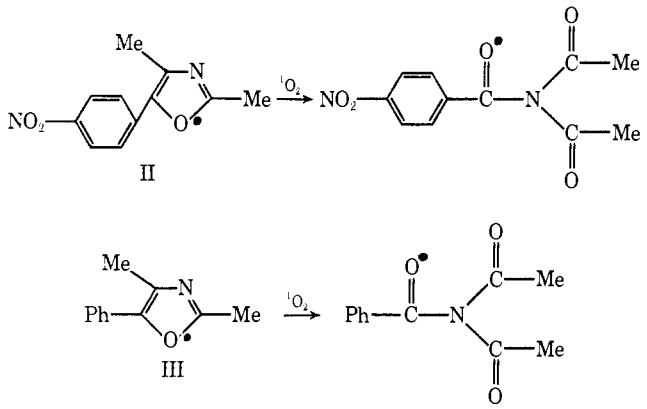
Chart II



II

III

Acknowledgments. This work was supported by National Institutes of Health Grant No. GM-13854.

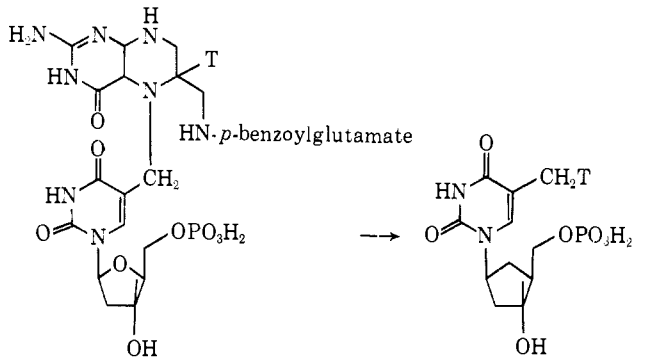
(20) NSF Predoctoral Fellow, 1970–1973.

H. H. Wasserman,* F. J. Vinick,[20] Y. C. Chang
Department of Chemistry, Yale University
New Haven, Connecticut 06520
Received July 5, 1972

A Chemical Model for Thymidylate Synthetase Catalysis

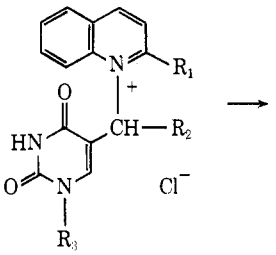
Sir:

The enzyme-catalyzed conversion of 2′-deoxyuridine 5′-phosphate (dUMP) to thymidine 5′-phosphate (**2**, TMP), a reductive methylation, utilizes formaldehyde as the carbon source and tetrahydrofolic acid (THFA) as the reducing agent.[1] In a definitive study of the mechanism of this enzymatic reaction Wahba and Friedkin proposed structure **1** as an intermediate. From their observations on the transfer of tritium from C-6 of THFA to the methyl group of TMP they suggested that **1** undergoes a 1,3 hydride shift. However, Gupta and Huennekins[2] reported that an analog of **1**, 5-thyminyltetrahydrofolic acid, was stable in air and did not undergo rearrangement to thymine when heated to 100° at pH 7.

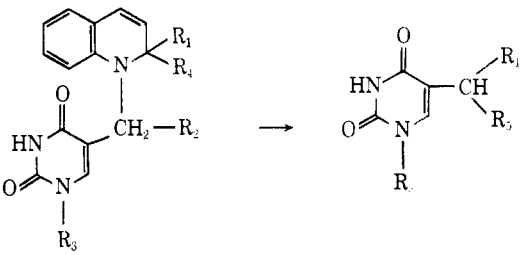


1 **2** + 7,8-dihydrofolic acid

This communication describes a chemical model in support of Wahba and Friedkin's mechanism for methylation and reduction leading to TMP catalyzed by the title enzyme. Treatment of 5-chloromethyluracil[3] with quinoline gave *N*-thyminylquinolinium chloride (**3a**, mp 278–280).[4] Reduction of **3a** with



