Res. 4, 353.
Riordan, J. F., and Vallee, B. L. (1972), *Methods Enzymol. 25*, 515.
Shlyapnikov, S. V., and Karpeisky, M. Y. (1969), *Eur. J. Biochem. 11*, 424.
Sokolovsky, M., Fuchs, M., and Riordan, J. F. (1970), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 7*, 167.
Sokolovsky, M., Harell, D., and Riordan, J. F. (1969), *Biochemistry 8*, 4740.
Sokolovsky, M., Riordan, J. F., and Vallee, B. L. (1966), *Biochemistry 5*, 3582.
Steers, E., Craven, G. R., and Anfinsen, C. B. (1965), *J. Biol. Chem. 240*, 2478.
Torchinsky, Y. M., Zufarova, R. A., Agalarova, M. B., and Severin, E. S. (1972), *FEBS (Fed. Eur. Biochem. Soc.) Lett. 28*, 302.
Turano, C., Giartosio, A., Riva, F., and Fasella, P. (1964), *Biochem. Biophys. Res. Commun. 16*, 221.
Watanabe, T., and Wada, H. (1971), *Biochem. Biophys. Res. Commun. 43*, 1310.

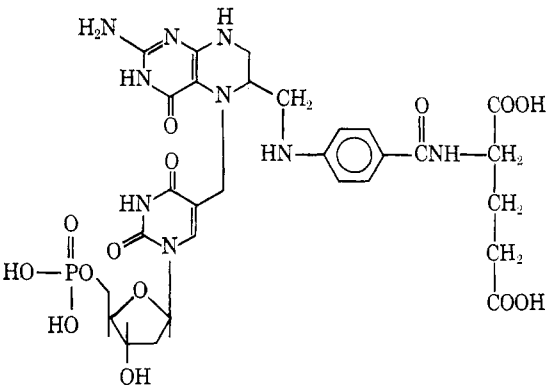
A Chemical Model for Thymidylate Synthetase Catalysis†

Raymond S. Wilson‡ and Mathias P. Mertes*

ABSTRACT: In the thymidylate synthetase catalyzed reductive methylation of 2′-deoxyuridine 5′-phosphate to give thymidine 5′-phosphate, 5-thymidylyltetrahydrofolic acid has been proposed as an intermediate which rearranges to give the observed products. However, an analog of the proposed intermediate, 5-thyminyltetrahydrofolic acid, was reported to be stable to air and did not undergo rearrangement to thymine when heated to 100° at pH 7 (Gupta, V. S., and Huennekens, F. M. (1967), *Biochemistry 6*, 2168). A chemical model is described in this paper which provides chemical precedence for the rearrangement of the proposed enzymatic intermediate. Heating 1,2-dihydro-*N*-thyminylquinoline to 200° under vacuum produced thymine and quinoline in 42 and 47% yields, respectively. The rearrangement could also be effected in refluxing solvents such as diglyme or water. When 1,2-dihydro-*N*-thyminylquinoline-*2,2-d*$_2$ was rearranged, thymine containing one deuterium in the methyl group was isolated. No exchange of the migrating deuterium with solvent occurred when the latter rearrangement was conducted in an aqueous medium. A crossover experiment using two deuterium-labeled model compounds provided evidence for both intramolecular and intermolecular rearrangements in these model compounds. In an extension of the model, 1,2,3,4-tetrahydro-*N*-thyminylquinoline was prepared in one step from uracil, formaldehyde, and 1,2,3,4-tetrahydroquinoline. This latter model compound produced thymine when heated to 250° under vacuum. This provides a chemical model in which uracil can be converted to thymine through a bridged intermediate similar to that proposed in the literature.

Thymidylate synthetase catalyzes the reductive methylation of dUMP[1] to give dTMP utilizing formaldehyde as the carbon source and H_4folate as the reducing agent (Friedkin, 1963; Blakely, 1967). The vital role of thymidylate synthetase in the biosynthesis of DNA has made it an attractive target enzyme in the chemotherapy of cancer (Hartmann and Heidelberger, 1961; Wolberg, 1969). Considerable interest has been focused upon determining the mechanism of this transformation which would greatly facilitate the rational design of inhibitors of the enzyme (Baker, 1967; Santi, 1967).

Extensive tritium labeling studies have shown that the hydrogen on C-6 of H_4folate is transferred exclusively to the methyl group of dTMP and does not exchange with the reaction medium during the transfer (Pastore and Friedkin, 1962; Lorenson *et al.*, 1967). A mechanism proposed by



1

Friedkin which is in agreement with the labeling studies involves the formation of 5-thymidylyltetrahydrofolic acid (**1**) as an intermediate which would then undergo rearrangement *via* a 1,3-hydride shift to give the observed products (Friedkin and Kornberg, 1957; Wahba and Friedkin, 1962).

The finding that thymidylate synthetase catalyzes the exchange of the 5-H of dUMP with water and that this occurs at maximum velocity only when all the components of the enzymatic reaction are present has been interpreted as supporting such a two-step mechanism (Lomax and Greenberg, 1967). However, 5-thyminyltetrahydrofolic acid, an analog

† From the Department of Medicinal Chemistry, University of Kansas, Lawrence, Kansas 66044. *Received January 22, 1973*. This work was supported by Research Grant No. CA 7522 and Career Development Award No. CA 10,739 (M. P. M.) from the National Institutes of Health and an NDEA Fellowship (R. S. W.).

‡ Present address: NIAMDD-LC, National Institutes of Health, Bethesda, Md. 20014.

[1] Abbreviations used are: dUMP, 2′-deoxyuridine 5′-monophosphate; dTMP, thymidine 5′-monophosphate; H_4folate, tetrahydrofolic acid.

of Friedkin's proposed intermediate **1**, was reported to be stable in air and did not undergo rearrangement to thymine when heated to 100° at pH 7 (Gupta and Huennekens, 1967).

Herein we report a series of models similar to the one described by Gupta and Huennekens except for the use of a quinoline for the pteridine. When these were heated at higher temperatures the expected products and labeling pattern were observed supporting Friedkin's mechanism for the reductive methylation of dUMP. A preliminary account of these results has appeared (Wilson and Mertes, 1972).

Materials and Methods

All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Infrared (ir) spectra were measured with a Beckman IR10, ultraviolet (uv) spectra with a Cary 14 recording spectrophotometer, nuclear magnetic resonance (nmr) spectra with a Varian Model T-60 and a Varian Model A-60, and mass spectra with a Varian CH-5. Microanalyses were run on an F&M-Model carbon, hydrogen, and nitrogen 185 analyzer. Routine identification of thymine was performed on silica gel thin layer chromatography (tlc) with chloroform–methanol (4:1) as eluent.

5-Chloromethyluracil-methyl-d (**2c**). 5-Formyluracil (Trahanousky *et al.*, 1965) (0.500 g, 3.6 mmol) was dissolved in 18 ml of boiling H_2O and then 15 ml of MeOH was added. As the cooling solution just began to turn cloudy, $NaBD_4$ (0.038 g, 0.9 mmol) was added with vigorous stirring. After 20 min of stirring at room temperature, 0.019 g (0.45 mmol) of additional $NaBD_4$ was added. A final fraction of $NaBD_4$ (0.005 g, 0.1 mmol) was added 20 min later and the mixture was stirred for an additional hour. The mixture was then evaporated to give 5-hydroxymethyluracil-*methyl-d* as a white solid.

