hydroxide, an indicator with a color change above *p*H 7 cannot be used, because of the lability of the silane hydrogen in the presence of excess base. Satisfactory titrations were obtained using brom thymol blue (*p*H 6.0–7.6). However, equally good results were obtained without an added indicator; the first appearance of bubbles of hydrogen in the solution gave an accurate end-point.

Experimental

1,1,3,3-Tetrachlorodisiloxane.—To 70 ml. of anhydrous ethyl ether was added 47 g. (0.35 mole) of trichlorosilane (Anderson Laboratories). This solution was cooled to −78° in a Dry Ice–acetone-bath and stirred vigorously while 3.4 g. (0.19 mole) of water was added from a buret over a period of 30 minutes. The mixture was then stirred for several hours while it warmed to room temperature. Fractional distillation of the solution was carried out using a 50-cm. column packed with stainless steel helices. The product distilled at 99–100° (763 mm.); a trace of chlorine-containing material boiling at 145° was also found (probably hexachlorotrisiloxane). The yield was 3.2 g., or 8.5%. Yields from similar runs varied from 5 to 10%. The product was a colorless mobile liquid with $n^{25}D$ 1.4075 and d^{25}_4 1.347. The molecular weight by the vapor-density method was 222 (calcd. 216).

Anal. Calcd. for $H_2Si_2OCl_4$: Cl, 65.67. Found: Cl, 65.6, 65.7.

The infrared absorption spectrum of tetrachlorodisiloxane vapor was obtained using a cell of 3-cm. path length with KBr windows. The spectrum in the sodium chloride region was measured with a Baird automatic recording infrared spectrophotometer (Fig. 1); the region from 450 to 650 cm.$^{-1}$ was studied with a Perkin–Elmer spectrophotometer using a KBr prism. Strong absorption bands in this region were found centered at 533 and 604 cm.$^{-1}$. For most of the absorption bands, analogies are found in the spectrum of $HSiCl_3$.[4,5] However, the bands at 871, 912 and 1128 cm.$^{-1}$ have no counterparts in the $HSiCl_3$ spectrum.

(4) T. C. Gibian and D. S. McKinney, THIS JOURNAL, **73**, 1431 (1951).

(5) C. A. Bradley, *Phys. Rev.*, **40**, 908 (1932).

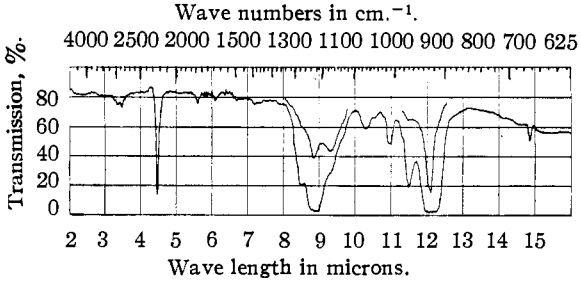

Fig. 1.—Infrared absorption spectrum of 1,1,3,3-tetrachlorodisiloxane. The lower line is at 40 mm., while the upper line is at 4 mm.

Partial Hydrolysis of Dichlorosilane.—The hydrolysis was carried out in the same way as that of trichlorosilane, except that *n*-propyl ether was used in place of ethyl ether. From 35 g. of dichlorosilane,[6] about 1 g. (4%) of product was obtained boiling over the range 70–81%. This material probably contained some *n*-propyl ether. Analysis gave 42.9% Cl; dichlorodisiloxane requires 48.2%.

In the course of this and other experiments with dichlorosilane, it was found that when a large quantity of the gas is vented in air, it inflames spontaneously and explosively. This is contrary to the findings of Stock,[7] who was working only with very small quantities. The liquid could be handled with relative safety if kept at −78°.

Partial Hydrolysis of Methyltrichlorosilanes.—The hydrolysis was performed in the same way as that of trichlorosilane. Only a trace of chlorine-containing material, boiling near 110°, was found.

Acknowledgment.—The author wishes to thank Dr. Eugene G. Rochow for his help and encouragement during the course of this work.

(6) The dichlorosilane was a gift from the Linde Air Products Co., of Tonawanda, N. Y.

(7) A. Stock and C. Somieski, *Ber.*, **52**, 718 (1919).

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COMMUNICATIONS TO THE EDITOR

THERMODYNAMIC AND KINETIC CONSTANTS FOR THE DIBORANE-BORINE EQUILIBRIUM

Sir:

We have succeeded in making a reliable estimate of the equilibrium constant for the dissociation of diborane into borine groups. The method is an indirect one. It is now clear why physical experiments designed to detect directly borine in diborane[1] have failed; at 155° and one atmosphere, the degree of dissociation of diborane as computed from the equilibrium constant given below is 1.63×10^{-5}, and at that temperature its general pyrolysis is fairly rapid.

The enthalpy change for the association reaction $2BH_3 = B_2H_6$ was deduced as follows. We measured the heats of reaction of trimethylamine with diborane and tetramethyldiborane, to produce the solid and liquid association products, respectively, using a precision vacuum ice calorimeter. These were corrected to the gas phase reactions and combined with the value given by Brown[2] for the heat of reaction of trimethylamine with boron trimethyl. Thus the three values (−17.3, −11.0, −17.6 kcal./mole of amine) demonstrate the effect of methyl substitution on the acidity of the boron unit, as well as on the strength of the (BH_2B) bridge. By making suitable corrections for inductive, steric and mesomeric contributions from the methyl groups as deduced from other data, we arrived at $\Delta H^\circ_{273} = -32\ ^{+1}_{-2}$ kcal. per mole of diborane, for the above reaction.

The entropy change for the association of two borines was computed. Based on the structure of diborane as reported by Hedberg and Schomaker,[3] its translational plus rotational entropy is 53.70 ±

(1) A. Stock and E. Kuss, *Ber.*, **56B**, 789 (1923); G. C. Pimentel and K. S. Pitzer, *J. Chem. Phys.*, **17**, 882 (1949).

(2) H. C. Brown, H. Bartholomay and M. D. Taylor, THIS JOURNAL, **66**, 435 (1944).

(3) K. Hedberg and V. Schomaker, *ibid.*, **73**, 1482 (1951).

TABLE I

EQUILIBRIUM CONSTANTS AND EQUILIBRIUM PRESSURES OF BORINE, FOR THE REACTION $2BH_3 = B_2H_6$

T, °K.	K (at.$^{-1}$)	K (m./cc.$^{-1}$)	P_{BH_3} (at.)	P_{BH_3} (mm.)
273	1.73×10^{18}	3.88×10^{22}	$1.48 \times 10^{-9}\sqrt{P_{at}}$	$4.1 \times 10^{-8}\sqrt{P_{mm}}$
300	8.57×10^{15}	2.11×10^{20}	$2.16 \times 10^{-8}\sqrt{P_{at}}$	$6.0 \times 10^{-7}\sqrt{P_{mm}}$
373	2.34×10^{11}	7.16×10^{16}	$4.14 \times 10^{-6}\sqrt{P_{at}}$	$1.1 \times 10^{-4}\sqrt{P_{mm}}$
473	2.54×10^{7}	0.99×10^{12}	$1.99 \times 10^{-4}\sqrt{P_{at}}$	$5.5 \times 10^{-3}\sqrt{P_{mm}}$

0.1 e.u./mole, at 300°K. and one atmosphere. Using the frequency assignments for diborane as given by Anderson and Barker[4] we computed a vibrational contribution of 2.34 ± 0.1 e.u. A planar structure was assumed for BH_3, with a B–H distance equal to 1.16 Å. Badger's rule was then used to estimate the stretching force constant,[5] and various interpolations made to estimate the bending and interaction force constants. These led to a total entropy for BH_3 at 300°K. and one atmosphere of 44.9 ± 0.2 e.u. Thus for the association reaction, $\Delta S^\circ_{300} = -33.78$ e.u./mole of diborane. To the precision dealt with here one may neglect the specific heat correction and consider the enthalpy change independent of the temperature. Thus, $\log (K_{eq})_{atm} = -7.38_4 + (6995/T)$. Typical values for four temperatures are listed in the table. Clearly the equilibrium concentrations of borine are too small to be detected by the usual physical techniques.