3a, $R_1 = R_2 = R_3 = H$
3b, $R_1 = R_2 = H$; $R_3 = CH_3$
3c, $R_2 = R_3 = H$; $R_1 = {}^2H$
3d, $R_1 = R_3 = H$; $R_2 = {}^2H$



4a, $R_1 = R_2 = R_3 = R_4 = H$
4b, $R_1 = R_2 = R_4 = H$; $R_3 = CH_3$
4c, $R_2 = R_3 = H$; $R_1 = R_4 = {}^2H$
4d, $R_1 = R_3 = R_4 = H$; $R_2 = {}^2H$

5a, $R_1 = R_2 = R_5 = H$
5b, $R_1 = R_5 = H$; $R_3 = CH_3$
5c, $R_1 = R_3 = H$; $R_5 = {}^2H$

$NaBH_4$ gave 1,2-dihydro-*N*-thyminylquinoline (**4a**, mp 190–205°, mass spectral peak matching: calcd 255.10069; found 255.10078.). Heating a neat sample of **4a** to 205° resulted in rearrangement to give quinoline (49%) and thymine (**5a**, 42%).[5] Alternatively **4a** was rearranged to give **5a** (24%) by refluxing in diglyme 90 min or a 3% yield of **5a** upon 4-hr reflux of an aqueous solution of **4a**. Another analog, 1,2-dihydro-*N*-(1-methylthyminyl)quinoline (**4b**, mp 155–160°), prepared by the same method as **4a** gave 5% of 1-methylthymine (**5b**).

Quinoline-*2-d*, prepared by decarboxylation of quinaldic acid–COOD, was used for the preparation of **3c** (mp 277–278°); $NaBD_4$ reduction of **3c** gave 1,2-dihydro-*N*-thyminylquinoline-*2,2-d*$_2$ (**4c**, mp 217–223° nmr (DMSO-d_6) δ 7.2 (s, 1 H, uracil C_6H), 3.85 (s, 2 H, NCH_2 uracil). Refluxing either a 50% dioxane solution or a diglyme solution of **4c** for 48 hr gave, respectively, an 18 and 34% yield of thymine-*methyl-d* (**5c**, mp 280–285°, nmr (DMSO-d_6) δ 1.75 (s, 2 H, uracil CH_2D); mass spectrum 70 eV *m/e* (relative intensity) 128 (13), 127 (100), 126 (13)). 1,2-Dihydro-*N*-thyminyl-*methyl-d*-quinoline (**4d**, mp 190–200°, nmr (DMSO-d_6) δ 3.9 (s, 1 H, NCHD uracil), mass spectrum 70 eV *m/e* 256) was prepared from 5-formyluracil *via* reduction with

(1) (a) A. J. Wahba and M. Friedkin, *J. Biol. Chem.*, 237, 3794 (1962); (b) M. Friedkin, *Annu. Rev. Biochem.*, 32, 185 (1963); (c) R. L. Blakely, "The Biochemistry of Folic Acid and Related Pteridines," Wiley, New York, N. Y., 1967.

(2) V. S. Gupta and F. M. Huennekins, *Biochemistry*, 6, 2168 (1967).

(3) (a) R. E. Cline, R. M. Fink, and K. Fink, *J. Amer. Chem. Soc.*, 81, 2521 (1959); (b) J. H. Burckhalter, R. J. Siewald, and H. C. Scarborough, *ibid.*, 82, 991 (1960).

