchard, Muthmann and Nagel, Cagliotti and Rosenheim *et. al.* The crystals are lemon yellow and crystallize both in needles and plates with a tendency for twinning.

Analyses:	Found		Calc. (for the comp. x = 0)
$(NH_4)_2O$	11.98 %	12.11 %	11.98 %
MoO_3	77.30 »	77.80 »	77.27 »
O	2.11 »	1.88 »	2.45 »
H_2O (by difference)	8.61 »	8.21 »	8.30 »
$(NH_4)_2O : MoO_3$	3.00 : 7	3.01 : 7	3 : 7

The mole ratio $(NH_4)_2O/MoO_3$ is constant = 3.0 : 7, but the oxygen content varies (*cf.* Muthmann and Nagel, Cagliotti). It is not easy to decide definitely if the compound loses part of its oxygen due to instability in the air, or if we have a solid solution range. Nor have we tried to determine any limits for the oxygen content exactly. We prefer to give the formula $3(NH_4)_2O \cdot 7MoO_3 \cdot (2-x)O \cdot 6H_2O$ $(x<0.5)$. A crystal structure determination will possibly yield a definite answer to this question.

A single crystal has been investigated. Weissenberg photographs (Cu*K*-radiation) around [100] were taken. The absence of $(h\ 0\ l)$ with l odd and of $(0\ k\ 0)$ with k odd indicate the spacegroup to be C_{2h}^5—$P2_1/c$. The monoclinic cell dimensions are $a = 10.7$ Å, $b = 10.2$ Å, $c = 30.0$ Å and $\beta = 106° \pm 0.5°$ which give $a : b : c = 1.05 : 1 : 2.94$. (We have interchanged the a and c axes in order to conform to the space group notation $P2_1/c$.) The value 1.05 for $a : b$ instead of 1.03 as found by Baerwald [2] may be attributed partly to difficulties in the crystallographic measurements, partly to inaccuracy in the determination of a solely from rotation photographs. The density has been determined by the swimming method to 2.71 (Baerwald gives without description of his method the value 2.975). These data give 3.94 units of $3(NH_4)_2O \cdot 7MoO_3 \cdot 2O \cdot 6H_2O$ and 2.3 units of $5(NH_4)_2O \cdot 12MoO_3 \cdot 3O \cdot 12H_2O$ in the

Table 2. Powder photographs taken with monochromatized CuKα-radiation.

$3(NH_4)_2O \cdot 7MoO_3 \cdot xO \cdot 4H_2O$; $3(NH_4)_2O \cdot 7MoO_3(2 - x)O \cdot 6H_2O$; $2(NH_4)_2O \cdot 5MoO_3 \cdot (5 - x)O \cdot 9H_2O$

I	$\sin^2\Theta$	I	$\sin^2\Theta$	I	$\sin^2\Theta$
v st	0.0071	v st	0.0063	v w	0.0036
m	0.0084	v st	0.0083	w	0.0044
st	0.0104	st	0.0106	v w	0.0059
st	0.0110	st	0.0110	v st	0.0068
v st	0.0117	st	0.0113	v st	0.0074
m	0.0139	st	0.0120	m	0.0085
st	0.0145	v w	0.0126	m	0.0089
m	0.0171	w	0.0130	st	0.0111
m	0.0176	st	0.0144	st	0.0130
m	0.0211	st	0.0168	v w	0.0134
w	0.0217	st	0.0208	m	0.0142
m	0.0231	m	0.0228	v w	0.0149
w	0.0244	v w	0.0244	v w	0.0171
v w	0.0250	m	0.0253	w	0.0193
v w	0.0269	m	0.0266	v w	0.0206
m	0.0284	st	0.0277	v w	0.0250
st	0.0297	v w	0.0281	w	0.0268
v w	0.0308	w	0.0288	w	0.0279
v w	0.0317	st	0.0297	m	0.0302
m	0.0341	w	0.0329	w	0.0361
m	0.0358	w	0.0353	w	0.0398
m	0.0383	w	0.0371	w	0.0425
m	0.0398	m	0.0405	w	0.0449
st	0.0409	m	0.0423	st	0.0469
st	0.0434	m	0.0438	w	0.0501
st	0.0460	v st	0.0451	m	0.0526
w	0.0467	v st	0.0478	w	0.0543
v st	0.0493	m	0.0532	w	0.0576
st	0.0519	w	0.0551	v w	0.0591
st	0.0531	v st	0.0562	v w	0.0605
m	0.0558	v w	0.0568	w	0.0653
v w	0.0580	w	0.0583	v w	0.0690
st	0.0593	st	0.0603	v w	0.0712
v st	0.0625	m	0.0619	v w	0.0741
v w	0.0640	m	0.0632	m	0.0789
m	0.0655	v w	0.0643	w	0.0833

cell. We have thus obtained a final confirmation of the formula $3(NH_4)_2O \cdot \cdot 7MoO_3 \cdot (2—x)O \cdot 6H_2O$ quite in the same way as the formula $(NH_4)_6Mo_7O_{24} \cdot \cdot 4H_2O$ was established by Sturdivant [10] and Lindqvist [11]. As it seemed very strange that $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ with $a = 8.38$ Å, $b = 36.12$ Å,

$c = 10.46$ Å and $\beta = 116° 0'$ [10] should give the same powder photographs as $3(NH_4)_2O \cdot 7MoO_3 \cdot (2—x)O \cdot 6H_2O$, as Cagliotti [8] states, we compared the powder photographs of the two compounds. They were quite different. As Cagliotti does not state the radiation used by him we have not been able to compare his values with ours which are given in Table 2.

