analysis, titration characteristics and melting point.[11] Thus it is concluded that this primary excretion product of Orinase (I) is 1-butyl-3-*p*-carboxyphenylsulfonyl-urea (II).

ADDED IN PROOF.—After submission of this paper, T. Dorfmueller, *Deut. med. Wochschr.*, **81**, 888 (1956), appeared, indicating the same finding on the structure of the Orinase excretion product.

(11) The authors wish to thank Susan Theal for the potentiometric titrations, James E. Stafford for the ultraviolet spectral studies, and Albert Lallinger for technical assistance.

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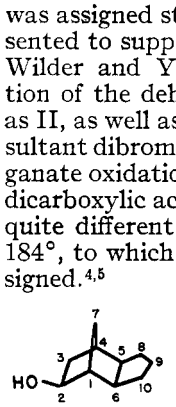
RECEIVED SEPTEMBER 4, 1956

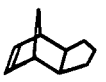
THE DEHYDRATION PRODUCT OF *exo*-TRIMETHYLENE-2-*exo*-NORBORNANOL[1]

Sir:

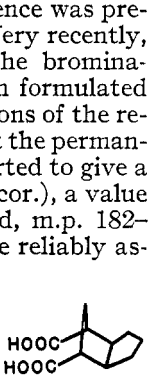
In 1948, Bruson and Riener[2] reported the phosphoric acid catalyzed dehydration of *exo*-trimethylene-2-*exo*-norbornanol (I). The olefinic product was assigned structure II, but no evidence was presented to support this assumption. Very recently, Wilder and Youngblood[3] examined the bromination of the dehydration product, again formulated as II, as well as studying several reactions of the resultant dibromide. It is significant that the permanganate oxidation of the olefin was reported to give a dicarboxylic acid of m.p. 162–163° (uncor.), a value quite different from that of the diacid, m.p. 182–184°, to which the structure III can be reliably assigned.[4,5]



I

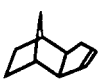


II

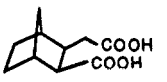


III

We wish to report evidence that the dehydration product (b.p. 760 mm.) 180.1°, n^{25}D 1.4985, when purified by distillation through an efficient column) has been incorrectly formulated as II, and in fact was *exo*-trimethylene-8-norbornene (IV). The infrared spectrum of the olefin in question was identical in all respects with that of an authentic sample of IV, b.p. 760 180.1°, n^{25}D 2.4985, whose structure can be considered to have been established rigorously.[5] Neither spectrum showed a band at 6.35 μ, possessed by all bicyclo[2.2.1]-2-heptene derivatives,[6] but rather absorbed at 6.18 μ, a value characteristic of the presence of a carbon–carbon double bond in an unstrained five membered ring. Permanganate oxidation of both samples of IV, produced by the two methods, gave the same diacid V, m.p.'s and mixed m.p. 165.1–165.6° (cor.). The dehydration product did not react with phenyl azide at room temperature indicating that it did not possess the norbornene structure.[7]



IV



V

Authentic *exo*-trimethylene-2-norbornene (II), b.p. (760 mm.) 176.0°, n^{25}D 1.4927, could be prepared easily by sodium ethoxide dehydrohalogenation of *exo*-trimethylene-2-*exo*-norbornyl iodide.[8] The spectrum of this hydrocarbon was completely different from that of IV and possessed the expected band at 6.35 μ. The phenyl azide adduct, which formed unusually rapidly, had m.p. 144.6–145.1. *Anal.* Calcd. for $C_{16}H_{19}N_3$: C, 75.85; H, 7.56; N, 16.59. Found: C, 76.09; H, 7.66; N, 16.84. Oxidation gave diacid III, m.p. 182.9–183.2°; the mixed m.p. with an authentic sample[4] of m.p. 182.8–183.2° was 183.0–183.3°.

Distillation of hydrocarbon II from phosphoric acid resulted in almost complete conversion into IV. Dehydration of other stereoisomers of alcohol I gave also the same product. Possible mechanistic interpretations of the above rearrangements as well as a discussion of some further reactions of hydrocarbons II and IV will be presented in future publications.

(1) It is suggested that the semi-trivial name "trimethylenenorbornane," numbered as in I, be utilized for the nomenclature of this series in a similar manner to that suggested for "bornane" and "norbornane," in the naming of other bicyclo[2.2.1]heptane derivatives ("Nomenclature for Terpene Hydrocarbons," No. 14, Advances in Chemistry Series, Am. Chem. Soc., Washington, D. C., 1955).

