Pyrimidines. VI. A Novel Degradation of 3-Methyl-4-thiouracil[1]

KYOICHI A. WATANABE, HERBERT A. FRIEDMAN, ROBERT J. CUSHLEY, AND JACK J. FOX

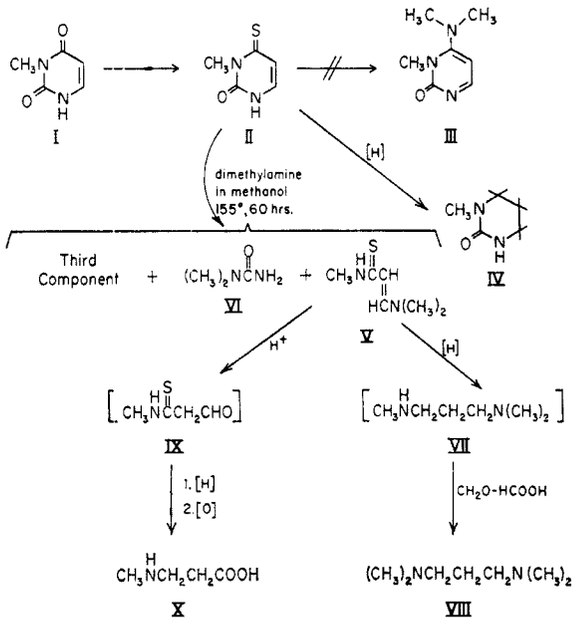
Division of Biological Chemistry, Sloan-Kettering Institute for Cancer Research, Sloan-Kettering Division of Cornell University Medical College, New York 21, New York

Received April 12, 1966

3-Methyl-4-thiouracil (II) underwent an unexpected degradation when treated with dimethylamine in methanol at 155° for 60 hr. Three products were produced, two of which were identified as N,N-dimethylurea (VI) and *trans*-β-dimethylaminothioacrylic acid methylamide (V). Catalytic reduction of V followed by N-methylation gave bis-1,3-dimethylaminopropane (VIII). Acid hydrolysis of the enamine V followed by catalytic reduction and then oxidation yielded β-methylaminopropionic acid (X). These chemical data along with nmr studies establish structure V. The structure of II was confirmed by reduction to the known N-methyl-N,N′-trimethyleneurea (IV). A plausible mechanism for the reaction of II → V and VI *via* an isocyanate intermediate is proposed.

In a previous paper, Ueda and Fox[2] attempted to prepare 3-methyl-4-dimethylamino-2(3H)-pyrimidinone (III) by reaction of 3-methyl-4-thiouracil (II) with dimethylamine (Scheme I). Instead of III, these authors obtained "sulfur containing products" from this reaction. The present paper deals with a detailed investigation into this reaction which, as will be shown, followed an unexpected course.

SCHEME I



In the previous report, compound II was prepared by selective thiation of 3-methyluracil (I). The structure of II was suggested by the similarity of its ultraviolet absorption spectrum to that for 4-thiouracils. In order to confirm this structure, the thiopyrimidine (II) was reduced with activated Raney nickel to the known[3] N-methyl-N,N′-trimethyleneurea (IV).

The reaction of 3-methyl-4-thiouracil (II) with dimethylamine in methanol was carried out in a sealed glass tube[4] at 155° for 60 hr. (At lower temperatures, *e.g.*, 110–130°, practically no reaction occurs.) After the reaction, the clear mixture exhibited a rather curious ultraviolet spectral behavior (Figure 1). The initial spectrum of the reaction mixture in water (curve A) showed maxima at 265 and 337 mμ. This spectrum changed during a 24-hr period to curve B which had completely lost the maximum at 337 mμ while the short wavelength maximum shifted to 260 mμ. This spectral change with time could be accelerated rapidly by addition of catalytic amounts of acid, whereas addition of alkali to the curve A solution prevented the conversion of the spectrum to curve B. Treatment of curve B solution with alkali gave curve C with a new maximum at 316 mμ. Acidification of C produced B. These spectral data suggested that the reaction mixture contained a compound which was unstable in water and in acid and which, after hydrolysis, gave a product whose spectrum was pH dependent.