We have also deduced an upper limit for the bimolecular rate constant (k') for the association of two borines. Burg[6] recently reported on the kinetics of the decomposition of borine carbonyl. He concluded that the mechanism of the reaction is as follows:

(a) $BH_3CO = BH_3 + CO \quad K'_{eq}$

(b) $BH_3 + BH_3CO \longrightarrow B_2H_6 + CO \quad k$

Hence, during the approach to equilibrium, both the inverse of (a), and step (b), must proceed faster than does the association:

(c) $2BH_3 \longrightarrow B_2H_6 \quad k'$

That is, the correctness of Burg's mechanism implies the inequality

$$k'(BH_3)^2 < k(BH_3)(BH_3CO)$$

Using the equilibrium condition on the first step of the borine decomposition (a), this reduces to

$$k' < k(P_{tot}/K'_{eq})^{1/2}$$

ΔH° for (a) was obtained by combining the ΔH° for the over-all borine carbonyl decomposition as given by Burg with our value for the heat of dissociation of diborane. The entropy change for step (a) was obtained by subtracting the computed entropy of H_3BCO—as based on the structural data of Gordy[7] and spectral data of Cowan[8]—from the now known entropy of borine and carbon monoxide.[9] Thus K'_{eq} was computed. The resulting condition on the rate constant is

$$k' < 5 \times 10^9 \text{ cc./mole/sec.}$$

Such a value for a second order rate constant suggests an activation energy of about 5 kcal./mole. It is indeed very interesting to compare the following rate constants:

$k = 1.98 \times 10^{6}$ cc./mole/sec.;	$E_a \cong 6.8$ kcal./mole
$k' < 5 \times 10^{9}$	$E_a \sim 5$
$k'' = 6.4 \times 10^{9}$	$E_a \cong 6$

The last value is for the displacement reaction

$$BH_3 + B_2D_6 = BH_3BD_3 + BD_3 \quad k''$$

as reported by Koski.[10]

(10) W. S. Koski, private communication.

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RECEIVED JANUARY 2, 1953

(4) W. E. Anderson and E. F. Barker, *J. Chem. Phys.*, **18**, 698 (1950).

(5) The force constant deduced is 3.38×10^5 dynes/cm. This compares with 3.21 and 3.42×10^5 reported for the B-H stretching force constant in H_3BCO and $B_3N_3H_6$, respectively.

(6) A. Burg, THIS JOURNAL, **74**, 3482 (1952).

(7) W. Gordy, H. Ring and A. Burg, *Phys. Rev.*, **78**, 512 (1950).

(8) R. D. Cowan, *J. Chem. Phys.*, **18**, 1101 (1950).

(9) H. L. Johnson and C. O. Davis, THIS JOURNAL, **56**, 271 (1934).

THERMODYNAMIC FUNCTIONS FOR SURFACES OF CRYSTALS

Sir:

Recently a method[1] was described for the determination of thermodynamic quantities of surfaces of solids which appears to be beautiful in its simplicity, and extremely general in its application. Besides the surface area, the measurements required are the differences in the heats of solution and in the heat capacities of a finely divided sample and one composed of very large crystals. It is regrettable that these authors omitted to call attention to an *essential pragmatic test* for the applicability of their method. Since the enthalpy, entropy and surface free energy are *extensive* properties their magnitudes per unit area should be *independent* of *crystallite size and shape.* The preliminary results quoted by Jura and Garland for magnesium oxide have not been subjected to this test. What, then, may we anticipate when a complete set of data becomes available for a well crystallized substance?

First let us assume that regardless of the temperature and crystallite size, the structure of the crystals is geometrically perfect,[2] and their surface is absolutely free of adsorbed gases. Nevertheless the thermodynamic functions for the surface for two samples with the same total area will differ *unless* they have the same proportions of area contributed by faces of different crystallographic indices. This is based on the established facts that faces with different indices have different characteristics for adsorption,[3] catalysis,[4] and reaction.[5]

(1) G. Jura and C. W. Garland, THIS JOURNAL, **74**, 6033 (1952).

(2) This is equivalent to the statement that such a structure is determined by the criterion of minimum enthalpy only.

(3) For example, T. N. Rhodin, Jr., THIS JOURNAL, **72**, 5691 (1950).

(4) O. Beeck, *Rev. Mod. Phys.*, **17**, 61 (1945).

(5) T. N. Rhodin, Jr., THIS JOURNAL, **73**, 3143 (1951).

Also, theoretical estimates indicate they differ in surface free energy.[6] One may argue with justification that precise values for the surface thermodynamic quantities for a polycrystalline sample cannot be conceptually defined without specifying a shape distribution function and proving that it is invariant with crystal size, for each substance studied. In addition, there are numerous experiments which suggest that the thermodynamic potential of material in small regions surrounding corners, edges and steps on crystal faces differs from that in geometrically plane surfaces. Since the number of such corners, edges, etc., is proportional to the number of crystals, whereas the total area depends on that number times the square of the linear extension of the crystals [so that for a mole of substance divided into n particles of uniform size, $S \alpha (M/\rho)^{2/3}n^{1/3}$], it is clear that any macroscopic quantity which depends on the thermodynamic potential will not be strictly extensive with respect to surface area. As a matter of fact, in a very interesting paper preceding the one under discussion, one of the authors (G. J.) and K. S. Pitzer[7] showed that at low temperatures a significant contribution to the specific heat of *very* small particles comes from the gross motion of the particles; *i.e.*, their heat capacity depends on the *number* of *particles*, in addition to the differences between the vibrational frequencies of atoms in the surface layers and those in the bulk crystal.

In a discussion of the thermodynamic properties of surfaces, one should recognize, however, that even under conditions approaching ideality in composition and configuration, the structures of crystals at temperatures other than 0°K. is determined by minimizing their total free energy.[8] This implies that there must be an entropy contribution arising from lattice defects. Such imperfections will affect the enthalpy as well (and hence the heat of solution), and the magnitude of the increment will depend on crystallite size; it is unlikely that the net effect of the various types of possible defects will in the aggregate depend precisely on the square of the linear extension of the crystals. Indeed, in most laboratory procedures for the preparation of crystals strains, distortions, minute amounts of impurity, etc., will be introduced.[9] Finally, the practical problem of the complete elimination of adsorbed gases will be difficult to solve. We may conclude that the application of the thermodynamic method of Jura and Garland to well crystallized materials will lead to values for thermodynamic functions which only in part are due to the presence of surface. Such magnitudes will be *extensive* with respect to surface area only when the method of preparation, crystal size and shape distributions, etc., are kept strictly unaltered. Such a restriction nullifies the power of the thermodynamic method.

The proposal of Jura and Garland does apply to liquids and glasses, for which the inherent randomness of internal structural minimizes the relative contributions of the very factors which vitiate the method for well crystallized materials.

It is a pleasure to acknowledge an interesting discussion of this paper with Prof. J. A. Krumhansl, Department of Physics.

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RECEIVED JANUARY 13, 1953

(6) R. Shuttleworth, *Proc. Phys. Soc.*, **A62**, 167 (1949); W. D. Harkins, *J. Chem. Phys.*, **10**, 268 (1942). Also, see the excellent summary "Use of Classical Macroscopic Concepts in Surface Energy Problems" by Conyers Herring, presented at a conference on "Structure and Properties of Solid Surfaces", September, 1952, Intn. Union Pure and Applied Physics.

(7) G. Jura and K. S. Pitzer, THIS JOURNAL, **74**, 6030 (1952).

(8) N. F. Mott and R. W. Gurney, "Electronic Processes in Ionic Crystals," Clarendon Press, Oxford, 1948, Chap. II.

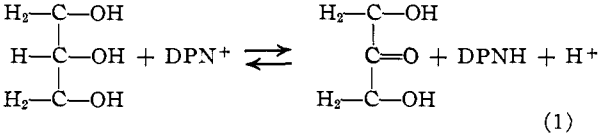
(9) See Chapter IV, ref. 8; W. Shockley, "Electrons and Holes in Semiconductors," D. Van Nostrand Co., New York, N. Y., 1950.