The crude 5-hydroxymethyluracil-*methyl-d* (0.500 g, 3.5 mmol) was dissolved in 5 ml of concentrated HCl and stirred at room temperature for 0.5 hr. The precipitate which appeared was collected on a sintered glass filter and washed with 2 ml of concentrated HCl and then with 20 ml of anhydrous ether to give 0.230 g (40%) of **2c** as a white powder. The crude product was used for subsequent reactions. For spectral analysis of the deuterium content, 0.025 g of white powder was refluxed in absolute EtOH for 10 min and allowed to stand overnight at room temperature to give the 5-ethoxymethyl derivative. Evaporation of the solvent afforded a white solid: mp 210–212° (lit. 212–214° (Carbon, 1960) for unlabeled 5-ethoxymethyluracil); nmr (Me_2SO-d_6) δ 11.1 (br, 2 H, NH), 7.4 (d, 1 H, $J_{1,6}$ = 6 Hz, C_6H), 4.1 (s, 1 H, CHD), 3.4 (q, 2 H, J = 7 Hz, OCH_2), 1.1 (t, 3 H, J = 7 Hz, CH_2CH_3); mass spectrum (70 eV) *m/e* (relative intensity) 171 (2), 142 (68), 127 (100), 126 (50), 99 (50), 83 (89).

Quinoline-2-d (**3b**). Quinaldic acid (1.00 g, 5.8 mmol) was added to D_2O (10 ml) and a catalytic amount of Na_2CO_3 (2 mg) was added. This was heated to near refluxing overnight under a N_2 atmosphere. The D_2O was then removed by lyophilization. Nmr ($CDCl_3$) indicated that the exchange was not complete, so another 10 ml of D_2O was added and again heated overnight. After removing the D_2O by lyophilization, the nmr ($CDCl_3$) indicated complete exchange of the carboxylic proton. This solid, quinaldic acid-*d* was placed in a distillation apparatus and immersed in a Wood's metal bath heated to 210–220°. After 2 min at this temperature the solid had completely melted and turned dark purple. The distillation apparatus was connected to an aspirator and the quinoline-*2-d* was allowed to distill into a flask immersed in Dry Ice and acetone, yielding 0.512 g of slightly orange quinoline-*2-d* (**3b**). The distillation flask and condenser were then washed with $HCCl_3$ (75 ml). The $HCCl_3$ was washed with 0.1 N NaOH (50 ml) and H_2O (50 ml) and dried (Na_2SO_4), and the solvent was removed to give an additional 0.100 g of slightly darker orange product; the total yield was 0.612 g (80%); mass spectrum (70 eV) *m/e* (relative intensity) 131 (23), 130 (100), 129 (38), 103 (47), 102 (33), 76 (25); nmr ($CDCl_3$) no absorption at δ 8.9, 8.3–7.0 (m, aromatic); ir (neat) 3080, 2240, 2260, 1300, and 760 cm^{-1}.

N-Thyminylquinolinium Chloride (**4a**). To a stirred solution of 5-chloromethyluracil (**2a**) (Giner-Sorolla and Medreck, 1966) (4.523 g, 0.028 mol) in 50 ml of anhydrous dimethylformamide was added 5.418 g (0.042 mol) of quinoline (**3a**). After stirring at room temperature for 48 hr, the precipitate was removed by filtration and washed well with Et_2O. This afforded 7.17 g (87%) of a white powder, mp 270.5–272°. Two recrystallizations from acetic acid–H_2O (10:1) gave 3.97 g (49%) of **4a** as colorless crystals: mp 278–280°; uv λ^{H_2O} 262 (ϵ 7130), 218 mμ (ϵ 6860); ir (KBr) 3030, 1710, and 1690 cm^{-1}; nmr (D_2O) δ 9.55 (br d, 1 H, $J_{2,3}$ = 5 Hz, $N^+{=}CH$), 9.25 (br d, 1 H, $J_{3,4}$ = 8 Hz), 8.6–7.3 (m, 6 H), 5.95 (s, 2 H, N^+CH_2).

Anal. Calcd for $C_{14}H_{12}N_3O_2Cl$: C, 57.98; H, 4.17; N, 14.49. Found: C, 58.05; H, 4.26; N, 14.45.

N-(1-Methylthyminyl)quinolinium Chloride (**4b**). 1-Methyl-5-chloromethyluracil (**2b**) (Santi and Pogolotti, 1971) (0.230 g, 0.0013 mol) was dissolved in 2 ml of anhydrous dimethylformamide with stirring under a N_2 atmosphere; quinoline (**3a**, 0.540 g, 0.0042 mol) was added in one portion. After stirring overnight at room temperature, the mixture was diluted with 10 ml of ether and the solid removed by filtration and then washed with more ether. This gave 0.330 g (85%) of white powder, mp 244–247°. Two recrystallizations from EtOH–EtOAc gave 0.200 g (51%) of **4b** as white crystals: mp 253.5–255°; uv λ^{EtOH} 270 (ϵ 8490), 318 mμ (ϵ 7430); ir (KBr) 3470, 3350, 3000, 1735, 1670, 1475, 1380, and 1350 cm^{-1}; nmr (D_2O) δ 9.67 (br d, 1 H, J = 5 Hz, $N^+{=}CH$), 9.35 (br d, 1 H, J = 8 Hz), 8.9–7.9 (m, 6 H), 6.2 (s, 2 H, NCH_2), 3.65 (s, 3 H, NCH_3).

Anal. Calcd for $C_{15}H_{14}N_3O_2Cl$: C, 59.26; H, 4.64; N, 13.82. Found: C, 59.18; H, 4.86, N, 13.95.

N-Thyminylquinolinium-2-d Chloride (**4c**). 5-Chloromethyluracil (**2a**, 0.512 g, 3.94 mmol) and quinoline-*2-d* (**3b**, 0.650 g, 4.05 mmol) were treated as described in the preparation of **4a** to give 0.950 g (83%) of **4c** as a white powder, mp 277–278°. An analytical sample was prepared by recrystallization from AcOH–H_2O (10:1) to give colorless crystals: mp 288–291°; nmr (D_2O) δ 9.3 (d, 1 H, J = 8 Hz), 8.8–8.0 (m, 6 H), 6.1 (s, 2 H, NCH_2); ir (KBr) 2900, 1720, and 1685 cm^{-1}.

Anal. Calcd for $C_{14}H_{11}DN_3O_2Cl$: C, 57.78; N, 14.44. Found: C, 58.08; N, 14.27.

N-Thyminyl-methyl-d-quinolinium Chloride (**4d**). Quinoline (**3a**, 0.200 g, 1.55 mmol) and 5-chloromethyluracil-*methyl-d* (**2c**, 0.205 g, 1.27 mmol), treated as described in the preparation of **4a**, gave 0.296 g (80%) of white powder, mp 273–275°. Recrystallization from AcOH–H_2O (10:1) gave an analytical sample of **4d** as pinkish crystals: mp 285–287°; nmr (D_2O) δ 9.55 (d of d, 1 H, $J_{2,4}$ = 1.5 Hz, $J_{3,4}$ = 8 Hz, C_4-H), 8.9–7.5 (m, 6 H), 6.0 (s, 1 H, $NCHD$); ir (KBr) 3030, 1715, and 1685 cm^{-1}.

Anal. Calcd for $C_{14}H_{11}DN_3O_2Cl$: C, 57.78; N, 14.44. Found: C, 57.42; N, 14.39.

