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## A Chemical Model for Thymidylate Synthetase Catalysis†

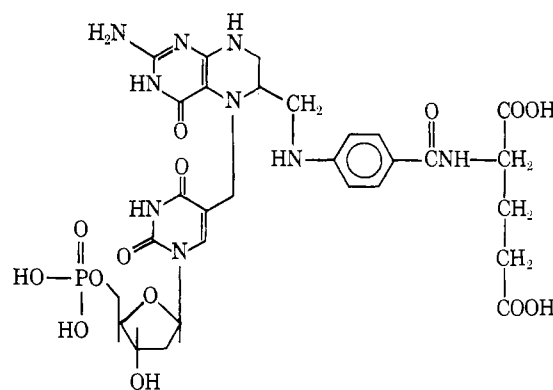
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**ABSTRACT:** In the thymidylate synthetase catalyzed reductive methylation of 2'-deoxyuridine 5'-phosphate to give thymidine 5'-phosphate, 5-thymidyltetrahydrofolic acid has been proposed as an intermediate which rearranges to give the observed products. However, an analog of the proposed intermediate, 5-thiminyltetrahydrofolic 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*-thiminylquinoline 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*-thiminylquinoline-2,2-*d*<sub>2</sub> 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*-thiminylquinoline 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.

**T**hymidylate synthetase catalyzes the reductive methylation of dUMP<sup>1</sup> to give dTMP utilizing formaldehyde as the carbon source and H<sub>4</sub>folate 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<sub>4</sub>folate 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



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Friedkin which is in agreement with the labeling studies involves the formation of 5-thymidyltetrahydrofolic 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-thiminyltetrahydrofolic acid, an analog

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<sup>1</sup> Abbreviations used are: dUMP, 2'-deoxyuridine 5'-monophosphate; dTMP, thymidine 5'-monophosphate; H<sub>4</sub>folate, 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<sub>2</sub>O and then 15 ml of MeOH was added. As the cooling solution just began to turn cloudy, NaBD<sub>4</sub> (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<sub>4</sub> was added. A final fraction of NaBD<sub>4</sub> (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<sub>2</sub>SO-*d*<sub>6</sub>)  $\delta$  11.1 (br, 2 H, NH), 7.4 (d, 1 H,  $J_{1,6}$  = 6 Hz, C<sub>6</sub>H), 4.1 (s, 1 H, CHD), 3.4 (q, 2 H,  $J$  = 7 Hz, OCH<sub>2</sub>), 1.1 (t, 3 H,  $J$  = 7 Hz, CH<sub>2</sub>CH<sub>3</sub>); 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<sub>2</sub>O (10 ml) and a catalytic amount of Na<sub>2</sub>CO<sub>3</sub> (2 mg) was added. This was heated to near refluxing overnight under a N<sub>2</sub> atmosphere. The D<sub>2</sub>O was then removed by lyophilization. Nmr (CDCl<sub>3</sub>) indicated that the exchange was not complete, so another 10 ml of D<sub>2</sub>O was added and again heated overnight. After removing the D<sub>2</sub>O by lyophilization, the nmr (CDCl<sub>3</sub>) 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 quino-

line-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<sub>3</sub> (75 ml). The HCCl<sub>3</sub> was washed with 0.1 N NaOH (50 ml) and H<sub>2</sub>O (50 ml) and dried (Na<sub>2</sub>SO<sub>4</sub>), 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<sub>3</sub>) no absorption at  $\delta$  8.9, 8.3–7.0 (m, aromatic); ir (neat) 3080, 2240, 2260, 1300, and 760 cm<sup>-1</sup>.

**N-Thyminyquinolinium 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<sub>2</sub>O. This afforded 7.17 g (87%) of a white powder, mp 270.5–272°. Two recrystallizations from acetic acid-H<sub>2</sub>O (10:1) gave 3.97 g (49%) of **4a** as colorless crystals: mp 278–280°; uv  $\lambda_{\text{max}}^{\text{EtOH}}$  262 ( $\epsilon$  7130), 218 m $\mu$  ( $\epsilon$  6860); ir (KBr) 3030, 1710, and 1690 cm<sup>-1</sup>; nmr (D<sub>2</sub>O)  $\delta$  9.55 (br d, 1 H,  $J_{2,3}$  = 5 Hz, N<sup>+</sup>=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<sup>+</sup>CH<sub>2</sub>).