The nmr spectrum of the reaction mixture (after removal of solvent and dissolution of the semicrystalline residue in DMSO-d_6 is shown in Figure 2a. Two pairs of doublets indicate four different olefinic protons. The five sharp resonance peaks at higher magnetic field represent the presence of several N-methyl groups. An additional broad peak was observed at δ 8.72. Addition of deuterium oxide gave spectrum 2b in which the methyl resonances centered at δ 2.97 (Figure 2a) had merged into a singlet. The downfield broad resonance peak at δ 8.72 disappeared immediately. Moreover, the olefinic doublets centered at δ 5.25 and δ 7.75 gradually diminished with the appearance of a new resonance peak at δ 7.75.

A pure compound of empirical formula $C_6H_{12}N_2S$ (V) was crystallized (from water) from this reaction. The ultraviolet absorption characteristics of V were identical with those shown in Figure 1. The nmr spectrum of V in DMSO-d_6 (Figure 2c) showed the presence of two nonequivalent protons attached to an internal double bond. The large coupling constant (J = 12 cps) indicates that they are vicinal. Integration of the spectrum showed that the methyl resonance doublet centered at δ 2.97 (secondary amine) was equivalent to three hydrogens whereas that at δ 2.88 (tertiary amine) represented six hydrogen atoms. The NH proton at δ 8.72 was also observed. These data suggested structure V for this product.[5]

Catalytic reduction of V with Raney nickel followed by N-methylation with formaldehyde–formic acid

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA 08748).

(2) T. Ueda and J. J. Fox, *J. Am. Chem. Soc.*, **85**, 4024 (1963).

(3) J. J. Fox and D. van Praag, *ibid.*, **82**, 486 (1960).

(4) Attempts to run this reaction in an open glass container enclosed in a steel bomb gave brown syrups from which 3-methyluracil (I) was obtained.

(5) Since the reaction was run at 155° for 60 hr, the thermodynamically more stable *trans* isomer is postulated. *trans* coupling constants for vinyl hydrogens have been reported from J = 9.5 to 22 cps (see A. A. Bothner-by in "Advances in Magnetic Resonance," Vol. 1, Academic Press Inc., New York, N. Y., 1965, p 265).

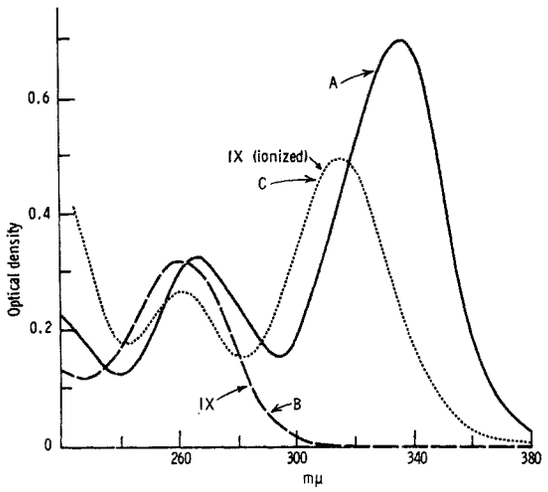


Figure 1.—Ultraviolet absorption spectrum of the reaction mixture. Curve A, reaction mixture taken immediately in water. Curve B, after 24 hr. Curve C, obtained by treatment of Curve B sample with alkali. Identical spectra (curves A, B, C) are given by similar treatment of compound V.

yielded the known[6] bis-1,3-dimethylaminopropane (VIII). Surprisingly, this same diamine was obtained directly when V was desulfurized with Raney nickel in methanol.[7] These data establish the skeleton of V as

$$C{-}N{-}C{-}C{-}C{-}N{<}^{C}_{C}$$

The enamine structure V is consonant with the ultraviolet data given in Figure 1. The alteration of curve A to B is best explained by the acid-catalyzed cleavage of V to the aldehyde (IX) and dimethylamine, a behavior characteristic of enamines.[8] The spectral curve B (Figure 1) is quite similar to that for thioacetamide[9] and N-methylthioacetamide. Curve C suggests an ionized form of IX which because of the presence of a conjugated system exhibits absorption above 300 mμ.