(2) H. A. Bruson and T. W. Riener, THIS JOURNAL, **70**, 2809 (1948).

(3) P. Wilder, Jr., and G. T. Youngblood, *ibid.*, **78**, 3795 (1956).

(4) H. A. Bruson and T. W. Riener, *ibid.*, **67**, 723 (1945).

(5) P. D. Bartlett and A. Schneider, *ibid.*, **68**, 6 (1946).

(6) Unpublished observations: *cf.*, P. R. Schleyer, paper presented at the 130th ACS Meeting, Atlantic City, N. J., Sept., 1956.

(7) K. Alder, G. Stein and W. Friedrichsen, *Ann.*, **501**, 1 (1933).

(8) The method used was analogous to that employed for the preparation of *exo*-dicyclopentadiene (P. D. Bartlett, and I. S. Goldstein, THIS JOURNAL, **67**, 2553 (1947)).

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FORMIMINO-TETRAHYDROFOLIC ACID AND METHENYLTETRAHYDROFOLIC ACID AS INTERMEDIATES IN THE FORMATION OF N^{10}-FORMYLTETRAHYDROFOLIC ACID

Sir:

In a previous communication[1] evidence was presented for the formation of 10-formyl-THF[2] from FIG and THF by purified extracts of *Clostridium cylindrosporum*, as shown by reaction (1)

$$\text{FIG} + \text{THF} \longrightarrow \text{10-formyl-THF} + \text{glycine} + \text{NH}_3 \quad (1)$$

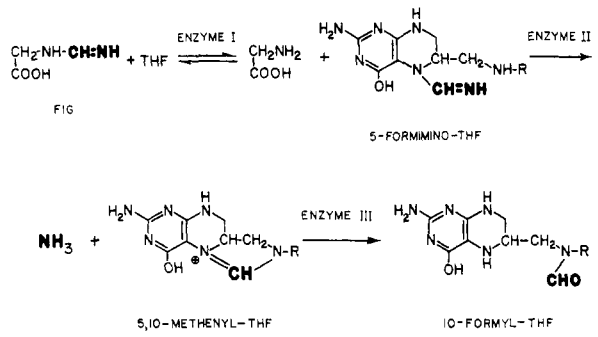
This over-all reaction has now been shown to be the sum of the three reactions, given by the equations.[3]

Enzymes I and II, acting together, are responsible for the formation of an intermediate in reaction (1) having an absorption maximum at 356 mμ and other spectral characteristics of 5,10-methenyl-THF. Evidence for the enzymatic formation of

(1) J. C. Rabinowitz and W. E. Pricer, Jr., THIS JOURNAL, **78**, 4176 (1956).

(2) Abbreviations used are: FIG, formiminoglycine; THF, tetrahydrofolic acid; 10-formyl-THF, N^{10}-formyltetrahydrofolic acid; 5-formyl-THF, N^5-formyltetrahydrofolic acid (leucovorin or citrovorum factor); 5-formimino-THF, N^5-formiminotetrahydrofolic acid; 5,10-methenyl-THF, the cyclic N^5-N^{10}-imidazolinium derivative of 5-formyl-THF, previously abbreviated as 5,10-formyl-THF[1] (anhydroleucovorin or anhydrocitrovorum factor); EDTA, ethylenediaminetetraacetic acid.

(3) R = benzoyl-L-glutamic acid.



this compound has already been presented.[1] Each of these two enzymes has been purified from the crude extract about 10-fold and is completely inactive in the following assay by itself. Either enzyme may be assayed by following the rate of increase in optical density at 356 mμ in the presence of THF, FIG, maleate buffer, EDTA and an excess of the other enzyme. The reaction rate is linear and is proportional to the enzyme concentration.

