Selective Photoreductions of Nucleic Acids and Their Building Stones. VIII. Photoreduction and Dimerization of 1,3-Dimethyluracil[1]

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Abstract: The photoreduction of 1,3-dimethyluracil (DMU) in the presence of sodium borohydride at 2537 Å, which requires much longer irradiation times than uridine and thymidine, leads to 5,6-dihydro-1,3-dimethyluracil (II) in addition to a dimeric product which was identified as 1,3-dimethyl-5,4′-[1′,3′-dimethyltetrahydropyrimidone-2′]uracil (III) on the basis of ir, nmr, and mass spectral data. Dimer III, which is the tetrahydro homolog of one of the irradiation products of uracil, was also obtained from dihydrodimethyluracil (II) with $NaBH_4$ in a novel type of light-independent condensation reaction, in addition to γ-(N,N′-dimethylureido)propanol-1 (V), the expected product of hydrogenolytic ring opening. The action of alkali, baryta, or alkaline borate on II produced only traces of III, in addition to γ-(N,N′-dimethylureido)propionic acid (IV). The oxidation product of I, 1,3-dimethyl-5-hydroxyuracil (VI), on catalytic hydrogenation gave the dihydro compound VIII, which was reduced by borohydride in methanol to the 4,5-*cis*-glycol IX and the 4-O-methyl ether X, characterized by the 5-O-acetates XI and XII. When 1,3-dimethyl-5,6-dideuteriouracil (XVII) was converted to the dimeric product XX by sodium borohydride, only two deuterium atoms were retained in a reaction which probably involves aldolization (XIII), crotonization (XIX), and stereospecific rearrangement of an exocyclic to an endocyclic double bond (XX).

Photoreduction of 1,3-Dimethyluracil. N_3-Substituted uracils undergo photohydration much more slowly than N_1- or unsubstituted uracils.[3] Similarly, in contrast to uridine[4] and thymidine,[4] their N_3-methylated derivatives were practically resistant to photoreduction in the presence of sodium borohydride.[5] However, 1,3-dimethyluracil underwent photoreduction when the usual irradiation time was extended fivefold. In a typical spectroscopic run a 10^{-4} *M* aqueous unbuffered solution of 1,3-dimethyluracil was irradiated with a Hanovia low-pressure mercury lamp (No. 87A-45, 2537 Å), in the presence of a tenfold excess of sodium borohydride. Aliquots were withdrawn at intervals and the optical density was measured at the respective absorption maxima (Figure 1). For control 1,3-dimethyluracil was irradiated *without sodium borohydride* and the rate of photohydration plotted. At the end of the photoreduction the original absorption maximum at 266 mμ had shifted to 258 mμ, when absorbance reached a constant value.

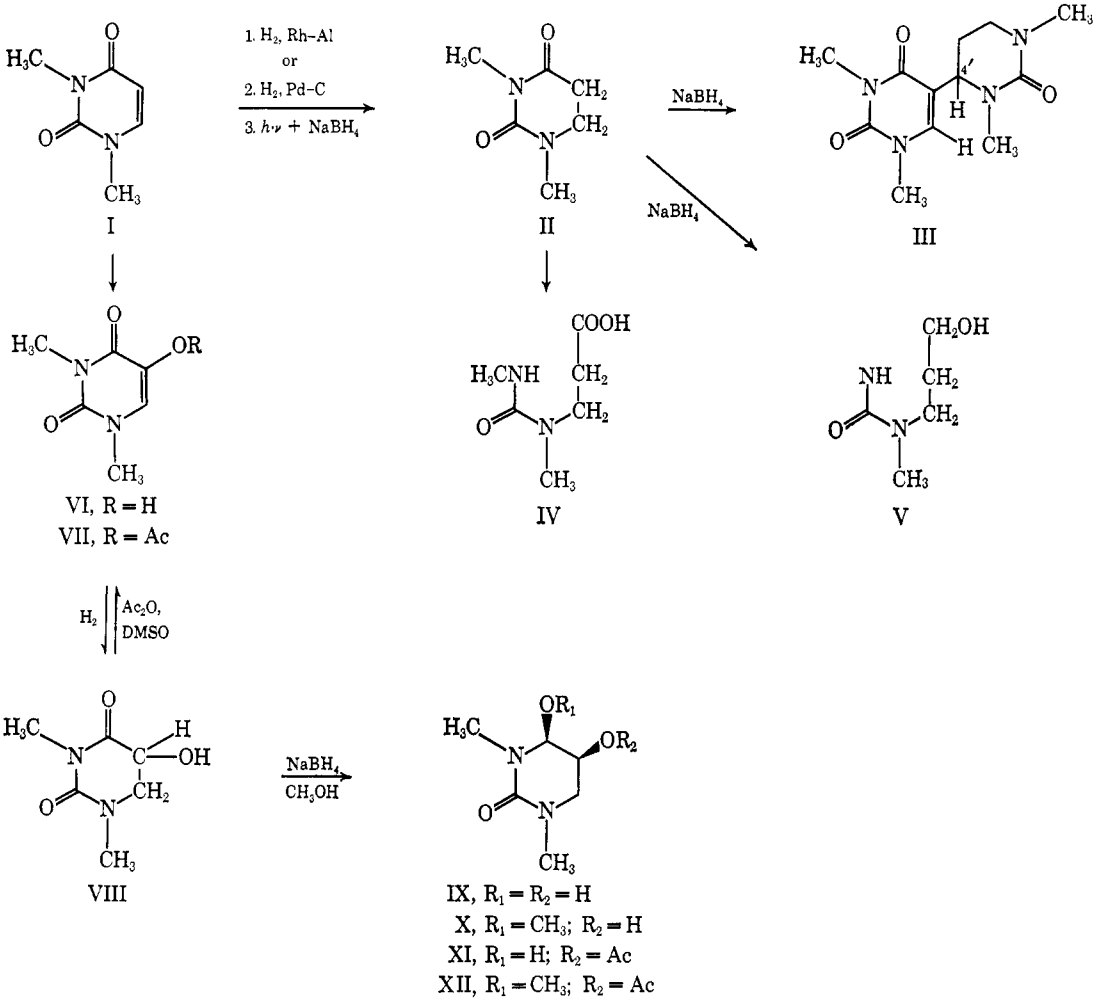
On a preparative scale a 2×10^{-3} *M* aqueous solution of 1,3-dimethyluracil (I) was irradiated in the presence of 5×10^{-3} mol of sodium borohydride for 10–12 hr at room temperature. After filtration through a column of Amberlite IRC-50 (H^+ form) the reaction mixture showed at least three major spots on silica gel tlc. Longer times of irradiation tended to increase the number of by-products. After separation by silica gel column chromatography two crystalline products were obtained. The major crystalline fraction, mp 55–56°, was identical with synthetic 1,3-dimethyl-5,6-dihydrouracil (II) obtained by catalytic reduction of 1,3-dimethyluracil (see Chart I).