1,2-Dihydro-N-thyminylquinoline (**5a**). *N*-Thyminylquino-

linium chloride (**4a**) (0.300 g, 1 mmol) was dissolved with heating in 3 ml of distilled H_2O and 10 ml of MeOH was added. $NaBH_4$ (0.075 g, 0.002 mol) was added in one portion and the mixture was stirred at room temperature under a N_2 atmosphere for 4 hr. The light yellow precipitate which appeared was removed by filtration and washed with several portions of MeOH under a constant stream of N_2. This gave 0.200 g (67%) of light yellow powder: mp 190–205°; uv λ^{EtOH} 351 (ϵ 2500), 262 (ϵ 7800), 231 mμ (ϵ 31,200); nmr (Me_2SO-d_6) δ 7.2 (s, 1 H, uracil C_6-H), 7.1–6.1 (m, 5 H), 5.7 (d of t, 1 H, $J_{2,3}$ = 3 Hz, $J_{3,4}$ = 10 Hz, NCH_2CH), 4.2 (m, 2 H, NCH_2CH), 3.95 (s, 2 H, NCH_2 uracil); mass spectrum (70 eV) *m/e* (relative intensity) 255 (2), 130 (100), 129 (91), 126 (17), 102 (30), 83 (9), 82 (9), 55 (17), 54 (4); mass spectrum peak matching calcd, 255.10069; found, 255.10078; ir (KBr) 3230, 1710, and 1670 cm^{-1}.

Anal. Calcd for $C_{14}H_{13}N_3O_2$: C, 65.87; H, 5.13; N, 16.46. Found: C, 65.64; H, 4.95; N, 16.48.

1,2-Dihydro-N-(1-methylthyminyl)quinoline (**5b**). A saturated solution of *N*-(1-methylthyminyl)quinolinium chloride (**4b**) (0.300 g, 1 mmol) in 2 ml of MeOH was treated by slowly adding $NaBH_4$ (0.075 g, 2 mmol) and the mixture was stirred for 1 hr under a N_2 atmosphere. After cooling the mixture in an ice bath, the white precipitate was collected by filtration and washed with several portions of cold MeOH under a constant stream of N_2. This gave 0.177 g (66%) of white powder; mp 155–160°; uv λ^{EtOH} 351 (ϵ 2780), 272 (ϵ 10,850), 231 mμ (ϵ 36,500); nmr (Me_2SO-d_6) δ 7.5 (s, 1 H, uracil C_6-H), 7.1–6.1 (m, 5 H), 5.65 (d of t, 1 H, $J_{2,3}$ = 3 Hz, $J_{3,4}$ = 10 Hz, NCH_2CH), 4.2 (m, 2 H, NCH_2CH), 3.95 (s, 2 H, NCH_2 uracil), 3.2 (s, 3 H, NCH_3); mass spectrum (70 eV) *m/e* (relative intensity) 269 (9), 139 (41), 131 (48), 130 (100), 129 (46), 96 (61), 55 (33), 42 (38); ir (KBr) 3020, 1713, and 1665 cm^{-1}.

Anal. Calcd for $C_{15}H_{15}N_3O_2$: C, 66.90; H, 5.61; N, 15.60. Found: C, 67.18: H, 5.60; N, 15.49.

1,2-Dihydro-N-thyminylquinoline-2,2-d_2 (**5c**). *N*-Thyminylquinolinium-*2-d* chloride (**4c**, 0.200 g, 0.67 mmol) dissolved in 4.4 ml of H_2O and 5 ml of MeOH was treated with $NaBD_4$ (0.043 g, 1.0 mmol) as described in the preparation of **5a** to give 0.149 g (87%) of **5c**: mp 217–223°; nmr (Me_2SO-d_6) δ 7.2 (s, 1 H, uracil C_6-H), 7.0–6.1 (m, 5 H), 5.6 (d, 1 H, $J_{3,4}$ = 10 Hz, NCD_2CH), 3.85 (s, 2 H, NCH_2 uracil); mass spectrum (70 eV) *m/e* (relative intensity) 257 (3), 132 (100), 131 (60), 130 (40); ir (KBr) 3200, 1690, and 1485 cm^{-1}.

1,2-Dihydro-N-thyminyl-methyl-d-quinoline (**5d**). *N*-Thyminyl-*methyl-d*-quinolinium chloride (**4d**, 0.100 g, 0.34 mmol) was dissolved in 1.5 ml of H_2O with gentle heating; 4 ml of MeOH was added followed quickly by the addition of $NaBH_4$ (0.025 g, 0.66 mmol). Treatment as in **5a** afforded 0.065 g (75%) of a yellow powder: mp 190–200°; nmr (Me_2SO-d_6) δ 7.2 (s, 1 H, uracil C_6-H), 7.1–6.1 (m, 5 H), 5.7 (d of t, 1 H, $J_{2,3}$ = 3 Hz, $J_{3,4}$ = 10 Hz, C_3-H), 4.2 (m, 2 H, C_2-H_2) 3.9 (s, 1 H, $NCHD$); mass spectrum (70 eV) *m/e* (relative intensity) 256 (2), 130 (100), 129 (96), 130 (18), 77 (18); ir (KBr) 3500, 1708, and 1670 cm^{-1}.

Rearrangement of 1,2-Dihydro-N-thyminylquinoline (**5a**).

METHOD A. An analytically pure sample of 1,2-dihydro-*N*-thyminylquinoline (**5a**, 0.153 g, 0.6 mmol) was placed in a microsublimation apparatus fitted with a trap immersed in Dry Ice–acetone. The system was evacuated and the sublimation apparatus immersed in a Wood's metal bath heated to 180°. The temperature was then raised to 205 ± 5° for for 2.5 hr. One half-hour after heating began, steam was passed through the condenser of the sublimation apparatus for the duration of the heating. After cooling the system, 0.036 g (49%) of a light yellow liquid was recovered from the trap. The ir spectrum of this compound was identical with that of commercial quinoline (Eastman Organic Chemicals). A white solid (0.032 g, 42%) was collected from the sublimation condenser. The ir spectrum of this compound was identical with a known sample of thymine. This solid was recrystallized from 2 ml of H_2O to give thymine (**6a**) as white crystals.

Anal. Calcd for $C_5H_6N_2O_2$: C, 47.62; H, 4.80; N, 22.22. Found: C, 47.82; H, 4.57; N, 21.96.

METHOD B. 1,2-Dihydro-*N*-thyminylquinoline (**5a**, 0.050 g, 0.2 mmol) was added to 3 ml of diglyme and the mixture was refluxed for 1.5 hr under an atmosphere of N_2.

METHOD C. 1,2-Dihydro-*N*-thyminylquinoline (**5a**, 0.054 g, 0.2 mmol) and 5 ml of H_2O were combined and heated to reflux for 4 hr under N_2.

Rearrangement of 1,2-Dihydro-N-(1-methylthyminyl)quinoline (**5b**). 1,2-Dihydro-*N*-(1-methylthyminyl)quinoline (**5b**, 0.089 g, 0.33 mmol) was placed in a pear-shaped flask and the closed system was flushed slowly with N_2 through a trap filled with $HCCl_3$. The reaction flask was immersed in a Wood's metal bath maintained at 205 ± 5° for 1.5 hr. After cooling, the yellow residue in the reaction vessel was extracted with two 30-ml portions of hot $HCCl_3$ and combined with the $HCCl_3$ from the trap. The combined $HCCl_3$ fractions were then adsorbed onto 2 g of silica and the resulting powder added to the top of a 10-g silica column packed with EtOAc. Elution with EtOAc produced 0.0025 g (6%) of quinoline (**3a**) in the second 20-ml fraction. In the eluent between 80 and 160 ml was 0.0022 g (5%) of 1-methylthymine (**6b**), identified by chromatographic and ir comparison with an authentic sample and by its mass spectrum: *m/e* (70 eV) 140. The starting material exhibited one spot on silica gel tlc; therefore, column-catalyzed breakdown was unlikely.

