10°, micro b. p. 26–27°, $n^{20}D$ 1.3569. [c] Traces of yellow lower layer formed. [d] 0.6 cc. hydrocarbon residue boiling above 10°, micro b. p. 29°, $n^{20}D$ 1.3610. [e] $n^{20}D$ 1.3575. [f] Traces of permanent gases formed. [g] 20.6% of total *n*-butane charge isomerized to *i*-butane. [h] *i*-Butane charge. [i] The heart cut of this fraction was 30×10^{-4} mole, $n^{20}D$ 1.3548. [j] The complete column analysis was 9×10^{-4} mole low boiling material, 410×10^{-4} mole i-C_4H_{10}, 62×10^{-4} mole n-C_4H_{10}, 34×10^{-4} mole *i*-pentane, 24×10^{-4} mole boiling higher than *i*-pentane. [k] Propane. [l] Aluminum chloride. [m] Methyl chloride. [n] No *i*-butane; no alkylate. After removal of volatile products, 0.920 g. of a white to light amber solid remained, m. p. 24°, stable to pumping at 10^{-5} mm. [o] Ethyl bromide. [p] Light yellow solution. *Ca.* 0.3 cc. light orange lower layer developed at end of run. [q] Total distillate boiling above *n*-butane, $n^{20}D$ 1.3800. Traces of low boiling material. [r] Light yellow solution. [s] Traces of low boiling material; butane fraction 47.7% isomerized. [t] *Ca.* 0.5 cc. deep red lower layer formed. [u] The complete analysis was as follows: traces of CH_4, 140×10^{-4} mole C_2H_6, 80×10^{-4} mole C_3H_8, 82×10^{-4} mole n-C_4H_{10}, 185×10^{-4} mole i-C_4H_{10}, 130×10^{-4} mole i-C_5H_{12}, 95×10^{-4} mole C_6H_{14} and higher, $n^{20}D$ 1.3779.

Summary

In the presence of aluminum bromide, methyl and ethyl bromide will alkylate butanes to give substantial yields of pentanes and hexanes, respectively, as well as some higher paraffins. The occurrence of the alkylation reaction substantiates a prediction based on the mechanism of paraffin isomerization previously presented.

BERKELEY 4, CALIFORNIA RECEIVED JULY 13, 1944

NOTES

Trimethylchlorosilane

BY W. F. GILLIAM AND ROBERT O. SAUER

Taylor and Walden[1] have recently reported the successful preparation of trimethylchlorosilane by direct chlorination of trimethylsilane. We obtained this chlorosilane in January, 1941, by the reaction of methylmagnesium chloride with a mixture of methylchlorosilanes[2] in ether solution.

Experimental

429.5 g. of a methylchlorosilane mixture[3] (b. p. 68.0–70.1°; 57.8% Cl; 2.75 moles dimethyldichlorosilane and 0.50 mole of methyltrichlorosilane) was dissolved in one liter of anhydrous ether and added to a 5-liter, three-neck flask fitted with a stirrer, an addition funnel, and a condenser cooled by a bath of acetone and solid carbon dioxide. To this solution was slowly added 500 cc. of a 4.1 *M* solution of methylmagnesium chloride in ether; the magnesium chloride precipitated as a fine sludge. The ether solution was separated and the ether removed by distillation; fractional distillation of the residue in a column of 40 theoretical plates gave five fractions totaling 38.7 g. (0.35 mole) of trimethylchlorosilane. The higher boiling constituents contained 159.2 g. (1.23 moles) of dimethyldichlorosilane, and 30.8 g. of an intermediate fraction. These materials were analyzed by the hydrolysis of approximately 1-g. samples and the titration of the liberated acid with *N*/2 sodium hydroxide. A sample of trimethylchlorosilane collected at 57.0° (748 mm.) gave 32.5, 32.6% Cl (calcd., 32.64% Cl). The dimethyldichlorosilane distilled at 70.0° (757 mm.) and gave 54.8, 54.8% Cl (calcd., 54.95% Cl).

Another sample of trimethylchlorosilane[3] upon careful fractional distillation in a column of 50 theoretical plates gave three consecutive fractions having the following properties: (a) b. p. 57.6–57.7° (760 mm.); d^{27}_{27} 0.8538; % Cl, 32.58, 32.59, 32.59; (b) b. p. 57.7° (760 mm.)-57.3° (749 mm.); d^{27}_{27} 0.8536; % Cl, 32.57, 32.56, 32.57; (c) b. p. 57.7° (760 mm.); d^{27}_{27} 0.8536, 0.8538; % Cl, 32.55, 32.57.

The vapor density of trimethylchlorosilane indicates this compound to be slightly associated at 100°. The result obtained by the Dumas method was 5.091 g./l. (S. T. P.) corresponding to a molecular weight of 114 (calcd., 108.6).

The molecular weight of this compound was also determined cryoscopically in cyclohexane (determined freezing point constant, 207). The following results show trimethylchlorosilane to exist as the dimer in this solvent at 6° (maximum concentration of solute, 0.3%): mol. wt., 223, 205, 216, 212 (calcd. for the dimer, 217.2).

RESEARCH LABORATORY
GENERAL ELECTRIC COMPANY
SCHENECTADY, NEW YORK RECEIVED AUGUST 4, 1944

(1) Taylor and Walden, THIS JOURNAL, **66**, 842 (1944).

(2) Gilliam, Liebhafsky and Winslow, *ibid.*, **63**, 801 (1941).

(3) Mr. W. J. Scheiber of this Laboratory kindly provided and distilled these materials.

The Solubility of Potassium Iodide in Sodium Hydroxide Solutions at 20°

BY H. DARWIN KIRSCHMAN[1] AND RICHARD POMEROY[1]

In former articles[2] we have presented the results of studies on the solubility of potassium iodide in potassium hydroxide solutions and of sodium iodide in sodium hydroxide solutions at 20°. The present paper extends these studies to the iodide of potassium in solutions of sodium hydroxide from 0 to 16.5 *N*.