Treatment of 3c sample (Figure 2) with a mixture of deuterioacetic acid–D_2O (Figure 2d) causes the disappearance of the olefinic proton doublets and the appearance of a new downfield resonance singlet at δ 7.75. This alteration of the nmr spectrum from 2c to 2d is consistent with the cleavage of V to IX and dimethylamine.[10]

Further confirmation of structure V was obtained as follows. Compound V was hydrolyzed in dilute acid and the resulting solution (containing IX) was desulfurized with Raney nickel and finally oxidized with potassium permanganate. A residue was obtained which by paper chromatographic examination (ninhydrin spray) in three solvent systems was identical with β-methylaminopropionic acid (X).

After compound V was separated from the reaction mixture, a second crystalline product was obtained. This product (VI) was identified as N,N-dimethylurea by its combustion analyses, melting point, and infrared spectrum.[11] The nmr spectrum of VI is given in Figure 2e.

It is clear from Figure 2a that the reaction mixture contains, in addition to V and VI, a third component which has olefinic protons centered at δ 5.57 and 7.38 (J = 7.5 cps) and a methyl resonance at δ 3.11. This third component could not be separated from N,N-dimethylurea (VI) (Figure 2f). Attempts to isolate the third component led to intractable tars. It is

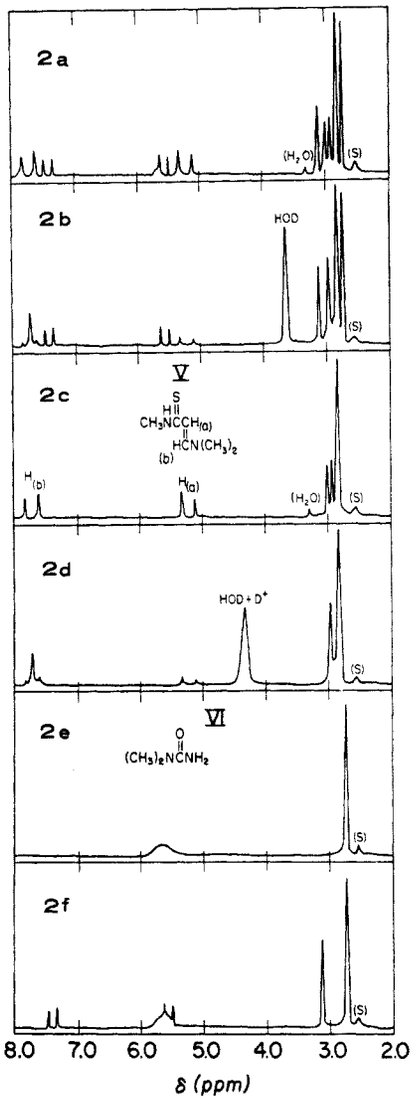


Figure 2.—Nmr spectra of reaction products taken in DMSO-d_6 on a Varian A-60 spectrometer using tetramethylsilane as an internal reference. Designation(s) at δ 2.52 refers to CD_3SOCD_2H. (2a) A mixture of reaction products. (2b) Addition of D_2O to sample 3a. (2c) Compound V. (2d) Addition of a mixture of CD_3COOD-D_2O to sample 3c. (2e) N,N-dimethylurea, VI. (2f) A mixture of the "third component" and N,N-dimethylurea.

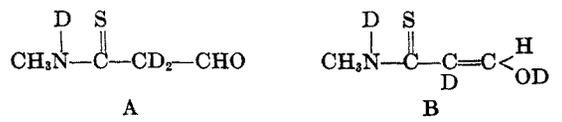
(6) H. T. Clarke, *J. Chem. Soc.*, **103**, 1689 (1913); L. Knorr and P. Roth, *Ber.*, **39**, 1420 (1906).

(7) The N alkylation of the dethiated intermediate was probably due to the presence of small amounts of formaldehyde and formic acid in the methanol.