*Anal.* Calcd for C<sub>14</sub>H<sub>12</sub>N<sub>3</sub>O<sub>2</sub>Cl: C, 57.98; H, 4.17; N, 14.49. Found: C, 58.05; H, 4.26; N, 14.45.

**N-(1-Methylthyminy)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<sub>2</sub> 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  $\lambda_{\text{max}}^{\text{EtOH}}$  270 ( $\epsilon$  8490), 318 m $\mu$  ( $\epsilon$  7430); ir (KBr) 3470, 3350, 3000, 1735, 1670, 1475, 1380, and 1350 cm<sup>-1</sup>; nmr (D<sub>2</sub>O)  $\delta$  9.67 (br d, 1 H,  $J$  = 5 Hz, N<sup>+</sup>=CH), 9.35 (br d, 1 H,  $J$  = 8 Hz), 8.9–7.9 (m, 6 H), 6.2 (s, 2 H, NCH<sub>2</sub>), 3.65 (s, 3 H, NCH<sub>3</sub>).

*Anal.* Calcd for C<sub>13</sub>H<sub>14</sub>N<sub>3</sub>O<sub>2</sub>Cl: C, 59.26; H, 4.64; N, 13.82. Found: C, 59.18; H, 4.86; N, 13.95.

**N-Thyminyquinolinium-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<sub>2</sub>O (10:1) to give colorless crystals: mp 288–291°; nmr (D<sub>2</sub>O)  $\delta$  9.3 (d, 1 H,  $J$  = 8 Hz), 8.8–8.0 (m, 6 H), 6.1 (s, 2 H, NCH<sub>2</sub>); ir (KBr) 2900, 1720, and 1685 cm<sup>-1</sup>.

*Anal.* Calcd for C<sub>14</sub>H<sub>11</sub>DN<sub>3</sub>O<sub>2</sub>Cl: C, 57.78; N, 14.44. Found: C, 58.08; N, 14.27.

**N-Thyminy-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<sub>2</sub>O (10:1) gave an analytical sample of **4d** as pinkish crystals: mp 285–287°; nmr (D<sub>2</sub>O)  $\delta$  9.55 (d of d, 1 H,  $J_{2,4}$  = 1.5 Hz,  $J_{3,4}$  = 8 Hz, C<sub>4</sub>-H), 8.9–7.5 (m, 6 H), 6.0 (s, 1 H, NCHD); ir (KBr) 3030, 1715, and 1685 cm<sup>-1</sup>.

*Anal.* Calcd for C<sub>14</sub>H<sub>11</sub>DN<sub>3</sub>O<sub>2</sub>Cl: C, 57.78; N, 14.44. Found: C, 57.42; N, 14.39.

**1,2-Dihydro-N-thyminyquinoline (5a).** N-Thyminyquino-

linium chloride (**4a**) (0.300 g, 1 mmol) was dissolved with heating in 3 ml of distilled H<sub>2</sub>O and 10 ml of MeOH was added. NaBH<sub>4</sub> (0.075 g, 0.002 mol) was added in one portion and the mixture was stirred at room temperature under a N<sub>2</sub> 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<sub>2</sub>. This gave 0.200 g (67%) of light yellow powder: mp 190–205°; uv  $\lambda_{\text{EtOH}}$  351 ( $\epsilon$  2500), 262 ( $\epsilon$  7800), 231 m $\mu$  ( $\epsilon$  31,200); nmr (Me<sub>2</sub>SO-*d*<sub>6</sub>)  $\delta$  7.2 (s, 1 H, uracil C<sub>6</sub>-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<sub>2</sub>CH), 4.2 (m, 2 H, NCH<sub>2</sub>CH), 3.95 (s, 2 H, NCH<sub>2</sub> 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<sup>-1</sup>.