A DPN SPECIFIC GLYCEROL DEHYDROGENASE FROM *AEROBACTER AEROGENES*[1]

Sirs:

A number of microörganisms have been shown to utilize glycerol as the sole carbon source.[2] The major product in the oxidation of glycerol in *Acetobacter suboxydans* has been shown to be dihydroxyacetone (DHA).[3] In *A. aerogenes* glycerol is oxidized past the DHA stage; the products are, in part, organic acids.[2b,4]

We have found that oxidation of glycerol in cell-free extracts of *A. aerogenes* requires diphosphopyridine nucleotide (DPN). This reaction can be followed spectrophotometrically at 340 mμ as shown in Fig. 1. Glycerol was incubated with DPN in the presence of dialysed cell-free extracts of *A. aerogenes*. After the reaction had approached equilibrium, DHA was added and the reaction that followed was due to the oxidation of the reduced DPN (DPNH) by the reduction of DHA to glycerol. Equation 1 expresses this relation as



(1)

Reaction 1 proceeds with glycerol in the presence of hydroxylamine which inhibits alcohol dehydrogenase activity.[5] This, therefore, indicates that the glycerol dehydrogenase is a separate enzyme distinct from alcohol dehydrogenase.

At comparable concentrations glyceraldehyde is reduced at a slower rate than DHA. This would suggest that DHA is the primary product of glycerol oxidation by DPN in *A. aerogenes*. Neither α- or β-glycerol monophosphates are oxidized by this enzyme preparation. Triphosphopyridine nucleotide (TPN) is inactive as a substitute for DPN in reaction 1.

(1) Contribution No. 37 of the McCollum-Pratt Institute. This work was supported in part by grants from the Rockefeller Foundation and the American Cancer Society as recommended by the Committee on Growth of the National Research Council.

(2)(a) H. R. Braak, Thesis, Delft, 1928; (b) R. E. Buchanan and E. I. Fulmer, "Physiology and Biochemistry of Bacteria," The Williams and Wilkins Company, Baltimore, Md., 1930, Vol. III, p. 248.

(3) A. J. Kluyver and F. J. G. DeLeeuw, *Tijdschr. Vergelijk. Geneesk.*, **10**, 170 (1924); L. A. Underkofler and E. I. Fulmer, THIS JOURNAL, **59**, 301 (1937).

(4) H. Kumagawa, *Biochem. Z.*, **131**, 156 (1922); A. C. Baskett and C. N. Hinshelwood, *Proc. Roy. Soc.* (*London*), **B138**, 75 (1951).

(5) N. O. Kaplan and M. M. Ciotti, *J. Biol. Chem.*, in press.

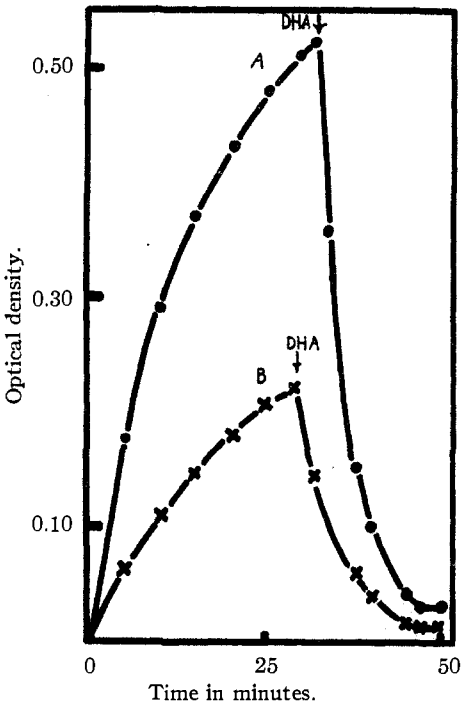


Fig. 1.—The reversible reaction catalyzed by glycerol dehydrogenase: curve A, the reaction mixture contained 100 μM. of potassium phosphate buffer, 0.5 μM. of DPN, 0.6 mg. of dialyzed *A. aerogenes* glycerol dehydrogenase preparation, and 500 μM. of glycerol in a total volume of 3.0 ml. at *p*H 9. The change in optical density was measured at 340 mμ with a Beckman model DU spectrophotometer at 23°. At the arrow 100 μM. of DHA was added. Curve B, the details for curve A apply here except 100 μM. of glycerol was used with 10 μM. of DHA added at the arrow.

During the course of this investigation an interesting non-enzymatic reaction between DHA and DPN was observed.[6] The product of this reaction has an ultraviolet spectrum identical to that of DPNH and possesses other properties of DPNH. That this product is not DPNH is shown by its failure to reduce acetaldehyde in the presences of yeast alcohol dehydrogenase. Further study has shown that the basic requirements for the reaction are an N-substituted nicotinamide structure (as in DPN), and α,β-hydroxy-keto-structure (as in DHA), and an alkaline medium. The details of this investigation and a discussion of the reaction mechanism will be reported elsewhere.

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RECEIVED DECEMBER 22, 1952

(6) A somewhat similar reaction using glyceraldehyde had been noticed previously by D. M. Needham, L. Siminovitch, and S. M. Rapkine, *Biochem. J.*, **49**, 113 (1951), who presumed that DPNH and glyceric acid were formed.

(7) Public Health Service Research Fellow of the National Heart Institute.

ADDITIONAL COMMENTS ON THERMODYNAMIC FUNCTIONS FOR SURFACES OF CRYSTALS

Sir:

In the preceding letter, S. H. Bauer has criticized a recent article by us. It appears that we were too brief in our discussion of the work which was presented. Our belief was that the data which we had obtained could not be considered final. We were careful to point out several factors which might change the final results. It was the hope of the senior author to reserve the discussion of some of the questions raised in Dr. Bauer's letter until better data were available. Since we knew that several years would be essential for a complete solution to all of the experimental problems involved in the determination, we thought that it would be advisable to publish that which we had done in the hope that these preliminary results would be of value and that others would think and work actively on this problem.

First, concerning the pragmatic test of the invariance of the results with respect to particle size and shape. For the past two years we have engaged in the design and construction of a calorimeter for the sole purpose of making these measurements with respect to the enthalpy. We have hopes that in a year we will be able to make a report of the results of this work. After this project is finished, we hope to make similar measurements of the heat capacity. These of course, will take even more time since the necessary experimental procedures are even more difficult than those involved in the enthalpy determination.

Bauer states that the results should be independent of size and shape. This, of course, will be true only if two conditions are fulfilled: (1) only one crystallographic face is present, and (2) if the particle is sufficiently large that the surface properties are not dependent on particle size. The first of these considerations was an overwhelming factor in the choice of MgO, since the structure is such that we would expect only the 100 face to appear at the temperatures at which the experiments are performed. The few poor electron photomicrographs that we have obtained with our sample of MgO indicated that this was so. Too few individual particles were observed, however, to state that this was certainly true for all of the particles. Since we considered these results as preliminary, we made no really serious efforts to obtain really good pic tures. Actually, it can be shown that under certain circumstances, even if more than one crystallographic face appears, it is possible to obtain the surface tension of each face from the same measurements and a knowledge of the crystal habit; provided that the future work shows that the proposed scheme of measurements is valid. The size at which it may be expected that the thermodynamic functions become size dependent, can be readily computed by the method of Lennard-Jones and Dent.[1]

The question naturally arises as to the possible specific effects mentioned by Bauer. First, the contribution to these functions of the gross motion of the particles studied by Jura and Pitzer[2]: at very low temperatures, say to about 10°K., this effect would be appreciable. However, at room temperature, this quantity is several orders of magnitude less than the observed experimental effects. There is complete justification in its neglect.

(1) J. E. Lennard-Jones and B. E. Dent, *Phil. Mag.* [7] **8**, 530 (1929).
(2) G. Jura and K. S. Pitzer, THIS JOURNAL, **74**, 6030 (1952).

The authors feel that the effect of defects on the measured entropy is also small by several orders of magnitude. The effect of edges and corners depends on the actual structure of the surface. It is simple to consider those systems in which only atomically plane surfaces are present. For the particles that were used in the experiment, assuming an average cube size, it is found that in round numbers there are 8 corner to 600 edge to 15,000 surface ions. The corners, obviously can be neglected. If it is assumed that the effect of the edge is twice that of the surface, then in order to detect the effect of edges with certainty, it would be necessary to make calorimetric measurements by a factor of ten better than now possible. If the surface is heterogeneous, then estimates of the effect of heterogeneity are impossible. However, if the degree of heterogeneity is independent of particle size, then the results which are obtained are valid. For very small particles, such as those which are used as catalysts, there is probably variation in the heterogeneity of the surface. These particles are smaller than those used in these experiments. It appears reasonable that as the particles grow, the distribution of energy over the surface would become independent of size.