3. $2(NH_4)_2O \cdot 5MoO_3 \cdot (5-x)O \cdot 9H_2O$ ($x<1.5$)

All efforts to get the compound richest in oxygen described by Muthmann and Nagel have been in vain. The solutions are very unstable and give in most cases $3(NH_4)_2O \cdot 7MoO_3 \cdot (2—x)O \cdot 6H_2O$. In a few preparations, however, we have obtained another compound with high but varying oxygen content. Plate-shaped and orangecoloured hexagons were formed by room temperature (lowest oxygen content) or low temperature (+ 4° C) (highest oxygen content) evaporation of paramolybdate solutions in H_2O_2, saturated at low temperature (+ 4° C). Some of the crystals showed signs of efflorescence while others proved to be stable. Some of the latter were picked out and analyzed:

Analyses:	Found	Calc. for the comp. $x = 0.63$
$(NH_2)_2O$	9.86 %	9.87 %
MoO_3	68.08 »	68.17 »
O_{active}	6.62 »	6.62 »
H_2O	15.47 »	15.35 »
$(NH_4)_2O : MoO_3$	2.00 : 5	

The mole ratio is thus surprisingly not 3 : 7 but 2 : 5 which would correspond to a formula $2(NH_4)_2O \cdot 5MoO_3 \cdot (5—x)O \cdot 9H_2O$. Such a type of molybdate has not been described before.

A single crystal investigation with CuK radiation indicates that the compound is monoclinic with $a = 12.9$ Å, $b = 18.6$ Å, $c = 10.7$ Å, $\beta = 109°$. The density was determined by the swimming method to be 2.50. The number of Mo atoms in the cell will then be 19.6. This gives a further support to the formula $2(NH_4)_2O \cdot 5MoO_3 \cdot (5—x)O \cdot 9H_2O$.

This compound gives powder photographs (Table 2) with weak lines, which are difficult to detect together with the lines of other phases. We have not tried to determine the limits of the oxygen content.

SUMMARY

The existence of a solid solution of peroxide oxygen in ammonium paramolybdate $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ has been proved. These crystals have been shown to be identical with those described by Moeller and Fock as $(NH_4)_2Mo_2O_8$. The lemon yellow ammonium permolybdate first obtained by Baerwald has been found to possess the formula $3(NH_4)_2O \cdot 7MoO_3 \cdot (2—x)O \cdot \cdot 6H_2O$ ($x<0.5$). It is monoclinic with $a = 10.7$ Å, $b = 10.2$ Å, $c = 30.0$ Å and $\beta = 106°$. The unit cell contains 4 units of the above formula. Space-group C_{2h}^5—$P2_1/c$.

We have also found an orange-coloured compound of a new type and with the formula $2(NH_4)_2O \cdot 5MoO_3 \cdot (5—x)O \cdot 9H_2O$ ($x < 1.5$). This is monoclinic with $a = 12.9$ Å, $b = 18.6$ Å, $c = 10.7$ Å, $\beta = 109°$. The unit cell contains 4 formula units.

An effort will be made to determine the crystal structures of these compounds.

This investigation has been supported by a grant, which is here gratefully acknowledged, from *Statens Naturvetenskapliga Forskningsråd.*

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Received October 6, 1949.

ACTA CHEMICA SCANDINAVICA 3 (1949) 1437–1448

Short Communication

Different Nitrogen Fractions in Normal and Low-Nitrogen Cells of Microorganisms

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In many papers, Virtanen and collaborators [1] have dealt with the dependence of the enzymatic activity of cells on their nitrogen content. The disappearance or the great decrease in activity of certain enzymes, accompanying the lowering of N-content, has been ascribed to the insufficient protein supply necessary for the building up of all the possible cell enzymes.

Lately, we have examined to what extent the content of different N-fractions has changed in low-nitrogen cells compared with normal nitrogen cells. The first object of research was *Pseudomonas fluorescens*. Low-nitrogen cells were produced according to Virtanen and Kokkola [2]. The nucleic acid nitrogen and the "protein nitrogen" insoluble in trichloracetic acid were primarily examined. A sample containing the same amount of nitrogen was taken from both normal and low-nitrogen cell masses, NaOH-solution was added to make the suspension 0.1 *N* to NaOH, and the suspension was shaken vigorously. After 10 min. standing, 50 % trichloracetic acid was added to make the solution 6.9 % with regard to trichloracetic acid, and the mixture kept in boiling water bath for 10 minutes. It was found in the control experiments that the amount of NA extracted in this way was at least 92 % of the amount extractable with 0.5 *N* NaOH in 1 hr in boiling water bath. However, in the latter stronger alkali extraction yellowish brownish compounds were formed which make the measurements uncertain and surely partly cause the higher absorption. The milder extraction was therefore adopted. Ahlström and v. Euler *et al.*[3] have used the same method for the splitting of nucleoproteides and for the extraction of nucleic acids.

The estimation of nucleic acids from the trichloracetic acid extracts, clarified by centrifugation, was made by determining the absorption spectrum between 240 and 300 mμ. The curves obtained (Fig. 1) were in fairly good agreement with the absorption curve found by Ahlström, v. Euler *et al.*[3] for purified desoxyribonucleic acid 99. Evidently the extract did not contain substances interfering with the absorption within the stated region, and the method is thus suitable for the determination of total nucleic acids. In calculating the nucleic acid content, the value a_{262} = 29.2 was used. A value of 14 % was taken for the N-content of nucleic acids. The amounts of different nitrogen fractions in normal and low-nitrogen bacterial masses are presented in Tables 1 and 2.