The results of our measurements are presented in Table I and Fig. 1.

Experimental

The solutions were equilibrated and analyzed as previously described except that the concentration of iodide was determined by titration with standard silver nitrate solution using eosine as an adsorption indicator. Equilibrium was more rapidly established than in the case of sodium iodide in sodium hydroxide solutions but less

(1) 117 East Colorado St., Pasadena 1, Calif.

(2) (a) Kirschman and Pomeroy, THIS JOURNAL, **65**, 1695 (1943); (b) Pomeroy and Kirschman, *ibid.*, **66**, 178 (1944).

TABLE I

SOLUBILITY AND DENSITY DATA AT 20°

NaOH, moles/liter	KI, moles/liter	Density, g./ml.
0.00	6.09	1.716
0.95	5.48	1.674
1.34	5.26	1.661
2.57	4.58	1.624
3.60	4.06	1.597
4.77	3.53	1.573
5.04	3.44	1.568
5.20	3.35	1.564
6.78	2.75	1.543
7.77	2.42	1.532
8.68	2.18	1.531
10.06	1.81	1.526
10.80	1.67	1.530
13.45	1.29	1.540
16.22	1.03	1.569
16.50	1.03	1.583

rapidly than with potassium iodide in potassium hydroxide solutions. The solid phase in equilibrium with solution 16.22 *N* in alkali was washed free from solution and dried. Analysis of the crystals gave 76.07% iodine (calcd. for KI 76.45%). The crystals were optically isotropic.

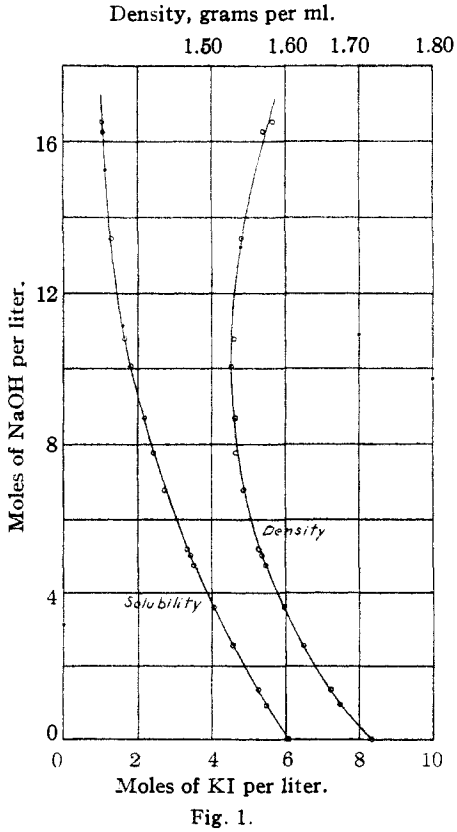

Fig. 1.

These observations, and the apparent continuity of the curves, indicate that potassium iodide was the only solid iodine compound in equilibrium with the solutions. The accuracy of the results is estimated to be 0.5%.

CONTRIBUTION FROM THE
CHEMISTRY LABORATORY
UNIVERSITY OF CALIFORNIA
AT LOS ANGELES
LOS ANGELES, CALIF.

RECEIVED APRIL 26, 1944

Isolation of Quercitrin and Quercetin from Goldenrod Material

BY JOHN D. GUTHRIE, ROBERT T. O'CONNOR, MACK F. STANSBURY AND THEODORE R. SAVICH

Quercitrin (3,3′,4′,5,7-pentahydroxyflavone-3-rhamnoside) and its aglucone, quercetin, were isolated from the acetone extractives of goldenrod, *Solidago leavenworthii*, T. and G. Since 15 to 20% of the dry goldenrod material, chiefly leaves, was soluble in acetone, a large quantity of material was available as a by-product from the acetone–benzene extraction process for obtaining rubber from goldenrod.

Experimental

Quercitrin.—Goldenrod leaf material that had been dried in a tray drier at 65° was ground to pass a 40-mesh screen, extracted with acetone in a Soxhlet-type metal extractor for forty-eight hours and the acetone removed from the extract by distillation. Quercitrin was isolated by extraction of the green, gummy material with 0.25 *N* sodium hydroxide, acidification of the extract with acetic acid, centrifugation, neutralization of the supernatant liquid with sodium hydroxide solution and precipitation of the quercitrin with lead acetate. After decomposition of the lead precipitate with hydrogen sulfide, some of the quercitrin was found in the filtrate and some adsorbed on the lead sulfide precipitate, from which it could be eluted with acetone. The quercitrin was purified by crystallization from dilute acetone. In a typical experiment 1.7 g. was obtained from 25 g. of the acetone-extractives. A solution in 95% ethanol had absorption maxima at 2600 and 3500 Å.

Anal. Calcd. for quercitrin, dry, $C_{21}H_{20}O_{11}$: C, 56.3; H, 4.7. Found after drying *in vacuo* at 170°, C, 56.2; H, 4.5.

The aglucone was obtained by hydrolysis of the quercitrin. Its absorption maxima were at 2560 and 3720 Å. in 95% ethanol.

Anal. Calcd. for quercetin, $C_{15}H_{10}O_7$: C, 59.6; H, 3.3. Found: C, 59.1; H, 3.4.

The hydrolyzate gave the Rosenthaler test for rhamnose and yielded the osazone of rhamnose. After recrystallization from dilute pyridine and dilute methanol, it melted at 184–185° (cor.); mixed melting point 184–185° (cor.). Nitrogen: found, 16.33; calcd., 16.36.