Rearrangement of 1,2-Dihydro-N-thyminylquinoline-2,2-d_2 (**5c**). To 3.2 ml of diglyme was added 0.037 g (0.14 mmol) of **5c** and the mixture was heated to reflux for 20 hr under N_2. After cooling, 5 ml of H_2O and 2 g of silica were added and the mixture was evaporated to a fine powder. The powder was added to the top of an 8-g silica column packed with benzene and eluted initially with 50 ml of benzene and 25 ml of benzene–MeOH (20:1). Then 10-ml fractions were collected while continuing elution with the latter solvent. Thymine-*methyl-d* (**6c**) was identified in fractions 1–9 by tlc. A sample from fraction 5 was submitted for mass spectral analysis: (70 eV) *m/e* (relative intensity) 128 (12), 127 (100), and 126 (14). Fractions 1–9 were combined and yielded 0.006 g (34%) of thymine-*methyl-d* (**6c**).

Rearrangement of 1,2-Dihydro-N-thyminylquinoline-2,2-d_2 (**5c**) *in Aqueous Medium.* To 10 ml of 50% aqueous dioxane through which N_2 was being bubbled was added **5c** (0.200 g, 0.78 mmol). The mixture refluxed for 2 days under N_2. The clear solution was then combined with 2 g of silica gel and evaporated to a fine powder. This was added to the top of a 30-g silica gel column packed with $HCCl_3$ and then eluted with 100 ml of the same solvent. Elution was continued using 100 ml of $HCCl_3$–MeOH (20:1) followed by $HCCl_3$–MeOH (10:1). The first 110 ml of the latter solvent contained quinoline (**3b**) and unidentified components. The next 30 ml of the same solvent contained what appeared to be thymine and a contaminant. The next 30 ml contained pure thymine-*methyl-d* (**6c**): 0.018 g (18%); mass spectrum (15 eV) *m/e* (relative intensity) 128 (13), 127 (100), 126 (13), 84 (8), and 56 (18). The following 30 ml of eluent contained what ap-

peared to be thymine and a contaminant, but no attempt was made to recover the thymine.

Crossover Experiment Utilizing 1,2-Dihydro-N-thyminyl-quinoline-2-d_2 (**5c**) *and 1,2- Dihydro-N-thyminyl-methyl-d-quinoline* (**5d**). Compound **5c** (0.050 g, 0.195 mmol) and compound **5d** (0.05 g, 0.195 mmol) were thoroughly mixed by triturating with a spatula. The finely powdered mixture was placed in the bottom of a microsublimation apparatus fitted with a Dry Ice–acetone trap on the side arm. The system was evacuated with a vacuum pump and then immersed in a Wood's metal bath heated to 155°. The temperature was increased to 205 ± 5° and held there for 2 hr. Steam was passed through the sublimation condenser throughout the heating period. After cooling, 0.013 g of white solid was recovered from the sublimation condenser and 0.020 g of quinoline was recovered from the side-arm trap. The white solid displayed tlc properties identical with thymine and was analyzed in the mass spectrometer: (70 eV) *m/e* (relative intensity) 128 (27), 127 (100), and 126 (38). After correction (Table II) this ratio calculates to give an approximate ratio of 2:7:1 for **6a**:**6c**:**6d**.

1,2,3,4-Tetrahydro-N-thyminylquinoline (**8**). Method A. 5-Chloromethyluracil (**2a**) (1.750 g, 11 mmol) was dissolved in anhydrous dimethylformamide (15 ml) and 1,2,3,4-tetraquinoline (**7**, 1.800 g, 13.5 mmol) was added in one portion. After stirring overnight at room temperature under a N_2 atmosphere, the mixture was poured into Et_2O (100 ml) and stirred for 30 min. The precipitate was collected by filtration, washed with Et_2O, and then slurried in MeOH (50 ml). To this was added 5 g of silica and the mixture was evaporated to a fine powder. This powder was added to the top of a 50-g silica column and the column was eluted with HCl_3 (250 ml). The solvent was then changed to $HCCl_3$–EtOH (20:1) and when **8** began to come off the column the solvent was changed to $HCCl_3$–EtOH (15:1). Elution was continued until **8** ceased coming off the column. Evaporation of the combined fractions containing **8** afforded 1.165 g (41%) of white powder: mp 235–237°; uv λ^{EtOH} 303 (ε 2620), 259 mμ (ε 17,800); nmr (CF_3COOH) δ 8.1 (s, 1 H, uracil C_6-H), 7.45 (s, 4 H, aromatic), 4.65 (s, 2 H, NCH_2 uracil), 3.8 (br, 2 H,) 3.1 (br, 2 H), 2.4 (br, 2 H); mass spectrum (70 eV) *m/e* (relative intensity) 257 (36), 133 (90), 132 (100), 118 (22), 117 (28), 83 (19), 82 (17); ir (KBr) 3080, 1725, and 1685 cm^{-1}.

Anal. Calcd for $C_{14}H_{15}N_3O_2$: C, 65.35; N, 5.88; N, 16.33. Found: C, 65.11; H, 5.73; N, 16.09.

Method B. Uracil (**9**, 1.12 g, 0.01 mol), paraformaldehyde (0.33 g, 0.011 mol), and 1,2,3,4-tetrahydroquinoline (**7**, 1.33 g, 0.01 mol) were combined in 75 ml of 95% EtOH. The mixture was heated to reflux for 2 days under N_2 with constant stirring. An additional 0.200 g (0.0067 mol) of paraformaldehyde was added and refluxing continued for another 24 hr. Silica column chromatography resolved 0.116 g (5%) of **8** and 70 mg of substance with chromatographic mobility slightly slower than **8**, mp 196–201°. This was shown to be 5-ethoxymethyluracil by its nmr, mass spectral, and chromatographic comparison with an authentic sample.

Rearrangement of 1,2,3,4-Tetrahydro-N-thyminylquinoline (**8**). A sample of **8** (0.150 g, 0.56 mmol) was placed in a microsublimation apparatus fitted with a Dry Ice–acetone trap on the side arm. The system was evacuated and immersed in a Wood's metal bath heated to 250 ± 5° where it was maintained for 3 hr. Steam was passed through the sublimation condenser for the duration of the heating period. After cooling the apparatus, 0.026 g of light yellow liquid was recovered from the Dry Ice–acetone trap. The ir and nmr spectra of this liquid were identical with the spectra of 1,2,3,4-tetrahydroquinoline (**7**, Eastman Organic Chemicals). From the sublimation condenser was recovered 0.022 g of white solid which appeared to be composed of thymine (**6a**) and **8**. The white solid was dissolved in a few drops of Me_2SO and streaked on a 2 mm thick 20 × 20 cm silica plate. After eluting with $HCCl_3$–EtOH (9:1), the band with R_F 0.24 was scraped from the plate and eluted with 100 ml of $HCCl_3$–EtOH (4:1). This afforded 0.007 g (10%) of white solid which on tlc appeared to be thymine (**6a**) plus a trace amount of **8**. Recrystallization from 1 ml of H_2O gave 0.001 g of white crystalline material, the ir of which was identical with that of known thymine.

5-Chloromethyl-3′,5′-di-O-p-toluyl-β-2′-deoxyuridine (**10**). A mixture of 0.350 g (0.6 mmol) of 5-benzyloxymethyl-3′,5′-di-*O*-*p*-toluyl-β-2′-deoxyuridine (Mertes and Shipchandler, 1971) and 15 ml of dry dioxane was stirred at room temperature and HCl gas was passed through an H_2SO_4 trap into the solution for 2 hr. The mixture was fitted with a $CaCl_2$ drying tube and stirred overnight at room temperature. After freezing the mixture in Dry Ice, the solvent was removed by lyophilization. The residue was washed with 50 ml of hot petroleum ether (bp 30–60°). This left 0.298 g (97%) of **10** as a white solid, mp 202–204° (lit. mp 206–207° (Brossmer and Rohm, 1967).