The results show that the percentage of the fraction soluble in trichloracetic acid is higher in low-nitrogen cells than in normal nitrogen cells. Nucleic acid nitro-

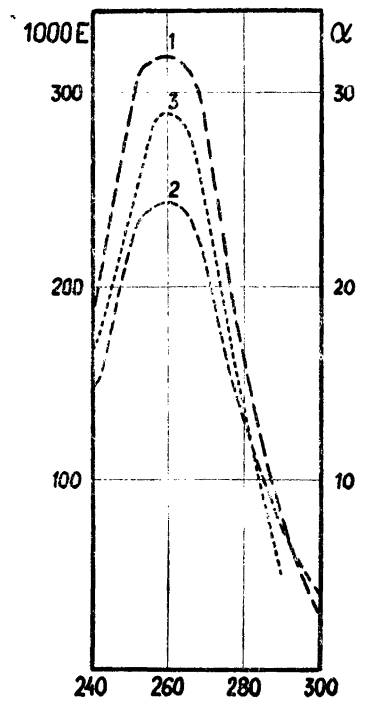

Fig. 1. Absorption spectra of extracts from normal N and low-N Ps. fluorescens and of a nucleic acid preparation by Ahlström, v. Euler et al.[3]

Extinction values
1 000 × E (Curves 1 and 2)
α (Curve 3)

Curve 1: Extract from normal N bacteria
» 2: » » low-N » »
» 3: DNA-preparation 99 (Ahlström, v. Euler et al.)

gen has decreased considerably more than total nitrogen, whereas no drop occurred in other soluble nitrogen, and accordingly, this nitrogen fraction, in per cent of total N, is in the low-nitrogen cells much higher than in the normal nitrogen cells. This nitrogen fraction comprises the amino acids and small peptides. The lack of nitrogen nutrition has the greatest effect on the decrease of the protein and nucleic acid fractions.

After hydrolysis with hydrochloric acid, amino acids were qualitatively determined

Table 1. Different nitrogen fraction in normal and low-nitrogen bacterial masses.

g per 100 g dry matter	Normal N bact. mass	Low-N bact. mass	Decrease %
1. Total N	12.7	7.8	38.6
2. "Protein N"	9.18	5.52	39.9
3. N soluble in trichloracetic acid	3.50	2.30	34.3
4. NA-N	2.27	1.05	54.7
5. Other soluble N	1.23	1.25	0

"Protein-N" = calc. difference between 1 and 3.
Other soluble N = calc. difference between 3 and 4.

Table 2. Different nitrogen fractions in % of total nitrogen.

% of total N	Normal N bact. mass	Low-N bact. mass	Difference %
"Protein N"	72.3	70.5	− 2.5
Soluble N	27.7	29.5	+ 6.5
NA-N	17.9	13.4	− 24.0
Other soluble N	9.7	16.0	+ 65.0

both in normal nitrogen and low-nitrogen bacterial masses by means of paper chromatography. No difference could be detected in the amino acid composition of the masses. The intensity of the colour spots, estimated by the eye, was also similar in both cases. Thus the sharp decrease in the protein content of cells does not cause changes in the amino acid composition of proteins that could be detected by means of paper chromatography.

The corresponding determinations as with *Ps. fluorescens* were also made with *Torula utilis*. Low-nitrogen yeast was prepared by driving, in a Kluyver-flask, a strong current of air through sugar nutrient solution (without combined nitrogen) in which normal-nitrogen yeast was suspended[4]. The analytical data appear from Table 3.

The nucleic acid content of low-nitrogen Torula has decreased approximately as much as in low-nitrogen *Ps. fluorescens*. Instead, the insoluble "protein-N" has

Direct Titration of Ammonia in Kjeldahl Determinations with Nickelammoniumsulphate Solutions as Absorbent

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In the Kjeldahl method for the determination of nitrogen in organic substances two distillation procedures are in common use. In the classical method the ammonia is distilled into a measured volume of a standard strong acid, and the excess acid is titrated with a standard base. This procedure gives a sharp end-point but requires two standard solutions, the base being rather unstable. In order to be able to titrate the ammonia directly with standard acid Winkler[1] suggested to absorb the ammonia in an excess of an exceedingly weak acid, boric acid. This method eliminates the need for a standard solution of a base, and for the accurate measurement of the absorbent, and it simplifies the calculation of the analysis, but it requires an additional solution in the form of an indicator-boric-acid-solution by which the end-point is matched and adjusted.

More than a year ago we introduced the boric acid method in our routine work, but we found that determinations according to this method were less accurate than determinations according to the classical method. The advantage of the direct titration over the back titration method was however so evident, that we looked for another absorbent, which would make a direct titration of the ammonia possible. It later occured to one of us, that a solution of some salt, whose cation forms metal ammine complexes[2], might suit our purpose. In order to obtain a titration, where the attainment of the end-point is not slow, salts have to be selected, whose cations form ammine compounds in fast reactions and are not hydrolysed into metal hydroxides.

In a macro-Kjeldahl determination 1—3 mval of ammonia are distilled off with 120 to 150 ml of water. Our preliminary in-

Table 3. Different nitrogen fractions in normal and low-nitrogen T o r u l a.

g per 100 g dry matter	Normal N *Torula*	Low-N *Torula*	Decrease %
Total N	9.5	5.7	40
"Protein N"	6.1	4.1	33
N soluble in trichloracetic acid	3.4	1.6	53
NA-N	1.4	0.65	53.5
Other soluble N	2.0	0.95	52.5

decreased in *Torula* noticeably less than total-N. Other soluble N, again, has decreased in *Torula* as much as the nucleic acid-N. In *Ps. fluorescens* this N-fraction has not lowered at all. Accordingly, different nitrogen fractions of microorganisms do not change quantitatively in the same way as their nitrogen content lowers.