*Anal.* Calcd for C<sub>14</sub>H<sub>13</sub>N<sub>3</sub>O<sub>2</sub>: 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<sub>4</sub> (0.075 g, 2 mmol) and the mixture was stirred for 1 hr under a N<sub>2</sub> 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<sub>2</sub>. This gave 0.177 g (66%) of white powder; mp 155–160°; uv  $\lambda_{\text{EtOH}}$  351 ( $\epsilon$  2780), 272 ( $\epsilon$  10,850), 231 m $\mu$  ( $\epsilon$  36,500); nmr (Me<sub>2</sub>SO-*d*<sub>6</sub>)  $\delta$  7.5 (s, 1 H, uracil C<sub>6</sub>-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<sub>2</sub>CH), 4.2 (m, 2 H, NCH<sub>2</sub>CH), 3.95 (s, 2 H, NCH<sub>2</sub> uracil), 3.2 (s, 3 H, NCH<sub>3</sub>); 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<sup>-1</sup>.

*Anal.* Calcd for C<sub>15</sub>H<sub>15</sub>N<sub>3</sub>O<sub>2</sub>: 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*<sub>2</sub> (5c).** *N*-Thyminylquinolinium-2-*d* chloride (**4c**, 0.200 g, 0.67 mmol) dissolved in 4.4 ml of H<sub>2</sub>O and 5 ml of MeOH was treated with NaBD<sub>4</sub> (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<sub>2</sub>SO-*d*<sub>6</sub>)  $\delta$  7.2 (s, 1 H, uracil C<sub>6</sub>-H), 7.0–6.1 (m, 5 H), 5.6 (d, 1 H,  $J_{2,3} = 10$  Hz, NCD<sub>2</sub>CH), 3.85 (s, 2 H, NCH<sub>2</sub> uracil); mass spectrum (70 eV) *m/e* (relative intensity) 257 (3), 132 (100), 131 (60), 130 (40); ir (KBr) 3200, 1690, and 1485 cm<sup>-1</sup>.

**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<sub>2</sub>O with gentle heating; 4 ml of MeOH was added followed quickly by the addition of NaBH<sub>4</sub> (0.025 g, 0.66 mmol). Treatment as in **5a** afforded 0.065 g (75%) of a yellow powder: mp 190–200°; nmr (Me<sub>2</sub>SO-*d*<sub>6</sub>)  $\delta$  7.2 (s, 1 H, uracil C<sub>6</sub>-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<sub>3</sub>-H), 4.2 (m, 2 H, C<sub>2</sub>-H<sub>2</sub>) 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<sup>-1</sup>.

*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 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<sub>2</sub>O to give thymine (**6a**) as white crystals.

*Anal.* Calcd for C<sub>8</sub>H<sub>8</sub>N<sub>2</sub>O<sub>2</sub>: 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<sub>2</sub>.

**METHOD C.** 1,2-Dihydro-*N*-thyminylquinoline (**5a**, 0.054 g, 0.2 mmol) and 5 ml of H<sub>2</sub>O were combined and heated to reflux for 4 hr under N<sub>2</sub>.

*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<sub>2</sub> through a trap filled with HCCl<sub>3</sub>. 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<sub>3</sub> and combined with the HCCl<sub>3</sub> from the trap. The combined HCCl<sub>3</sub> 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*<sub>2</sub> (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<sub>2</sub>. After cooling, 5 ml of H<sub>2</sub>O 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*<sub>2</sub> (5c) in Aqueous Medium.* To 10 ml of 50% aqueous dioxane through which N<sub>2</sub> was being bubbled was added **5c** (0.200 g, 0.78 mmol). The mixture refluxed for 2 days under N<sub>2</sub>. 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<sub>3</sub> and then eluted with 100 ml of the same solvent. Elution was continued using 100 ml of HCCl<sub>3</sub>–MeOH (20:1) followed by HCCl<sub>3</sub>–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-thyminyllquinoline-2- $d_2$  (5c) and 1,2-Dihydro-N-thyminyllmethyl-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  $\pm$  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-thyminyllquinoline (8).** METHOD A. 5-Chloromethyluracil (**2a**) (1.750 g, 11 mmol) was dissolved in anhydrous dimethylformamide (15 ml) and 1,2,3,4-tetrahydroquinoline (**7**, 1.800 g, 13.5 mmol) was added in one portion. After stirring overnight at room temperature under a N<sub>2</sub> atmosphere, the mixture was poured into Et<sub>2</sub>O (100 ml) and stirred for 30 min. The precipitate was collected by filtration, washed with Et<sub>2</sub>O, 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<sub>3</sub> (250 ml). The solvent was then changed to HCl<sub>3</sub>-EtOH (20:1) and when **8** began to come off the column the solvent was changed to HCl<sub>3</sub>-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°;  $\lambda^{EtOH}$  303 ( $\epsilon$  2620), 259 m $\mu$  ( $\epsilon$  17,800); nmr (CF<sub>3</sub>COOH)  $\delta$  8.1 (s, 1 H, uracil C<sub>8</sub>-H), 7.45 (s, 4 H, aromatic), 4.65 (s, 2 H, NCH<sub>2</sub> 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<sup>-1</sup>.