In view of the preceding considerations the authors still feel that the ideas presented in the original paper are valid.

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RECEIVED JANUARY 30, 1953

SYNTHESIS AND CONFIGURATION OF DIHYDROSPHINGOSINE[1,2]

Sir:

Dihydrosphingosine is one of the four stereoisomeric forms of 1,3-dihydroxy-2-aminoöctadecane.[3] Recently several papers have appeared describing synthetic methods for obtaining such compounds.[4,5,6,7] However, none of these methods provides evidence as to the stereochemistry of the product and indeed most of them would be presumed to yield a mixture of the two racemic forms.

We have approached the synthesis by a different route. The two racemic α-amino-β-hydroxystearic acids were prepared and characterized as the *threo* and *erythro* isomers by comparison of the properties of a number of derivatives with the corresponding derivatives of threonine (*threo*) and allothreonine (*erythro*). The assignment of configuration was based on the complete agreement in the relative behavior of the C_{18} isomers as compared to the C_4 isomers.[8] Thus the characteristic shifts in infrared spectra from threonine to allothreonine and the relative melting point and solubility behavior of the two isomers and their derivatives are duplicated exactly in the C_{18} series. Furthermore the interconversion and isomerization of the 2-phenyloxazoline derivatives of threonine and allothreonine[9] are given by the C_{18} isomers under identical conditions. These data leave little or no doubt as to the configuration of the two α-amino-β-hydroxystearic acids. This work will be reported in detail shortly.

The methyl esters of the two amino acids were reduced to the corresponding 1,3-dihydroxy-2-aminooctadecanes with lithium aluminum hydride. The N-benzoyl methyl esters under similar conditions yielded the corresponding N-benzyl derivatives, which were readily debenzylated by catalytic reduction. The melting points of these substances are summarized in Table I.

TABLE I

Each of the compounds listed gave C, H, and N analyses agreeing with the theoretical.

	Threo series m.p., °C.	*Erythro* series m.p., °C.
α-Amino-β-hydroxystearic acid	205–206	217–220
N-Benzoyl	92–95	174–176
Methyl ester	77–78	71–73
Methyl ester, N-benzoyl-	86–88	97–99.5
1,3-Dihydroxy-2-aminoöctadecane	99.5–100.5	84–86
N-Benzyl-	55–56	62.5–63.5
Triacetyl-	65–66	90–92
Tribenzoyl-	...	144–45
N-Acetyl-	104.5	120–121

Reduction of the methyl α-amino-β-hydroxysterates over Raney nickel also gave excellent yields of the corresponding bases with no evidence of inversion. These results thus establish with some certainty the configuration of the two DL-1,3-dihydroxy-2-aminoöctadecanes.

Determination of the configuration of the natural compound was made possible by a striking difference in properties of the two racemic bases. The *erythro* base gave readily a tribenzoyl derivative (m.p. 144–145°) in quantitative yield on treatment with benzoyl chloride and pyridine. Under similar conditions natural dihydrosphingosine gives a tribenzoyl derivative melting at 144–145°. In marked contrast to this behavior we have found that the *threo* base gives only a dibenzoyl derivative and have not been able to obtain a tribenzoyl derivative with pyridine and benzoyl chloride under any of a variety of conditions. When a mixture of the *threo* and *erythro* bases was benzoylated only the tribenzoyl derivative of the *erythro* form was obtained. This result affords an explanation for the fact that all of the synthetic 1,3-dihydroxy-2-aminoöctadecane preparations described in the literature gave a tribenzoyl derivative melting at about 146°.

On the basis of these data it is tentatively con-

(1) This investigation was supported in part by a research grant (RG 2031) from the National Institutes of Health, Public Health Service.

(2) Part of the material in this paper was taken from the thesis submitted to the Graduate College of the University of Illinois by J. Bradley Harrison in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Chemistry.

(3) H. E. Carter, F. J. Glick, W. P. Norris, and G. E. Phillips, *J. Biol. Chem.*, **170**, 285 (1947).

(4) G. I. Gregory and T. Malkin, *J. Chem. Soc.*, 2453 (1951).

(5) N. Fisher, *Chem. and Ind.*, 130 (1952).

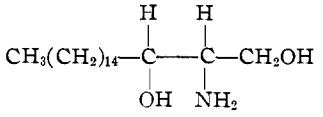
(6) C. A. Grob, E. F. Jenny and H. Utzinger, *Helv. Chim. Acta*, **34**, 2249 (1951).

(7) C. A. Grob and E. F. Jenny, *ibid.*, **35**, 2106 (1952).

(8) J. Bradley Harrison, Ph.D. thesis, University of Illinois, 1952.

(9) D. F. Elliott, *J. Chem. Soc.*, 62 (1950).

cluded that natural dihydrosphingosine has the *erythro* configuration. In conjunction with previous data on the configuration of the amino carbon of dihydrosphingosine[10] it would appear that dihydrosphingosine is *erythro*-D-1,3-dihydroxy-2-aminoöctadecane.



Recently Grob and Jenny[7] resolved the DL-1,3-dihydroxy-2-aminoöctadecane melting at 100° and concluded that one of the enantiomorphic forms was identical with natural dihydrosphingosine. Unfortunately no acyl derivatives of the resolved base were reported. However in the light of our data it seems probable that the resolved base was actually a diastereoisomer of natural dihydrosphingosine.

Resolution of the *erythro* base is being investigated and the results of this study will be reported shortly.

(10) H. E. Carter and C. G. Humiston, *J. Biol. Chem.*, **191**, 727 (1951).

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RECEIVED JANUARY 10, 1953

THE REACTIVITY OF BRIDGEHEAD BROMIDES

Sir:

The peculiar situation existing in a bicyclic system with a bridgehead halide is pertinent to the hypothesis of hyperconjugation, planarity and degree of substitution as they are related to the energy of carbonium ions and to the hypothesis of solvation at the rear as it relates to rate of solvolysis. Encouraged by the implications of these theoretical considerations and dissuaded of the opinion that bicyclic halides are generally unreactive[1] by detection of reactivity in 4-chlorocamphane (I),[2] we have synthesized by various methods 1-bromobicyclo[2.2.1]heptane (II), 1-bromo-8,8-dimethylbicyclo[2.2.2]octane (III) and 1-bromobicyclo[2.2.2]octane (IV) and wish to report initial results of the investigation of their reactivities, which is continuing.

II reacts with aqueous silver nitrate at 150° for two days to give 1-hydroxybicyclo[2.2.1]heptane whereas III and IV react at room temperature in 4 hr. giving 1-hydroxy-8,8-dimethylbicyclo[2.2.2]-octane and 1-hydroxybicyclo[2.2.2]octane, respectively. The rate of ethanolysis of III is first order in III and independent of ethoxide ion concentration and the product is 1-ethoxy-8,8-dimethylbicyclo[2.2.2]octane. The first order rate constants for hydrolysis in 70% (by vol.) aqueous dioxane at 100.0° and 131.2° are 1.49 and 19.8 $\times$ 10^{-5} sec.$^{-1}$ for III and 0.68 and 9.33 $\times$ 10^{-6} sec.$^{-1}$ for IV. By way of comparison, the calculated rate of hydrolysis of *t*-butyl bromide at 100° in 80% aqueous alcohol is 0.82 sec.$^{-1}$.[3]

The increased reactivity of 1-bromobicyclo-[2.2.2]octane as compared with that of 1-bromobicyclo[2.2.1]heptane appears to us to be experimental evidence supporting the hypothesis that a tetrahedral (sp^3) carbonium ion is of higher energy than some other configuration, most probably the planar (sp^2).[4] Relative to the effect of substitution, the operation of hyperconjugation and the ability to become solvated, the carbonium ions derived from II and IV are comparable, but relative to the amount of energy required to deviate from the tetrahedral configuration, the highly constrained ion from II is at a disadvantage compared to the more flexible ion from IV.[5]

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RECEIVED JANUARY 8, 1953

(1) P. D. Bartlett and L. H. Knox, THIS JOURNAL, **61**, 3184 (1939); P. D. Bartlett and S. G. Cohen, *ibid.*, **62**, 1183 (1940); P. D. Bartlett and E. S. Lewis, *ibid.*, **72**, 1005 (1950).