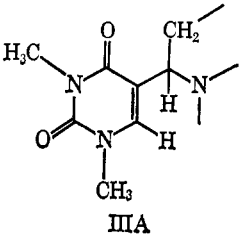
Dimerization of 1,3-Dimethyluracil. The second product, mp 186–186.5°, analyzed for a dimer, $C_{12}H_{18}N_4O_3$, and showed a molecular ion peak at 266 in the mass spectrum. The chromophore at 271 mμ (log ε 3.95) is characteristic of a 2,4-dioxopyrimidine moiety. The ir spectrum had no bands in the N–H and O–H stretching region. The structure of dimer III was proven by its nmr spectrum (Figure 2) which shows the olefinic proton of a 5-substituted uracil. A characteristic triplet at 4.54 ppm shows long-range spin–spin coupling with the olefinic doublet as was ascertained by spin decoupling. The splitting constant suggests this coupling to be allylic. These findings are supported by the mass spectrum (Figure 3) and favor the partial structure IIIA, which then is easily supplemented to III. The fragmentation pattern of III is typical of a 5-substituted 1,3-dimethylpyrimidinone.[6] The initial step (pathway A, III → IIIb) involves the loss of mass 57 (CH_3NCO) from the molecular ion in a retro-Diels–Alder type of reaction followed by loss of a proton (IIIc) and carbon monoxide (IIId). While 1,3-dimethyluracil did not give the M − CH_3 peak at 70 eV,[6] the mass spectrum of III showed 251 (M − CH_3) as the base peak. This suggests pathway B beginning with demethylation in the 1,3-dimethyltetrahydropyrimidone moiety. The resulting ion (IIIe, one of two tautomeric forms of a positively charged amide ⇄ imidole) again loses mass 57 (CH_3-NCO) to give peak 194 (IIIf).

The C-6 proton of uridine derivatives is generally more deshielded than its C-5 counterpart, and the difference between both chemical shifts averages 1.5 ppm.[7] Table I summarizes the chemical shifts of 1,3-dimethyl-

(1) Preceding paper: B. Witkop, *Photochem. Photobiol.*, 7, 813 (1968).
(2) Associate in the Visiting Program of the U. S. Public Health Service, 1966–1969.
(3) S. Y. Wang and J. C. Nnadi, *Science*, in press.
(4) P. Cerutti, Y. Kondo, W. R. Landis, and B. Witkop, *J. Am. Chem. Soc.*, 90, 771 (1968); Y. Kondo and B. Witkop, *ibid.*, 90, 764 (1968).
(5) Y. Kondo and B. Witkop, unpublished data.
(6) J. M. Rice, G. O. Dudek, and M. Barber, *J. Am. Chem. Soc.*, 87, 4569 (1965).
(7) H. P. M. Fromageot, B. E. Griffin, C. B. Reese, J. E. Sulston, and D. R. Trenthan, *Tetrahedron*, 22, 705 (1966), and literature cited therein.

Chart I





IIIA

pyrimidinones and the dihydro bases in 0.5–1.0 *M* deuteriochloroform solution.

The chemical shift of the olefinic proton of III according to Table I is in line with that of the C_6 proton of uracil and thymine. Dimer III is best represented as 1,3 - dimethyl-5,4′-(1′,3′-dimethyltetrahydropyrimidine-2′-one)uracil.

The allylic coupling constant would suggest that the

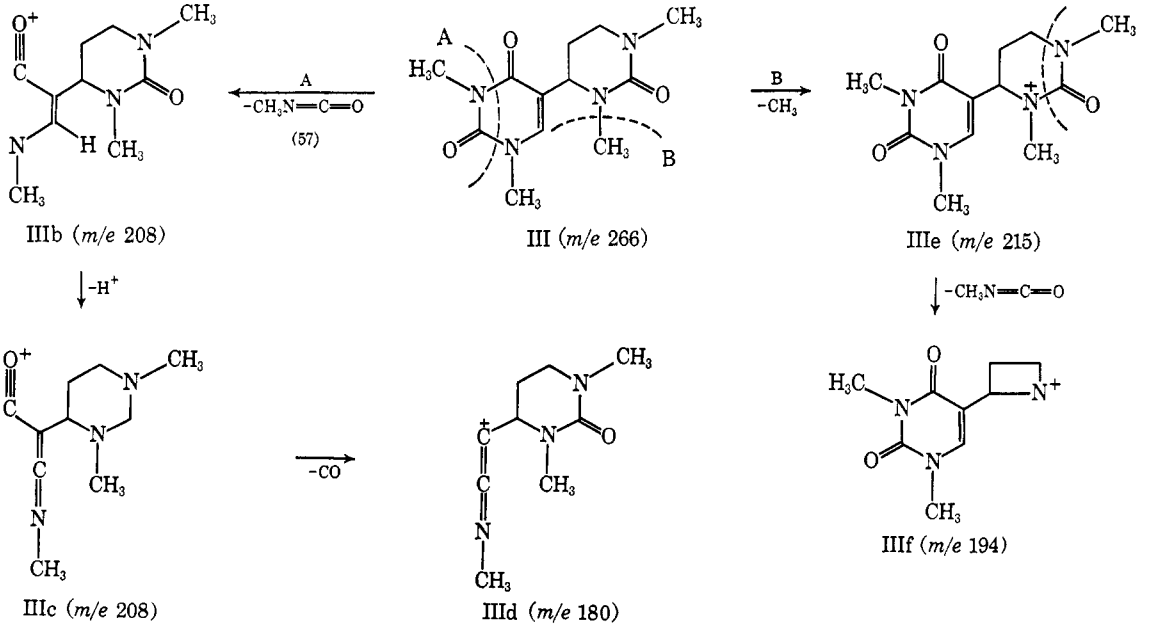
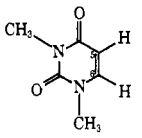
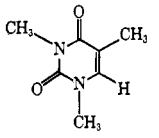
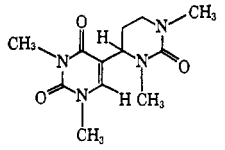
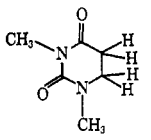
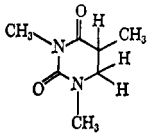


