TABLE I

EVIDENCE FOR THE ENZYMATIC FORMATION OF 5-FORMIMINO-THF AND ITS CONVERSION TO 5,10-METHENYL-THF

The incubation mixture, containing 25 μmoles of Na FIGLU, 4.8 μmoles of *dl*-THF, 60 μmoles of mercaptoethanol, 10 μmoles of K phosphate buffer (*p*H 7.2) and 100 γ of enzyme I (liver) in a final volume of 1 ml., was incubated at room temperature in an atmosphere of helium. At various times 0.05-ml. aliquots were diluted to 1 ml. with a 0.01 *M* phosphate buffer (*p*H 7.2)–0.05 *M* mercaptoethanol mixture, and the optical density determined immediately (line A). Simultaneously a similar dilution was made in another cuvette containing an excess of enzyme II (*C. cylindrosporum*), and the optical density determined after 3 minutes (line B). Line C presents the result obtained when the dilution was made in 2.3% perchloric acid, and read after 60 minutes at 365 mμ. The ΔO.D.365 mμ reported in these experiments has been corrected for an initial value of 0.103. This value did not show any change during the incubation period in simultaneous control experiments without FIGLU.

	Δ Optical density (365 mμ)[c]						
Incubation time min.	2.5	6	9	15	20	25	30
A. Enzyme I[a]	.010	.030	.030	.100	.130	.150	
B. Enzyme I[a] + Enzyme II[b]	.115	.280	.360	.490	.595	.595	
C. EnzymeI[a] + Acid							.600

[a] Source liver: the liver preparations still contain some enzyme II activity, which accounts for the slow increase in optical density on line A. The presence of enzyme II in various preparations was conveniently assayed by measuring the formation of 5,10-methenyl-THF in incubation mixtures containing an excess of formiminoglycine and enzyme I from *C. cylindrosporum*. The rate of increase at 365 mμ was proportional to the amount of enzyme II added; no ΔO.D.$_{365}$ mμ was observed in the absence of enzyme II. [b] Source: *C. cylindrosporum:* no Δ O.D.$_{365}$ mμ was observed in a comparable experiment when FIGLU and THF were incubated with this enzyme II, in the absence of enzyme I. [c] The absorption maximum was 355 mμ in line B and 350 mμ in line C, 365 mμ was arbitrarily used for following the changes in optical density reported in Table I and Fig. 1.

zyme III (from liver or *C. cylindrosporum*)[10], and in their slow non-enzymatic conversion to 10-formyl-THF at neutral *p*H, which is markedly accelerated by phosphate.

These observations on the formation of 10-formyl THF by a system involving formiminoglutamic acid explain the accumulation of formiminoglutamic acid in folic-deficient rats, and indicate a pathway for the previously-described incorporation of the isotope from (2-C^{14}-imidazole) histidine into various "one-carbon" compounds.[11]

Further work is necessary to determine the relationship of the enzymatic steps described here to the enzymatic conversion of FIGLU to formamide (in *Aerobacter aerogenes*[12a] and in *C. tetanomorphum*[12b]) and to formylglutamic acid (in *Pseudomonas*[1]).

[10] Although the enzymatic product is completely converted to 10-formyl-THF by enzyme III, only 50% of the synthetic 5,10-methenyl-THF is converted, presumably due to the racemic nature of the latter.

[11] J. C. Reid and M. O. Landefeld, *Arch. Biochem.*, **34**, 219 (1951); D. B. Sprinson and D. Rittenberg, *J. Biol. Chem.*, **198**, 655 (1952); M. Toporek, L. L. Miller and W. F. Bale, *ibid.*, **198**, 839 (1952).

[12] (a) B. Magasanik and H. R. Bowser, *J. Biol. Chem.*, **213**, 571 (1955); (b) J. T. Wachsman and H. A. Barker, *J. Bacteriol.*, **69**, 83 (1955).

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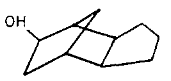
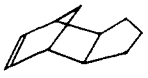
THE DEHYDRATION OF 9-HYDROXYTETRAHYDRO-*exo*-DICYCLOPENTADIENE

Sir:

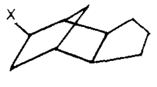
In 1948 Bruson and Riener[1] reported the dehydration of 9-hydroxytetrahydro-*exo*-dicyclopentadiene[2] (I) with phosphoric acid and suggested that the product of the reaction was 1,2-dihydro-*exo*-dicyclopentadiene (II). We have recently prepared an authentic sample of II by the action of alcoholic potash or sodium amide in liquid ammonia upon either 9-chloro-(III) or 9-bromotetrahydro-*exo*-dicyclopentadiene[3] (IV) and have found this olefin to be different from that obtained by Bruson and Riener. The structure of II was established (1) by the ready formation of a phenyl azide adduct, m.p. 140–140.5° (uncor.) (reported[4] 142°); (2) by catalytic hydrogenation to the known tetrahydro-*exo*-dicyclopentadiene; (3) by permanganate oxidation in acetone solution to the dicarboxylic acid[5] V, the *trans*-acid of which was shown to be identical with the *trans*-acid from the oxidation of 9-ketotetrahydro-*endo*-dicyclopentadiene[6]; and (4) by its identity with the olefin produced upon pyrolysis of the quaternary ammonium hydroxide VI.



I

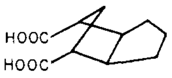


II

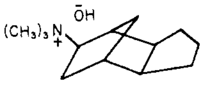


III, X = Cl
IV, X = Br

We also wish to report that the olefin which Bruson and Riener prepared by the dehydration reaction has been shown to be 9,10-dihydro-*exo*-dicyclopentadiene (VII). The infrared spectrum of this olefin is identical in every respect with an authentic sample of VII which was first described by Bruson and Riener[5] and later investigated by Bartlett and Schneider.[7] Like olefin II, that of Bruson and Riener was converted readily into tetrahydro-*exo*-dicyclopentadiene, but, unlike II, gave no adduct with phenyl azide even after four days.



V



VI

VII

Another observation of interest here is the smooth conversion of 1,2-dihydro-*exo*-dicyclopentadiene (II) into the 9,10-dihydroisomer (VII) in the presence of phosphoric acid at elevated temperature.

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

[2] Previously referred to as hydroxytetrahydro-*nor*-dicyclopentadiene (*ibid.*, **79**, 727 (1945)).

[3] G. T. Youngblood and P. Wilder, Jr., *J. Org. Chem.*, in press.

[4] K. Alder and G. Stein, *Ann.*, **504**, 240 (1933).

[5] H. A. Bruson and T. W. Riener, THIS JOURNAL, **67**, 723 (1945).

[6] K. Alder and G. Stein, *Ann.*, **485**, 223 (1931).

[7] P. D. Bartlett and A. Schneider, THIS JOURNAL, **68**, 6 (1946).