When enzyme I alone is incubated with FIG and THF, a product is formed which shows properties consistent with 5-formimino-THF. There is no release of ammonia nor are there any spectral changes in the region 260 to 400 mμ accompanying this reaction. However, a product is formed which, when treated with acid, yields 5,10-methenyl-THF and an equivalent amount of ammonia. The product formed by enzyme I becomes labeled when NH=C^{14}H—NH—CH$_2$—COOH is the substrate, but not when NH=CH—NH—CH$_2$—C^{14}OOH is used, thus confirming the transfer of the formimino group as shown in the scheme. The enzymatic reaction is readily reversible (Fig. 1) with an equilibrium constant of about 0.2. The 5-formimino rather than the 10-formimino structure is assigned to the product because the stability to oxygen and the rate of conversion of the product to 5,10-methenyl-THF by acid correspond to those shown by 5-formyl-THF and are distinct from 10-formyl-THF.[4]

TABLE I

DEGRADATION OF 5-FORMIMINO-THF BY ENZYME II

Tubes containing 50 μmoles of FIG, 5.0 μmoles of *dl*-THF (weight basis), 125 μmoles of maleate buffer at *p*H 7.0, 25 μmoles of EDTA, 31.5 γ of enzyme I and water to make to 5.0 ml. were flushed with helium and incubated at room temperature for 10 min. Enzyme II, equivalent to 8.8 γ of protein was then added.

Time after addition of enzyme II, min.	5,10-methenyl-THF,[a] μmoles	NH_3,[b] μmoles
4	0.73	0.74
10	1.19	1.27

[a] 5,10-Methenyl-THF was determined from the optical density of an aliquot at 356 mμ using a molar extinction value of 22,000.[4] Under the conditions used, 5,10-methenyl-THF is degraded non-enzymatically at a very slow rate (less than 0.01 μmoles in 10 min.).[1] [b] The reaction mixture was passed over a 2-ml. column of XE K$^+$. The column was washed with water and the adsorbed NH_3 was eluted with KOH and determined by nesslerization. Values have been corrected for 0.27 μmole of NH_3 found in a blank tube from which enzyme II, which contained no detectable NH_3 itself, was omitted.

(4) The stability in oxygen has been cited by May, *et al.*, THIS JOURNAL, **73**, 3067 (1951), and by Cosulich, *et al.*, *ibid.*, **74**, 3252 (1952), as characteristic of N-5 substituted tetrahydropteridines; in contrast, tetrahydropteridines not substituted in this position, such as THF, 10-formyl-THF and 2-amino-4-hydroxy-6-methyltetrahydropteridine, are very labile to oxygen.

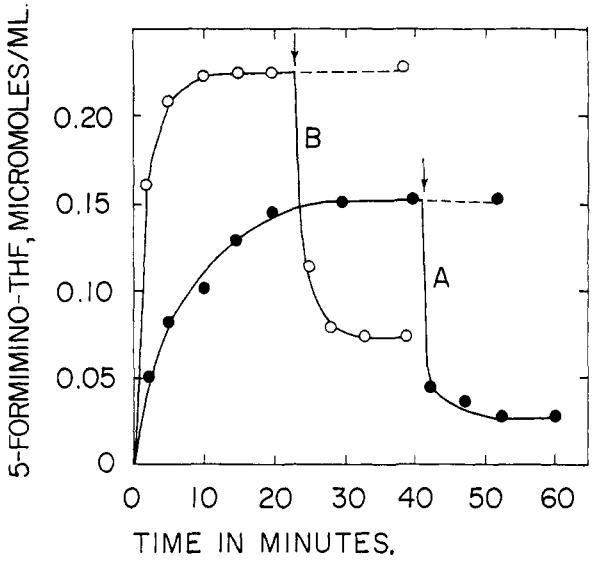


Fig. 1.—Components as indicated below were incubated at room temperature in tubes flushed with helium. Aliquots were removed at the times indicated, diluted in 0.24 *N* HCl, and heated at 100° for 50 sec. to convert the enzymatic product to 5,10-methenyl-THF. The optical density at 350 mμ was then determined using the Beckman model DU spectrophotometer. Values obtained in control tubes incubated without enzyme have been subtracted. Glycine was added as indicated by the arrow to give a final concentration of 16.6 μmoles per ml. (The broken lines indicate values obtained in the absence of glycine.) Curve A: 2.0 μmoles of FIG; 16.6 μmoles of maleate buffer at *p*H 7.0; 2.0 μmoles of EDTA; 3.3 μmoles of 2-mercaptoethanol; enzyme I equivalent to 6.9 γ of protein; and 0.22 μmole of THF,[1] when determined by this assay in the presence of an excess, 50 μmoles, of FIG, using a value of 22,000 for the molar extinction coefficient of 5,10-methenyl-THF formed,[5] or 0.66 μmole of *dl*-THF on a weight basis. Curve B: 20.0 μmoles of FIG and other additions as above.