1,2,3,4-Tetrahydro-N-[1-(2′-deoxy-3′,5′-di-O-p-toluyl-β-D-ribofuranosyl)thyminyl]quinoline (**11**). A mixture of 5-chloromethyl-3′,5′-di-*O*-*p*-toluyl-β-2′-deoxyuridine (**10**, 0.413, 0.8 mmol), 1,2,3,4-tetrahydroquinoline (**7**, 0.213 g, 1.6 mmol), and 0.2 ml of triethylamine was stirred in 5 ml of dioxane under an atmosphere of N_2 for 2 days. Filtration of the precipitate followed by washing with 2 ml of dioxane gave 0.103 g of triethylamine hydrochloride as white crystals, mp 247–252°. The filtrate was evaporated to a viscous oil and added to the top of a 40-g silica column packed with $HCCl_3$–petroleum ether (bp 30–60°) (2:1) and then eluted with 100 ml of the same solvent. The solvent was changed to $HCCl_3$–petroleum ether (4:1) and the first 100 ml of this solvent yielded nothing; however, the next 50 ml yielded 1,2,3,4-tetrahydroquinoline (**7**). Continued elution with the same solvent produced a mixture of 1,2,3,4-tetrahydroquinoline (**7**) and **11** in the next 50 ml followed by 0.471 g (96%) of **11** in the next 240 ml. Evaporation gave **11** as a tan glass: mp 100–105°; nmr (CCl_4) δ 7.85 (d, 4 H, J = 8 Hz, *p*-toluyl α-H), 7.15 (m, 5 H, uracil C_6-H and *p*-toluyl β-H), 7.0–6.1 (m, 5 H, tetrahydroquinoline aromatic H and C_1'-H), 5.3 (m, 1 H), 4.15 (m, 5 H), 3.3 (m, 2 H), 2.7 (m, 4 H), 2.4 (s, 6 H, *p*-toluyl CH_3), 1.95 (m, 2 H); mass spectrum (80 eV) *m/e* (relative intensity) 610 (10), 609 (28), 133 (79), 132 (100), 129 (45), 119 (86), 91 (79), 81 (83); ir (KBr) 2965, 1715, and 1670 cm^{-1}; uv λ^{EtOH} 301 (ε 2660), 260 (ε 21,900), 252 (ε 22,030), 245 (ε 21950), and 222 mμ (ε 20,130).

Anal. Calcd for $C_{35}H_{35}N_3O_7$: C, 68.95; H, 5.79; N, 6.89. Found: C, 68.98; H, 6.10; N, 6.68.

1,2,3,4-Tetrahydro-N-[1-(2′-deoxy-β-D-ribofuranosyl)-thyminyl]quinoline (**12**). To 12 ml of absolute MeOH was added the ditoluyl compound **11** (0.576 g, 0.95 mmol) and the solution was made distinctly alkaline by the addition of freshly prepared sodium methoxide. After stirring for 3 days at room temperature, the pH was adjusted to about 6–7 with Dowex 50W. The resin was removed by filtration and washed with 10 ml of MeOH. The combined filtrates were evaporated to a semisolid, dissolved in the minimum amount of $HCCl_3$, and added to the top of a 40-g silica column packed with

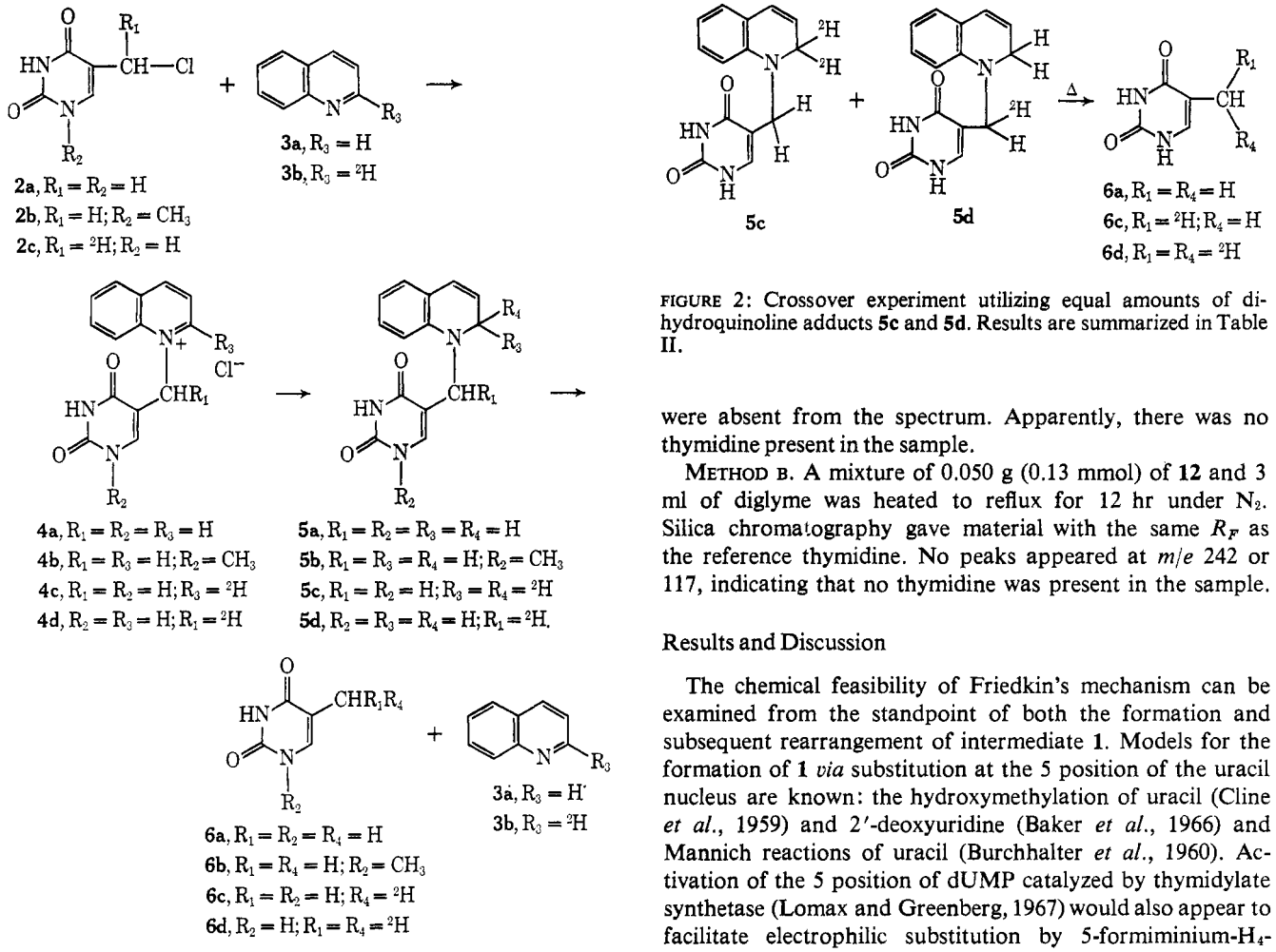


FIGURE 1: Preparation and rearrangement of dihydroquinoline model compounds. All experimental conditions for synthetic procedures are described under Materials and Methods. Conditions for rearrangement are found in Table I.