Table I. Chemical Shifts of 1,3-Dimethyl- and 1,3-Dimethyl-5,6-dihydropyrimidone Bases ($CDCl_3$)[a]

Structure	N-CH_3	C_6-H	C_5-H	C-CH_3
	3.37 3.43	7.20 (d, J = 8)	5.73 (d, J = 8)	
	3.37 3.38	7.01 (q, J = 1)		1.94 (d, J = 1)
	3.36 3.44 2.94 2.98	6.90 (d, J = 1)		
	3.06 3.19	3.39 (m)	2.74 (m)	
	3.06 3.17	3.25 (m)	2.78 (m)	1.26 (d, J = 7)

[a] J values in cycles per second (cps).

angle between the plane between C_4, C_5, C_6 and the adjacent C_4'-H bond should lie between 60 and 110°.[8]

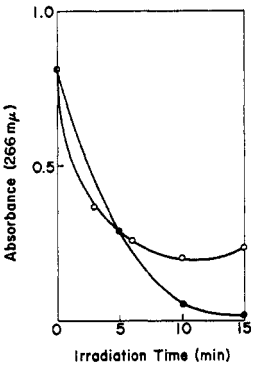


Figure 1. Heat-reversible photohydration of 1,3-dimethyluracil (λ_{max} 266 mμ) in aqueous solution (—●—●—) and photoreduction in the presence of sodium borohydride (10^{-4} M aqueous solution) (—○—○—).

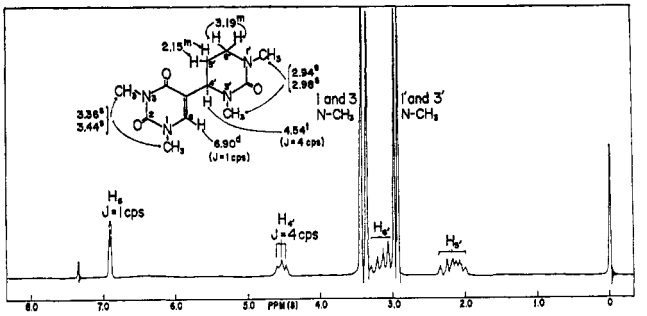


Figure 2. The nmr spectrum of dimer III in deuteriochloroform.

(8) D. J. Collins, J. J. Hobbs, and S. S. Sternhell, *Australian J. Chem.*, **16**, 1030 (1963).

Access to this double bond may be hindered, as evidenced by the failure of reduction with a rhodium–alumina catalyst under 20 lb for 14 hr.

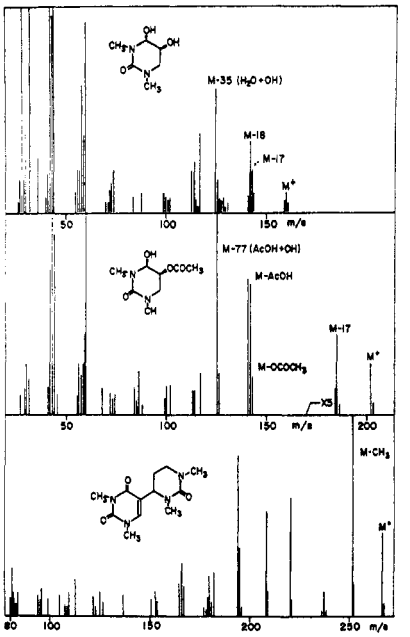
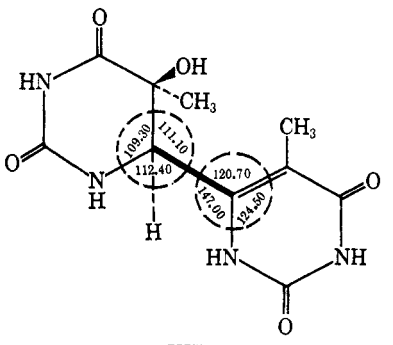


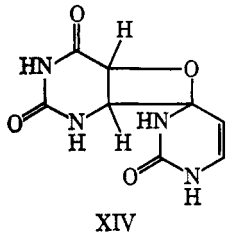
Figure 3. Mass spectra of 1,3-dimethyl-*cis*-4,5-dihydroxy-2-oxohexahydropyrimidine (IX), its 5-O-acetate (XI), and dimer III.

Exact data on the stereochemistry of an analogous hydroaromatic–aromatic pyrimidine dimer have recently become available. X-Ray crystallography of the so-called T–T adduct[9] resulting from irradiation of

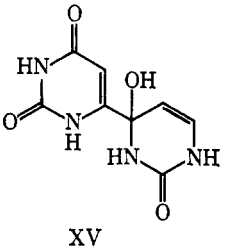
(9) A. J. Varghese and S. Y. Wang, *Science*, **160**, 186 (1968).