**Anal.** Calcd for C<sub>14</sub>H<sub>15</sub>N<sub>3</sub>O<sub>2</sub>: C, 65.35; N, 5.88; O, 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<sub>2</sub> 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-thyminyllquinoline (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  $\pm$  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<sub>2</sub>SO and streaked on a 2 mm thick 20  $\times$  20 cm silica plate. After eluting with HCl<sub>3</sub>-EtOH (9:1), the band with  $R_F$  0.24 was scraped from the plate and eluted with 100 ml of HCl<sub>3</sub>-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<sub>2</sub>O 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-toluyll- $\beta$ -2'-deoxyuridine (10).** A mixture of 0.350 g (0.6 mmol) of 5-benzoyloxymethyl-3',5'-di-O-p-toluyll- $\beta$ -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<sub>2</sub>SO<sub>4</sub> trap into the solution for 2 hr. The mixture was fitted with a CaCl<sub>2</sub> 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-toluyll- $\beta$ -D-ribofuranosyl)thyminyll]quinoline (11).** A mixture of 5-chloromethyl-3',5'-di-O-p-toluyll- $\beta$ -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<sub>2</sub> 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 HCl<sub>3</sub>-petroleum ether (bp 30–60°) (2:1) and then eluted with 100 ml of the same solvent. The solvent was changed to HCl<sub>3</sub>-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<sub>4</sub>)  $\delta$  7.85 (d, 4 H,  $J$  = 8 Hz,  $p$ -toluyll  $\alpha$ -H), 7.15 (m, 5 H, uracil C<sub>6</sub>-H and  $p$ -toluyll  $\beta$ -H), 7.0–6.1 (m, 5 H, tetrahydroquinoline aromatic H and C<sub>1'</sub>-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,  $p$ -toluyll CH<sub>3</sub>), 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<sup>-1</sup>;  $\lambda^{EtOH}$  301 ( $\epsilon$  2660), 260 ( $\epsilon$  21,900), 252 ( $\epsilon$  22,030), 245 ( $\epsilon$  21950), and 222 m $\mu$  ( $\epsilon$  20,130).

**Anal.** Calcd for C<sub>38</sub>H<sub>35</sub>N<sub>3</sub>O<sub>7</sub>: 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- $\beta$ -D-ribofuranosyl)thyminyll]quinoline (12).** To 12 ml of absolute MeOH was added the ditoluyll 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 HCl<sub>3</sub>, and added to the top of a 40-g silica column packed with