The *Pseudomonas* masses were prepared by Miss Ulla Winkler.

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Received December 15, 1949.

vestigations were performed with 1 to 10 mval of cupric ion, zink ion, cobalt ion, cadmium ion and nickel ion. Under these conditions only 10 mval nickel ion in the form of nickelsulphate proved to be suitable. This method was adopted for routine work and used for several months. Using methyl red as indicator the green color of the nickel ion does not interfere with the detection of the end-point. If the titration is not carried out within about one hour after the distillation, the attainment of the end-point becomes rather slow, owing to the formation of minute amounts of a precipitate (nickel hydroxide ?).

Since the hydrolysis is depressed by ammonium ion, the same investigations were later on repeated with the addition of up to 20 mval of ammonium ion. Even with 20 mval of ammonium ion hydroxides are precipitated in solutions of cupric ion and zink ion. Suitable amounts of ammonium ion depress the hydrolysis of cobalt ion and cadmium ion, so that they might so far also be used. The amount of ammonium ion required is however greatest with cobalt ion, much smaller with cadmium ion, and smaller still with nickel ion. Moreover the red color of the cobalt ion interferes with the observation of the end-point using methyl red as indicator. Cadmium salt solutions are appreciably more acid than nickel salt solutions, so that methyl red cannot be used as indicator. We found that nickel ion was definitely best suited to our purpose. Of the different nickel salts we choose $NiSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$, since this salt is easily obtained chemically pure [3] and furthermore contains the two components in a proportion suitable for our purpose. In our routine work, where about 1 mval of ammonia is distilled off with about 130 ml of water 20 ml of 0.2 M nickelammoniumsulphate are used as absorbent. If the amount of ammonia is greater, proportionally more nickelammoniumsulphate solution has to be used. Under these conditions nickel hydroxide is not precipitated even on standing for hours. During distillation the pH is only raised from 5.1 to 7.6, and the volatility of the ammonia at room temperature is negligibly small; the receiver flasks may stand unstoppered for hours without measurable loss of ammonia. Nevertheless the ammonia may be titrated sharply with methyl red as indicator. With a mixture of methyl red and methylene blue the end-point is still more easily recognized. Contrary to the boric acid method a comparison solution is unnecessary.

Table 1. pH measurements with glass electrode. Total volume: 150 ml.

	H_2O	$NiSO_4 \cdot (NH_4)_2SO_4$ 0.2 *M* 20 ml	$CdSO_4$ 0.2 *M* $(NH_4)_2SO_4$ 0.4 *M* 20 ml
H_2O	—	5.05	4.80
NH_3, 1 mval	10.6	7.6	7.5
NH_4Cl, 1 mval	5.43	5.08	4.80
End-point of methyl red	5.45	5.08	5.20

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Received December 21, 1949.

A Method for Quantitative Determination of Tetraethylthiuram Disulphide (Antabuse, Abstinyl) and Its Reduced Form, Diethyldithiocarbamic Acid, as Found in Excreta

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In recent papers by danish authors the action of antabuse (*t*etraethyl*t*hiuram *d*isulphide, here called T.T.D.) has been studied and a method for its determination was developed [1]. T.T.D. was determined as the amount of sulphur present in ether extracts from the sample to be tested, using the common gravimetric method of determining sulphate in solution. This method has some disadvantages, however, as it is rather laborious and not specific as other ether-soluble sulphur compounds may interfere.

A new rapid and more specific method was developed, based on the intense colour of the cupric diethyldithiocarbamate, which makes it possible to trace even small amounts of T.T.D. and also offers a possibility to determine its reduced form, which can be expected to occur in the excreta.

1. *Determination of tetraethylthiuram disulphide.* A solution of T.T.D. in an organic solvent, *e. g.* benzene, carbon tetrachloride or chloroform, reacts with *cuprous* iodide, probably according to

$$CuI + (C_2H_5)_2N\cdot CS\cdot S\cdot S\cdot CS\cdot N(C_2H_5)_2 \rightarrow$$

$$(C_2H_5)_2N\cdot CS\cdot S\cdot Cu\cdot S\cdot CS\cdot N(C_2H_5)_2 + \tfrac{1}{2}I_2$$

The solution has an intense brown-yellow colour suited for photometry [2]. The reaction is fast and seems to proceed practically quantitatively. Cupric diethyldithiocarbamate isolated from the solution showed the correct m. p. (193—194°)[2]. Positive iodine reaction with starch solution can be obtained, but the iodine liberated does not interfere with the photometric determinations as described below.

Cuprous chloride and bromide also react at first with T.T.D. to give the brown-yellow cupric salt but subsequent reactions (not studied as yet) change the colour of the solutions.

Procedure. The sample (urine or a water suspension of faeces) is extracted with a known volume of carbon tetrachloride and centrifuged, if necessary, to get a clear carbon tetrachloride layer. The carbon tetrachloride is withdrawn, shaken with 0.5 g of pulverized cuprous iodide for 5—10 minutes and filtered. The extinction can be determined in a Pulfrich photometer, filter S 66 (violet), 2 cm cuvettes. A reference solution is prepared from an antabuse solution of known concentration. When the extinction is plotted against the concentration a straight line through the origine is obtained.