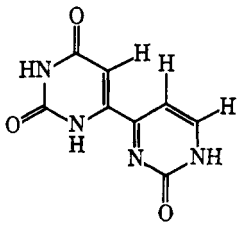
XIII



XIV



XV

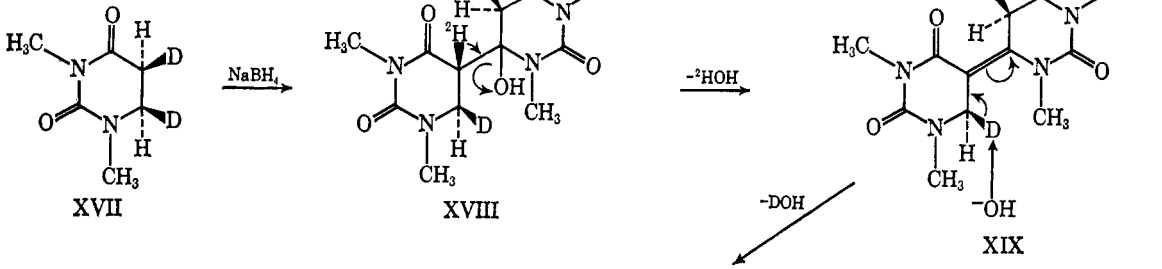


XVI

An easier route to dimer III, operative independent of light, was the treatment of 1,3-dimethyl-5,6-dihydrouracil (II) with sodium borohydride in aqueous solution. The oily reaction product showed two major spots on tlc. After column chromatography on silica gel the crystalline product was identical with dimer III in every respect.

The expected product of hydrogenolytic ring opening, γ-(N,N′-dimethylureido)propanol-1 (V), was obtained as colorless oil after purification by silica gel column chromatography. Spectroscopically V was characterized by a ureido group at 1623 cm^{-1} in the ir spectrum and lack of other carbonyl absorption. The nmr spectrum of V displayed two N-methyl groups and a sequence of three methylene groups at 1.69 ppm (quintet, J = 6 cps), 3.45 ppm (t, J = 6 cps), and 3.59 ppm (t, J = 6 cps), respectively.

In order to study the mechanism of the remarkable dimerization of II → III, 5,6-di-^{2}H-1,3-dimethyluracil (XVII) was prepared and converted to dimer XX which contained only two deuterium atoms according to nmr and mass spectra. This result is rationalized in terms of aldolization to XVIII, loss of (heavy) water to XIX, and rearrangement of the exocyclic to the endocyclic double bond (XIX → XX) with stereospecific removal of deuterium. The driving force in this rearrangement is the aromatization and attainment of planarity of the uracil moiety. This unprecedented dimerization by sodium borohydride is not brought about by alkali, baryta, or alkaline borate. Under too alkaline conditions ring opening to IV is observed with only trace amounts of dimer.



XVII XVIII XIX

XX

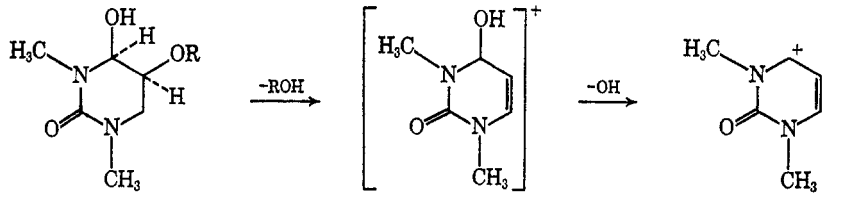
thymine in frozen aqueous solution has established the structure XIII.[10]

In the hydrated thymine ring the methyl and pyrimidine substituents are *trans* to each other. Dehydration to the fully aromatic dimer is easily achieved by acid.[11]

Another analog of dimer III is the novel uracil photodimer XVI from the irradiation of frozen aqueous uracil solutions.[12] It may arise *via* the oxetan XIV[13] and the hydrate XV.

Dihydroxytetrahydrouracils. 1,3-Dimethyl-5-hydroxyuracil (VI)[14] was catalytically reduced to 1,3-dimethyl-5-hydroxy-5,6-dihydrouracil (VIII) with rhodium on alumina. VIII was oxidatively acetylated to 1,3-dimethyl-5-acetoxyuracil (VII), mp 151–152.5°, with dimethyl sulfoxide and acetic anhydride.[15] Acetate VII was obtained directly by acetylation of VI. Hydrolysis of the acetate VII to VI was easily effected by ammonium hydroxide.

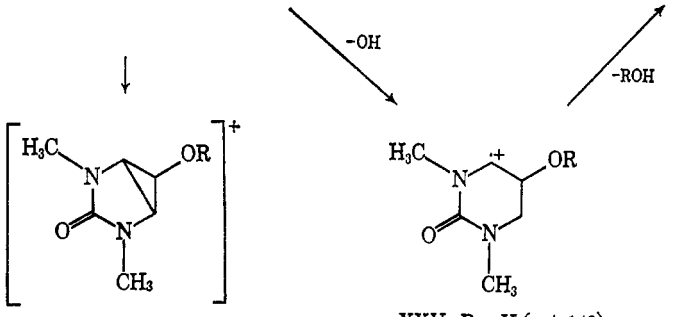
(10) I. Karle, personal communication.
(11) *Cf.* A. J. Varghese and S. Y. Wang, *Science*, **156**, 955 (1967); *Biochem. Biophys. Res. Commun.*, **29**, 543 (1967).
(12) M. N. Khattak and S. Y. Wang, *Science*, in press.
(13) *Cf.* R. S. Stafford and J. E. Donnellan, Jr., *Proc. Natl. Acad. Sci. U. S.*, **59**, 822 (1968).
(14) S. Y. Wang, *J. Am. Chem. Soc.*, **80**, 6196 (1958).
(15) J. D. Albright and L. Goldman, *ibid.*, **89**, 2416 (1967).