$HCCl_3$–petroleum ether (bp 30–60°) (2:1). Elution with $HCCl_3$–petroleum ether and $HCCl_3$–acetone resolved the products. The combined weight of all the monoprotected nucleosides was 0.075 g and 0.210 g (59%) of **12** was obtained as a glassy solid. A small amount crystallized from $HCCl_3$ as tan crystals: mp 157–160°; nmr ($CDCl_3$) δ 7.4 (s, 1 H, uracil C_6-H), 7.2–6.1 (m, 5 H, aromatic and C_1'-H), 4.3 (br, 1 H, C_3'-H), 4.2 (s, 2 H, uracil C_5-CH_2), 3.9 (br, 1 H, C_4'-H), 3.4 (m, 4 H, C_5'-H and tetrahydroquinoline C_2-H), 2.8 (m, 2 H, tetrahydroquinoline C_4-H), 2.1 (m, 4 H, C_2'-H and tetrahydroquinoline C_3-*H*); mass spectrum (80 eV) (relative intensity) 373 (6), 133 (85), 132 (100), 129 (53), 118 (26) 83 (48); ir (KBr) 3400, 1720, and 1645 cm^{-1}; uv λ^{EtOH} 304 (ϵ 2560), 260 mμ (ϵ 13,050).

Anal. Calcd for $C_{19}H_{23}N_3O_5$: C, 61.11; H, 6.21; N, 11.25. Found: C, 61.30; H, 6.07; N, 11.47.

Attempted Rearrangement of 1,2,3,4-Tetrahydro-N-[1'-(2'-deoxy-β-D-ribofuranosyl)thyminyl]quinoline (**12**). METHOD A. Heating 0.050 g (0.13 mmol) of **12** *in vacuo* to 160° failed to give any change. After heating to 200°, examination of the residue in the flask by tlc revealed several components. Thick layer chromatography resolved a band with an R_F similar to thymidine. This gave about 0.002 g of a glassy solid that was examined by mass spectroscopy. The peaks at *m/e* of 242 and 117 which are characteristic of thymidine

FIGURE 2: Crossover experiment utilizing equal amounts of dihydroquinoline adducts **5c** and **5d**. Results are summarized in Table II.

were absent from the spectrum. Apparently, there was no thymidine present in the sample.

METHOD B. A mixture of 0.050 g (0.13 mmol) of **12** and 3 ml of diglyme was heated to reflux for 12 hr under N_2. Silica chromatography gave material with the same R_F as the reference thymidine. No peaks appeared at *m/e* 242 or 117, indicating that no thymidine was present in the sample.

Results and Discussion

The chemical feasibility of Friedkin's mechanism can be examined from the standpoint of both the formation and subsequent rearrangement of intermediate **1**. Models for the formation of **1** *via* substitution at the 5 position of the uracil nucleus are known: the hydroxymethylation of uracil (Cline *et al.*, 1959) and 2'-deoxyuridine (Baker *et al.*, 1966) and Mannich reactions of uracil (Burchhalter *et al.*, 1960). Activation of the 5 position of dUMP catalyzed by thymidylate synthetase (Lomax and Greenberg, 1967) would also appear to facilitate electrophilic substitution by 5-formiminium-H_4-folate, the proposed reactive form of 5,10-methylene-H_4-folate (Benkovic *et al.*, 1969; Kallen and Jencks, 1966).

However, chemical precedence for the reductive elimination of dTMP from **1** appears to be lacking and even tends to be contradicted by the reported stability of 5-thyminyl-H_4folate (Gupta and Huennekens, 1967). Therefore, we chose to examine a model compound which would contain the portion of compound **1** directly involved in the reductive elimination.

Initially a model was designed which would provide the reductive driving force necessary for a nonenzymatic rearrangement. Based on steric similarity, fewer functional groups, and its known reducing power (Braude *et al.*, 1960), 1,2-dihydroquinoline was chosen to represent the pteridine ring of **1** in the model compounds **5** (Figure 1). The functionalities of H_4folate which are omitted in the model would probably have some influence on the rearrangement, but the nature and significance of these effects are not readily apparent.

Heating the air-sensitive **5a** under vacuum at 205° produced thymine (**6a**) and quinoline (**3a**) in 42 and 47% yields, respectively[2] (Figure 2). Other conditions (Table I) were found which could effect the rearrangement, the mildest being aqueous reflux; however, the isolated yield was low (3%). The 1-methylthyminyl analog **5b** was examined to compare the effect of substituion at the 1 position of the uracil ring as found in compound **1**. This compound rearranged to give 1-methylthymine (**6b**), however, in lower yield.

[2] A substituted 1-methyl-1,2-dihydroquinoline derivative has been reported to yield methane and the substituted quinoline on heating (Meisenheimer and Schutze, 1923).

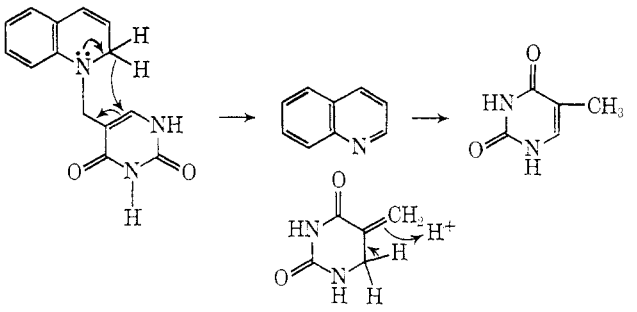


FIGURE 3: Possible low-energy pathway for rearrangement of dihydroquinoline model compounds; label experiments do not support this model.

The dideuterio compound **5c** was prepared as shown in Figure 1. Rearrangement of **5c** in refluxing diglyme produced thymine (**6c**) shown to contain one deuterium by mass spectral analysis. In the nmr spectrum of this thymine the methyl group and the C-6 hydrogen integrated in the ratio of 2:1 respectively. This indicates that the deterium is in the methyl group, a result in accord with Friedkin's studies.

Rearrangement of the dideuterio compound **5c** in refluxing aqueous dioxane produced thymine (**6c**) quantitatively labeled with deuterium. None of the deuterium is transferred to the exchangeable positions. Furthermore, the migrating hydrogen is a hydride or possibly a radical rather than a proton since it is not exchanged with the solvent (Schellenberg, 1970). These results are also in agreement with the labeling studies in the enzymatic system.

To examine the question of intramolecular *vs.* intermolecular rearrangement a crossover experiment was performed, Figure 2, using equal amounts of **5c** and the analog containing one deuterium on the thyminyl group, **5d**, prepared as shown in Figure 1. In a purely intramolecular rearrangement, all of the thymine produced would contain one deuterium (**6c**) because the third hydrogen of the thymine methyl group has been shown to come from C-2 of the dihydroquinoline moiety.

However, in a purely intermolecular rearrangement a more complex distribution of label would result. Notice that transfer of a deuterium from **5c** to the thyminyl of another molecule of **5c** would give monolabeled thymine. But if the same deuterium were transferred to the thyminyl of a molecule of **5d** a dilabeled thymine would result. Similarly, transfer of a hydrogen from **5d** to the thyminyl of another molecule of **5d** would yield monolabeled thymine, but transfer of that hydrogen to a molecule of **5c** would yield unlabeled thymine.