2. *Determination of diethyldithiocarbamic acid.* The reduced form of T.T.D. is the diethyldithiocarbamic acid, which at the pH values in question is present in ionized form. In this form it is water-soluble but insoluble in organic solvents. Thus it is possible to separate the two forms: when extracting with carbon tetrachloride, the diethyldithiocarbamate ions remain in the water phase while the disulphide is transferred to the carbon tetrachloride phase. The sodium diethyldithiocarbamate is used as reagent in the common method for micro determination of cupric ions [3] and we have made use of a reversal of this method.

Procedure: Cupric sulphate solution, sodium citrate solution (buffer) and a suitable volume of carbon tetrachloride are added to a defined amount of the sample (urine or a water suspension of faeces) or the water phase left from the

extraction procedure under 1. The mixture is shaken thoroughly until all the complex cupric salt is dissolved in the carbon tetrachloride and centrifuged. The carbon tetrachloride solution is then filtered and the extinction is determined as under 1.

3. *Application of the method.* The method was used to determine T.T.D. qualitatively and quantitatively in experiments on rabbit and man.

Rabbits were kept in metabolism cages and urine was sampled as a rule during 24 hours periods. T.T.D. in varying doses was given as a suspension by stomach tube. In one rabbit 0.3 g T.T.D. was given on three successive days. 27 per cent of the T.T.D. given was recovered in the urine in the reduced form while only traces of unaltered T.T.D. were found. In another rabbit, given 0.05 g T.T.D. in a single dose, 12 per cent of the dose given was recovered in the reduced form. 48 hours after the dosage practically no T.T.D. could be recovered in the urine. A third rabbit was given 0.1 g T.T.D. on three successive days and killed 6 hours after the last dosage. 3 per cent of the T.T.D. given was recovered in the urine in the reduced form. The unaltered form of T.T.D. was found in the contents of the digestive tract. No T.T.D. could be traced in the blood serum or in the liver.

Two healthy men were given T.T.D. per os during three days (1 + 0.5 + 0.5 g). On the third day urine and faeces were sampled. No T.T.D. could be traced in the urine. In the faeces 117 respectively 56 mg of unaltered T.T.D. were recovered and only minute amounts of the reduced form (0.5 and 0.2 mg respectively).

A closer study of the accuracy of the methods, their applicability under different conditions *etc.* is in progress.

The authors are indebted to Aktiebolaget Pharmacia for financial support.

A Simple "X-Ray Colorimeter"

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When performing a quantitative microchemical analysis often the problem of determining small amounts of an element with a high atomic number in the presence of elements with low atomic numbers arises. A typical case is that of a protein reacting with a heavy metal such as silver. By determining the amount of silver bound to a protein the number of the silverbinding groups in the protein can be calculated. In the example mentioned the quantitative estimation by ordinary methods of analysis of the small amounts of silver bound to the organic substance offers difficulties.

This paper describes a method permitting the rapid determination of a high atomic element bound to an organic compound. As the absorption of X-rays increases with the fourth power of the atomic number, an organic compound to which a small amont of a high atomic element is bound gives greater absorption of X-rays than the organic compound alone. This is the same principle as utilized in the determination of tetraethyl lead in gasoline[1].

The principle of the method can be seen from Figure 1. Primary X-rays are generated in the X-ray tube A. Philips' commercial diffraction unit was used as a source of X-rays. The voltage of the X-ray tube and the filtering of the X-rays may be

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adjusted for different samples. The X-rays are filtered in B, collimated into two beams in C and pass through the two cuvettes 1 and 2 at D. The X-rays transmitted by the cuvettes strike the light proof fluorescent screen, F, which is attached to the photomultiplier G. By the shutter, E, (or a rotating sector) the radiation transmitted by the cuvette 1 or 2 can be cut off alternately. The photomultiplier used is a R.C. A 931 tube with a Patterson fluorescent screen. The high voltage for the multiplier tube is taken from the A.C.-line by means of the common stabilized D.C. power supply. The voltage on each dynode is 90 volts. The photocurrent from the anode is measured with a spot light galvanometer.

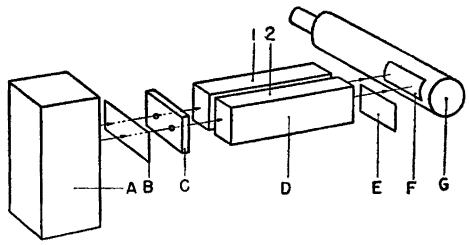

Fig. 1. Schematic drawing of the experimental apparatus.

A. Head of the X-ray diffraction unit.
B. Filter.
C. Pinhole diaphragm.
D. Cuvettes 1 and 2.
E. Shutter or rotating sector.
F. Fluorescent screen.
G. Photomultiplier tube.

When adjusting the apparatus the two cuvettes, 1 and 2, are filled with the same solution, the untreated organic compound in its solution, which is used as a blank. The X-ray beams and the photomultiplier are then adjusted so that the X-rays transmitted by 1 give the same photocurrent as those transmitted by 2. This is checked by alternately cutting off the beams with the shutter E. To the contents of the cuvette 1 different amounts of the high atomic element are added. The absorption of X-rays is greater in cuvette 1 and the difference between the intensities of the radiation transmitted by 1 and 2 is recorded by reading the galvanometer deflections. As an example, in Fig. 2, two curves for $AgNO_3$ and $CuSO_4$ as measured against water are reproduced. In that manner the apparatus is calibrated for the element or substance being determined.