XXI, R = H (*m/e* 160)
R = Ac (*m/e* 202)

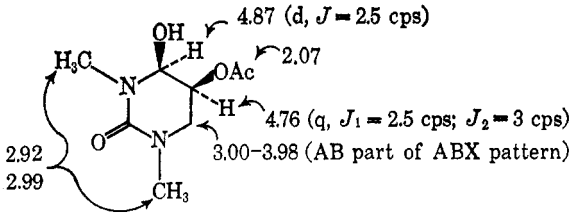
XXII, (*m/e* 142)

XXIII (*m/e* 125)



XXIV, R = H (142)
R = Ac (185)

XXV, R = H (*m/e* 143)
R = Ac (*m/e* 185)



XXVI

When 1,3-dimethyl-5-hydroxy-5,6-dihydrouracil (X) was reduced with sodium borohydride in 50% aqueous methanol the glycol IX, rather than a ring-opened alcohol, was obtained. After column chromatography on silica gel two compounds were isolated.

The major product IX, with a molecular ion peak at *m/e* 160, had a ureido group at 1616 cm^{-1} (ir) and two secondary alcoholic methines at 4.65 ppm (nmr). Acetylation of IX gave crystalline monoacetate XI, mp 111.5–112°, molecular ion peak *m/e* 202. The nmr spectrum of monoacetate XI had the secondary alcoholic methines separated at 4.76 and 4.87 ppm, respectively. 1,3-Dimethyl-2-oxo-*cis*-4,5-dihydroxyhexahydropyrimidine (IX) and its O-acetate belong to the interesting group of tetra- and hexahydropyrimidines derived from thym(id)ine,[16] N^4-acetylcytidine,[17] 4-thiouridine,[18] and uridine,[19] which are of interest as (potential) inhibitors of enzymes, such as cytidine deaminase.

The fragmentation patterns of IX and XI (Figure 3) showed intense peaks at *m/e* 142 and 125 for the loss of (ROH) and (ROH + OH) from the molecular ion, respectively. The loss of H_2O from the glycol IX involves 1,2 (XXI → XXII → XXIII; XXI → XXV → XXIII) and not 1,3 dehydration.[20]

The minor $NaBH_4$-reduction product from VIII turned out to be the O-methyl ether X which was acetylated to the crystalline, hygroscopic acetate XII. The oxygen functions in the glycol IX and its derivatives (X, XI, XII) are in the *cis* (equatorial–axial) conformation. This conclusion is based on the nmr spectrum (XXVI) of the monoacetate XI in which the secondary methine groups at C-4 and C-5 show up as a doublet (J = 2.5 cps) at 4.76 ppm and as a quartet (J_1 = 2.5 cps; J_2 = 3 cps) at 4.87 ppm, respectively. These coupling constants prove the *cis* conformation for the C-4 and C-5 protons.

(16) Y. Kondo and B. Witkop, *J. Amer. Chem. Soc.*, **90**, 764 (1968).
(17) N. Miller and P. Cerutti, *ibid.*, **89**, 2767 (1967).
(18) P. Cerutti, J. W. Holt, and N. Miller, *J. Mol. Biol.*, **34**, 505 (1968).
(19) A. R. Hanze, *J. Am. Chem. Soc.*, **89**, 1843 (1967).
(20) C. G. MacDonald, J. S. Shannon, and G. Sugowdz, *Tetrahedron Letters*, 807 (1963); H. Budzikiewicz, Z. Pelah, and C. Djerassi, *Monatsh.*, **95**, 158 (1964).

Experimental Section

General Procedures. Melting points are uncorrected and were taken on a Büchi apparatus. Nuclear magnetic resonance spectra were obtained on a Varian A-60 spectrometer using tetramethylsilane as an internal standard (TMS = 0.0 ppm). Ultraviolet and infrared absorption spectra were recorded on a Cary recording spectrophotometer, Model 14, and a Perkin-Elmer Infracord spectrophotometer, Model 137B, respectively. Thin layer chromatograms were Uniplate (silica gel G, 250 μ thick, Analtech, Inc.) and developed with the following ascending solvent systems: solvent A, chloroform–methanol, 7:3; solvent B, chloroform–methanol, 9:1; solvent C, ethyl acetate.

Irradiation. For irradiation a U-shaped Hanovia low-pressure mercury lamp, No. 87A-45, with an intensity of 4.3 W at 2537 Å was used. The light source was surrounded by a cylindrical water cooled quartz jacket (inner diameter 4.5 cm, width 1.5 cm). The distance of the two semicircular quartz cells from the outside of the cooler jacket was 1 cm. Irradiations were carried out at room temperature.

Photoreduction of 1,3-Dimethyluracil (I). A. Spectrometric Scale. A 10^{-4} M aqueous solution of 1,3-dimethyluracil was irradiated in the presence of a tenfold excess of sodium borohydride. Aliquots were withdrawn at 3, 6, 10, 15, 20, and 30 min, and the optical density was measured at the respective absorption maxima. This experiment was repeated twice. The average of the three values was plotted as shown in Figure 1.

B. Preparative Scale. A solution of 280 mg of 1,3-dimethyluracil in 1000 ml of water (2×10^{-3} M) was irradiated in the presence of 185 mg (5×10^{-3} M) of sodium borohydride as described under General Procedures. After irradiation for 10–12 hr the photolyzed solution was passed through a column of Amberlite IRC-50 (H^+ form) and lyophilized. The residue was taken up in methanol and the methanol solution evaporated to dryness. The colorless semicrystalline residue showed at least three major and four minor spots on tlc in solvent B. The photolyzed mixture was chromatographed on a column of silica gel (15 × 300 mm) and the

column was eluted with chloroform (300 ml). The chloroform eluate was evaporated and the residue crystallized from a mixture of ether–petroleum ether (bp 40–45°) to afford colorless needles, mp 54–55°. This material was identical with authentic 1,3-dimethyl-5,6-dihydrouracil with regard to ir spectrum and mixture melting point.

The remaining material on the column was eluted with a mixture of chloroform–methanol (95:5). Fractions 15–35 were pooled and purified by chromatography on a silica gel column. Crystallization from ether gave colorless prisms, mp 186°. This material was identical with 1,3-dimethyl-5,4′-(1′,3′-dimethyltetrahydropyrimidin-2′-one)uracil which was obtained by the reaction of 1,3-dimethyl 5,6-dihydrouracil with sodium borohydride (*vide infra*).