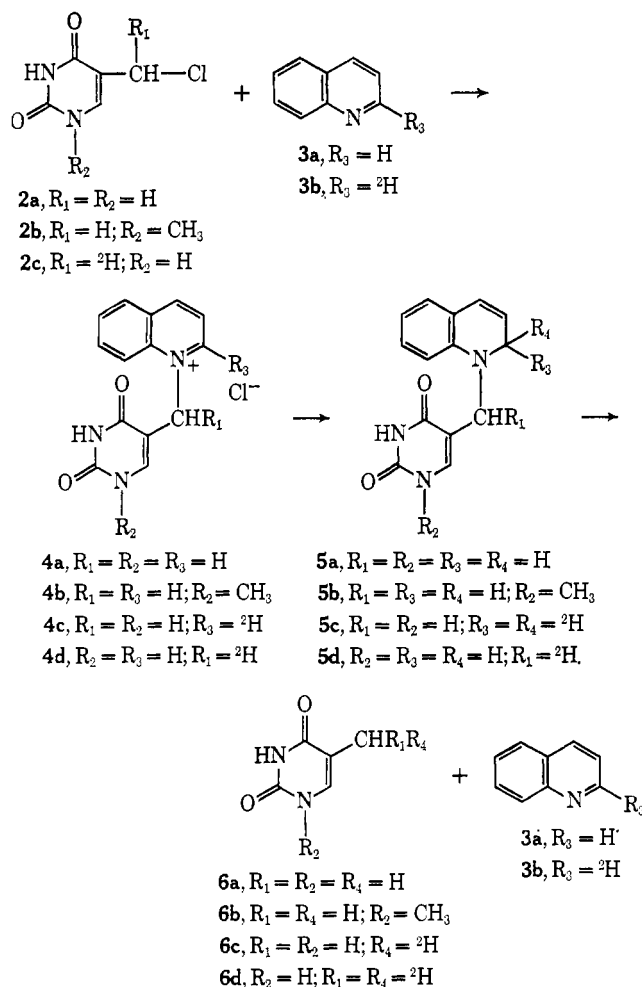


FIGURE 1: Preparation and rearrangement of dihydroquinoline model compounds. All experimental conditions for synthetic procedures are described under Materials and Methods. Conditions for rearrangement are found in Table I.

$CHCl_3$ -petroleum ether (bp 30–60°) (2:1). Elution with  $CHCl_3$ -petroleum ether and  $CHCl_3$ -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  $CHCl_3$  as tan crystals: mp 157–160°; nmr ( $CDCl_3$ )  $\delta$  7.4 (s, 1 H, uracil C<sub>6</sub>-H), 7.2–6.1 (m, 5 H, aromatic and C<sub>1'</sub>-H), 4.3 (br, 1 H, C<sub>3'</sub>-H), 4.2 (s, 2 H, uracil C<sub>5</sub>-CH<sub>2</sub>), 3.9 (br, 1 H, C<sub>4'</sub>-H), 3.4 (m, 4 H, C<sub>5'</sub>-H and tetrahydroquinoline C<sub>2</sub>-H), 2.8 (m, 2 H, tetrahydroquinoline C<sub>4</sub>-H), 2.1 (m, 4 H, C<sub>2'</sub>-H and tetrahydroquinoline C<sub>3</sub>-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  $\lambda_{EtOH}^{max}$  304 ( $\epsilon$  2560), 260  $m\mu$  ( $\epsilon$  13,050).

Anal. Calcd for  $C_{19}H_{23}N_5O_5$ : 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- $\beta$ -D-ribofuranosyl)thymine]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

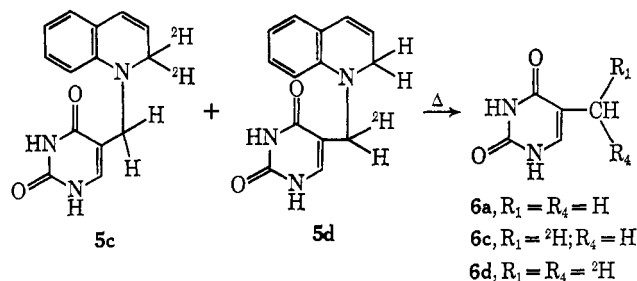


FIGURE 2: Crossover experiment utilizing equal amounts of dihydroquinoline adducts **5c** and **5d**. Results are summarized in Table II.

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-thymine- $H_4$ -folate (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  $H_4$ folate 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<sup>2</sup> (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-methylthymine analog **5b** was examined to compare the effect of substitution 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.