Any intermolecular transfer of deuterium from **5c** has an equal probability of reacting with a molecule of **5c** or **5d** thereby producing equal amounts of monolabeled and dilabeled thymine. Similarly, transfer of hydrogen from **5d** has an equal probability of reacting with a molecule of **5c** or **5d** giving equal amounts of unlabeled and monolabeled thymine. This leads to the relationship that the sum of the amount of unlabeled (**6a**) plus the amount of dilabeled thymine (**6d**) (*m/e* 126 and 128) should equal the amount of monolabeled thymine (**6c**) (*m/e* 127) in a purely intermolecular reaction. For the simplest case where hydrogen and deuterium are transferred at the exact same rate, unlabeled, monolabeled, and dilabeled thymine would be produced in a ratio of 1:2:1. Any difference in the rates of transfer of hydrogen and deuterium or a combination of intramolecular and intermolecular reactions would therefore have a predictable effect on the distribution of label in the product thymine based on the preceding rationale.

The results of the crossover experiment are summarized in Table II. The observed values for unlabeled (**6a**), monolabeled (**6c**), and dilabeled thymine (**6d**) (*m/e* 126, 127, and 128, respectively) in the mass spectrum are corrected to account for the P − 1 and P + 1 peaks observed in the spectrum of thymine obtained by rearranging **5c** or **5d** alone. The corrected values then appear in the ratio of 29:94:13 for *m/e* 126, 127, and 128, respectively.

Since unlabeled (**6a**) and dilabeled (**6d**) thymine could only arise from intermolecular reactions, it must be concluded that this type of reaction did occur. The amount of monolabeled thymine (**6c**) produced intermolecularly should equal the sum of the amount of unlabeled plus the amount of dilabeled thymine, or 29 plus 13 which gives the relative value of 42. Subtracting this from the total value of 94 for monolabeled thymine gives 52, the relative amount of monolabeled thymine which presumably had to arise from intramolecular rearrangement.

TABLE I: Rearrangements of Model Compounds.

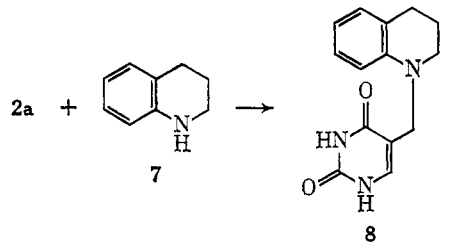
Compd	Conditions	Yield (%)[a] Thymine	Quinoline
5a	205°, *in vacuo*, 2.5 hr	42	49
5a	Refluxing diglyme, N_2 atmosphere, 1.5 hr (161°)	24	NI[b]
5a	Refluxing H_2O, N_2 atmosphere, 4 hr	3	NI
5b	205°, N_2 atmosphere, 1.5 hr	5[c]	6
8	250°, *in vacuo*, 3 hr	10	NI[d]

[a] Isolated yield. No attempts were made to maximize the yields. [b] Not isolated. [c] Product here is 1-methylthymine. [d] None of the expected product, 3,4-dihydroquinoline, was recovered.

TABLE II: Crossover Experiment Using **5c** and **5d**.

	Rel Proportion of Product Thymine: Unlabeled **6a**	Monolabeled **6c**	Dilabeled[a] **6d**
Expected for purely intramolecular rearrangement	0	1	0
Expected for purely intermolecular rearrangement[b]	1	2	1
Obsd in crossover expt of **5c** plus **5d**	38	100	27
Obsd values after correcting for background[c]	29	94	13

[a] Unlabeled (*m/e* 126); monolabeled (*m/e* 127); dilabeled (*m/e* 128). [b] For the case in which hydrogen and deuterium are transferred at the same rate. [c] Corrected for average P + 1 contribution of 15% of the molecular ions and for a P − 1 contribution of 9% seen in mass spectra of thymine obtained by rearranging **5c** or **5d** alone.



;URE 4: Preparation of 1,2,3,4-tetrahydro-*N*-thyminylquinoline

The crossover experiment provides evidence for both ramolecular and intermolecular rearrangements in the odel compounds. Under the conditions of the experiment, e heating of **5c** and **5d** without solvent, the intermolecular ocess is not unexpected; therefore, the extent of the intra-olecular reaction is considered to be a minimum value. iedkin's intramolecular 1,3-hydride shift mechanism is asonable in light of these results.

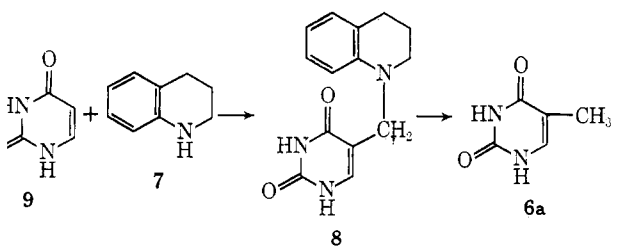
Mechanistically, an intramolecular rearrangement of **5** ght be envisioned as proceeding through the six-membered nsition state wherein the hydrogen is transferred to C-6 the uracil ring. Tautomerization of the initial product uld then give thymine (**6**) (Figure 3). However, the finding at none of the deuterium is exchanged when **5c** is rearranged an aqueous medium and that the nmr spectrum of the oduct indicates all of the deuterium to be in the methyl up rules out this mechanism.

Presumably, a 1,3 transfer of hydrogen directly to the thylene bridge occurs during the intramolecular rear-angement. The available evidence does not allow distinc-n between a concerted or a stepwise mechanism for the rrangement and therefore speculation about transition te conformational requirements tends to be inconsequential.

The model compound **5a** has the driving force of aromatiza-n during the rearrngement. Since compound **1** lacks this ving force, the tetrahydroquinoline model compound **8** s examined (Figure 4).

In addition to the method shown in Figure 4, the tetra-droquinoline model **8** could be prepared in one step from acil by combining uracil (**9**), formaldehyde, and 1,2,3,4-rahydroquinoline (**7**) in refluxing ethanol (Figure 5). Com-und **8** also produced thymine (**6a**) when heated under cuum; however, heating to 250° was required and the yield s lower. This provides a model system wherein uracil n be converted to thymine *via* an intermediate similar to mpound **1**.

The nucleoside analog, compound **12**, also was prepared d examined for rearrangement to thymidine (Figure 6). o thymidine was isolated following thermal decomposition **12**; presumably the high temperature required for decom-sition of **4** decomposed any thymidine produced.



;URE 5: Preparation of tetrahydroquinoline adduct **8** in one step om uracil and subsequent rearrangement to thymine.

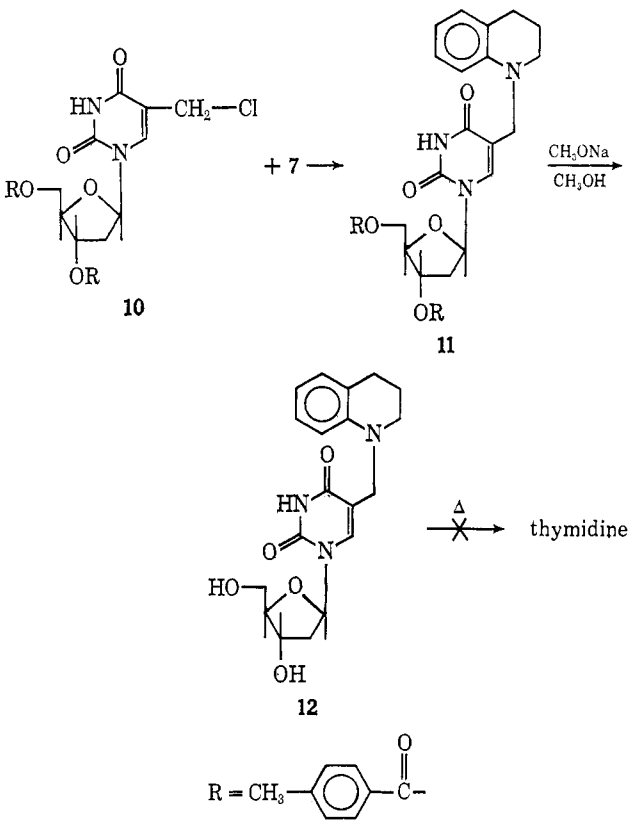


FIGURE 6: Preparation of nucleoside model compound **12**.