The third major spot (R_f 0.0 in solvent B) has so far not been isolated in a pure state.

1,3-Dimethyl-5,6-dihydrouracil (II). A. A solution of 1,3-dimethyluracil (1.34 g) in 50 ml of water was hydrogenated in the presence of 0.61 g of 10% palladium on carbon at room temperature in a Paar hydrogenator under 37 lb pressure. Aliquots were taken at intervals in order to follow the reduction by ultraviolet absorption. The absorption maximum at 266 mμ disappeared during 13 hr. After removal of the catalyst the filtrate was lyophilized. Recrystallization from a mixture of ether–petroleum ether (bp 30–60°) gave colorless needles, mp 54.5–56°.

Anal. Calcd for $C_6H_{10}N_2O_2$: C, 50.69; H, 7.09; N, 19.71. Found: C, 50.74; H, 7.14; N, 19.60.

The following data were obtained: ir (Nujol) (cm^{-1}): 1707 and 1695 (sh) (carbonyl), 1658 (cyclic ureido group); nmr ($CDCl_3$) (ppm): 2.74 (A_2 part of A_2B_2 pattern) (–CO*CH*$_2$*CH*$_2$N<), 3.06 (>NCH$_3$), 3.19 (>NCH$_3$), 3.39 (B_2 part of A_2B_2 pattern) (–COCH$_2$-*CH*$_2$N<).

B. A solution of 1,3-dimethyluracil (1.495 g) in 50 ml of water was hydrogenated in the presence of 5% rhodium on alumina (0.6 g) at a pressure of 22 lb. The characteristic uv absorption at 266 mμ disappeared completely after 1 hr. The catalyst was removed and the filtrate lyophilized. The residue crystallized from ether to afford colorless needles, mp 53–55°.

1,3-Dimethyl-5,6-dideuteriouracil (XVII). 1,3-Dimethyluracil was reduced catalytically in an atmosphere of deuterium gas as described above. Recrystallization from a mixture of ether–cyclohexane gave colorless prisms, mp 53–55°, in a yield of 93%. *Anal.* Calcd for $C_6H_8D_2N_2O_2$: mol wt, 144. Found: mol wt, 144 (mass spectrum).

The following data were obtained: ir (Nujol) (cm^{-1}): 1704 (carbonyl) and 1658 (cyclic ureido group); nmr (D_2O) (ppm): 2.77 d, J = 6.5 cps) (–COC*H*DCHDN<), 3.06 (>NCH$_3$), 3.18 (>NCH$_3$), 3.32 (d, J = 6.5 cps) (–COCHDC*H*DN<).

Conversion of 1,3-Dimethyl-5,6-dihydrouracil (II) with Sodium Borohydride to 1,3-Dimethyl-5,4′-(1′,3′-dimethyltetrahydropyrimidin-2′-one)uracil (III) and γ-(1,3-Dimethylureido)propanol-1 (V). A solution of 1.933 g (1.3×10^{-2} mol) of 1,3-dimethyl-5,6-dihydrouracil in 200 ml of water was reduced with 1.083 g (2.60×10^{-2} mol) of sodium borohydride for 2 hr. The resulting solution was worked up as described in the preceding experiments. The reduced product, a viscous oil, was dissolved in a small amount of methylene chloride, and ether added until the solution became turbid. On standing crystals deposited. The crude crystalline fraction (1.355 g) was poured on a silica gel column and eluted with a mixture of chloroform–methanol (95:5). Fractions 16–30 were pooled and evaporated to dryness *in vacuo*. Recrystallization from a mixture of acetone–ether afforded colorless prisms, mp 186–186.5°; R_f 0.49 in solvent B; soluble in chloroform, methanol, benzene, acetone, and water; sparingly soluble in ether and insoluble in petroleum ether.

Anal. Calcd for $C_{12}H_{18}N_4O_3$: C, 54.12; H, 6.81; N, 21.04, mol wt, 266. Found: C, 54.71; H, 6.24; N, 20.82; mol wt, 266 (mass spectrum).

The following data were obtained: ir (Nujol) (cm^{-1}): 1704 (α,β-unsaturated carbonyl), 1672 (sh), 1658 (cyclic ureido), 1639 (conjugated double bond); uv, $\lambda_{max}^{H_2O}$ (mμ): 204 (log ε 4.27), 271 (log ε 3.95); $\lambda_{max}^{0.01\ N\ NaOH}$ (mμ): 271 (log ε 3.95); nmr ($CDCl_3$) (ppm): 2.15 (m) (2 H) (–CH$_2$*CH*$_2$CH<), 2.94 (>NCH$_3$), 2.98 (>NCH$_3$), 3.19 (m) (2 H) (>NC*H*$_2$CH$_2$–), 3.36 (>NCH$_3$), 3.44 (>NCH$_3$), 4.54 (broad t, J = 4 cps) (1 H) (>N$^+$*CH*CH$_2$–), 6.90 (d, J = 1 cps) (1 H) (olefinic proton).

The mother liquor was evaporated to dryness *in vacuo*. The viscous oil was chromatographed on a column of silica gel (20 × 250 mm) and the column eluted with chloroform–methanol (95:5) (6-ml fractions). Fractions 32–48 were pooled, evaporated, and rechromatographed over silica gel (20 × 300 mm) in the same solvent system. Fractions 36–49 showed a homogeneous spot (R_f 0.80) by tlc in the solvent system chloroform–methanol (8:2). After the removal of solvent under reduced pressure pure γ-(N,N′-dimethylureido)propanol-1 was obtained as a colorless oil in a yield of 13.8%.

Anal. Calcd for $C_6H_{14}N_2O_2$: C, 49.30; H, 9.65; N, 19.17; mol wt, 146. Found: C, 49.20; H, 9.34; N, 18.82; mol wt, 146 (mass spectrum).