Quercetin.—Goldenrod leaf material that had been dried at 65° was extracted in a large jacketed extractor by percolation with acetone at 50° for twenty-four to thirty-six hours. After recovery of the acetone by distillation, the extractives were melted by heating to about 140° and poured into cans. Quercetin was isolated from 47 kg. of the green, gummy acetone-extractives by extraction with boiling water, hydrolysis of the quercitrin in the hot water extract by adding sulfuric acid in the amount of 1% and heating. The crude product which separated weighed 4.3 kg. and contained about 58% quercetin. It was purified by solution in alcohol, precipitation with water, recrystallization from 80% alcohol, and fractional precipitation from acetone with petroleum ether. The yield of purified quercetin was 780 g.

Anal. Calcd. for $C_{15}H_{10}O_7$: C, 59.6; H, 3.3. Found: after drying at 170° *in vacuo*, C, 59.2; H, 3.8.

The pentaacetate was prepared and melted at 200° (cor.). The mixed melting point with a known sample of the pentaacetate of quercetin was 200° (cor.). Sando gives 194 to 196° for the melting point of the pentaacetate of quercetin.[1] The absorption curves of the two samples of the pentaacetate in 95% ethanol were practically identical. The absorption maxima were at 2530 and 2990 A. with extinction coefficients (E, g. per liter, 1 cm.) of 40.0 and 34.5, respectively. Quercetin was regenerated from the pentaacetate preparations. The absorption curves of the regenerated quercetin preparations in 95% ethanol were practically identical. The maxima were at 2570 and 3750 Å. with extinction coefficients of 67.0 and 75.5, respectively, calculated on the dry basis. Grinbaumówna and Marchlewski give 2555 and 3755 Å. for the absorption maxima of quercetin.[2]

The micro-analyses were made by G. Warren Buckaloo and Lawrence E. Brown.

(1) C. E. Sando, *J. Biol. Chem.*, **117**, 45 (1937).

(2) R. Grinbaumówna and L. Marchlewski, *Biochem. Z.*, **290**, 261 (1937).

BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY
AGRICULTURAL RESEARCH ADMINISTRATION
U. S. DEPARTMENT OF AGRICULTURE
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2100 ROBERT E. LEE BOULEVARD
NEW ORLEANS, LOUISIANA RECEIVED JULY 22, 1944

Observations on the Rare Earths. LII. The Preparation of Rare Earth Bromates from the Perchlorates

BY HOWARD E. KREMERS[1] AND THERALD MOELLER

In the preparation of rare earth bromates by metathetical reaction between rare earth sulfates and barium bromate,[2] significant quantities of rare earth materials are occluded by the precipitated barium sulfate. Furthermore, the method is complicated by the limited solubility of barium bromate.[2] Reactions between rare earth perchlorates and the more soluble potassium bromate overcome these objections, the precipitated potassium perchlorate showing less tendency to occlude rare earth salts than barium sulfate because of its somewhat greater solubility and consequent slower rate of precipitation. Bromates are also more readily prepared in this fashion than by treatment of rare earth oxides or hydroxides with bromic acid and are suited to fractional crystallization.

Experimental

Nearly neutral rare earth perchlorate solutions, prepared from yttrium group oxides by action with perchloric acid and containing the equivalent of 15 to 20% rare earth oxide, were treated with powdered potassium bromate and the resulting mixtures boiled for one hour. After being cooled to 15°, the suspensions were filtered and the residues washed with saturated potassium perchlorate solution until the washings were rare earth-free. These precipitates generally contained about 0.1% rare earth calculated as oxide, and never more than 0.5%.

Each filtrate was systematically fractionally crystallized to six fractions after fifteen crystallizations. Analyses of these fractions by standard methods showed the most insoluble fractions to consist of potassium bromate with traces of potassium perchlorate, the middle fractions to consist of rare earth bromates with traces of potassium bromate, and the most soluble fractions to consist of rare earth bromates with traces of rare earth perchlorates.

During the course of the fractionation, small amounts of potassium perchlorate and basic rare earth bromates precipitated. The latter never amounted to more than 1% of the total fraction, and such precipitations did not prove objectionable.

The preparation of rare earth bromates from perchlorates is more convenient and rapid than the preparation involving barium bromate, but the removal of by-products is not as complete. Since potassium bromate and perchlorate rapidly concentrate in the most insoluble fractions while remaining traces of perchlorate are carried through to the most soluble fractions, fractional crystallization is not impaired. Avoidance of loss of rare earth material in the initial precipitation constitutes the chief recommendation for the method.

(1) Present address, Lindsay Light and Chemical Company, West Chicago, Illinois.

(2) James, THIS JOURNAL, **30**, 182 (1908).

NOYES CHEMICAL LABORATORY
UNIVERSITY OF ILLINOIS
URBANA, ILLINOIS RECEIVED JULY 10, 1944

Sterols from Peruvian Guano

BY JOHN KRUEGER

The sterol present in comparatively large amounts in Peruvian guano and provisionally called "guanosterol" or "guanosterine"[1] is really cholesterol as shown by the m. p. and m. m. p. of both the sterol and its acetate. Marker[2] has shown that the sterol present in largest amount in chicken feces is sitosterol. The cholesterol present in guano reflects the diet of the marine birds which produce the deposits.

Procedure.—Three pounds of Peruvian guano was stirred with 4 liters of ethanol at 40–50° for several hours, and the mixture then allowed to stand overnight. The residue was filtered and the filtrate was evaporated. The residue from the evaporation was refluxed with excess alcoholic sodium hydroxide, diluted with water, then extracted into ether. The pale tan sterol obtained by evaporation of the washed ether solution was recrystallized from 30 cc. of ethanol to yield 4.0 g. of cholesterol of m. p. 142° which showed no depression in m. m. p. when mixed with cholesterol. The acetate, prepared in the usual way, melted at 113° and showed no depression in m. m. p. when mixed with cholesteryl acetate.

(1) del Aguila, *Bol. soc. Quim. Peru*, **4**, 199–200 (1938) (*C. A.*, **33**, 2270 (1939)).