(2) W. v. E. Doering and E. F. Schoenewaldt, *ibid.*, **73**, 2333 (1951).

(3) K. A. Cooper and E. D. Hughes, *J. Chem. Soc.*, 1183 (1937).

(4) Professor G. E. Kimball, Columbia University (private communication) has suggested that a tetrahedral carbonium ion in which only 1/4 of the 2s orbital is occupied will be approximately 24 kcal. (1/4 of the energy required to promote a 2s electron of carbon to a 2p orbital [A.G. Shenstone, *Phys. Rev.*, **72**, 411 (1947)]) higher in energy than a planar carbonium ion in which the entire 2s orbital is occupied.

(5) The strain energy required to obtain a planar ion from IV is estimated to be 6 kcal. [G. E. Humphrey and R. Spitzer, *J. Chem. Phys.*, **18**, 902 (1950)].

(6) Department of Chemistry, Yale University, New Haven, Connecticut.

PENTAVALENT OSMIUM

Sir:

When ammonium hexabromoösmate (IV) is added slowly to anhydrous ethylenediamine at 10°, the exothermal reaction yields a red solution from which micaceous pink plates of (I) separated. This contained apparently three molecules of the base and two ionized bromine atoms per atom of osmium, and acted as a weak monoacid base when titrated with hydrobromic acid to yield green needles of (II). Substance (II) contained one atom of ionized bromine more than (I), into which it was changed by alkali. Both (I) and (II) were diamagnetic, and did not show the reducing properties to be expected of Os(II) or Os(III). On reduction with sodium hydrosulfite, colorless solutions resulted which, on addition of sodium iodide, gave bright yellow plates (III) of tris-ethylenediamineosmium(III) iodide. This is concluded from its similarity to the hexammine[1] $Os(NH_3)_6I_3$, its reduction of silver nitrate, and its paramagnetism, (1.6 B.M.). Found: Os, 24.3; N, 10.8; I, 48.5. Calcd. for $Os(en)_3I_3 \cdot 2H_2O$: Os, 24.20; N, 10.69; I, 48.46. Therefore (I) and (II) are Os(IV) complexes, two or one protons, respectively, having been lost from the ethylenediamine as in the gold complexes of Block and Bailar.[2] These magnetic moments are consistent with the usual experience of Os(IV) compounds, in which in conflict with Hund's rule all of the 5d electrons are paired leaving a vacant orbital.

(1) F. P. Dwyer and J. W. Hogarth, *J. Proc. Roy. Soc. N.S.W.*, **85**, 113 (1951).

(2) B. P. Block and J. C. Bailar, THIS JOURNAL, **73**, 4722 (1951).

Found: (I) (for corresponding iodide): Os, 30.3; N, 13.55; I, 41.0. Calcd. for $(Os(en\text{-}H)_2en)I_2$, (en-H=$NH_2CH_2CH_2NH$[1]): Os, 30.56; N, 13.52; I, 40.87. Found (II): Os, 28.9; N, 12.64; Br, 36.3. Calcd. for $(Os(en\text{-}H)en_2)Br_3\cdot 3H_2O$: Os, 28.69; N, 12.68; Br, 36.20.

In anhydrous ethylenediamine at 100° in the absence of air, (I) dissolved to a red solution which became intense green in color. The green solid (IV) precipitated by alcohol lost ethylenediamine very easily with reversion to (I) and could not be obtained pure. It appeared to be $(Os(en\text{-}H)_2\text{-}en_2)I_2$. In such an 8-covalent complex, (d^4sp^3 bonds) two electrons must be promoted to the 7s or 6d orbitals—presumably the former, since (IV) was diamagnetic. In the air (IV) rapidly oxidized to yield two greenish brown substances (V), (VI) separated by crystallization from methanol and ether. The less soluble (V) was paramagnetic, (1.78 B.M.) and is probably a pentavalent osmium compound—the first recorded. Found: Os, 25.4; N, 14.7; I, 33.6; H_2O, 9.6. Calcd. for $(Os(en\text{-}H)_3\text{-}en)I_2\cdot 4H_2O$: Os, 25.25; N, 14.87; I, 33.74; H_2O, 9.57. (VI) was diamagnetic (no unpaired electrons), and appears to be an 8-covalent hexavalent osmium compound. Found: Os, 25.8; N, 15.3; I, 34.2. Calcd. for $(Os(en\text{-}H)_4)I_2\cdot 3H_2O$: Os, 25.91; N, 15.25; I, 34.60. Dilute aqueous solutions of (V) and (VI) were brown and green, respectively, reacted alkaline and accepted up to two equivalents of acid. They were interconvertible by oxidizing and reducing agents.

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RECEIVED JANUARY 10, 1953

THE COENZYME FUNCTION OF THIAMINE PYROPHOSPHATE IN PENTOSE PHOSPHATE METABOLISM

Sir:

One of the products of pentose phosphate cleavage by enzymes from red cells,[1] bacteria and yeast,[2] liver[3] and plant sources[4] has been identified as triose phosphate. The two-carbon fragment, however, has not been characterized; glycolaldehyde, the expected product, does not accumulate or react in the presence of such enzyme preparations.[2,5]

In a previous report[3] we have described the formation of sedoheptulose phosphate from pentose phosphate with an enzyme preparation from rat liver. A similar conversion has now been observed with a highly purified spinach enzyme preparation. This preparation contains substantial amounts of thiamin pyrophosphate (ThPP) in a bound form which can be separated by precipitation of the protein with ammonium sulfate at low *p*H. The inactive enzyme obtained in this manner can be almost completely reactivated by the addition of ThPP.

The ThPP content of a purified enzyme preparation, determined manometrically with the carboxylase assay of Lohmann and Schuster[6] was found to be 0.8×10^{-2} micromole per mg. of protein. On the assumption that the molecular weight is about 100,000, each mole of protein contained about 0.8 mole of ThPP. Since the enzyme preparation had been purified about 100-fold from spinach leaf extracts by a procedure which included several ammonium sulfate fractionations, dialysis, fractionation with acetone and absorption on calcium phosphate gel and elution, it is evident that the coenzyme is not readily dissociated from the protein. Essentially complete separation of the coenzyme was obtained at *p*H 3 in the presence of ammonium sulfate (45% saturated). With 10^{-4} *M* ThPP (Table I) the activity obtained represented

TABLE I

THE THPP REQUIREMENT OF THE ACID-PRECIPITATED ENZYME

In Experiment 1 pentose phosphate splitting activity was followed spectrophotometrically by measuring triose phosphate formation according to Racker.[7] The absorption cell contained 3.4×10^{-4} *M* ribulose-5-phosphate, 5.8×10^{-6} *M* reduced DPN, 0.05 mg. of crude rabbit muscle fraction containing α-glycerophosphate dehydrogenase and triose phosphate isomerase and 0.004 mg. of spinach enzyme. The total volume was 1.06 ml. In Experiment 2 sedoheptulose phosphate formation was followed with the orcinol reaction.[3] The incubation mixture contained 5×10^{-3} *M* ribulose-5-phosphate and 0.5 mg. of acid-precipitated enzyme. The total volume was 0.5 ml. Glycylglycine, buffer, 0.01 *M*, *p*H 7.4, was present in both experiments. The temperature was 25°. $MgCl_2$ and ThPP, when added, were 2×10^{-3} and 10^{-4} *M*, respectively.

Enzyme	ThPP	$MgCl_2$	Experiment 1 Triose P[a]	Experiment 2 Sedoheptulose P[b]
Original	−	−	8.1×10^{-3}	
Acid treated	+	+	6.0×10^{-3}	
Acid treated	−	+	0.8×10^{-3}	
Acid treated	+	−	1.7×10^{-3}	
Acid treated	−	−		0
Acid treated	+	+		1.1

[a] Micromoles formed per minute. [b] Micromoles formed in thirty minutes. The reaction at this time was essentially complete.