The product formed by enzyme I yields equivalent amounts of ammonia and 5,10-methenyl-THF when treated with enzyme II (Table I). 5-Formyl-THF is inactive with either enzyme.

The enzymatic conversion of synthetic 5,10-methenyl-THF to 10-formyl-THF has already been described,[1] and it has been found that the product of enzyme II also acts as a substrate for enzyme III, as shown in the scheme. The product formed by enzyme III is converted to 5,10-methenyl-THF in the presence of acid and is destroyed by exposure to oxygen in a manner identical to that observed with 10-formyl-THF.

It is suggested that enzymes I, II, and III be called respectively: FIG formimino transferase[6]; formimino-THF cyclodeaminase; and methenyl-THF cyclohydrolase.

(5) G. R. Greenberg, L. Jaenicke and M. Silverman, *Biochim. et Biophys. Acta*, *17*, 589 (1955).

(6) An analogous enzyme which catalyzes the transfer of the formimino group of formiminoglutamic acid to THF has been purified from rabbit liver (Taber and Rabinowitz, accompanying communication); this enzyme is inactive with FIG.

Sagers, *et al.*,[7] on the basis of exchange experiments with glycine-2-C^{14} and FIG, have postulated the occurrence of a formimino transferring enzyme and the formation of formimino-THF. Miller and Waelsch[8] on the basis of kinetic experiments have suggested that 10-formimino-THF may be an intermediate in the formation of 10-formyl-THF from formiminoglutamic acid and THF.

(7) R. D. Sagers, J. V. Beck, W. Gruber and I. C. Gunsalus, THIS JOURNAL, **78**, 694 (1956).

(8) A. Miller and H. Waelsch, *Arch. Biochem. and Biophys.*, **63**, 263 (1956).

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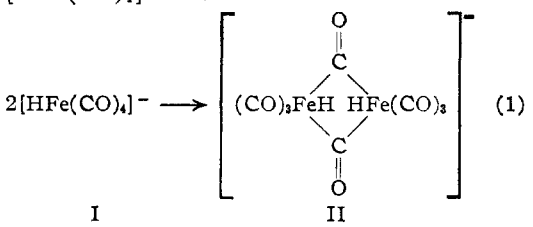
RECEIVED SEPTEMBER 6, 1956

CHEMISTRY AND CATALYTIC PROPERTIES OF THE IRON PENTACARBONYL–AQUEOUS ALKALI SYSTEM

Sir:

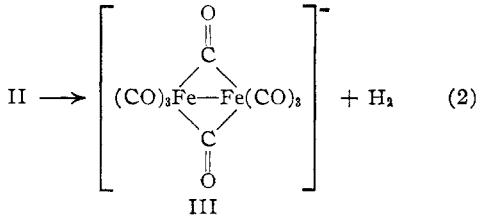
Solutions obtained by treating $Fe(CO)_5$ with aqueous alkali have intriguing chemical and catalytic properties, which include the conversion of olefins to the next higher alcohols,[1] the reduction of nitrobenzene to aniline,[2] and acetylene to ethylene.[3] We have found that these properties can be explained and new reactions predicted on the basis of the existence of a dimeric ion (II), formed from two $[HFe(CO)_4]^-$ ions:[4]



$$2[HFe(CO)_4]^- \longrightarrow [(CO)_3FeH\ HFe(CO)_3]^- \quad (1)$$

I II

Complex II decomposes according to equation (2):



$$II \longrightarrow [(CO)_3Fe{-}Fe(CO)_3] + H_2 \quad (2)$$

III

In the presence of a hydrogen acceptor, II acts as a hydrogen donor (equation 3)

$$II + \text{acceptor} \longrightarrow \text{acceptor}\cdot H_2 + III \quad (3)$$