(2) Marker and Shabica, THIS JOURNAL, **62**, 2523 (1940).

RESEARCH DEPARTMENT
THE EDWAL LABORATORIES, INC.
CHICAGO 5, ILLINOIS RECEIVED JULY 10, 1944

Electrophoresis of Rat Sera[1]

BY CHOH HAO LI

In the last few years, many investigators[2] have studied the electrophoresis of animal and human sera, but apparently no studies with rat sera have

(1) Aided by grants from the University of California Research Board, the Josiah Macy, Jr., Foundation, New York City, and General Mills, Inc., Minneapolis, Minnesota.

(2) Referred to, for example, by H. Svenson, *J. Biol. Chem.*, **139**, 805 (1941).

Fig. 1.

Fig. 2.

Fig. 1.—Normal rat serum, diluted 1:1. Exposures taken on the descending boundary.

Fig. 2.—Hypophysectomized rat serum, diluted 1:1. Exposures taken on the descending boundary.

been reported using the optical techniques of Longsworth[3] or Svensson-Philpot.[4] Recently Levin[5] found a decrease of serum-albumin concentration in rats following hypophysectomy; his conclusions were based on the results obtained by chemical analysis. It appeared, therefore, of some interest to study the electrophoretic behavior of sera of normal and hypophysectomized rats.

Male rats forty days of age of the Long–Evans strain were used. The post-operative period following hypophysectomy was two weeks and the completeness of the operation was ascertained carefully at autopsy by examination of the sella turcica. The animals were maintained on the usual diet of this Laboratory *ad libitum*. Blood was taken directly from the heart and was usually obtained from 3 or more rats for one sample. No significant difference in protein concentration was observed in normal and hypophysectomized rat sera as found by Levin.[5]

Electrophoresis experiments were carried out in the apparatus of Tiselius[6] with the optical arrangement of Longsworth.[3] The sera were usually diluted with an equal volume of the buffer and dialyzed against 2 liters of the same buffer for at least three days. The buffer was prepared from sodium diethylbarbiturate with *p*H 8.5 and ionic strength 0.10. In most cases the period of electrolysis was one hundred twenty minutes with a potential gradient of about 6 volts per cm. Since under these circumstances the false boundary was not fully separated from the slowly moving real component the concentration–distribution measurements were obtained from the descending boundary only.

(3) L. G. Longsworth, THIS JOURNAL, **61**, 529 (1939).

(4) J. St. L. Philpot, *Nature*, **141**, 283 (1938); H. Svensson, *Kolloid-Z.*, **87**, 181 (1939).

(5) L. Levin and J. H. Leathem, *Am. J. Physiol.*, **136**, 306 (1942).

(6) A. Tiselius, *Trans. Faraday Soc.*, **33**, 524 (1937).

A typical electrophoresis pattern of normal rat serum is shown in Fig. 1. There are not less than five components. According to the conventional designation they may be called, in the order of decreasing mobilities, albumin, x, α-, β-, and γ-globulins. The x component[7] is also found in mice serum.[9] The sharp and high β-globulin peak has been found in all normal rat sera thus far examined. As in the rabbit serum, the albumin concentration is about 75% of the total protein which is in contrast with the sera obtained from horse, cow and swine.[2] This means that the albumin–globulin ratio is about 3.0.

In the sera of hypophysectomized rats, there are no indications of the x component. This appears to be a characteristic[10] of such sera in contrast with sera obtained from normal rats. As shown in Fig. 2, it is evident that the globulin concentration is higher than in the normal rat. The albumin–globulin ratio from five samples of hypophysectomized rat sera[11] averaged 1.33.

Table I summarizes the albumin and globulin concentration distribution data in the normal and hypophysectomized rat sera. Since the separation of the globulin components was imperfect, no attempts were made to calculate the percentage composition of each globulin. Due to the high concentration of protein, no accurate mobilities could be obtained; the mobility deter-

TABLE I

PER CENT. COMPOSITION OF ALBUMIN AND GLOBULIN IN NORMAL AND HYPOPHYSECTOMIZED RAT SERA

No. of rats	Body weight at bleeding	Relative concentration Albumin	Globulin	Albumin/ globulin
		Normal Rats		
5	140	72.0	28.0	2.58
4	138	70.8	29.2	2.42
3	141	70.4	29.6	2.38
5	138	79.2	20.8	3.80
				Mean 2.80
		Hypophysectomized Rats		
5	124	53.5	46.5	1.15
4	122	61.5	38.5	1.60
5	125	56.5	43.5	1.30
3	125	55.2	45.8	1.21
4	124	58.4	41.6	1.40
				Mean 1.33

(7) Some authors (ref. 8) have denoted the x component as α_1-globulin.

(8) See, for example, L. G. Longsworth, *Chem. Rev.*, **30**, 323 (1942).

(9) J. Bourdillon and E. H. Lennette, *J. Expt. Med.*, **72**, 11 (1940).

(10) In a private communication, D. H. Moore has not been able to find this difference between normal and hypophysectomized rat sera in Levin's samples. The discrepancy between their results and ours may depend on the dilution of sera used.

(11) L. Levin has kindly sent us a sample of hypophysectomized rat serum. The albumin–globulin ratio is found to be 1.5. Levin has obtained a ratio of 1.2 from the sample using the salt fractionation method. It may be added that we were not able to find the x component with this sample.

mination is therefore purposely omitted. However, the mobilities were used for identification of the components.

It will be noted that the albumin–globulin ratio is distinctly lower in the hypophysectomized rat serum in comparison with normal serum. The percentage lowering of this ratio is about 53.0. This is somewhat higher than that found by Levin,[5] who obtained a percentage lowering of 46.0.

Addendum.—While this note was in the hands of the Editors, an article by Moore, *et al.*,[12] appeared in which they found that the normal rat serum lacks the α-globulin. It may be noted in Fig. 1 that the appearance of this component is evident. It is possible that the sera they used are too dilute to escape the detection of a small concentration of the α-globulin component.