TABLE II

THE FORMATION OF PENTOSE PHOSPHATE AND HEPTULOSE PHOSPHATE FROM L-ERYTHRULOSE AND TRIOSE PHOSPHATE

The reaction mixture contained 0.02 *M* L-erythrulose,[8] 0.004 M hexosediphosphate as a source of triose phosphate, 0.047 mg. of crystalline muscle aldolase and 0.6 unit of resolved enzyme in a total volume of 0.79 ml. In the complete system 10^{-4} *M* ThPP and 10^{-3} *M* $MgCl_2$ were added. Pentose and heptulose were determined in the orcinol reaction. Amounts are in micromoles.

Time, minutes	No ThPP		Complete system	
	Pentose	Heptulose	Pentose	Heptulose
30	0	0	1.2	1.2
60	0	0	1.8	1.6
120	0	0	2.3	2.4
180	0	0	2.5	2.9

(1) Z. Dische, *Naturwiss.*, **26**, 252 (1938).

(2) E. Racker, in W. D. McElroy and B. Glass, "Phosphorus Metabolism," The Johns Hopkins Press, Baltimore, Md., 1951, Vol. I, p. 147.

(3) B. L. Horecker and P. Z. Smyrniotis, THIS JOURNAL, **74**, 2123 (1952).

(4) B. Axelrod, in W. D. McElroy and B. Glass, "Phosphorus Metabolism," The Johns Hopkins Press, Baltimore, Md., 1952, Vol. II, p. 79.

(5) Z. Dische, in W. D. McElroy and B. Glass, "Phosphorus Metabolism," The Johns Hopkins Press, Baltimore, Md., 1951, Vol. I, p. 195.

(6) K. Lohmann and P. Schuster, *Biochem. Z.*, **294**, 188 (1937).

(7) E. Racker, *J. Biol. Chem.*, **167**, 843 (1947).

(8) Kindly furnished by Dr. G. C. Mueller of the McArdle Memorial Laboratory, Madison, Wis.

71% of the original activity. Only partial reactivation occurred in the absence of Mg^{++} ions.

The spinach enzyme also catalyzes the reaction of L-erythrulose and D-glyceraldehyde-3-phosphate to form a mixture of pentose phosphate and heptulose phosphate. Neither product is formed in the absence of ThPP (Table II).

While the mechanism of sedoheptulose phosphate formation is not yet known, the reactivity of erythrulose in this system suggests that it may be formed by the reactions

2 D-ribulose-5-phosphate $\rightleftharpoons$ L-erythrulose + 2 D-glyceraldehyde-3-phosphate (1)

L-erythrulose + D-glyceraldehyde-3-phosphate $\longrightarrow$ sedoheptulose-7-phosphate (2)

An alternative mechanism, supported by the requirement for ThPP for sedoheptulose phosphate synthesis from erythrulose, would be a condensation of ribose phosphate with an active two-carbon fragment. Pentose phosphate isomerase is still present in the enzyme preparation and the participation of ribose phosphate has not been excluded. In either case an activated form of glycolaldehyde, formed in the cleavage of pentose phosphate, would undergo an acyloin condensation. The synthesis of acetoin in such reactions is known to require ThPP.[9,10] The name transketolase, suggested by Racker, de la Haba and Leder[11] is consistent with this formulation. In the presence of spinach enzyme pentose formation from erythrulose is observed with other aldehydes, such as D-glyceraldehyde and L-glyceraldehyde-3-phosphate.

(9) M. Silverman and C. H. Werkman, *J. Biol. Chem.*, **138**, 35 (1941).

(10) D. E. Green, W. W. Westerfeld, B. Vennesland and W. E. Knox, *J. Biol. Chem.*, **145**, 69 (1942).

(11) E. Racker, G. de la Haba and I. G. Leder, THIS JOURNAL, **75**, 1010 (1953).

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RECEIVED JANUARY 30, 1953

THIAMINE PYROPHOSPHATE, A COENZYME OF TRANSKETOLASE

Sir:

In a note by Horecker and Smyrniotis[1] previous work on enzymes concerned in the breakdown of pentose phosphate is quoted. We have isolated from baker's yeast a crystalline enzyme which catalyses the cleavage of ribulose-5-phosphate with the formation of D-glyceraldehyde-3-phosphate, identified by means of glyceraldehyde-3-phosphate dehydrogenase free of triose isomerase. The cleavage of ribulose-5-phosphate[2] occurs only on the addition of an "acceptor aldehyde" such as ribose-5-phosphate, glycolaldehyde, or glyceraldehyde. The enzyme was also found to decarboxylate hydroxypyruvate in the presence of an "acceptor aldehyde." With D-glyceraldehyde-3-phosphate (formed from fructose-1,6-diphosphate by aldolase) as acceptor aldehyde, the decarboxylation of hydroxypyruvate led to the formation of ribulose-5-phosphate.[3] The pentose phosphate was isolated as an alcohol insoluble barium salt and determined by two independent tests as shown in Table I. Similar results were obtained when DL-glyceraldehyde-3-phosphate (Concord Laboratories) was used instead of fructose-1,6-diphosphate and aldolase.

TABLE I

ENZYMATIC FORMATION OF RIBULOSE-5-PHOSPHATE FROM HYDROXYPYRUVATE AND TRIOSE PHOSPHATE

0.5 mg. of purified yeast transketolase (22,000 units per mg. protein) was used in these experiments. Carbon dioxide was measured manometrically. In Expt. 1, the reaction mixture (2 ml.) contained 100 micromoles of potassium phosphate (*p*H 6.5), 5 micromoles of fructose-1,6-diphosphate, 20 micrograms of aldolase, 12 micromoles of $MgCl_2$, 20 micrograms of ThPP and about 30 micromoles of sodium hydroxypyruvate. In Expt. 2, 100 micromoles of tris-(hydroxymethyl)-aminomethane (*p*H 6.9) was used instead of potassium phosphate and the concentration of fructose-1,6-diphosphate was increased to 10 micromoles. The vessels were incubated at 37° for 75 minutes in Expt. 1 and 175 minutes in Expt. 2. Deproteinization with 5% trichloroacetic acid was followed by the isolation of an alcohol-insoluble barium salt which was analyzed colorimetrically as well as spectrophotometrically. In the latter test transketolase free of pentose isomerase was used and triose phosphate formation was measured with either glycolaldehyde, glyceraldehyde, or ribose-5-phosphate as "acceptor aldehydes."

Expt.	CO_2 liberation, micromoles	Isolated ribulose-5-phosphate, micromoles	
		Orcinol reaction	Spectrophotometric
1	4.9	1.8	1.6
2	4.0	3.1	2.9

TABLE II

THIAMINE PYROPHOSPHATE REQUIREMENT OF TRANSKETOLASE

The enzyme preparation was dialyzed against 1000 volumes of 0.6% Versene in 0.02 *M* potassium phosphate of *p*H 7.4 for 20 hours and then against 1000 volumes of 0.6% Versene in 0.9% KCl for another 20 hours. The enzyme was assayed by measuring triose phosphate formation from ribulose-5-phosphate in the presence of ribose-5-phosphate as "acceptor aldehyde."

Enzyme preparation	Additions to test system	Activity (units per ml.)
Undialyzed	.....	50,000
Dialyzed for 40 hours	.....	2,000
	3 μM. $MgCl_2$	5,000
	50 μg. ThPP and 3 μM. $MgCl_2$	43,000
Dialyzed for 40 hours then left in icebox for 24 hours	.....	500
	3 μM. $MgCl_2$	1,000
	50 μg. of ThPP	7,000
	50 μg. of ThPP and 3 μM. of $MgCl_2$	40,000

Since the formation of ribulose-5-phosphate represents a ketol condensation, and no free glycolaldehyde is formed, one must assume the formation of an "active glycolaldehyde" which condenses with the "acceptor aldehyde" to form a ketosugar. The enzyme may therefore be termed a transketolase.

(1) B. L. Horecker and P. Z. Smyrniotis, THIS JOURNAL, **75**, 1009 (1953).

(2) We wish to thank Dr. B. L. Horecker for a gift of ribulose-5-phosphate.

(3) A similar reaction catalyzed by rabbit muscle mince has been described by S. Akabori, Kihachiro Uehara and I. Muramatsu, *Proc. Japan Academy*, **28**, 39 (1952).