The following data were obtained: ir (liquid film) (cm^{-1}): 3442 (NH and –OH), 1623 (ureido), 1059 (ν_{CO}); nmr ($CDCl_3$) (ppm): 1.69 (quintet, J = 6 cps) (–CH$_2$*CH*$_2$CH$_2$OH), 2.79 (>NCH$_3$), 2.87 (>NCH$_3$), 3.45 (t, J = 6 cps) (>NC*H*$_2$CH$_2$–), 3.59 (t, J = 6 cps) (–*CH*$_2$OH), 3.90 (broad) (>NH and –OH), mass (*m/e*): 146 (M^+), 101 (M – C_2H_5O), 88, 58 (·CH$_3$NHCO·), 45 (C_2H_5O), 44 (base peak).

Subsequent eluted fractions contained 300 mg of oily substances, which showed several spots on tlc.

Condensation of 1,3-Dimethyl-5,6-dideuteriouracil (XXVII) to 1,3-Dimethyl-5,4′-(1′,3′-dimethyl-5′,6′-dideuteriotetrahydropyrimidin-2′-one) (XX) with Sodium Borohydride. The procedure described above was applied to 2.16 g (1.5×10^{-2} mol) of 1,3-dimethyl-4,6-dideuteriouracil and 1.134 g (3×10^{-2} mol) of sodium borohydride in 250 ml of water. The colorless oily reaction product was chromatographed on a column of silica gel (20 × 300 mm) with 200 ml of chloroform and 350 ml of chloroform–methanol (95:5). After recovery of 0.543 g of starting material, fractions 53–60 gave 0.871 g of crude dimer and fractions 71–89 gave 0.30 g of a colorless oil. Fractions 53–60 were rechromatographed on a column of silica gel (20 × 320 mm) (chloroform–methanol, 95:5). The new fractions 12–30 crystallized from ether. Recrystallization from a mixture of acetone–ether gave colorless prisms, mp 185°. The nmr spectrum showed: 2.10 (broad m) (–CHDC*H*DCH<), 928 (>NCH$_3$), 3.05 (>NCH$_3$), 3.20 (broad) (>NC*H*DCHD–), 3.36 (>NCH$_3$), 3.44 (>NCH$_3$), 4.50 (broad) (<NC*H*CHD–), 6.88 (olefinic proton).

γ-(1,3-Dimethylureido)-5,6-dideuteriopropanol-1. Fractions 71–89 were purified by preparative tlc to give the deuterated hydrogenolysis product as a colorless oil in a yield of 13.7%

γ-(1,3-Dimethylureido)propionic Acid (IV). A solution of 1 g of 1,3-dimethyl-5,6-dihydrouracil in 25 ml of 0 5 *N* aqueous baryta was left at room temperature for 48 hr. The solution was evaporated and the barium salt of acid IV was recrystallized from water to give colorless needles, mp 196–196.5°.

Anal. Calcd for $(C_6H_{11}N_2O_3)_2Ba \cdot H_2O$: C, 30.43; H, 5.17. Found: C, 29.95; H, 5.39.

The following data were obtained: ir (Nujol) (cm^{-1}): 3436 (>NH and H_2O), 1639 (sh) (ureido), 1618 (carboxylate); nmr (D_2O) (ppm): 2.37 (t, J = 7.3 cps) (–CH$_2$*CH*$_2$COO·0.5Ba), 2.68 (>N-CH$_3$), 2.84 (>NCH$_3$), 3.48 (t, J = 7.3 cps) (–ND*CH*$_2$CH$_2$–). The aqueous solution of the barium salt was neutralized with 10% sulfuric acid and the precipitate, barium sulfate, was removed by filtration through a Celite layer. The supernatant was lyophilized to give a crystalline residue which was recrystallized from a mixture of methanol–ethyl acetate to yield colorless needles, mp 73–75°.

Anal. Calcd for $C_6H_{12}N_2O_3$: C, 44.99; H, 7.55; N, 7.49. Found: C, 44.71; H, 7.59; N, 7.30; mol wt, 160 (mass spectrum).

When the dihydrouracil II was left in excess 1.0 *N* sodium carbonate, diethylamine, or alkaline borate buffer (pH 10) for 5–24 hr at 20° starting material was recovered quantitatively.

1,3-Dimethyl-5-acetoxyuracil (VII). By Oxidation of 1,3-Dimethyl-5-hydroxy-5,6-dihydrouracil (VIII). To the stirred solution of 270 mg of 1,3-dimethyl-5-hydroxy-5,6-dihydrouracil (VIII) in 3 ml of dry dimethyl sulfoxide was added dropwise 2 ml of acetic anhydride. After standing for 24 hr at room temperature the reaction mixture was evaporated *in vacuo* at 50°. The residue crystallized on trituration with ether. Recrystallization from a mixture of methylene chloride and ether gave 147 mg of colorless prisms, mp 151–152.5°.

Anal. Calcd for $C_8H_{10}N_2O_4$: C, 48.48; H, 5.09; N, 14.14; mol wt, 198. Found: C, 48.43; H, 5.02; N, 14.24; mol wt, 198 (mass spectrum).

The following data were obtained: ir (Nujol) (cm^{-1}): 1773 (enol acetyl), 1706 (carbonyl), 1672 (conjugated double bond), 1642 (cyclic ureido group), 1198 (ν_{CO}); nmr ($CDCl_3$) (ppm): 2.29 (*CH*$_3$CO), 3.36 (>NCH$_3$), 3.41 (>NCH$_3$), 7.21 (olefinic proton); uv, λ_{max}^{EtOH} (mμ): 208 (log ε 4.14), 270.5 (log ε 4.13).

Hydrolysis of Acetate VII. A solution of acetate VII (100 mg) in 25% NH_4OH was heated on a steam bath for 30 min and then evaporated to dryness. The crystalline residue was recrystallized twice from acetone to afford colorless needles, mp 201°.

Anal. Calcd for $C_6H_8N_2O_3$: C, 46.15; H, 5.16; N, 17.94. Found: C, 46.18; H, 5.06; N, 17.81.

The melting point of this material was undepressed on admixture with 1,3-dimethyl-5-hydroxyuracil (VI) (mp 201–201.5°) obtained by published methods.[13] The two compounds were completely identical with regard to ir, nmr spectra, and R_f values on tlc.