(12) D. H. Moore, L. Levin and J. H. Leathem, *J. Biol. Chem.*, **153**, 349 (1944).

INSTITUTE OF EXPERIMENTAL BIOLOGY
UNIVERSITY OF CALIFORNIA
BERKELEY, CALIF. RECEIVED MAY 22, 1944

The Determination of Water in Formic Acid

BY J. MITCHELL, JR., AND WALTER HAWKINS

In a previous publication from this Laboratory[1] the Karl Fischer reagent was not recommended for the determination of water in the presence of formic acid, presumably because of dehydration of the acid. Later studies on this system have indicated that under normal conditions this interference is not appreciable, amounting to only a fraction of a per cent. in high concentrations of formic acid.

Experimental

Aqueous solutions of the acid were prepared by adding various amounts of water to Eastman Kodak Company formic acid. Weighed samples were analyzed for water by direct titration with Karl Fischer reagent and for free acid by titration with standard alkali. Results are summarized in the following table.

THE DETERMINATION OF WATER IN FORMIC ACID

Water, wt. %		Acid, wt. %		Total, wt. %
89.85	± 0.05	10.22	± 0.01	100.07
70.30	0.10	29.76	0.02	100.06
26.1	0.1	74.0	0.2	100.1
14.80	0.00	85.20	0.00	100.00
1.55	0.05	98.80	0.02	100.35

(1) Smith, Bryant and Mitchell, THIS JOURNAL, **61**, 2407 (1939).

AMMONIA DEPARTMENT
E. I. DU PONT DE NEMOURS & CO., INC.
WILMINGTON, DELAWARE RECEIVED MAY 31, 1944

The van der Waals Constant "a" from C_p/C_v Measurements

BY R. E. RUNDLE

By an improved resonance method Clark and Katz[1] have succeeded in obtaining accurate measurements of γ, (C_p/C_v), as a function of pressure for a number of gases. They find experimentally that for simple gases the variation of γ with pressure is linear, and they show that this is the expected behavior of a gas whose equation of state is $PV = RT + BP$. It is also interesting to note that a linear dependence of γ on pressure is to be expected for a van der Waals gas at moderate pressures, and that from the slope of the curve, γ *vs.* P, the van der Waals constant a can be determined.

For a substance whose properties are a function of P and T only[2]

$$C_p - C_v = T\left(\frac{\partial V}{\partial T}\right)_P\left(\frac{\partial P}{\partial T}\right)_V \qquad (1)$$

For a mole of van der Waals gas

$$(P + a/V^2)(V - b) = RT \qquad (2)$$

$$\left(\frac{\partial P}{\partial T}\right)_V = \frac{R}{V - b} \qquad (3)$$

$$\left(\frac{\partial V}{\partial T}\right)_P = \frac{R}{P - a/V^2 + 2ab/V^3} \qquad (4)$$

Substituting (2), (3) and (4) in (1), and ignoring $2ab/V^3$ with respect to a/V^2

$$C_p - C_v = R\frac{P + a/V^2}{P - a/V^2} \qquad (5)$$

In the term a/V^2 it suffices to use the molal volume from the perfect gas equation. Then

$$C_p - C_v = R\frac{(RT)^2 + aP}{(RT)^2 - aP}, \text{ or} \qquad (6)$$

$$\gamma = \left(\frac{R}{C_v} + 1\right) + \frac{R}{C_v}\frac{2aP}{(RT)^2} + \ldots \qquad (7)$$

where the coefficients of higher powers of P are small, so that the extra terms may be ignored at moderate pressures. It is to be noted that C_v is independent of pressure for a van der Waals gas, so that $C_v = R/(\gamma_0 - 1)$, and the dependence of γ on pressure is linear.

If terms in higher powers of P are necessary, the term in b cannot be ignored. In this case

$$\gamma = 1 + R/C_v\left[1 + \frac{2aP}{(RT)^2} + \frac{2a}{(RT)^3}(a/RT - b)P^2 + \ldots\right] \qquad (8)$$

For certain gases, Clark and Katz find that γ at constant T must be expressed in terms of an equation of the form

$$\gamma = \gamma_0 + C_1P + C_2P^2 + \ldots$$

but the correlation with equation (8) is not good. Apparently the approximation of a real gas by the van der Waals equation is not sufficient to make the coefficient of P^2 in (8) significant. It appears, however, that the coefficient of P can be used to calculate a, just as in equation (7).

Equation (7) has been applied to data of Clark and Katz[1,3] to obtain a for a number of gases. In the table these values of a are compared with

(1) Clark and Katz, *Can. J. Research*, **18A**, 23 (1940); **21A**, 1 (1943).

(2) Lewis and Randall, "Thermodynamics," McGraw-Hill Book Co., New York, N. Y., 1923, p. 136.

(3) Clark and Katz, *Can. J. Research*, **19A**, 111 (1941).

those calculated from critical data.[4] The agreement is good for the simpler gases for which equation (7) is valid. For the gases CO_2, N_2O and SO_2, not well represented by the van der Waals equation, the values of a from the critical constants are not satisfactory at temperatures and pressures far from the critical conditions, and hence cannot be expected to agree well with the values of a calculated from other data. It is interesting that PVT data[5] for carbon dioxide at pressures and temperatures more nearly comparable with those of Clark and Katz yield values of a in the neighborhood of 4 to 6, while similar calculations from sulfur dioxide data[6] give values of a as high as 10–15. The values of a from (7) are probably as reliable as any for gases which show large deviations from van der Waals behavior, and like other values of a for such gases, they are useful only over limited ranges of pressure and temperature.

Values of γ at zero pressure can be obtained from molecular and spectral data, and equation (7) should provide a simple, approximate correction to moderate pressures. But it is evident from the table that if values of a are taken from critical data, equation (7) is accurate only for gases which are well represented by the van der Waals equation.