(8) J. A. West, *J. Chem. Educ.*, **40**, 194 (1963); L. F. Fieser and M. Fieser, "Advanced Organic Chemistry," Reinhold Publishing Corp., New York, N. Y., 1961, p 498.

(9) D. Rosenthal and T. I. Taylor, *J. Am. Chem. Soc.*, **79**, 2684 (1957).

(10) It is assumed that IX has the form of either A or B or an average showing rapid equilibration of A and B due to CD_3COOD. Such compounds are known to exist mostly in enolic form B: J. Hine in "Physical Organic Chemistry," McGraw-Hill Book Co., Inc., New York, N. Y., 1956, p 231.

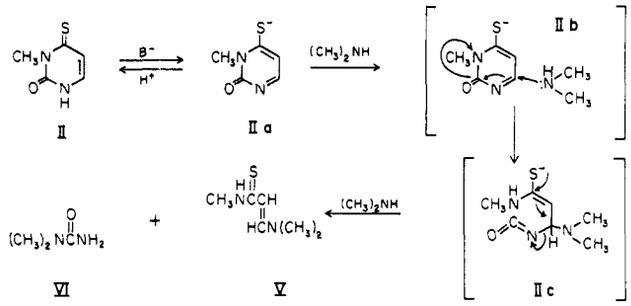


(11) N. B. Colthup, L. H. Daly, and S. E. Wiberly, "Introduction to Infrared and Raman Spectroscopy," Academic Press Inc., New York, N. Y., 1964, p 384.

possible that the third component is a β-methoxyacrylic acid derivative since the methyl resonance of methyl vinyl ether occurs at δ 3.14.[12]

A plausible mechanism for the reaction of II → V is given in Scheme II. The formation of the monoanion IIa in base is expected from the ultraviolet spectral data previously reported.[2] Nucleophilic attack by dimethylamine on the 6 position to give IIb would then be followed by the formation of isocyanate IIc.[13,14]

SCHEME II



Compound V (*trans*-β-dimethylaminothioacrylic acid methylamide) is formed from IIc by the elimination of the isocyanate group which would react simultaneously with dimethylamine to form N,N-dimethylurea (VI). This mechanism receives some support from the fact that 1,3-dimethyl-4-thiouracil,[15] which cannot form the monoanion IIa, fails to react with dimethylamine under the conditions used to convert II to V.[16]

The fact that II was not converted to the 4-dimethylamino derivative III (Scheme I) may be due to steric hindrance caused by the bulky methyl groups. In this regard, Russell, *et al.*,[17] observed that 5-methyl-2,4-pyrimidinedithiol failed to react with secondary amines of all types under conditions in which 2,4-pyrimidinedithiol was easily converted to the corresponding 4-dialkylamino-2-pyrimidinethiol. They concluded that their reaction was blocked by steric hindrance. In compound II, the presence of a methyl group on position 3 is essentially sterically equivalent to a methyl group on position 5. In support of this hypothesis, 1-methyl-4-thiouracil[18] which lacks steric hindrance at the 3 and 5 positions, reacts with dimethylamine in methanol under milder conditions (115° for 19 hr) to yield the known[19] 1-methyl-4-dimethylamino-2(1H)-pyrimidinone.

To our knowledge, the breakdown of a pyrimidine derivative by the sequence II → V and VI has not hitherto been reported.

Experimental Section[20]

N-Methyl-N,N'-trimethyleneurea (IV) from II.—Compound II[2] (2.0 g) was dissolved in ethanol (400 ml) and refluxed with *ca.* 20 g of activated Raney nickel. Aliquots were taken at intervals in order to monitor the reduction by spectrophotometry. The absorption maximum at 320 mμ immediately shifted to 305 mμ which remained essentially constant during 2 hr. Additional Raney nickel (20 g) was added and the mixture refluxed for 2 hr. The maximum at 305 mμ disappeared during this period, but absorption at 260 mμ was still observed. Further addition of Raney nickel (20 g) and continued refluxing for 2 hr was necessary to remove selective absorption in the 250–300-mμ region. The catalyst was removed by filtration and the filtrate was evaporated to dryness under reduced pressure. The residue, upon crystallization from ethanol, afforded 1.57 g (98%) of colorless crystals, mp 87–89° (lit.[3] 86–89°). The infrared spectrum of the picrate of IV was identical with that of an authentic sample. The melting point of the picrate (133–135°) was not depressed on admixture with an authentic specimen.[3]