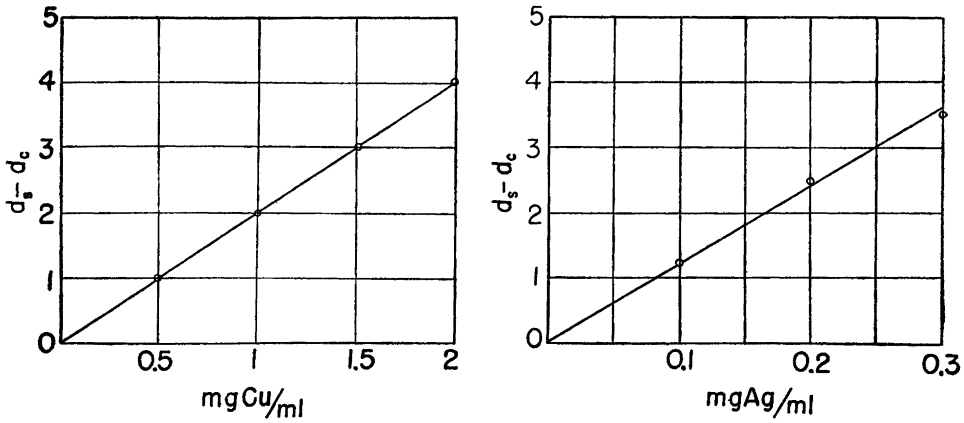

Fig. 2. The difference in galvanometer deflection between the sample, d_s, and the blank, d_c, for solutions of silver nitrate and copper sulfate. The sensitivity of the galvanometer is not the same in the two cases.

After the calibration the contents of the cuvette 1 are replaced by the organic compound which has combined with the high atomic element. The difference in absorption is recorded and the amount of the substance or element to be analyzed can be taken from the calibration curve. When the organic compound is an aqueous solution of low protein content the blank may be water without affecting the analytical results.

To simplify the technique a modification was introduced. The shutter E was designed as a rotating sector which alternately cut off the beams transmitted by 1 and 2. The sector was driven by a synchronous motor allowing for equal time period of transmission of the rays from 1 and 2. The photocurrents from 1 and 2 were amplified and connected with an oscillograph. The apparatus was adjusted with the same solutions, *e. g.* water, in the two cuvettes so that the oscillograph beam was balanced. When the contents of 1 were replaced by a substance with slightly higher absorption capacity the balance previously observed on the oscillographic screen was disturbed. With a microburette or pipette the solution in 2 was titrated with the same element as in 1 until balance was observed again. The amount of high atomic substance added to 2 was equal to the amount of the same substance present in 1. The end point of the titration is easily seen and if too much high atomic element is added in 2 the balanced oscillographic beam is dusturbed but in the opposite direction to what would be observed before starting the titration. Balance can then be obtained again by "back titration" in cuvette 1. To get the correct amount of substance to be added the amount added to 1 is substracted from that added to 2.

The method described has been used to determine the amount of silver that under certain experimental conditions is bound to a protein. It is obvious that the method can be applied only in such cases where there are no high atomic substances present in the sample except the one being analyzed. Where the method can be used it is a rapid tool for the quantitative determination of small amounts of an element or substance. An optically inhomogeneous solution can be analyzed as well as a homogeneous one. The chemical combination of the high atomic element is unimportant for the determination, as the absorption of X-rays takes part in the electron shells close to the atomic nucleus. The method is also applicable to solids, gases, powders etc. when the sample containers are properly designed.

1. Doxey, G. A. *Electronics.* (1949) p. 87. *The instrument manual 1949.* London (1949) p. 95.

A Mercurimetric Modification of Zacherl and Krainick's Micro Halogen Determination Method

KARIN PÄÄBO AND MAX ROTTENBERG

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In the course of synthetic work involving halogenated paraffin compounds the necessity arose of having at hand a rapid, simple and accurate method for the routine micro-determination of halogen in such compounds. A number of well established and generally adopted micro methods for the determination of chlorine and bromine are described in the standard literature [1, 2].

For our purposes titrimetric methods appeared to be most suitable because quick information was one of the features aimed at. Among the titrimetric methods the one first described by Zacherl and Krainick [3] was chosen because firstly, it apparently requires no particular experi-

After the calibration the contents of the cuvette 1 are replaced by the organic compound which has combined with the high atomic element. The difference in absorption is recorded and the amount of the substance or element to be analyzed can be taken from the calibration curve. When the organic compound is an aqueous solution of low protein content the blank may be water without affecting the analytical results.

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ence, and secondly, it avoids such expensive and elaborate apparatus as are in general only used by specialized microanalytical laboratories. In several runs using tetrabromostearic acid as a test substance, however, that method gave unsatisfactory results in our hands. In considering possible sources of error, it was thought that an acidimetric-alkalimetric system in such high dilutions might in itself be too susceptible for use in the ordinary organic laboratory, particularly because even traces of acidic or basic combustion products from substances containing sulphur or nitrogen might cause very serious errors. We therefore replaced the indirect alkalimetric by a direct mercurimetric procedure which allows to use one titrimetric solution only instead of two, and which avoids any steaming of flasks and boiling of the solutions to be titrated.

It will be seen in the experimental part that with halogenated hydrocarbons and fatty acids, as well as with nitrogenous substances such as arginine hydrochloride, the proposed method gave correct values within the usual limits of error. With the sulphur containing substance, S-benzylthiuronium chloride, however, the results were quite unsatisfactory under the conditions tried as yet.

Alicino *et al.*[4] very recently published an excellent iodometric procedure for the microdetermination of bromine; but since by mercurimetry both chlorine and bromine can be determined with the same equipment the present method might in many instances still represent a welcome supplement with the limitations mentioned.