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

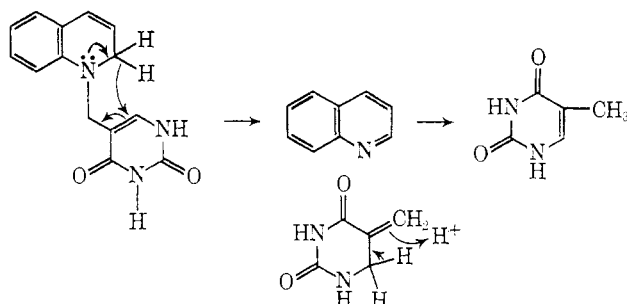


FIGURE 3: Possible low-energy pathway for rearrangement of dihydroquinoline model compounds; label experiments do not support this model.

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 deuterium 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 thyminy 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 thyminy of another molecule of **5c** would give monolabeled thymine. But if the same deuterium were transferred to the thyminy of a molecule of **5d** a dilabeled thymine would result. Similarly, transfer

TABLE I: Rearrangements of Model Compounds.

Compd	Conditions	Yield (%) <sup>a</sup>	
		Thymine	Quinoline
<b>5a</b>	205°, <i>in vacuo</i> , 2.5 hr	42	49
<b>5a</b>	Refluxing diglyme, N <sub>2</sub> atmosphere, 1.5 hr (161°)	24	NI <sup>b</sup>
<b>5a</b>	Refluxing H <sub>2</sub> O, N <sub>2</sub> atmosphere, 4 hr	3	NI
<b>5b</b>	205°, N <sub>2</sub> atmosphere, 1.5 hr	5 <sup>c</sup>	6
<b>8</b>	250°, <i>in vacuo</i> , 3 hr	10	NI <sup>d</sup>

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

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

	Rel Proportion of Product Thymine		
	Un-labeled <b>6a</b>	Mono-labeled <b>6c</b>	Di-labeled <b>6d</b> <sup>a</sup>
Expected for purely intramolecular rearrangement	0	1	0
Expected for purely intermolecular rearrangement <sup>b</sup>	1	2	1
Obsd in crossover expt of <b>5c</b> plus <b>5d</b>	38	100	27
Obsd values after correcting for background <sup>c</sup>	29	94	13

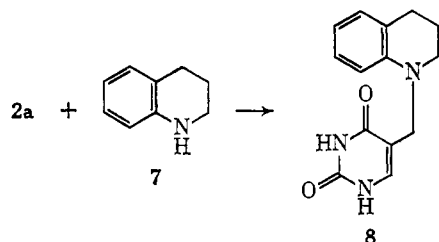
<sup>a</sup> Unlabeled (*m/e* 126); monolabeled (*m/e* 127); dilabeled (*m/e* 128). <sup>b</sup> For the case in which hydrogen and deuterium are transferred at the same rate. <sup>c</sup> Corrected for average *P* + 1 contribution of 15% of the molecular ions and for a *P* - 1 contribution of 9% seen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

of a hydrogen from **5d** to the thyminy 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.

FIGURE 4: Preparation of 1,2,3,4-tetrahydro-*N*-thyminylquinoline 8.