Reaction of 3-Methyl-4-thiouracil (II) with Dimethylamine.—A solution of II (3.05 g, 21.5 mmoles) in 20% methanolic dimethylamine (80 ml) was placed in a glass tube which was sealed and heated at 155° for 60 hr. The tube was cooled and opened and the reaction mixture was evaporated to a syrup under reduced pressure at about 40°. The syrup was dissolved in 20 ml of benzene and the solvent was removed by evaporation. The residue was again dissolved in 20 ml of benzene and concentrated to dryness. This procedure was repeated until a semicrystalline residue was obtained. The nmr spectrum of this residue is shown in Figure 2a. The semicrystalline residue was recrystallized from 30 ml of hot water. Pale yellow leaflets of V were obtained, 0.75 g (25%), mp 149–150°.

Anal. Calcd for $C_6H_{12}N_2S$: C, 50.00; H, 8.33; N, 19.44; S, 22.22. Found: C, 50.08; H, 8.34; N, 19.32; S, 22.21.

The mother liquor from recrystallization of V was evaporated to dryness *in vacuo* at ~40° to a syrup. This syrup was further dried azeotropically several times with benzene after which the residue solidified. The residue was triturated with a 1:3 mixture of dichloroethane and xylene (20 ml). The insoluble crystalline material was filtered and recrystallized from ethanol–petroleum ether. The yield of colorless cubes of N,N-dimethylurea (VI) was 250 mg, mp 183–184° (lit.[21] 182–184°).

Anal. Calcd for $C_3H_8N_2O$: C, 40.91; H, 9.09; N, 31.82. Found: C, 40.73; H, 8.90; N, 31.67.

The dichloroethane–xylene extracts were evaporated to dryness. The dark brown residue was dissolved in hot xylene (20 ml) and cooled gradually to room temperature. A brown crystalline precipitate formed which was recrystallized from xylene (20 ml). The yield of brown, recrystallized product was about 120 mg, mp 70–110° dec. The nmr spectrum of this product (Figure 2f) indicated that it was a mixture of at least two components, one of which was N,N-dimethylurea. Attempts to decolorize this precipitate with charcoal failed. The product was slowly converted to a dark tar after several days.

Bis-1,3-dimethylaminopropane (VIII) from V. Method A.—A mixture of V (402 mg, 2.8 mmoles) and Raney nickel (10 g, washed with ethanol) in 70 ml of ethanol was stirred for 2 hr. The catalyst was removed by filtration and washed with 50 ml of water. The combined filtrate and washings were adjusted to pH 1 with 1 *N* hydrochloric acid and the solution was evaporated to dryness. A syrup (VII) was obtained which could not be crystallized from ethanol. The syrup was dissolved in 40 ml of water and the aqueous solution was adjusted to pH 14 by 1 *N* sodium hydroxide. The free amine (VII) was extracted with two 20-ml portions of ether. The extracts were dried over anhydrous sodium sulfate. The ether was removed by evaporation under reduced pressure at room temperature. The syrup of VII thus obtained was dissolved in a 1:1 mixture of formic acid and formalin (2 ml) and refluxed overnight. The reaction mixture was diluted with water (2 ml) and treated dropwise with a methanolic solution of picric acid until precipitation of the picrate of VIII ceased. The picrate was filtered and washed with ether. The yield of picrate (VIII) was 910 mg (55%), mp 205–207° (Clarke[6] reported mp 207°).

Anal. Calcd for $C_7H_{18}N_2 \cdot 2C_6H_3N_3O_7$: C, 38.78; H, 4.08; N, 19.05. Found: C, 38.92; H, 4.25; N, 18.92.

(12) R. T. Hobgood, Jr., G. S. Reddy, and J. H. Goldstein, *J. Phys. Chem.*, **67**, 110 (1963).