The activity of a partially purified transketolase from *E. coli* was doubled by the addition of thiamine pyrophosphate (ThPP). No requirement for ThPP was found with a twice recrystallized preparation of the yeast enzyme, but extensive dialysis against a Versene–KCl solution caused nearly complete inactivation. Addition of magnesium chloride and of ThPP to the dialyzed enzyme restored the activity, as shown in Table II. The crystalline yeast enzyme shows no aldolase, triose isomerase or pentose isomerase activity.

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RECEIVED JANUARY 30, 1953

BIS-CYCLOPENTADIENYL DERIVATIVES OF SOME TRANSITION ELEMENTS

Sir:

The structure suggested[1] for bis-cyclopentadienyliron(II)[2] (ferrocene), in which the iron atom is symmetrically placed between two cyclopentadienyl rings, has been confirmed by X-ray crystal structure measurements.[1b,3] The original proposals[1] of this formulation were coupled with the suggestion that the electronic structure of the iron atom attains an inert gas configuration, and this idea could also be extended to the ruthenium analog $C_{10}H_{10}Ru$[4] and to the bis-cyclopentadienylcobalt(III) (cobalticinium) ion $[C_{10}H_{10}Co]^+$,[1b,5] which is isoelectronic with ferrocene.

In addition to the objection that a high negative charge would be placed on the central metal atom, the aromatic properties[6] of ferrocene make it seem most unlikely that all the π electrons of the cyclopentadienyl rings can be involved in the filling of the orbitals of the metal atom. More definite evidence against this view has now been obtained.

Bis-cyclopentadienylnickel(II) has been prepared by the reaction of cyclopentadienylmagnesium bromide with nickel(II) acetylacetonate. (*Anal.* Calcd. for $C_{10}H_{10}Ni$: C, 63.6; H, 5.3; Ni, 31.0. Found: C, 63.7; H, 5.5; Ni, 31.0). It forms dark green crystals from ligroin which decompose slowly even in absence of air and light; it sublimes above 130° but decomposes below the melting point. Solutions in an alcoholic supporting electrolyte show a polarographic anodic wave at −0.08 volt *versus* the saturated calomel electrode. The yellow solutions obtained by oxidation contain the bis-cyclopentadienylnickel(III) ion, $[C_{10}H_{10}Ni]^+$, and give precipitates with silicotungstic acid, Reinecke's salt, potassium triiodide, etc., as do the ferricinium,[1] ruthenicinium[4] and cobalticinium[5] ions. Aqueous solutions of the bis-cyclopentadienylnickel(III) ion are rather unstable, and decompose in a few minutes. An unstable dark brown crystalline picrate is obtained by mixing ether solutions of bis-cyclopentadienylnickel (II) and picric acid in presence of air (*Anal.* Calcd. for $C_{16}H_{12}N_3O_7Ni$: Ni, 14.1. Found Ni, 14.0).

On the above view, the nickel atom in bis-cyclopentadienylnickel(II) should have two electrons in excess of the krypton structure, which would be expected to occupy the 5s orbital. Magnetic susceptibility measurements show, however, that bis-cyclopentadienylnickel(II) has two unpaired electrons ($\chi^{25°}_{mol.} = +3440 \times 10^{-6}$ c.g.s.u. corrected for diamagnetic contribution, $\mu_{eff} = 2.88$ B.M.). In our view, this fact may be best accommodated by assuming that in these bis-cyclopentadienyl compounds, the metal ion utilizes three of the electrons from each of the two cyclopentadienyl anions, forming bonds involving the *s* and two *d* orbitals of the metal. In the case of Ni(II), the formation of $C_{10}H_{10}Ni$ would involve promotion of two electrons to the 4p orbitals, which must be singly occupied. Further, we have been unable to obtain a bis-cyclopentadienyl derivative of Cu(II), which has only one *d* orbital available, and cyclopentadienyl derivatives of the zinc group show properties of typical organo-metallic compounds. We have, however, been able to prepare bis-cyclopentadienyl compounds of titanium, zirconium and vanadium, where sufficient electrons are not available for completion of an inert gas configuration of the metal atom.

Bis-cyclopentadienyltitanium(IV) dibromide has been prepared by the reaction of excess cyclopentadienylmagnesium bromide with titanium tetrachloride in toluene solution. It forms dark red crystals, m.p. 240–243°, from toluene (*Anal.* Calcd. for $C_{10}H_{10}TiBr_2$: C, 35.6; H, 3.0; Ti, 14.2; Br, 47.3. Found: C, 36.0; H, 3.1; Ti, 14.3; Br, 47.3) and is diamagnetic ($\chi^{25°}_{mol} = -145 \times 10^{-6}$ c.g.s.u.). It is to some extent hydrolyzed by water, giving a yellow solution, which gives precipitation reactions similar to those of other bis-cyclopentadienyl metal ions. A crystalline picrate (m.p. 139–141°, explodes) has been isolated (*Anal.* Calcd. for $C_{22}H_{14}N_6O_{14}Ti$: Ti, 7.55. Found: Ti, 7.48). Aqueous perchlorate solutions show a polarographic cathodic wave at −0.44 volt *versus* the saturated calomel electrode. Controlled potential reduction, or reduction using a Jones reductor, produces a green solution containing the bis-cyclopentadienyltitanium(III) ion which shows a polarographic anodic wave at −0.44 volt.

The almost colorless bis-cyclopentadienylzirconium(IV) dibromide (*Anal.* Calcd. for $C_{10}H_{10}ZrBr_2$, C, 31.5; H, 2.7; Zr, 24.0; Br, 41.9. Found: C, 31.4; H, 2.7; Zr, 23.9; Br, 42.0) (m.p. 260° C. dec.) was prepared from zirconium tetrachloride and cyclopentadienylmagnesium bromide. Aqueous solutions of this compound show no polarographic reduction wave.

Vanadium tetrachloride reacts similarly, forming a dark green, rather unstable, ligroin soluble, bromide (*Anal.* Calcd. for $C_{10}H_{10}VBr_2$: V, 14.9; Br, 46.9. Found: V, 14.8; Br, 47.1) and a pale green, ligroin insoluble chloride (*Anal.* Calcd. for $C_{10}H_{10}VCl_2$: C, 47.6; H, 4.0; V, 20.2; Cl, 28.2. Found:

(1) (a) G. Wilkinson, M. Rosenblum, M. C. Whiting and R. B. Woodward, THIS JOURNAL, **74**, 2125 (1952); (b) E. O. Fischer and W. Pfab, *Z. Naturforschung*, **7B**, 377 (1952).

(2) (a) T. J. Kealy and P. L. Pauson, *Nature*, **168**, 1039 (1951); (b) S. A. Miller, J. A. Tebboth and J. F. Tremayne, *J. Chem. Soc.*, 632 (1952).

(3) (a) P. F. Eiland and R. Pepinsky, THIS JOURNAL, **74**, 4971 (1952); (b) J. D. Dunitz and L. E. Orgel, *Nature*, **171**, 121 (1953).

(4) G. Wilkinson, THIS JOURNAL, **74**, 6146 (1952).

(5) G. Wilkinson, *ibid.*, **74**, 6148 (1952).

(6) R. B. Woodward, M. Rosenblum and M. C. Whiting, *ibid.*, **74**, 3458 (1952).

C, 47.2; H, 4.0; V, 20.3; Cl, 28.0). Bis-cyclopentadienylvanadium(IV) dichloride is soluble in chloroform, ethyl acetate and alcohol; it decomposes on heating above 250°. In water it forms a green unstable solution which gives the precipitation reactions typical of bis-cyclopentadienyl metal ions. A dark green picrate (*Anal.* Calcd. for $C_{22}H_{14}N_6O_{14}V$: N, 12.8; V, 7.6. Found: N, 12.8; V, 7.7) has been precipitated from this solution. Bis-cyclopentadienylvanadium(IV) dichloride is paramagnetic with one unpaired electron ($\chi_{mol}^{23°C} = +1600 \times 10^{-6}$ c.g.s.u., corrected for diamagnetic contribution; $\mu_{eff} = 1.95$ B.M.).

