Synthesis of 5-Hydroxyalkylpyrimidines from Lactones[1,2]

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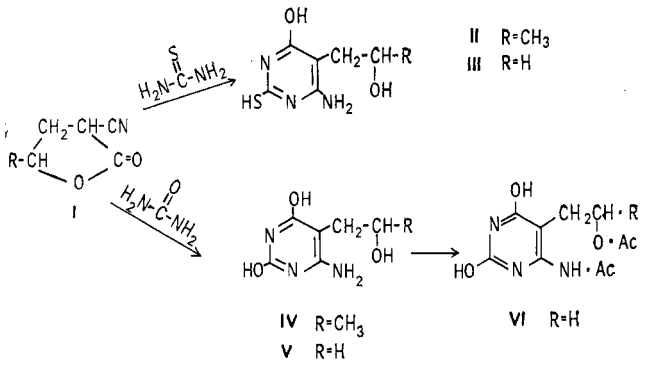
The use of lactones in the synthesis of pyrimidines has been explored, and the method has been applied to the synthesis of a series of 5-hydroxyalkylpyrimidine analogs of naturally occurring compounds.

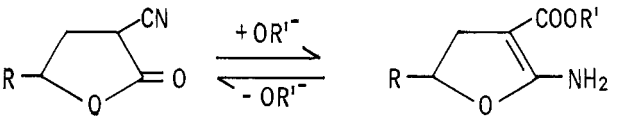
A number of naturally occurring pyrimidines possess a mono- or a polyhydroxyalkyl group attached to position 5 of the pyrimidine ring. Of these, the first isolated was 5-hydroxymethylcytosine present in the nucleic acid of the T-even coliphages as a nucleotide glucosylated on the side chain.[3] Subsequently, Kallen, *et al.*,[4] reported the occurrence of 5-hydroxymethyluracil in the DNA of *Bacillus subtilis* bacteriophage SP8, and Tanaka, *et al.*,[5] isolated an antibiotic (Bacimethrin) from *Bacillus megatherium*, which was shown[6] to be 4-amino-5-hydroxymethyl-2-methoxypyrimidine. The more complex 5-ribosyluracil (pseudouridine), to which no specific biological role is yet attributed, was found to be a normal component of RNA.[7]

We undertook the synthesis of a series of 5-(2-hydroxyalkyl)pyrimidines analogous to the foregoing. In addition we studied some transformations of substituents on the pyrimidine ring in the presence of such alkyl side chains.

Condensation products of epoxides with ethyl cyanoacetate have been used in the synthesis of 2,4,6-trisubstituted-5-hydroxyalkylpyrimidines. Schrage and Hitchings[8] prepared 2,6-diamino-5-(2-hydroxyethyl)-4-hydroxypyrimidine from α-cyano-γ-butyrolactone and guanidine, and Davoll[9] found that glycide condenses with ethyl cyanoacetate to give a product which reacts directly with guanidine or thiourea to give the corresponding pyrimidines, *i.e.*, 4-amino-5-(2,3-dihydroxypropyl)-2,6-dihydroxy- and 4-amino-5-(2,3-dihydroxypropyl)-6-hydroxy-2-mercaptopyrimidine.



Similar procedures were used in the synthesis of pyrimidines II to V. α-Cyano-γ-valerolactone was prepared by the method of Glickman and Cope.[10] A modified procedure of Feofilactov and Onishchenko[11] was used for the synthesis of α-cyano-γ-butyrolactone. Condensations of the lactones with thiourea were satisfactory and the products were readily isolated. With urea the reaction required several days to give appreciable yields.[12]



VII R=H, R'= C_2H_5

VIII R=CH_3, R'= C_2H_5

Attempts to convert V to a 5-hydroxyethyluracil *via* thiation of the 6-position were unsuccessful, but there was some evidence that its diacetyl derivative (VI) could be thiated. Use of the sodium derivative of the α-hydroxymethylenebutyrolactone (IX) did permit the synthesis of pyrimidines unsubstituted at position 6.