(13) Isocyanate intermediates in the 3-methylcytosine rearrangement had been postulated.[14]

(14) T. Ueda and J. J. Fox, *J. Org. Chem.*, **29**, 1762, 1770 (1964).

(15) G. B. Elion and G. H. Hitchings, *J. Am. Chem. Soc.*, **69**, 2138 (1947).

(16) 3-Methyluracil also failed to react with dimethylamine in methanol at 155°.

(17) P. B. Russell, G. B. Elion, E. A. Falco, and G. H. Hitchings, *J. Am. Chem. Soc.*, **71**, 2279 (1949).

(18) J. J. Fox, D. van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *ibid.*, **81**, 178 (1959).

(19) G. W. Kenner, C. B. Reese, and A. R. Todd, *J. Chem. Soc.*, 855 (1955).

(20) Melting points were taken on a Thomas–Hoover capillary melting point apparatus and are corrected. Analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich., and by Galbraith Labs, Inc., Knoxville, Tenn. Nmr spectra were taken on a Varian A-60 spectrometer with tetramethylsilane as an internal reference.

(21) F. Kurzer, *Org. Syn.*, **32**, 61 (1952).

The infrared spectrum of this picrate was identical with that of an authentic specimen.[6]

B. Direct Method.—To a solution of V (288 mg, 2 mmoles) in reagent grade methanol (50 ml) was added methanol-washed, activated Raney nickel (*ca.* 5 g) and the mixture was stirred for 2 hr. The Raney nickel was filtered and washed with water (30 ml). The combined filtrate and washings were adjusted to pH 1 with 1 *N* hydrochloric acid, then evaporated to dryness under reduced pressure. A slightly yellow, crystalline residue was obtained which was recrystallized from ethanol. Pale yellow needles of the dihydrochloride of VIII (248 mg, 58%) were obtained, mp 302°.

The picrate of VIII, mp 205–207°, was readily obtained by addition of a methanolic solution of picric acid to the aqueous solution of the dihydrochloride. The infrared spectra and melting points of the dihydrochloride and picrate of VIII were identical with those of authentic samples prepared from 1,3-dibromopropane and dimethylamine.[6] The melting points of these salts were not depressed by admixture with the authentic samples[6] of the corresponding salts.

β-Methylaminopropionic Acid (X) from V.—Compound V (633 mg, 4.4 mmoles) was dissolved in 130 ml of a 1:1 mixture of ethanol and 0.1 *N* hydrochloric acid. Hydrolysis of IX was followed spectrophotometrically. Within 15 min the absorption maximum at 337 mμ completely disappeared. Raney nickel (30 g) was then added and the mixture was stirred for 30 min. The catalyst was filtered and washed with a 1:1 mixture of ethanol and water (50 ml). The filtrate and washings were combined and evaporated to dryness. The residue was dissolved in 100 ml of water and treated with 1.2 g of potassium permanganate and 5 ml of 1 *N* sodium hydroxide. The mixture was shaken for 2 hr after which it was treated with methanol until the red color of permanganate disappeared. Precipitated manganese dioxide was removed by filtration through a pad of diatomaceous earth. The filtrate was evaporated to dryness to a white solid. The solid was dissolved in 10 ml of water followed by addition of ethanol (20 ml). Precipitated inorganic material was removed by filtration. The filtrate was evaporated to a glass, which still contained considerable amount of impurities and could not be crystallized. The glass was subjected directly (Table I) to paper chromatographic examination (Whatman No. 1, descending method) and was identical in $R_{sarcosine}$ values with authentic X.

TABLE I

Solvent system[a]	$R_{sarcosine}$: Glassy product (X)	$R_{sarcosine}$: Authentic (X)[b]	$R_{sarcosine}$: β-Dimethylaminopropionic acid[c]
A	2.0	2.0	4.1
B	1.5	1.5	1.6
C	Front	Front	Front

[a] Solvent system A = 2,6-lutidine, ethanol, water, and diethylamine (55:25:20:2); B = upper layer of the following mixture: *n*-butanol, water, and acetic acid (50:50:6); C = phenol (400 ml), water (100 ml), and 8-hydroxyquinonoline (50 mg). [b] Prepared by condensation of β-bromopropionic acid with methylamine [P. Handler, M. L. C. Bernhein, and J. R. Klein, *J. Biol. Chem.*, **138**, 215 (1941)]. [c] Prepared by reaction of β-bromopropionic acid with dimethylamine [U. S. Patent 2,203,009 (1937)].