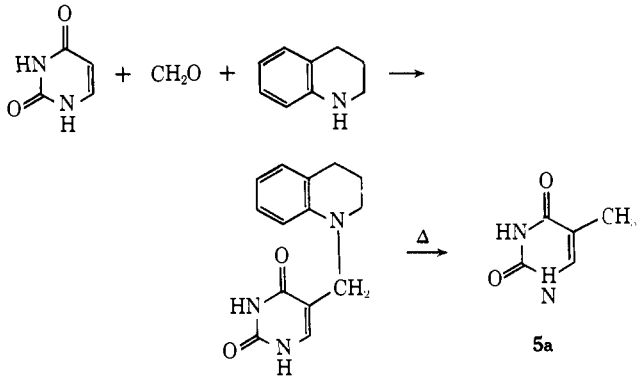
(4) All compounds with the exception of **4c** and **4d** had acceptable carbon, hydrogen, and nitrogen analyses. All compounds had the expected ir, uv, nmr, and mass spectral patterns. Thymine and its derivatives (**5d–c**) were identified by melting point, ir, and tlc.

(5) A substituted 1-methyl-1,2-dihydroquinoline derivative has been reported to yield methane and the substituted quinoline on heating: J. Meisenheimer and M. Schutze, *Ber.*, 56, 1353 (1923).

$NaBD_4$ to the alcohol, conversion to 5-chloromethyluracil-*methyl-d*, reaction with quinoline to give **3d** (mp 273–275°), and $NaBH_4$ reduction to **4d**. As in the example of **4c**, compound **4d** after heating to 205° gave thymine-*methyl-d* (**5c**, mass spectrum *m/e* (relative intensity) 128 (10), 127 (100), 126 (5)).

A crossover experiment wherein **4c** and **4d** were mixed and heated was run to establish an intramolecular process in the rearrangement. If the rearrangement went by an intramolecular process only thymine-*methyl-d* would be formed. An intermolecular process, assuming H and ^2H rearranged at the same rate, should lead to thymine, thymine-*methyl-d*, and thymine-*methyl-d*$_2$ in a ratio of 1:2:1. The mixed reaction of **4c** and **4d**, heated to 205° for 2 hr, gave thymine-*methyl-d* isolated in 6% yield (mass spectrum 70 eV *m/e* (relative intensity) 128 (27), 127 (100), 126 (38)).[6] Correcting for background it appears that the major portion of the product is derived from an intermolecular process. However, even in a neat melt of **4c** and **4d**, an intramolecular reaction does occur.

To complete the chemical model, a two-step reaction, alkylation and reduction, was performed. The first step, methylation, was accomplished by refluxing uracil, paraformaldehyde, and 1,2,3,4-tetrahydroquinoline for 72 hr in 75% ethanol to yield 1,2,3,4-tetrahydro-*N*-thyminylquinoline (**6**, 5%, mp 235–237°).



6 **5a**

Reduction to give thymine (**5a**, 10%) was done by heating **6** to 250° for 3 hr. Direct treatment of 5-hydroxymethyluracil with 1,2,3,4-tetrahydroquinoline failed to give thymine; a 3% yield of **6** was isolated.

Since the formation of thymine from uracil, formaldehyde, and a reducing agent, analogous to THFA, is chemically feasible through a bridged intermediate (**4a–d**), Friedkin's proposal employing structure **1** appears to be the most reasonable interpretation of the enzyme mechanism. An alternative mechanism, THFA reduction of the 5-hydroxymethyl derivative of dUMP, could not be confirmed in these models.

Acknowledgments. This work was supported by research Grant No. CA 7522 and Career Development Award CA 10,739 (MPM) from the National Institutes of Health and an NDEA Fellowship (R. S. W.).

(6) Authentic samples of thymine, thymine-*methyl-d*, and thymine-*methyl-d*$_2$ gave relative intensity backgrounds of the P + 1 *m/e* mass spectral peak of 10–18% of the P peak.