TABLE I

VALUES OF a IN ATM. L.^{2}/MOLES^2

Gas	a (from eq. 7)	a (critical data)
A	1.57	1.345
H_2	0.234	0.244
N_2	1.63	1.390
CO_2	5.02	3.59
N_2O	6.80	3.78
SO_2	19.2	6.71

(4) Lange, "Handbook of Chemistry," fourth ed., Handbook Publishers, Inc., Sandusky, Ohio, 1941, pp. 1307–1309.

(5) Cooper and Maass, *Can. J. Research*, **4**, 283 (1931).

(6) Cooper and Maass, *ibid.*, **4**, 495 (1931).

DEPARTMENT OF CHEMISTRY
IOWA STATE COLLEGE
AMES, IOWA

RECEIVED JULY 5, 1944

[CONTRIBUTION FROM ALLERGEN INVESTIGATIONS, BUREAU OF AGRICULTURAL AND INDUSTRIAL CHEMISTRY, AGRICULTURAL RESEARCH ADMINISTRATION, U. S. DEPARTMENT OF AGRICULTURE, AND THE ALLERGY CLINIC OF PROVIDENCE HOSPITAL, WASHINGTON, D. C.]

The Chemistry of Allergens. X. Comparison of Chemical and Immunological Properties of CB-1A Preparations from Domestic Castor Beans and Brazilian Castor Bean Pomace[1,2]

BY JOSEPH R. SPIES, E. J. COULSON AND HENRY STEVENS

The allergenic fraction, CB-1A, was originally isolated from a domestic variety of castor beans.[3] Before application of the isolation procedure, it was necessary to shell, grind and defat the seeds. In investigations involving castor beans it is necessary to curtail handling as much as possible because of the hazard associated with the primary toxalbumin, ricin[4,5] and because of the possibility of acquiring sensitivity to the castor bean allergen by continued exposure to dust.[6] In an effort to simplify the procedure and to lessen the hazards involved in isolating a large quantity of CB-1A, an examination of Brazilian castor bean pomace was undertaken. The pomace was used directly without ether extraction, and a preliminary heat treatment was employed to detoxify the ricin.

A yield of 0.45% CB-1A was obtained from the pomace as compared with 1.8% previously obtained from one lot of shelled, defatted, domestic castor beans.[3,7] Results in Table I show the close similarity in chemical composition of the CB-1A obtained from the two sources.

TABLE I

COMPARISON OF CHEMICAL COMPOSITION OF CB-1A FROM SHELLED, DOMESTIC CASTOR BEANS AND FROM BRAZILIAN CASTOR BEAN POMACE

Determination[a]	Composition in % of CB-1A from Domestic castor beans[b]	Brazilian castor bean pomace
Nitrogen	18.4	18.2
Nitrogen pptd. by 5% trichloroacetic acid at 20 ± 0.1°	30.7[c]	39.4
Sulfur	2.33	2.36
Carbohydrate	3.12	3.10
Arginine	26.6	26.6
Cystine	5.0	4.1
Tyrosine	1.1	1.1
Tryptophan	0.0	0.0

[a] Analyses are expressed on an ash-water-free percentage basis. The authors are indebted to Dorris C. Chambers for the microanalytical determinations. Amino acid determinations are expressed on the basis of per cent. of the total nitrogen in the form of the given amino acid. Tyrosine was determined by Lugg's procedure, *Biochem. J.*, **31**, 1422 (1937); **32**, 775 (1938). Other methods were the same as those used in Paper VIII.[3] [b] Isolation of this sample of CB-1A is described in Paper VIII.[3] [c] It was reported in Paper VIII[3] that 22.5% of the nitrogen of CB-1A from domestic castor beans was precipitated by 5% trichloroacetic acid. This value was in error owing to inadvertent use of a lower concentration of trichloroacetic acid.

The samples of CB-1A from both sources were immunologically equivalent. Both samples were equally potent in producing contractions in excised uterine strips of sensitized guinea pigs by the Schultz–Dale technique, using the multiple in-

(1) Not copyrighted.

(2) For Article IX of this series see Spies, Coulson, Chambers, Bernton and Stevens, THIS JOURNAL, **66**, 748 (1944).

(3) Spies and Coulson, *ibid.*, **65**, 1720 (1943).

(4) Stillmark, "Arbeiten des Pharmakologischen Instituts Zu Dorpat," 1889; *Chem. Zentr.*, [2] **60**, 978 (1889).

(5) Osborne, Mendel and Harris, *Am. J. Physiol.*, **14**, 259 (1905).

(6) Figley and Elrod, *J. Am. Med. Assoc.*, **90**, 79 (1928); Vaughn, *J. Allergy*, **1**, 474 (1930); Coca, Walzer and Thommen, "Asthma and Hay Fever in Theory and Practice," Charles C. Thomas, Baltimore, 1931, pp. 42, 175–176, 405.

(7) The lower yield of CB-1A from the pomace is attributed to natural differences which occur in the content of plant constituents from different sources or species and is not attributed to the slightly modified isolation procedure used.

crement titration method.[8] That both samples of CB-1A possessed the same specificity was demonstrated by complete cross-neutralization of excised uterine strips from appropriately sensitized guinea pigs when tested by the Schultz–Dale method.

When diluted $1:10^6$ (near threshold dilution) both samples of CB-1A gave cutaneous reactions of equal intensity on a castor bean-sensitive subject. A threshold quantity of 0.001 mγ of each sample of CB-1A was required to produce positive passive transfer reactions using serum from a castor bean-sensitive subject.[9]

Experimental[10]

Isolation of CB-1A from Brazilian Castor Bean Pomace.—Brazilian castor bean pomace was obtained from a commercial source in the United States. The sample consisted of broken shells and crushed seeds. The pomace was ground to a coarse powder in a hand grinder in a hood.[11] Experiment showed that the yield of CB-1A obtained directly from the pomace was the same as that obtained after ether extraction.