1-Methyl-4-dimethylamino-2(1H)-pyrimidinone.[19]—A mixture of 1-methyl-4-thiouracil[18] (2.0 g, 0.014 mole) in 20% methanolic dimethylamine was heated at 115° for 19 hr in a sealed glass tube. The almost colorless reaction mixture was evaporated *in vacuo* to dryness to a solid which was crystallized from ethyl acetate. Colorless needles (0.85 g) deposited, mp 175–177° [not lowered on admixture with 1-methyl-4-dimethylamino-2(1H)-pyrimidinone which was prepared by the procedure of Kenner, *et al.*[19]] The ultraviolet absorption spectral characteristics were also identical with those reported.[19] An additional 500 mg of this product was obtained from the mother liquors (total yield 1.35 g, 63%).

Acknowledgment.—The authors are indebted to Dr. George B. Brown for his warm and continued interest.

Synthesis of 5-Hydroxyalkylpyrimidines from Lactones. II. 5-Monohydroxycyclopentylpyrimidines[1]

JOHN D. FISSEKIS AND BARBARA A. MARKERT

Division of Biological Chemistry, Sloan-Kettering Institute for Cancer Research, New York 21, New York

Received June 24, 1965

The synthesis of 5-hydroxycyclopentylpyrimidines, including those of uracil and cytosine, is described. Their stereochemistry and the significance of their spectra with respect to those of other hydroxyalkylpyrimidines are discussed.

We have reported[2] the synthesis of a number of 5-hydroxyalkylpyrimidines from γ-lactones, and have now extended this synthesis to a group of pyrimidines possessing a monohydroxycycloalkyl group at the 5 position. Of these compounds the 5-hydroxycyclopentyluracil (7) bears structural similarity to pseudouridine. It also serves as a model for the several 5-hydroxyalkyluracils which have been obtained as degradation products of pseudouridine.[3] In these cycloaliphatic model compounds the number of conformations possible is considerably fewer than in those with an acyclic 5 substituent. Consequently a study of its physical properties may serve to elucidate the role of hydrogen bonding in the previously observed[4] spectral differences between thymine and 5-hydroxymethyluracil and between α- and β-pseudouridines.

These hydroxycycloalkylpyrimidines may also be useful in biological studies, as have similar analogs of purine nucleosides where 9-hydroxycyclopentyl or cyclohexyl derivatives have been used successfully in obtaining information concerning the nature of the functional group which is required for binding to the active site of various enzymes.[5] Such studies with adenosine deaminase led to the conclusion that the hydroxyl group at C′-2 makes a significant contribution to the binding whereas the hydroxy groups at C′-3 and the hydroxymethyl group at C′-4 make only a small contribution to binding to that enzyme. An unexpected finding[5a] was that the stereochemistry of the hydroxyl group at the 2′ position of the cycloaliphatic ring is not critical to the binding. On the other hand

(1) This investigation was supported by Public Health Service Research Grant No. CA-03190-09 and the Maude K. Irving Memorial Grant for Cancer Research from the American Cancer Society.

(2) J. D. Fissekis, A. Myles, and G. B. Brown, *J. Org. Chem.*, **29**, 2670 (1964): manuscript I of this series.

(3) R. W. Chambers and V. Kurkov, *Biochemistry*, **3**, 326 (1964).

(4) R. W. Chambers, V. Kurkov, and R. Shapiro, *ibid.*, **2**, 1192 (1963).

(5) (a) H. J. Schaeffer, S. Marathe, and V. Alks, *J. Pharm. Sci.*, **53**, 1368 (1964); (b) H. J. Schaeffer, K. K. Kaistha, and S. K. Chakraborti, *ibid.*, **53**, 1371 (1964).