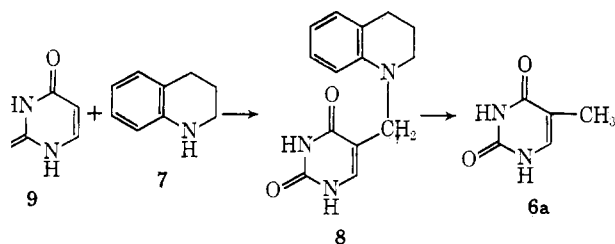
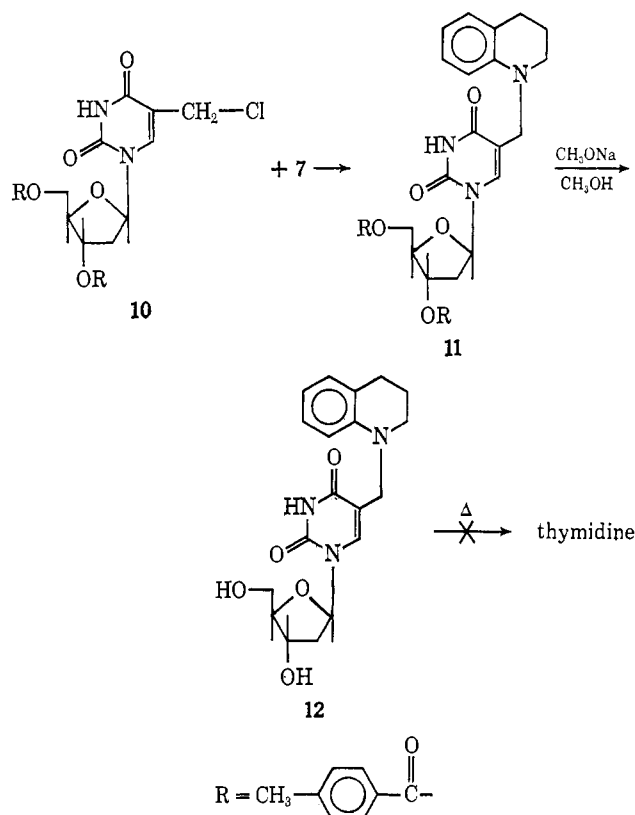
The crossover experiment provides evidence for both intramolecular and intermolecular rearrangements in the model compounds. Under the conditions of the experiment, the heating of **5c** and **5d** without solvent, the intermolecular process is not unexpected; therefore, the extent of the intramolecular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

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

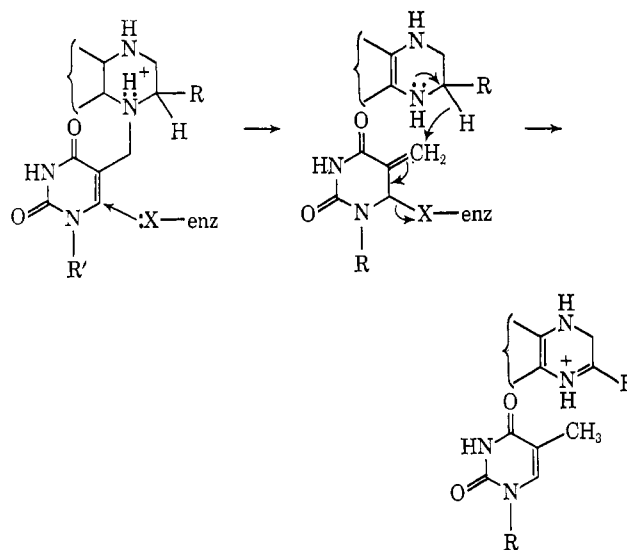
Presumably, a 1,3 transfer of hydrogen directly to the ethylene bridge occurs during the intramolecular rearrangement. The available evidence does not allow distinction between a concerted or a stepwise mechanism for the rearrangement and therefore speculation about transition state conformational requirements tends to be inconsequential. The model compound **5a** has the driving force of aromatization during the rearrangement. Since compound **1** lacks this driving force, the tetrahydroquinoline model compound **8** is examined (Figure 4).

In addition to the method shown in Figure 4, the tetrahydroquinoline model **8** could be prepared in one step from uracil by combining uracil (**9**), formaldehyde, and 1,2,3,4-tetrahydroquinoline (**7**) in refluxing ethanol (Figure 5). Compound **8** also produced thymine (**6a**) when heated under vacuum; however, heating to 250° was required and the yield is lower. This provides a model system wherein uracil can be converted to thymine *via* an intermediate similar to compound **1**.

The nucleoside analog, compound **12**, also was prepared and examined for rearrangement to thymidine (Figure 6). No thymidine was isolated following thermal decomposition of **12**; presumably the high temperature required for decomposition of **4** decomposed any thymidine produced.

FIGURE 5: Preparation of tetrahydroquinoline adduct **8** in one step from 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- $H_4$ folate, to the enzyme is an important consideration. Maximum overlap of the  $\pi$  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  $\pi$  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.<sup>3</sup>

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.

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<sup>3</sup> For a discussion of the "strain" and "distortion" theories of enzyme catalysis see Jencks (1969).