A preliminary heat treatment of the castor beans, to destroy the ricin toxicity,[12] was carried out as follows: To 3 kg. of ground castor bean pomace was added 6 l. of distilled water. The mixture was heated in an autoclave to 85–92° and maintained at that temperature for one and one-half hours. The suspension was then cooled slightly and an additional 12 l. of water was added. The procedure for isolating CB-1A was then essentially the same as that previously described,[3] except that water extracts from 12 kg. of pomace were combined and worked up together. A further convenient modification was the substitution of pressure filtration through a Seitz sterilizing pad, instead of centrifugation in the Sharples supercentrifuge, to clarify solutions at corresponding points in the procedure.

From a total of 39.9 kg. of pomace, worked up in four lots, 181.3 g. (air dried) of CB-1A was obtained. The four samples of CB-1A were combined, dissolved in 2 l. of water and reprecipitated with five volumes of ethanol at 5°. The recovered CB-1A was dried in a vacuum over calcium chloride. Before analysis the dried CB-1A was ground to pass a 100-mesh sieve and equilibrated with air.

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(8) The dilution of each sample of CB-1A required to produce 2 + contractions in the uterine muscles of sensitized guinea pigs was $1:1.83 \times 10^{11}$.

(9) Details of this method of comparing castor bean allergenic fractions are given in Table III of Paper IX.[3] The serum (W) used for this comparison gave positive passive transfer reactions when diluted $1:10^3$. *Cf.* Coca and Grove, *J. Immunol.*, **10**, 445 (1925). The authors are indebted to Dr. Harry S. Bernton for this serum and to Dorris C. Chambers for the clinical tests.

(10) The authors acknowledge the technical assistance of James H. Shimp in the isolation of CB-1A from the pomace.

(11) Ground pomace is commercially available.

(12) Stillmark[4] and later workers have shown that moist heat destroys the toxicity of ricin. Osborne, Mendel and Harris observed that heating at 60–80° coagulates ricin. Unpublished experiments in this Laboratory have shown that heating water extracts of castor beans destroys their toxic action but an ulcer-producing factor remains. Thus guinea pigs survived subcutaneous injection of 174 M. L. D. of nitrogen of a clarified water extract of castor beans that had been heated for one hour at 77–80°. However, ulcers developed at the site of the injection. Carmicheal (*Soc. Exptl. Biol. Med.*, **24**, 5 (1926)) previously reported that ricin solutions were detoxified by sodium ricinoleate but that ulcers always formed at the site of the injection. In protocols of later work by Carmicheal (*J. Pharmacol.*, **35**, 193 (1929); *ibid.*, **35**, 223 (1929)) it is apparent that ulcers sometimes formed on injection of ricin solutions detoxified by other means. Further work is needed to clarify the nature of this ulceration factor produced by detoxifying ricin solutions or castor bean extracts.

The Sulfonation of Acetophenone

BY E. H. WOODRUFF

It was reported[1] that the alkali fusion of acetophenone disulfonyl chloride gave *m*-hydroxybenzoic acid, confirming the statement of Suter and Weston[2] and that only one sulfonic acid group had entered the ring. Weston and Suter,[3] in a more detailed study, isolated only salicylic acid from the fusion of their acid chloride, showing it to be acetophenone 2,α-disulfonyl chloride.

To clarify this contradiction a further examination of the experimental data (not previously reported) yields the following information:

When added to cooled chlorosulfonic acid and then heated at 110° acetophenone yields an ether-insoluble compound, m. p. 195–196° (from carbon tetrachloride), identical with that previously reported.[3,4] This material on fusion gives salicylic acid. If, however, acetophenone is added to chlorosulfonic acid already heated to 110°, upon pouring onto ice almost no insoluble precipitate is obtained. Upon working up the aqueous solution a disodium disulfonate is obtained which on fusion with alkali gives *m*-hydroxybenzoic acid. Thus the *m*-hydroxybenzoic acid does not result from the fusion of the disulfonyl chloride, as the previous report from this Laboratory would indicate, but from another water-soluble product of the sulfonation. The aqueous solution from which the disulfonyl chloride was obtained has not been worked up in a similar manner. It would appear, however, that acetophenone may sulfonate in either the ortho or meta position when treated with chlorosulfonic acid. A low temperature during the mixing of the reactants favors the formation of the ortho derivative. Whether the meta isomer is formed during the process giving the best yield of the ortho isomer has not been demonstrated but in view of the low yield of the ortho isomer it is not unlikely that such is the case. The behavior of acetophenone toward sulfonation appears to duplicate that of nitration[5] where both the ortho and meta nitroacetophenones are formed.

Experimental

Acetophenone 2-α-Disulfonyl Chloride.—This was prepared by the addition of acetophenone to chlorosulfonic acid kept at room temperature and then heated to 110° for one hour[4]; yield 21.5%, m. p. 195–196° uncor., crystallized from carbon tetrachloride.

Acetophenone 3-α-Disulfonic Acid Disodium Salt.—To 1 kg. (8.60 moles) of chlorosulfonic acid heated to 110° with stirring, 168 g. (1.4 moles) of acetophenone was added over a period of one hour. The temperature rose to 120° during the addition and was kept there an additional hour. After cooling to 10°, the material was added to 4 kg. of cracked ice. One hundred cc. of chloroform was added and the solution filtered with suction. Upon heating the solution to 80° with a current of steam, 1880 g. (5.8 moles)

(1) Woodruff, THIS JOURNAL, **64**, 2859 (1942).

(2) Suter and Weston, *ibid.*, **61**, 233 (1939).

(3) Weston and Suter, *ibid.*, **61**, 389 (1939).

(4) Riesz and Frankfurter, *Monatsh.*, **50**, 68 (1928).

