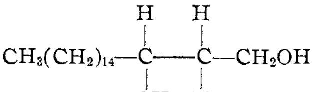
cluded that natural dihydrosphingosine has the *erythro* configuration. In conjunction with previous data on the configuration of the amino carbon of dihydrosphingosine[10] it would appear that dihydrosphingosine is *erythro*-D-1,3-dihydroxy-2-aminoöctadecane.



$$\mathrm{CH_3(CH_2)_{14}}-\overset{\mathrm{H}}{\underset{\mathrm{OH}}{\mathrm{C}}}-\overset{\mathrm{H}}{\underset{\mathrm{NH_2}}{\mathrm{C}}}-\mathrm{CH_2OH}$$

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.

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

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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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[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 3/4 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)].

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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.

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[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 $(Os(en\text{-}H)_2en)I_2$, $(en\text{-}H{=}NH_2CH_2CH_2NH^1)$: 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 $(Os(en\text{-}H)_2en_2)I_2$. 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 $(Os(en\text{-}H)_3en)I_2{\cdot}4H_2O$: 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^{-5} *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 Pentose	No ThPP Heptulose	Complete system Pentose	Complete system 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

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(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.

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

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