Evidence for the existence of II and III is based on these observations: (a) On standing, the light aqueous solution containing I becomes dark red, even in the absence of oxygen,[5] and slowly gives off hydrogen. Ether extraction yields a dark-red pyrophoric solid. The iron and sodium content of this solid indicates a mixture of $NaHFe_2(CO)_8$, (IV), and $H_2Fe_2(CO)_8$, (V), *i.e.*, the acid salt and free acid corresponding to III. (b) A freshly prepared solution of II absorbs at 4750 Å., while a solution of IV and V absorbs at 5350 Å. When the solution of II is allowed to stand the band at 4750 Å. gradually is replaced by the band at 5350 Å. (c) Acidification of an aqueous solution of the mixture of IV and V proceeds according to equation (4)[6]

$$[Fe_2(CO)_8]^- + 2H^+ \longrightarrow H_2 + \tfrac{2}{3}[Fe(CO)_4]_2 \quad (4)$$

III iron tetracarbonyl

(d) Equation 3 shows that reduction is achieved by transferring hydrogen from II to the substrate. The reducing properties of these solutions were previously attributed[7] to the oxidation-reduction potential

$$3[HFe(CO)_4]^- \longrightarrow [Fe(CO)_4]_3 + 3H^+ + 6e,\ E_0 = 0.35 \text{ volt} \quad (5)$$

However, there is no evidence that iron tetracarbonyl, $[Fe(CO)_4]_3$, is formed in alkaline solutions from $[HFe(CO)_4]^-$. The action of oxidizing agents (acceptors) on alkaline solutions containing $[HFe(CO)_4]^-$ ions always leads to dark-red solutions which yield the tetracarbonyl only on acidification.[8] The need for this acidification is not apparent from (5) but follows from (3) and (4).

In view of the structural resemblance between III and dicobalt octacarbonyl, we predicted that III, or solution in which III can be formed, should catalyze the isomerization of olefinic double bonds and the addition of carbon monoxide and hydrogen to olefins in a manner similar to dicobalt octacarbonyl.[9] These predictions proved to be correct. When 1-hexene was shaken with a solution containing $[HFe(CO)_4]^-$ ion for 24 hours at room temperature, 90% was isomerized to 2-hexene and 3-hexene. Treatment of excess cyclopentene with an aqueous solution containing $[HFe(CO)_4]^-$ ions at 155° and 160 atmospheres of CO yielded 33% of cyclopentanecarboxaldehyde. This is the first report of an iron catalyzed conversion of an olefin to the next higher aldehyde.

The conversion[1] of olefins to the next higher alcohols in the $Fe(CO)_5$-aqueous alkali system can now be explained as taking place in two steps, *i.e.*, formation of the next higher aldehyde followed by hydrogenation to the alcohol. That aldehydes are reduced by solutions that catalyze the conversion of olefins to alcohols was demonstrated by treating benzalde-

(1) W. Reppe and H. Vetter, *Ann.*, **582**, 133 (1953).

(2) German Patent to I. G. Farbenindustrie Akt.-Ges., 441,179 of January 18, 1925.

(3) H. W. Sternberg, R. A. Friedel, R. Markby and I. Wender, THIS JOURNAL, **78**, 3621 (1956).

(4) The presence of $[HFe(CO)_4]^-$ ions in solutions obtained by treating $Fe(CO)_5$ with aqueous alkali was shown by P. Krumholz and H. M. A. Stettiner, *ibid.*, **71**, 3035 (1949).

(5) These solutions were considered to be stable (see W. Hieber and F. Leutert, *Z. anorg. allgem. Chem.*, **204**, 145 (1932)) and the darkening was attributed to traces of oxygen or oxidizing agents. However, dimer formation accounts for the darkening in the absence of oxygen.

(6) Acidification of the monomeric $NaHFe(CO)_4$ and $Na_2Fe(CO)_4$ leads to an entirely different reaction. Iron hydrocarbonyl is liberated, which decomposes (see W. Hieber and H. Vetter, *Z. anorg. allgem. Chem.*, **212**, 145 (1933)) according to

$$2H_2Fe(CO)_4 \longrightarrow 2H_2 + Fe(CO)_5 + Fe(CO)_3$$

In this case 1 mole of hydrogen and $1/2$ mole of $Fe(CO)_5$ and $Fe(CO)_3$ are obtained for each atom of iron present. No iron tetracarbonyl is formed.

(7) W. Hieber and W. Huebel, *Z. Elektrochem.*, **57**, 331 (1953).

(8) W. Hieber, *Z. anorg. allgem. Chem.*, **204**, 165 (1932).

(9) I. Wender, S. Metlin, S. Ergun, H. W. Sternberg and H. Green field, THIS JOURNAL, **78**, 5401 (1956).