Experimental. The mercurimetric titrations were carried out essentially according to Kolthoff[5], using diphenylcarbazide as an indicator. The mercuric nitrate solution was 0.01 *N* and was standardized against NaCl (C. P., dried and ignited). Samples corresponding to 2—5 ml of 0.01 *N* solution were taken for each analysis. After combustion and draining, 1 ml of 7 *N* HNO_3 and 4 drops of indicator (1 % alcoholic solution) were added to the halide solution (usually about 20 ml) which was then titrated with mercuric nitrate to a bright violet colour. Sharp end-points were always obtained in this way. The method was first tried with analytically pure tetrabromostearic acid as test substance. Some typical results follow.

Substance	Sample mg	ml $Hg(NO_3)_2$ 0.01 *N* $f = 1.009$	% hal. found	% hal. calc.
Tetrabromostearic acid	6.53	4.31	53.20	53.28 Br
	5.59	3.69	53.20	
	6.39	4.21	53.07	
	6.47	4.27	53.19	
	6.10	4.03	53.24	
Tetrabromostearic acid methyl ester [1]	5.33	3.44	52.02	52.05 Br
	5.26	3.41	52.32	
	4.93	3.18	52.17	
Pentabromoheptadecane [2]	7.18	5.60	62.87	62.93 Br
	5.85	4.22	62.63	
Arginine monohydrochloride [3]	7.22	3.38	16.76	16.83 Cl
	4.35	2.03	16.69	
	6.99	3.23	16.52	
	6.60	3.11	16.89	

[1] Gravimetrically found 52.24 % Br (by Mr. Grossmann, Copenhagen).

[2] Gravimetric analysis gave 62.94 % (Grossmann).

[3] Merck preparation, analyzed.

When this work was completed, a paper by Kirsten[6] on a similar titration procedure came to our notice. This author recommends the use of an alcoholic solution of mercuric nitrate which, however, is unstable and must be freshly prepared every day. Our aqueous 0.01 *N* solutions, in contrast can be used directly and are fairly stable (in the course of one month the factor dropped from 1.015 to 0.99). It is only necessary to check the titer against standard NaCl.

The Dipole Moment of Pyocyanine

KAI ARNE JENSEN and CARL HENRIK HOLTEN

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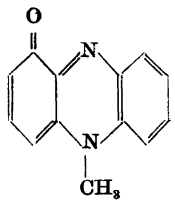
Pyocyanine, the blue colouring matter of the bacillus *Serratia marcescens (Bacillus pyocyaneus)* was shown by Wrede and Strack [1] to be an N-methyl derivative of 1-hydroxyphenazine and could be synthesized by treatment of 1-hydroxyphenazine with dimethyl sulphate. A bimolecular formula was originally assumed for pyocyanine, but the work of Michaelis [2], Elema [3] and Kuhn [4] established the monomolecular formula. Finally Hillemann [5] has shown that it is the nitrogen atom furthest away from the hydroxyl group which is methylated, so that a conventional formula for pyocyanine may be satisfactorily written as I.



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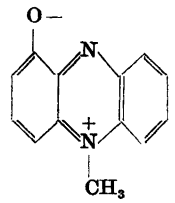

II

The intense colour of pyocyanine, however, suggests that resonance structures of the type II may be of great importance for this compound, as are similar structures for the triphenylmethane and cyanine dyestuffs. Compounds of these types have been shown to possess very large dipole moments [6]. We have measured the dipole moment of pyocyanine, and found it also to be abnormally large.

Pyocyanine was prepared according to Surrey [7]. It was purified by dissolution in chloroform and precipitation with petroleum ether, when it had a melting point of 131–32°.

Pyocyanine is very slightly soluble in the solvents usually employed for dipole moment measurements. We only succeeded in making measurements in dioxane solutions, and even in this solvent the solubility was so low, that only an approximate value of the dipole moment could be determined.

The dielectric constants of dioxane and the solutions were measured, and the molecular polarization was measured, using the formula:

$$P_M = \frac{1000}{c}\left[\frac{\varepsilon_{12}-1}{\varepsilon_{12}+2} - \frac{\varepsilon_1 - 1}{\varepsilon_1 + 2}\right]$$

where c is the molar concentration of the solute and ε_1 and ε_{12} are the dielectric constants of the solvent and the solution (*cf.* Jensen and Nygaard [8]). The dipole moment was calculated from the formula:

$$\mu = 0.01273\ \sqrt{P_M \cdot T}$$

The following values were obtained:

This work is part of an investigation supported by *Statens Naturvetenskapliga Forskningsråd.*

1. Pregl-Roth, *Quantitative organische Mikroanalyse* 5th ed., Vienna (1947).
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3. Zacherl, M. K., and Krainick, H. G. *Mikrochemie* **11** (1932) 61.
4. Alicino, J. F., Crickenberger, A., and Reynolds, B. *Anal. Chem.* **21** (1949) 755.
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6. Kirsten, W. *Mikrochemie* **34** (1949) 149.

Received December 8, 1949.

c	$\Delta\varepsilon$	$\varepsilon_{\text{Dioxane}}^{25}$	P_M	μ
0.00415	0.0247 ± 0.0005	2.2103	1000 ± 20	7.0 D
0.00208	0.0130 ± 0.0005		1058 ± 40	
0.00104	0.0065 ± 0.0005		1058 ± 80	

The accuracy with which P_M can be determined in these very dilute solutions is too small to allow any reliable extrapolation to zero concentration. The solutions measured are, however, so dilute that P_M has probably already reached its maximum value. The true dipole moment can therefore not be much larger than 7.0 D, the value calculated from P_M of the most concentrated solution.

