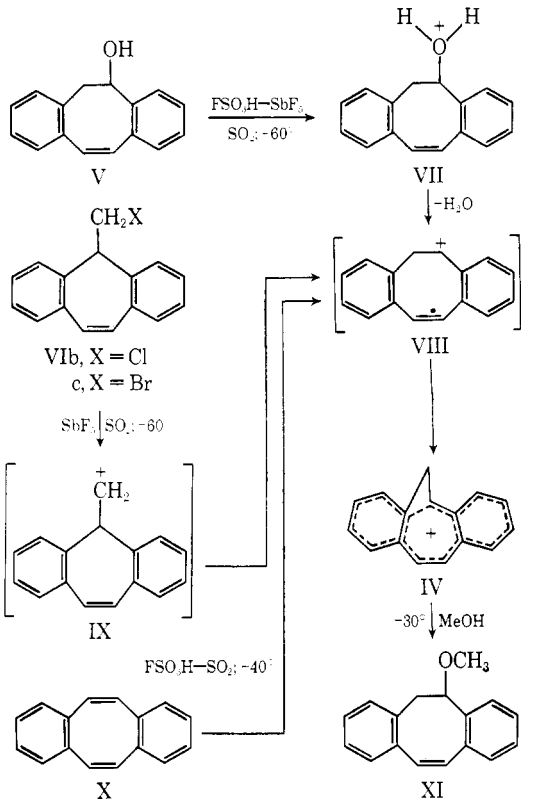
part (two lines) at 2.75 (due to a solvent effect; the nmr spectrum of V in CCl_4 shows the typical octet of an AB part). The nmr spectrum of VIb (Figure 1c) emphasizes the symmetry of the molecule; the signals of the aromatic (δ 6.98) and olefinic (δ 6.60) protons are considerably narrower and an AB_2 pattern (δ 3.40–4.40) illustrates the equivalence of the two H_B protons. The nmr spectrum of the dibenzohomotropylium cation IV (Figure 1b) exhibits dramatic changes in comparison with the spectra of the precursors. There are three major factors supporting the homotropylic structure: the striking alteration of the ABX and AB_2 patterns, the significant modification of the aromatic absorption, and the disappearance of the signals in the olefinic region.



Both ABX (Figure 1a) and AB_2 (Figure 1c) patterns become an AMX one in the spectrum of the cation IV. A large chemical shift difference ($\Delta\delta$ = 3.2 ppm) between the protons H_A (δ 1.5) and H_M (δ 4.7) is observed. As already pointed out[1-3] this difference could not be accounted for by the effect of the aromatic rings alone, and the shielding of H_A is mainly caused by the homoaromatic ring current (H_A lies above this ring while H_M is situated in the deshielding zone). The coupling constants ($J_{AM} = J_{MX}$ = 8.6 Hz and J_{AX} = 11.4 Hz) are also in agreement with previous observations.[2] H_X (δ 8.90) experiences a similar deshielding as the corresponding proton in the cation II (δ 7.92).[2] The assignments of the chemical shifts and coupling constants in the AMX system of IV were confirmed by double-resonance experiments. The dissymmetry created in the cation IV and the alteration of the electronic structure results also in the significant diversification of the aromatic lines (δ 7.2–9.3) due to alteration of both shielding and coupling constants. Finally the integration of the signals of the olefinic protons in the deshielded aromatic region strongly supports the homoaromatic character of the central ring.

Quenching of the FSO_3H–SbF_5–SO_2 or SbF_5–SO_2 solutions of the cation IV with methanol gave a high yield of methyl ether XI. The nmr spectrum of XI is very similar to that of the alcohol V and shows a splitting of the olefinic signal as an effect of the presence of the more anisotropic methoxy group.