This sodium derivative, previously mentioned by Korte and Machleidt,[13] was prepared from butyrolactone and methylformate in dry ether in the presence of sodium methoxide. Its enolic nature is indicated by the strong ultraviolet absorption of an aqueous solution of its sodium salt (λ_{max} 277.5 mμ), and the absence of selective absorption in acid. With guanidine hydrochloride in absolute ethanol, IX gave compound X. 4-Hydroxy-5-(2-hydroxyethyl)-2-mercaptopyrimidine (XI) was prepared by reaction of IX with saturated aqueous thiourea.[14] This pyrimidine was subsequently used in the preparation of 5-hydroxyethyluracil (XII). IX and urea gave XII, but in poor yield. The preferred route to XII was the methylation of the 2-mercapto analog (XI) with methyl iodide in alkali followed by acid hydrolysis of the resulting 4-hydroxy-5-(2-hydroxyethyl)-2-methylmercaptopyrimidine (XIII) (see Scheme I).

(1) This investigation was supported in part by funds from the Maude K. Irving Memorial Grant for Cancer Research from the American Cancer Society and from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant CA 03190-08).

(2) A preliminary report was presented at the 145th National Meeting of the American Chemical Society, New York, N. Y., Sept., 1963, p. 9C.

(3) (a) G. R. Wyatt and S. S. Cohen, *Nature*, **170**, 1072 (1952); (b) G. R. Wyatt and S. S. Cohen, *Biochem. J.*, **55**, 774 (1953); (c) I. R. Lehman and E. A. Pratt, *J. Biol. Chem.*, **235**, 3254 (1960).

(4) R. G. Kallen, M. Simon, and J. Marmur, *J. Mol. Biol.*, **5**, 248 (1962).

(5) (a) F. Tanaka, S. Takeuchi, N. Tanaka, H. Yonehara, H. Umezama, and Y. Sumiki, *J. Antibiotics* (Tokyo), *Ser. A.*, **14**, 161 (1961); (b) T. Nishimura and N. Tanaka, *ibid.*, **16**, 179 (1963).

(6) H. C. Koppel, R. H. Springer, R. K. Robins, and C. C. Cheng, *J. Org. Chem.*, **27**, 3614 (1962).

(7) (a) W. E. Cohn, *J. Biol. Chem.*, **235**, 1489 (1960); (b) W. S. Adams, F. Davis, and M. Nakatani, *Am. J. Med.*, **28**, 726 (1960); V. M. Ingram and J. G. Pierce, *Biochemistry*, **1**, 580 (1962); (d) A. Dlugajczyk and J. J. Eiler, *Federation Proc.*, **22**, 470 (1963).

(8) A. Schrage and G. H. Hitchings, *J. Org. Chem.*, **16**, 1153 (1951).

(9) J. Davoll, *J. Chem. Soc.*, 131 (1960).

(10) S. A. Glickman and A. C. Cope, *J. Am. Chem. Soc.*, **67**, 1012 (1945).

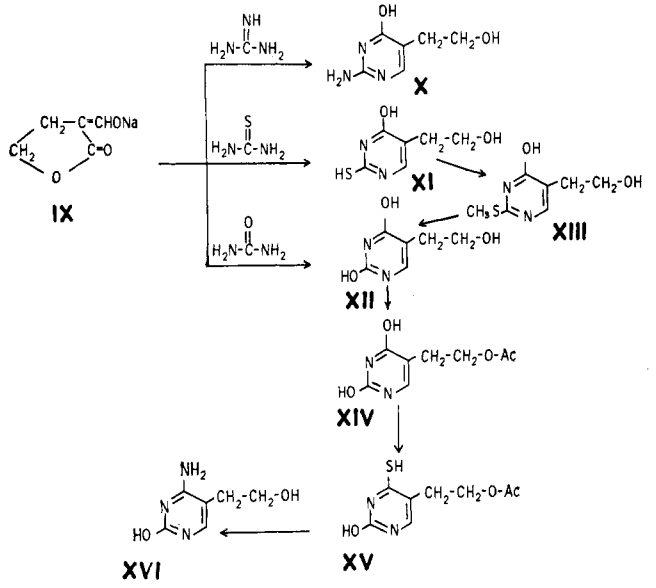
(11) V. V. Feofilactov and A. S. Onishchenko, *J. Gen. Chem. USSR*, **9**, 304 (1939).