Raymond S. Wilson, Mathias P. Mertes*
Department of Medicinal Chemistry
The University of Kansas
Lawrence, Kansas 66044

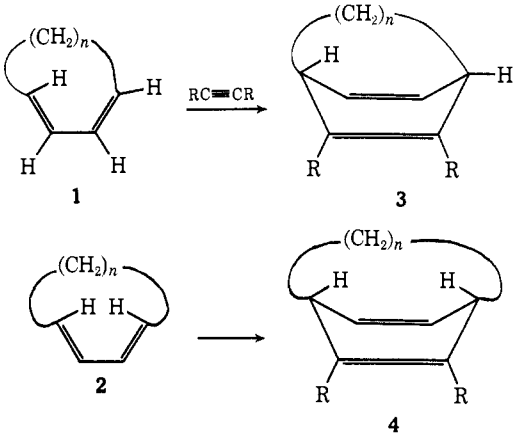
Received June 16, 1972

A Diels–Alder Approach to Inside–Outside Bicyclics[1]

Sir:

The synthesis and study of inside–outside (in–out) and inside–inside (in–in) bicyclics has been almost entirely limited to macrobicyclic systems having nitrogens at both bridgeheads.[2,3] In the form of their quaternary salts, these diazamacrobicyclics can exist in all three possible stereoisomeric forms. Since bridgehead stereochemistry has been achieved in these systems *via* reversible protonation, their method of synthesis is not readily extrapolated to the preparation of similar carbocyclic systems. We now wish to report that carbocyclic inside–outside bicyclics may be prepared through the addition of a suitable dienophile to a monocyclic *cis,trans*-1,3-diene.

In principle, concerted 2 + 4 Diels–Alder addition of dienophiles to cyclic conjugated *cis,trans*- and *trans,trans*-dienes, such as **1** and **2**, should yield inside–



outside bicyclics (**3**) and inside–inside bicyclics (**4**), respectively. In practice, this approach is complicated by the tendency of certain small cyclic 1,3-dienes, which have at least one trans double bond, to undergo 2 + 2 addition of dienophiles[4] *via* the formation of diradical intermediates. In order to avoid this complication, we chose *cis,trans*-1,3-cyclododecadiene[5,6] (**5**), which has a relatively strain-free trans double bond, and perfluoro-2-butyne (**6**), which is a powerful dienophile but a poor participator in free-radical 2 + 2 additions,[7] as our reactants. When a 2.5:1 mixture of **6**:**5** was heated in a sealed tube at 150° for 42 hr, we obtained 78% of **7** and 16% of **8**, in addition to 2% of unreacted

(1) Paper XXX on "The Chemistry of Bent Bonds." For the previous paper in this series, see P. G. Gassman and T. Nakai, *J. Amer. Chem. Soc.*, **94**, 5497 (1972).

(2) (a) H. E. Simmons and C. H. Park, *ibid.*, **90**, 2428, 2429, 2431 (1968); *Chem. Eng. News.*, 46 (July 3, 1967); C. H. Park and H. E. Simmons, U. S. Patent 3,531,468 (1970); *Chem. Abstr.*, **74**, 13189w (1971); (b) H. E. Simmons, C. H. Park, R. T. Uyeda, and M. F. Habibi, *Trans. N. Y. Acad. Sci.*, **32**, 521 (1970); (c) J. M. Lehn, J. P. Sauvage, and B. Dietrich, *J. Amer. Chem. Soc.*, **92**, 2916 (1970); *Tetrahedron Lett.*, 2885, 2889 (1969); (d) see also B. Metz, D. Moras, and R. Weiss, *Chem. Commun.*, 217 (1970).

(3) For exceptions, see ref 2b.

(4) P. G. Gassman, H. P. Benecke, and T. J. Murphy, *Tetrahedron Lett.*, 1649 (1969).

(5) *cis,trans*-1,3-Cyclododecadiene was prepared from commercially available *trans*-cyclododecene by *N*-bromosuccinimide bromination, thermal dehydrobromination, and purification by chromatography on activity 1 basic alumina. This known[6] diene gave ir, nmr, and uv spectra consistent with the assigned structure.

(6) A. W. Fawcett and J. Harris, *J. Chem. Soc.*, 2673 (1954); British Patent 1,023,540 (1966); *Chem. Abstr.*, **64**, 19447g (1966).

(7) P. G. Gassman and K. T. Mansfield, *J. Amer. Chem. Soc.*, **90**, 1517 (1968).