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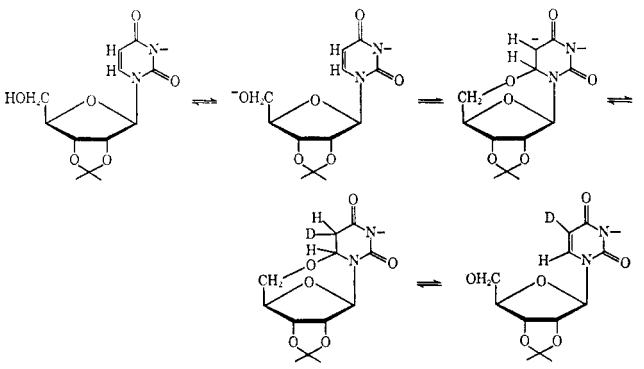
Model Studies of Thymidylate Synthetase. Neighboring-Group Facilitation of Electrophilic Substitution Reactions of Uracil Furanosides

Sir:

We report herein the initial results of our model studies of thymidylate synthetase. This enzyme catalyzes the reductive methylation of 2′-deoxyuridine 5′-monophosphate (dUMP) to give thymidine 5′-monophosphate,[1] and the exchange of the 5-H of dUMP for those of water.[2] We have observed that certain electrophilic reactions of uracil furanosides analogous to those catalyzed by the enzyme proceed exclusively by rate-determining nucleophilic attack of an ionized hydroxyl of the furanoside upon the heterocycle to form transient 6,5′- or 6,2′-anhydro nucleoside intermediates.

We initially observed that when 2′,3′-O-isopropylideneuridine (**1**) is treated with MeONa (0.474 *N*)–MeOD at 60.0°, the 5-H of the pyrimidine is exchanged for deuterium by an apparent first-order process ($t_{1/2}$ = 2.84 hr); in the absence of base no exchange is observed after as long as 2 weeks. Under identical conditions, the 5-H of 5′-deoxy-2′,3′-O-isopropylideneuridine (**2**) and 1-methyluracil (**3**) are stable for as long as 2 weeks. These data suggest that the formation of 2′,3′-O-isopropylideneuridine-5-*d* proceeds by anchimeric assistance of the 5′-oxy anion of **1**, as depicted in Scheme I. The 6,5′-anhydro cyclonucleoside inter-

Scheme I



(1) M. Friedkin, *Ann. Rev. Biochem.*, **32**, 185 (1963).
(2) M. I. S. Lomax and G. R. Greenberg, *J. Biol. Chem.*, **242**, 1302 (1967).

mediate is reminiscent of the 6,5′-cyclic episulfides formed from 5′-thiouridine and its acetonide.[3] It also bears analogy to the 6,2′-anhydro nucleosides proposed[4] as intermediates in the base-catalyzed hydrolysis of the N_3–C_4 amide of the 1-β-D-arabinofuranosides of 5-fluorouracil and 5-fluorocytosine.

In Table I are listed the half-lives and rates relative to uridine (**4**) of 5-H exchange for 0.20 *M* solutions of certain uracil-1-β-D-furanosides and 1-substituted uracils in NaOMe (0.474 ± 3%)–MeOD at 60.0°.[5] The involvement of oxy-anion participation in the rate-determining step suggests that the relative rates of 5-H exchange of these derivatives will be governed by the acidities of the participating hydroxyl group as well as proximity and orientation effects. With the exception of the 2′,3′-*cis*-glycol system of ribofuranosides,[6] p*K* values of the hydroxyl groups of furanosyl nucleosides cannot be assigned with any certainty. However, since it is unreasonable that isolated hydroxyl groups of the furanoside ring differ by more than 1 p*K* unit, rates of 5-H exchange that differ by a minimum factor of 10 must be attributed to entropy differences in entering the transition states leading to cyclonucleoside intermediates. It follows that structural differences remote from the participating hydroxyl which affect the conformation[7] of the furanoside ring and/or its stereochemical relationship to the heterocycle[8] will be reflected in the rate of 5-H exchange.

Table I. 5-H Exchange of 1-Substituted Uracils in MeONa–MeOD[a]

No.	Compd	$t_{1/2}$, hr	k_{rel}
1	2′,3′-O-Isopropylideneuridine	2.84	67
2	5′-Deoxy-2′,3′-O-isopropylideneuridine	...[b]	...
3	1-Methyluracil	...[b]	...
4	Uridine	192	1.0
5	2′-Deoxyuridine	178	1.1
6	5′-Deoxyuridine	...[b]	...
7	1-β-D-Arabinofuranosyluracil	2.58	76
8	5′-Deoxy-1-β-D-arabinofuranosyluracil	1.27	152
9	1-(3-Hydroxypropyl)uracil	31.4	6.1

[a] See ref 5 for specifications. [b] 5-H exchange was not observed after as long as 2 weeks.