The infrared absorption spectra of bis-cyclopentadienylnickel(II) and the bis-cyclopentadienyl dibromides of titanium, zirconium and vanadium are similar to those of ferrocene[1a] and ruthenocene.[4]

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RECEIVED JANUARY 15, 1953

THE ACCUMULATION OF ACETYLMETHYLCARBINOL (3-HYDROXY-2-BUTANONE) BY ACETATE-REQUIRING MUTANTS OF *NEUROSPORA CRASSA*[1]

Sir:

The biological oxidation of pyruvate to acetate occurs in a number of steps, the first of which apparently includes decarboxylation of the pyruvate with the formation of a two-carbon–enzyme complex at the oxidation level of acetaldehyde.[2] This C_2-enzyme complex is then oxidized, as a complex, after which the oxidation product can be hydrolyzed to give acetic acid. Evidence for the first step has been obtained by Schweet, *et al.*,[3] who showed that enzyme preparations forming acetate can also form acetylmethylcarbinol (AMC-3-hydroxy-2-butanone) presumably by reaction of the C_2-enzyme complex at the acetaldehyde oxidation stage.

Additional evidence relating to the initial stages of pyruvate oxidation has been obtained using a series of mutants of *Neurospora crassa* which require acetate for growth. These mutants are deficient in their ability to oxidize pyruvic acid.[4] The acetate-requiring strains 50–6[4], S34, S48, S48+sp, S210[5] accumulate a volatile substance giving a positive Voges–Proskauer reaction on standing. This substance has been identified as AMC on the basis of the following criteria: (a) distillates of media in which acetate mutants have grown give a positive Voges–Proskauer reaction; (b) no reaction is observed on treating distillates of media with hydroxylamine and nickelous chloride but a heavy red precipitate characteristic of nickel dimethylglyoxime is obtained after oxidation with ferric chloride[6] and redistillation; (c) the 2,4-dinitrophenylhydrazones of the substances, obtained by the method of Green, *et al.*,[7] from distillates of the culture media of S48 and S48+sp. (grown six days in 20 ml. of minimal medium[8] containing 20 mg. of acetic acid as the sodium salt), gave decomposition points similar to that reported for the dinitrophenylhydrazone of diacetyl (317°, reported 315°) and there was no depression of the decomposition point on mixing the derivative from S48+sp with dinitrophenylhydrazone prepared from an authentic sample of diacetyl.

Neurospora metabolizes pyruvate via a carboxylase as well as by an oxidative system.[4] This carboxylase reaction is involved in ethanol production. That the carboxylase system is not involved in the production of AMC by acetate mutants is indicated by Table I. Acetate requiring strains with both high and low carboxylase activities accumulate large amounts of AMC. Acetate independent strains do not accumulate significant amounts of AMC regardless of their alcohol production or carboxylase activity.

TABLE I

ACCUMULATION OF ACETYLMETHYLCARBINOL BY MUTANTS OF *Neurospora*

Strains grown 4 days on minimal medium[8] containing 20 mg. of acetic acid as the sodium salt. AMC was determined by the method of Westerfeld, *et al.*,[9] alcohol was determined by the method of Friedmann and Klass.[10] Carboxylase was assayed as the μl. of CO_2 evolved in 10 minutes at *p*H 5.3 from 9×10^{-2} *M* pyruvate at 37°. The extract used for carboxylase assay was the supernatant fraction of a mycelial homogenate.

	Strain			
	Acetate-requiring		Acetate-independent	
	S48	S48+sp	8a	50-8
Dry weight, mg.	38.3	28.3	71.2	87.6
Acetylmethylcarbinol accumulated g./mg., dry wt.	175	450	0.9	0.2
Ethanol accumulated mg./mg. dry wt.	0.64	0.07	1.25	0.32
Carboxylase activity μl. CO_2/10 min./mg. N	156	16	285	101

The acetate requiring mutants of *Neurospora* are apparently blocked in pyruvic acid oxidation after the formation of a C_2-enzyme complex at the acetaldehyde oxidation stage. As a result of the block preventing oxidative metabolism of the complex, the complex reacts with a C_2 compound at the acetaldehyde oxidation stage or with pyruvate to give AMC. This mechanism is consistent with, and supports, present ideas of the initial stages of pyruvate oxidation. Since at least three genetically different acetate requiring mutants accumulate AMC further studies of the biochemical differences among these mutants can be expected to help elucidate the details of pyruvate oxidation.

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CHEMICAL INTERACTIONS OF AMINO COMPOUNDS AND SUGARS. VI.[1] THE REPEATING UNIT IN BROWNING POLYMERS[2]

Sir:

Individual browning reactions were effected between glycine and each of the radioactive sugars L-arabinose-1-C^{14}, D-xylose-1-C^{14}, and D-glucose-1-C^{14}, as well as between each of these sugars in their inactive forms and glycine-1-C^{14} and glycine-2-C^{14}, respectively. The aqueous solutions, 0.125 *M* in sugar and 1.25 *M* in glycine, were heated under a nitrogen stream at 95° (short of reflux) for 90 hr. and the evolved carbon dioxide was collected as barium carbonate. The polymer selected for study was the water-soluble, non-dialyzable, brown solid fraction[1] obtained in 20–45% yields by dialysis, using a cellulose membrane, followed by concentration, precipitation with dioxane and drying under reduced pressure (0.5 mm.) at 56° for 20 hr. Elementary analyses were carried out on the polymers. The observable specific activities[3] of the polymers and of the radioactive reactants were used to calculate the relative number of each type of labeled carbon atom in the polymers and also the percentage recovery of the sugar number-one carbons in the polymers. The amount of barium carbonate arising from the glycine carbons was calculated from the activities of "infinitely-thick" plates, employing an experimentally determined factor relating these activities to the glycine activities. The amounts of carbon dioxide arising from the sugar number-one carbons were calculated from the maximum specific activities of the barium carbonate and of the sugar samples.

In agreement with Mackinney and co-workers,[4] who employed uniformly labeled D-glucose and glycine-1-C^{14}, we find that essentially all (90–100%) of the carbon dioxide formed originated in the glycine carboxyl group. The ratio of sugar number-one carbon:glycine methylene carbon:nitrogen was established as 1:1:1 in all of our polymers. The empirical formulas, relative to the glycine methylene group, were calculated and found to be $C_{6.0}H_{6.4}O_{2.0}N_{1.0}(CH_2)(CO_2H)_{0.5}$ for the glycine reaction product from D-glucose and $C_{5.0}H_{4.7}O_{1.5}N_{0.8}(CH_2)(CO_2H)_{0.3}$ for each of the products from the two pentoses. The pentose-glycine polymers were essentially identical. All three polymer fractions appeared to have arisen from an equimolar reaction between the reducing sugar and the amino acid effected without apparent carbon chain scission of the sugar, with partial decarboxylation of the amino acid, and with nearly complete trimolar dehydration of the sugar. The repeating unit for the D-glucose–glycine polymer very closely approximates $C_6H_6O_2N(CH_2)(CO_2H)_{0.5}$ while that for the pentose–glycine polymers approximates $C_5H_4ON(H_2O)_{0.5}(CH_2)(CO_2H)_{0.3}$.

Illustrative of the method used in deriving these formulas, the reaction of D-glucose-1-C^{14} (630 counts/min./mg.) and glycine yielded a polymer (610 counts/min./mg.) of analysis: C, 56.17; H, 5.60; N, 8.87. The dilution factor (D. F.) was then: D. F. = (count on polymer) (%C of reactant in labeled position)/(count on active sugar) (%C in polymer) = 0.115. Two more D. F. values, 0.060 and 0.127, were obtained from the reaction of D-glucose with glycine-1-C^{14} and glycine-2-C^{14}, respectively. Combination of these values with the empirical formula $C_{7.40}H_{8.78}NO_{2.90}$ and adjustment to unit glycine methylene carbon gave: CH_2, 1; CO_2H, 0.47; C from C_1 of D-glucose, 0.91; total C from D-glucose, 6.00; total polymer C, 7.47; N, 1.01; H, 8.86; O, 2.93; and thus $C_{6.00}H_{6.39}O_{2.01}N_{1.01}(CH_2)(CO_2H)_{0.47}$.

These repeating units approach the sugar dehydration stage represented by a 2-furaldehyde-glycine condensation product, for the pentose system, and by a 5-(hydroxymethyl)-2-furaldehyde–glycine condensation product for that from the hexose, but do not require that the furan rings are necessarily present in their intact cyclized forms.

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