As expected the dipole moment of pyocyanine is rather large, showing that a highly dipolar structure plays an important rôle in determining the actual state of the molecule. Although there are other possible dipolar resonance structures, the only one of importance is probably that pictured as II. On the basis of the known bond lenghts and valency angles, a dipole moment of approximately 24 D can be calculated for this structure. Since the dipole moment of the single bond C—N is only small, the dipole moment of the ortho quinoid structure I should be almost exclusively determined by the moments of the bonds >C = O and >C = N—. The first is about 2.5 D and the second is 1.6 D[9], so that the dipole moment of structure I may be taken as $\sqrt{2.6^2 + 1.6^2 + 2.6 \times 1.6} = 3.6$ D. If is assumed that the resulting dipole moment can be calculated from the formula [10]:

$$\mu = (1-x_{II})\mu_I + x_{II}\mu_{II}$$

where μ is the dipole moment found and μ_I and μ_{II} are the moments calculated for structure I and II, the fractional contribution of the polar structure should be $x_{II} = 0.17$.

1. Wrede, F., and Strack, E. *Z. physiol. Chem.* **140** (1924) 1; **142** (1925) 103; **177** (1928) 177; **181** (1929) 58; *Ber.* **62** (1929) 2051.

The Dipole Moment of Sempervirine

KAI ARNE JENSEN

Chemical Laboratory, University of Copenhagen, Copenhagen, Denmark

According to Prelog [1] sempervirine, the brown alkaloid of *Gelsemium sempervirens*, has the structure I. Woodward and Witkop[2], however, have presented evidence in favour of describing the molecule in terms of a resonance hybrid of structures II and III. This proposal accounts very well for the chemical and physical properties of sempervirine, but it remains to be shown that sempervirine has the high dipole moment required, if structure III makes a significant contribution to the state of the molecule.

2. Friedheim, E., and Michaelis, L. *J. Biol. Chem.* **91** (1931) 355.
Michaelis, L. *Ibid.* **92** (1931) 211.
Michaelis, L., Hill, E. S., and Schubert, M. P. *Biochem. Z.* **255** (1932) 66.
3. Elema, B. *Rec. trav. chim.* **50** (1931) 796, 806.
4. Kuhn, R., Schön, K., and Valko, E. *Ber.* **68** (1935) 1537.
5. Hillemann, H. *Ber.* **71** (1938) 46.
6. Maryott, A. A., and Acree, S. F. *J. Research Natl. Bur. Standards* **38** (1947) 505.
Brooker, L. G. S., Sprague, R. H., Smyth, C. P., and Lewis, G. L. *J. Am. Chem. Soc.* **62** (1940) 1116.
Brooker, L. G. S., White, F. L., Keyes, G. H., Smyth, C. P., and Oesper, P. F. *Ibid.* **63** (1941) 3192.
Brooker, L. G. S., and Sprague, R. H. *Ibid.* **63** (1941) 3203, 3214.
7. Surrey, A. R. *Org. Synth.* **26** (1946) 86.
8. Jensen, K. A., and Nygaard, B. *Acta Chem. Scand.* **3** (1949) 479.
9. Everard, K. B., and Sutton, L. E. *J. Chem. Soc.* (1949) 2318.
10. Smyth, C. P. *J. Am. Chem. Soc.* **63** (1941) 57.
Kushner, L. M., and Smyth, C. P. *Ibid.* **71** (1949) 1403.

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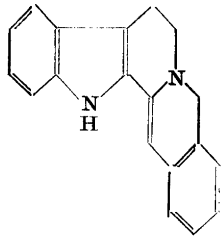

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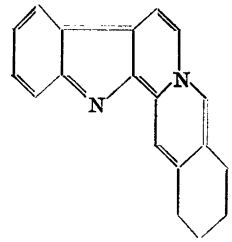

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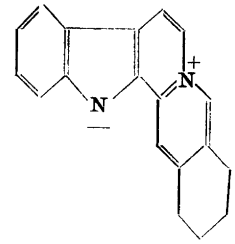

III

Sempervirine is very slightly soluble in most solvents. The dielectric constant of almost saturated solutions in dioxane and benzene (which have a very intense golden colour), however, differ measurable from those of the pure solvents. On the basis of these measurements the dipole moment of sempervirine has been estimated to be 7—8 D, a very high value, indicating that the structure III is of definite importance for the actual state of the molecule. The reaction of sempervirine with alkyl halides (Woodward and Witkop, *l. c.*) is analogous to the reaction of unambiguous dipolar ions with alkyl halides (*cf.* Jensen and Friediger [3]).

The dipole moment was determined as described in a previous publication [4].

Table 1. Dipole moment of sempervirine ($C_{19}H_{16}N_2 \cdot H_2O$) in dioxane at 22° C.

Molar conc.	ε	$\varepsilon_{\text{Dioxane}}$	P_M	μ
0.001711 (4.962 mg/10 ml)	2.2250	2.2095	1532	8.5 D
0.000851 (2.468 mg/10 ml)	2.2170	2.2095	1504	

Table 2. Dipole moment of sempervirine in benzene at 22° C.

Molar conc.	ε	$\varepsilon_{\text{Benzene}}$	P_M	μ
0.00076 (2.200 mg/10 ml)	2.2840	2.2785	1186	7.5 D

I wish to thank professor Prelog, Zürich, for a gracious gift of sempervirine.

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