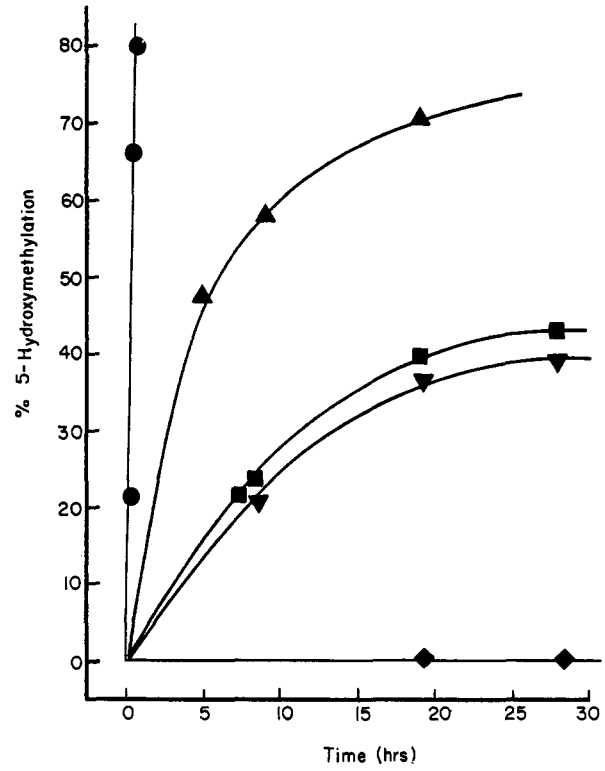


Figure 1. Extent of 5-hydroxymethylation of 1-substituted uracils: ●, **1**; ▲, **9**; ■, **4**; ▼, **5**; ◆, **3**. All reactions were kept at 60° and were 0.2 *M* in substrate, 3.4 *M* in formaldehyde, and 0.5 *N* in NaOH.

The only participating nucleophiles in the ribofuranosyl nucleosides (**1**, **4**, and **5**) are derived from the 5′-hydroxyls, as borne out by the stability of the 5-H of the 5′-deoxy nucleosides **2** and **6**. The 67-fold rate enhancement observed for **1** as compared to **4** and **5** can best be accounted for by the rigidity and skewing[7,9] imposed upon the furanoside ring by the acetonide group. A related effect has been reported[3c] in the formation of the 6,5′-cyclic episulfides from 5′-thiouridine and 5′-thio-2′,3′-*O*-isopropylideneuridine.

The twofold increase in the rate of 5-H exchange that is observed for the 5′-deoxyarabinoside (**8**) as compared to **7** identifies the 2′-"up" oxy anion as the participating group in the arabinofuranosyl nucleosides. It is also apparent that the 5′-oxy anions of the ribofuranosides **4** and **5** are much less favorable for cyclonucleoside formation than are the 2′-oxy anions derived from the arabinofuranosyl nucleosides **7** and **8**. These results are in accord with Fox and coworkers,[4] who have observed the formation of a 6,2′-cyclonucleoside with 1-β-D-arabinofuranosyl-5-fluorouracil but were unable to detect the 6,5′-cyclonucleoside derived from 5-fluorouridine. Oxy-anion participation is also evident in the case of 1-(3-hydroxypropyl)uracil (**9**) which undergoes 5-H exchange about six times faster than do the ribofuranosides **4** and **5**.

Cyclonucleoside intermediates have also been implicated in the known[10] base-catalyzed hydroxymethylation of 1-β-D-furanosyluracils, a reaction closely related to the enzyme-catalyzed formation[11] of a thymidylyltetrahydrofolic acid intermediate. Whereas 1-methyluracil (**3**) is isolated unchanged after treatment with formaldehyde in 0.5 *N* NaOH at 60° for as long as 1 week, the uracil furanosides **1**, **4**, and **5**, as well as

(3) (a) B. Bannister and F. Kagan, *J. Am. Chem. Soc.*, **82**, 3363 (1960); (b) R. W. Chambers and V. Kurkov, *ibid.*, **85**, 2160 (1963); (c) E. J. Reist, A. Benitez, and L. Goodman, *J. Org. Chem.*, **29**, 554 (1964).

(4) J. J. Fox, N. C. Miller, and R. J. Cushley, *Tetrahedron Letters*, 4927 (1966).