1,3-Dimethyl-5-acetoxyuracil (VII). A mixture of 1,3-dimethyluracil (I) (300 mg), acetic anhydride (2 ml), and dry pyridine (5 ml) was kept at 50° for 12 hr and then evaporated to dryness *in vacuo*. The residue was dissolved in methylene chloride. The solution after filtration through a silica gel column (10 × 50 mm) and removal of the solvent gave colorless crystals. Recrystallization from a mixture of methylene chloride–ether gave 257 mg of colorless prisms, mp 150–151.5°.

Anal. Calcd for $C_8H_{10}NO_4$: C, 48.48; H, 5.09; N, 14.14. Found: C, 48.03; H, 4.60; N, 14.52.

Reduction of 1,3-Dimethyl-5-hydroxy-5,6-dihydrouracil (VIII) with Sodium Borohydride. To a stirred solution of 1.0 g of 1,3-dimethyl-5-hydroxy-5,6-dihydrouracil (VIII) in 100 ml of 50% aqueous methanol was added 250 mg of sodium borohydride. After 30 min another 250 mg of sodium borohydride was added and the mechanical stirring continued for 2 hr. The resulting solution was passed through an Amberlite IRC-50 (H^+ form) column in order to remove the sodium ion. The filtrate was lyophilized and the residue dissolved in *methanol* and evaporated *in vacuo*. This procedure was repeated three or four times until all the boric acid had been removed. The reduction product was chromatographed on a column of silica gel (20 × 280 mm), the column was eluted with a mixture of chloroform and methanol (8:2), and fractions of 6 ml were collected.

Fractions 15–30 (600 mg) were collected and purified twice on silica gel and finally by preparative thin layer chromatography to give the glycol XII as a homogeneous amorphous powder (R_f 0.51; solvent A), mol wt, 160 (mass spectrum). The following were obtained: ir (liquid film) (cm^{-1}): 3448 (NH), 1618 (ureido); nmr (CD_3OD) (ppm): 2.92 (>NCH_3), 2.98 (>NCH_3), 3.0–3.8 (m) (>NCH_2CH–), 4.67 (m) (2>$CHOH$).

Acetate. The acetate was prepared with acetic anhydride in pyridine. Recrystallization from ether gave colorless prisms, mp 111.5–112°.

Anal. Calcd for $C_8H_{14}N_2O_4$: C, 47.52; H, 6.98; N, 13.86; mol wt, 202. Found: C, 47.55; H, 6.91; N, 13.78; mol wt, 202 (mass spectrum).

The following data were obtained: ir (Nujol) (cm^{-1}): 3311 (–OH), 1742 (acetyl), 1645 (sh), 1629 (ureido); nmr ($CDCl_3$) (ppm): 2.07 (CH_3CO–), 2.92 (>NCH_3), 2.99 (>NCH_3), 3.00–3.98 (m) (>NCH_2CH–), 4.76 (q, J_1 = 2.5 cps, J_2 = 3 cps), 4.87 (d, J = 2.5 cps), 5.35 (broad) (OH).

Fractions 12–14 (400 mg) were purified by preparative tlc. The band corresponding to R_f 0.55 (solvent B) was separated, eluted, and acetylated with acetic anhydride in pyridine. The hydroscopic acetate formed colorless prisms: ir (liquid film) (cm^{-1}): 1742 (acetyl), 1650 (ureido), 1230 (acetyl), 1080 and 1742 (ν_{C-O}); nmr ($CDCl_3$) (ppm): 2.08 (acetyl), 2.95 (>NCH_3), 3.08 (>NCH_3), 3.47 (–OCH_3), 3.50 (m) (>NCH_2CH–), 4.41 (q, J_1 = 1.7 cps, J_2 = 2.8 cps) (>CHO), 4.96 (q, J_1 = 2.8 cps, J_2 = 5 cps) (>CHO).

Studies on Models for Tetrahydrofolic Acid. I. The Condensation of Formaldehyde with Tetrahydroquinoxaline Analogs

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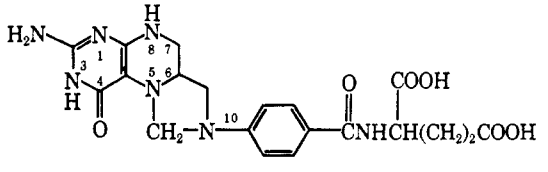
Abstract: To investigate the mechanisms of tetrahydrofolic acid catalyzed one carbon unit transfers, we have synthesized several tetrahydroquinoxaline analogs. A kinetic investigation of the condensation with formaldehyde of one of these models reveals the intermediacy of the iminium cation as a steady-state species and the importance of general catalysis in formation of the imidazolidine ring, the latter a model for 5,10-methylenetetrahydrofolic acid. The relevance of these results to the mechanism of one carbon unit transfers and the importance of certain structural and electronic features in the actual cofactor are discussed.

The intermediacy of N^5,N^{10}-methylenetetrahydrofolic acid in the transfer of one carbon unit at the oxidation level of formaldehyde in various enzymic reac-



tions including the interconversion of glycine and serine and the formation of 5-hydroxymethyldeoxycytidylic acid and deoxythymidylic acid illustrates its importance in biosynthesis.[2] The molecular arena comprised of the 5-nitrogen of the reduced pyrazine ring and the 10-nitrogen of the *p*-aminobenzamide residue also provide many challenging mechanistic questions concerned with the actual pathway of the carbon transfer, challenges further complicated by the instability of the actual cofactor.

We have approached this problem by designing model analogs predicated on the desirability of simplifying experimental problems through removal of several "nonessential" dissociable groups subject, however, to the following considerations.

It is apparent from the extensive work of Baker, *et al.*,[3] on analogs of tetrahydrofolic acid designed to act as nonclassical antimetabolites against dihydrofolate reductase and the structure–activity analysis of their inhibition by the method of Hansch[4] that a major function of the pyrimidine ring of the pteridine moiety is the binding of the cofactor to the enzyme. This is

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