The formation of the tetrahydroquinoline adduct **8** in one step from uracil and the rearrangements of both **8** and dihydroquinoline adduct **5** to give thymine (**6**) provide chemical precedence for the formation and rearrangement of compound **1**. The high reaction temperatures required for the rearrangements of **5** and **8** indicate high energies of activation for these processes. Presumably, if compound **1** is a true intermediate in the thymidylate synthetase catalyzed reac-

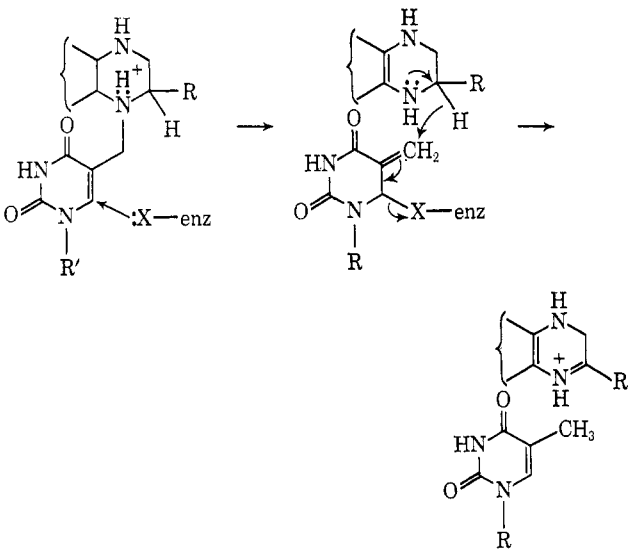


FIGURE 7: Possible manner in which thymidylate synthetase might assist in the rearrangement of intermediate **1**. Initially bond cleavage is assisted by addition of an enzyme nucleophile to the 6 position of the uracil ring followed by a reductive elimination of the enzyme nucleophile.

tion, the enzyme could provide the catalysis necessary to overcome a high energy barrier in the rearrangement.

At least two possibilities whereby the enzyme could facilitate the rearrangement of compound **1** seem worthy of discussion. In the first case the binding of the two reactant molecules, dUMP and 5-formiminium-H_4folate, to the enzyme is an important consideration. Maximum overlap of the π orbitals involved in the new bond formation requires the uracil and pteridine rings to be situated in parallel planes on the enzyme surface. However, following bond formation to give compound **1**, the methylene carbon would be rehybridized to sp^3 with the result that the uracil and pteridine rings could no longer maintain the parallel plane π overlap spatial relationship. If intermediate **1** remained bound to the enzyme through the same binding sites as did the original reactants, then considerable deformation of the enzyme would have to occur. The tendency for the enzyme to return to its original conformation while still binding **1** might then provide the driving force for the breakdown of **1** to the products of the reaction. This could be envisioned as either putting a strain on the bond about to be broken or by forcing the molecule into a transition state like conformation.[3]

A second possibility is suggested by numerous studies indicating that the enzyme contains a nucleophile capable of adding to the 6 position of the uracil ring of dUMP (Santi and Brewer, 1965; Kalman, 1971; Santi and McHenry, 1972; Wataya and Hayatsu, 1972). As indicated in Figure 7, the addition of a nucleophile to position 6 of compound **1** might facilitate the cleavage of the carbon–nitrogen bond followed by a reduction of the intermediate methylene compound.

In summary, model compounds were employed to demonstrate reactions similar to both the formation and rearrangement of compound **1**. This provides the chemical precedence previously lacking to support Friedkin's model.

[3] For a discussion of the "strain" and "distortion" theories of enzyme catalysis see Jencks (1969).

References

Baker, B. R. (1967), Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Active-Site, New York, N. Y., Wiley.

Baker, B. R., Schwan, T. J., and Santi, D. V. (1966), *J. Med. Chem. 9*, 66.

Benkovic, S. J., Benkovic, P. A., and Comfort, D. R. (1969), *J. Amer. Chem. Soc. 91*, 5270.

Blakely, R. L. (1967), The Biochemistry of Folic Acid and Related Pteridines, New York, N. Y., Wiley.

Braude, E. A., Hannah, J., and Linstead, S. R. (1960), *J. Chem. Soc.*, 3249.

Brossmer, R., and Rohm, E. (1967), *Hoppe-Seyler's Z. Physiol. Chem. 348*, 1431.

Burckhalter, J. H., Seiwald, R. J., and Scarborough, H. C. (1960), *J. Amer. Chem. Soc. 82*, 991.

Carbon, J. A. (1960), *J. Org. Chem. 25*, 1731.

Cline, R. E., Fink, R. M., and Fink, K. (1959), *J. Amer. Chem. Soc. 81*, 2521.

Friedkin, M. (1963), *Annu. Rev. Biochem. 32*, 185.

Friedkin, M., and Kornberg, A. (1957), *in* The Chemical Basis of Heredity, McElroy, W. D., and Glass, H. B., Ed., Baltimore, Md., John Hopkins Press, p 609.

Giner-Sorolla, A., and Medrek, L. (1966), *J. Med. Chem. 9*, 97.

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

Hartmann, K. U., and Heidelberger, C. (1961), *J. Biol. Chem. 236*, 3006.

Jencks, W. P. (1969), Catalysis in Chemistry and Enzymology, New York, N. Y., McGraw-Hill, p 295.

Kallen, R. G., and Jencks, W. P. (1966), *J. Biol. Chem. 241*, 5851.

Kalman, T. I. (1971), *Biochemistry 10*, 2567.

Lomax, M. I. S., and Greenberg, G. R. (1967), *J. Biol. Chem. 242*, 1302.

Lorenson, M. Y., Maley, G. F., and Maley, F. (1967), *J. Biol. Chem. 242*, 3332.

Meisenheimer, J., and Schutze, M. (1923), *Ber. 56*, 1353.

Mertes, M. P., and Shipchandler, M. T. (1971), *J. Heterocycl. Chem. 8*, 133.

Pastore, E. J., and Friedkin, M. (1962), *J. Biol. Chem. 237*, 3802.

Santi, D. V. (1967), *J. Heterocycl. Chem. 4*, 475.

Santi, D. V., and Brewer, C. F. (1968), *J. Amer. Chem. Soc. 90*, 6236.

Santi, D. V., and McHenry, C. S. (1972), *Proc. Nat. Acad. Sci. U. S. 69*, 1855.

Santi, D. V., and Pogolotti, A. L. (1971), *J. Heterocycl. Chem. 8*, 265.

Schellenberg, K. A. (1970), *in* Pyridine Nucleotide-Dependent Dehydrogenases, Sund, H., Ed., New York, N. Y., Springer-Verlag.

Trahanousky, W. S., Young, L. B., and Brown, B. L. (1965), *J. Org. Chem. 32*, 3865.

Wahba, A. J., and Friedkin, M. (1962), *J. Biol. Chem. 237*, 3794.

Wataya, Y., and Hayatsu, H. (1972), *Biochemistry 11*, 3583.

Wilson, R. S., and Mertes, M. P. (1972), *J. Amer. Chem. Soc. 94*, 7182.

Wolberg, W. H. (1969), *Cancer Res. 29*, 2137.