(5) Experiments were performed in boron-free containers kept at a temperature constancy of ±0.1°; the NaOMe titer did not change more than 2% during the extent of the reactions. Deuterium analyses of neutralized aliquots were performed with a Varian HA-100 spectrometer by a procedure that will be described in a subsequent report. Rate data were calculated from plots of the log of the concentration of the 5-H pyrimidine *vs.* time (8–12 points), which were strictly linear for 1–2 half-lives.

(6) R. M. Izatt, J. H. Rytting, L. D. Hansen, and J. J. Christensen, *J. Am. Chem. Soc.*, **88**, 2641 (1966).

(7) L. D. Hall, *Adv. Carbohydrate Chem.*, **19**, 51 (1964).

(8) T. R. Emerson, T. J. Swan, and T. L. V. Ulbricht, *Biochemistry*, **6**, 843 (1966); R. J. Cushley, K. A. Watanabe, and J. J. Fox, *J. Am. Chem. Soc.*, **89**, 394 (1967).

(9) R. J. Abraham, L. D. Hall, L. Hough, and K. A. McLauchlan, *J. Chem. Soc.*, 3699 (1962).

(10) K. H. Scheidt, *Tetrahedron Letters*, 1031 (1965); B. R. Baker, T. J. Schwan, and D. V. Santi, *J. Med. Chem.*, **9**, 66 (1966).

(11) M. Friedkin, *Fed. Proc.*, **18**, 230 (1959); F. M. Huennekens, *Biochemistry*, **2**, 151 (1963).

1-(3-hydroxypropyl)uracil (**9**), are hydroxymethylated at the 5 position of the heterocycle. Moreover, careful monitoring of the progress of the reaction (Figure 1) has demonstrated that the relative rates of hydroxymethylation parallel those of the base-catalyzed 5-H exchange, supporting the existence of cyclonucleoside intermediates.

The data presented herein offers a reasonable chemical rationalization of the catalytic role of thymidylate synthetase.[12] It is suggested that this reaction may involve activation of the 5 position of 2′-deoxyuridine 5′-monophosphate toward electrophilic attack by addition of a nucleophilic group of the enzyme to the 6 position which can be eliminated in a subsequent step. Further investigations of possible mechanistic features of this enzyme are in progress.

Acknowledgment. This work was supported by Public Health Service Research Grant No. CA-10499. We also express our gratitude for the helpful suggestions of B. R. Baker and T. C. Bruice.

(12) Our data do not support a previous proposal[2] which involves covalent bond formation between the 5 position of dUMP and the enzyme or the cofactor, 5,10-methylenetetrahydrofolic acid.

(13) National Science Foundation Predoctoral Trainee, 1966–present.

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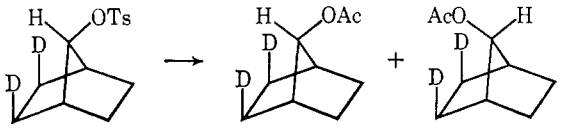
The 7-Norbornyl Cation. Nonclassical or Nonplanar?

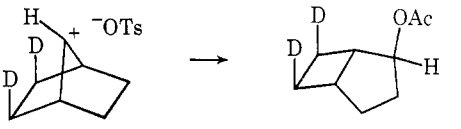
Sir:

The acetolysis of *exo,exo*-2,3-dideuterio-*anti*-7-tosyloxybicyclo[2.2.1]heptane (**1**) was recently reported to yield a mixture of **2** and **3** with 90% retention of stereochemistry.[1,2] Among the possible mechanistic explanations suggested for this unusual case of retention of stereochemistry were formation of a delocalized carbonium ion[1] and competitive frontside and backside displacement by solvent on the initially formed ion pair.[2] A definitive choice could not be made between these alternate mechanisms due to the limited experimental data. We report here the details of an extensive study of this intriguing system which indicate that competitive frontside and backside displacement on an initially formed ion pair is an inadequate explanation of the observed phenomenon.