(5) Reese, *Chem. Rev.*, **14**, 90 (1934).

of hydrated barium hydroxide was added. The barium sulfate was removed and 250 g. of sodium carbonate added. Upon evaporation to dryness, 375 g. of crude sodium salt was obtained.

m-**Hydroxybenzoic Acid.**—One hundred twenty-nine and six-tenths grams (0.4 mole if pure) of the above salt was fused with a mixture of 200 g. of sodium hydroxide and 168 g. of potassium hydroxide at 310° for two hours. After cooling the solid was dissolved in 800 cc. of water and 700 cc. of concentrated hydrochloric acid added. On cooling the aqueous solution was extracted with three 200-cc. portions of ether. The ether on evaporation gave 20 g. (36%) of crude acid m. p. 172–182°; recrystallized twice from benzene–ether, m. p. 202° uncor.

When treated with methyl sulfate and alkali *m*-methoxybenzoic acid was obtained, m. p. 101–102°; neutral equivalent calcd., 152.0; found, 150.0.

THE UPJOHN COMPANY
KALAMAZOO, MICHIGAN

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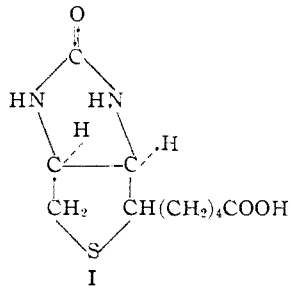
COMMUNICATIONS TO THE EDITOR

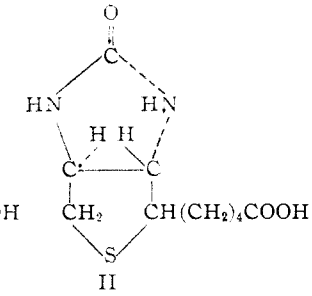
BIOTIN. III. *cis* and *trans* FORMS RELATED TO *dl*-BIOTIN

Sir:

It was stated in a previous publication[1] that it was believed that compounds having two five-membered saturated heterocyclic nuclei fused through adjacent carbon atoms, as in biotin, would exist only in *cis* forms of the rings, as in structure I.



I

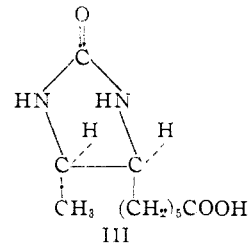


II

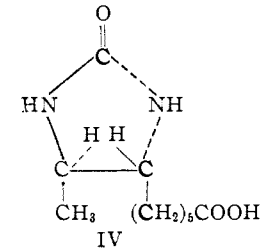
Originally two racemates related to biotin, namely, *dl*-biotin, m. p. 232° (*Anal.* Calcd. for $C_{10}H_{16}N_2O_3S$: C, 49.16; H, 6.60; N, 11.46. Found: C, 49.33; H, 6.39; N, 11.68), and *dl*-allobiotin, m. p. 194–196° (*Anal.* Calcd. for $C_{10}H_{16}N_2O_3S$: C, 49.16; H, 6.60; N, 11.46. Found: C, 49.36; H, 6.50; N, 11.39), were obtained, one of which yielded biotin on resolution. The series of reactions[2] which led to the formation of these racemates is described in another communication. A third racemate, *dl-epi*allobiotin (decomposes without melting starting at 195°) (*Anal.* Calcd. for $C_{10}H_{16}N_2O_3S$: C, 49.16; H, 6.60; N, 11.46. Found: C, 49.23; H, 6.75; N, 11.21), having the structure of biotin has been derived from the reduction product of the dehydro isomer[2] melting at 162–163° The other reduction product from this isomer yielded *dl*-allobiotin.

Thus, three racemates corresponding to six of the eight theoretically possible isomers are known. It is evident that one or two of the known racemic pairs must have a *trans* configuration of its nitrogen atoms as represented by structure II.

This new racemate has been correlated to *dl*-allobiotin[2] by hydrogenolysis[3] with Raney nickel catalyst. Both the new racemate and *dl*-allobiotin gave the same desthio derivative, III or IV, which is called *dl*-desthioallobiotin; m. p. 165–166° (*Anal.* Calcd. for $C_{10}H_{18}N_2O_3$: C, 56.05; H, 8.47; N, 13.08. Found: C, 55.86; H, 8.25; N, 12.76.



III



IV

dl-Biotin gave *dl*-desthiobiotin which also melted at 165–166° (*Anal.* Calcd. for $C_{10}H_{18}N_2O_3$: C, 56.05; H, 8.47; N, 13.08. Found: C, 56.04; H, 8.52; N, 13.22). However, these two compounds showed a mixed melting point depression of twenty degrees. Dr. Jacob L. Stokes of this Laboratory found that *dl*-desthioallobiotin was inactive for the growth of yeast, while *dl*-desthiobiotin was one-half as active as *d*-desthiobiotin.[4]

From these results it is evident that the new racemate is epimeric at carbon atom 2 of *dl*-allobiotin; therefore, it will be called *dl-epi*allobiotin.

The fact that *d*-desthiobiotin methyl ester[3] and *d*-desthiobiotin ($[\alpha]^{31}D$ +10.4 (*c*, 1.7525 in 0.1 *N* sodium hydroxide)) have a low but definite optical activity is evidence that inversion of the nitrogen atoms did not take place during the hydrogenolysis. Furthermore, the latter compound does not agree in melting point[4] or mixed melting point with either of the *dl* derivatives.

(1) Paper I, Harris, Wolf, Mozingo and Folkers, *Science*, **97**, 447 (1943).

(2) Harris, *et al.*, Paper II, THIS JOURNAL, **66**, 1756 (1944).

(3) du Vigneaud, *et al.*, *J. Biol. Chem.*, **146**, 475 (1942); Mozingo, *et al.*, THIS JOURNAL, **65**, 1013 (1943).

(4) Melville, Dittmer, Brown and du Vigneaud, *Science*, **98**, 497 (1943).