1 **2** **3**

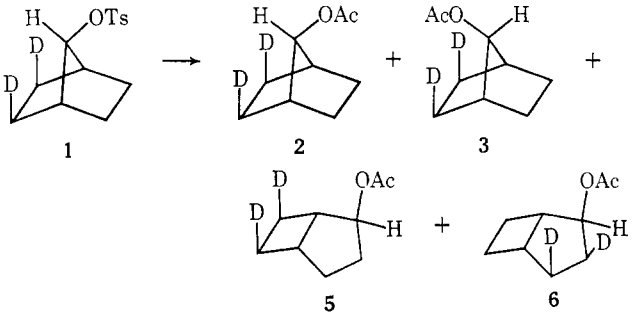
As demonstrated by Winstein and coworkers, the acetolysis of 7-tosyloxybicyclo[2.2.1]heptane yields small amounts (3–5%) of *exo*-2-acetoxybicyclo[3.2.0]heptane in addition to 7-acetoxybicyclo[2.2.1]heptane.[3] If a single intermediate, such as ion pair **4**, was involved in this reaction, only the alkyl chain *trans*-antiparallel to the leaving tosylate function should migrate since the orbital arrangement is unsuitable for migration of the "*cis*" alkyl chain. By utilizing the deuterium labels



4 **5**

on **1** we should be able to test whether **4** is the sole intermediate involved in the formation of **2** and **3**, since if **4** were the only intermediate, **5** should be the only rearranged acetate.

When **1** was solvolyzed in acetic acid buffered with sodium acetate, a mixture of 7-acetoxybicyclo[2.2.1]-heptane (97% of the product) and *exo*-2-acetoxybicyclo-[3.2.0]heptane (3% of the product) was obtained. The mixture of **2** and **3** was separated from the 2-acetoxybicyclo[3.2.0]heptane by preparative vpc. Analysis of these samples by quantitative infrared spectroscopy showed that the ratio **2**:**3** was (90 ± 3):(10 ± 3), as previously reported.[1,2] However, *the 2-acetoxybicyclo[3.2.0]heptane did not consist of a single deuterium-labeled isomer as would be predicted if* **4** *were the only precursor.* Instead, quantitative infrared analysis *vs.* authentic samples of **5** and **6**[4] showed that this sample consisted of a mixture of 95 ± 3% **5** and 5 ± 3% **6**. The presence of **6** in the reaction media precludes the intermediacy of a single ion pair such as **4**.[5] The formation of **6** from an ion-pair precursor would require the internal return of **4** to **7**[6] and ionization of **7** to **8**. Since it has been shown[2] that internal return of **8** to **7** should be two to three times faster than collapse with solvent, the intermediacy of **8** should be reflected



1 **2** **3** **5** **6**

(1) P. G. Gassman and J. M. Hornback, *J. Am. Chem. Soc.*, **89**, 2487 (1967).

(2) F. B. Miles, *ibid.*, **89**, 2488 (1967); **90**, 1265 (1968).

(3) S. Winstein, F. Gadient, E. T. Stafford, and P. E. Klinedinst, Jr., *ibid.*, **80**, 5895 (1958).

(4) The details of the synthesis of **5** and **6** will be presented in a full paper on this topic. The analyses were carried out by preparing authentic mixtures of **2** and **3** and of **5** and **6** starting with pure samples of **2**, **3**, **5**, and **6**. The methods of preparation of **2**, **3**, **5**, and **6** were all different, and the synthetic routes used precluded the possibility of any contamination of any of these samples by small amounts of any of the other materials being analyzed. Since **2** and **1**, and **3** and **7**, had common precursors, respectively, it follows that **1** and **7** were also epimerically pure.

The authentic mixtures used for the quantitative infrared analysis were made up in 5% increments. Two different peaks in the authentic mixtures were compared with the same peaks from the solvolysis mixture. Both visual comparison of the spectra and plots of concentration *vs.* peak intensity ratios were used in the determinations. Since the change in the peak intensity ratios was relatively large, the experimental error was minimized. We feel that the actual error in the determinations was considerably less than the stated ±3%.

(5) In order to ensure that an unexpected deuterium isotope effect was not playing a part in determining the product composition, the epimeric tosylate **7** was solvolyzed under the same conditions as **1**. It gave **2** and **3** in the ratio (10 ± 3):(90 ± 3), respectively, and **5** and **6** in the ratio (5 ± 3):(95 ± 3), respectively. Since identical results were obtained from the two epimerically pure tosylates, the solvolysis of **7** served as a check both of the method of analysis and of the experimental results.

(6) For a recent example of tosylate inversion see A. Streitwieser, Jr., and T. D. Walsh, *J. Am. Chem. Soc.*, **87**, 3686 (1965).