(12) Factors other than the lack of reactivity of urea and its extensive decomposition during the long refluxing period may be involved. F. Korte and K. Trautner [*Chem. Ber.*, **95**, 281 (1962)] have shown that, under such conditions, both α-cyano-γ-butyrolactone and α-cyano-γ-valerolactone undergo a base-catalyzed reversible rearrangement to 2-amino-3-carbethoxy-4,5-dihydrofurans (VII and VIII).

(13) F. Korte and H. Machleidt, *ibid.*, **88**, 136 (1955).

(14) H. L. Wheeler and L. M. Liddle, *Am. Chem. J.*, **40**, 547 (1908).

SCHEME I



The 5-hydroxyethyluracil (XII) served as starting material for the synthesis of the corresponding cytosine derivative. When the hydroxyl group in the side chain was protected by acetylation, the resulting product (XIV) could be thiated to XV under the controlled conditions of Mizuno, *et al.*[15] Only the use of 0.5 mole of phosphorus pentasulfide, based upon the formula P_2S_5, gave a satisfactory yield of the 4-thio derivative (XV). This mercapto compound was then treated with ammonia in methanol at 100° to yield 5-hydroxyethylcytosine (XVI). The ultraviolet spectral properties and ionization constants of 5-hydroxyethyl- and 5-hydroxymethyluracils are similar (see Fig. 1), as are those of the corresponding cytosines (Fig. 2, Table I).

Chambers, Kurkov, and Shapiro[16a] proposed that differences between the spectra of thymine and 5-hydroxymethyluracil at pH 12 could be attributed to a hydrogen bond between the 5-hydroxymethyl group and the 4-carbonyl group in the latter.[16b] By analogy they argued that the thymine-like spectrum of pseudouridine B (α-furanosyl) and the hydroxymethyluracil-like spectrum of pseudouridine C (β-furanosyl) could likewise be explained by stereochemical features which render the H-bond unlikely in the former. In the 5-(2-hydroxyethyl)uracil such an H-bond is sterically permissible, but its spectrum is similar to those of uracil and thymine and not to that of 5-hydroxymethyluracil.

Experiemntal

Melting Points.—All melting points were determined with a Fisher-Johns apparatus and are uncorrected.

Paper Chromatography.—Ascending technique on Whatman No. 1 paper was used with the solvents: A, *n*-BuOH–HOAc–H_2O (12:3:5); B, *n*-BuOH–H_2O (85:15); and C, *i*-PrOH–H_2O–NH_4OH (7:2:1).

α-Cyano-γ-butyrolactone.—A solution of 11.6 g. (0.5 g.-atom) of sodium in 250 ml. of ethanol was cooled to 5°, and 58 g. (0.5 mole) of ethylcyanoacetate was added dropwise with vigorous stirring which was continued overnight without further cooling. To this suspension of the sodium derivative was added 28 g. (0.63 mole) of ethylene oxide in 50 ml. of absolute ethanol and the mixture was heated to 45° to yield a light amber solution which was kept for approximately 6 hr. at 45–50°, and then at room temperature overnight. After the solvent had been evaporated under vacuum, 100 ml. of ice–water and 50 ml. of concentrated hydrochloric acid were added to the residue. The resulting solution was continuously extracted with ether for 72 hr., the ether extract was concentrated under vacuum, and the residue was fractionated. After redistillation, 24 g. (44%) of a colorless sirup boiling at 115–117° at 0.18 mm. was recovered.[17]

6-Amino-4-hydroxy-5-(2-hydroxyethyl)-2-mercapto- and 6-Amino-4-hydroxy-5-(β-hydroxy-*n*-propyl)-2-mercaptopyrimidine (III and II).—To 1.15 g. (50 mg.-atoms) of sodium dissolved in 160 ml. of dry ethanol, 2.3 g. (30 mmoles) of thiourea and 30 mmoles of the appropriate α-cyanolactone were added. The reaction mixture was refluxed with stirring for 18 to 20 hr., then taken to dryness under vacuum, and the residue was dissolved in 25 ml. of cold water. This solution was extracted twice with 10-ml. portions of ether, acidified with 3 ml. of glacial acetic acid, and cooled. The product that precipitated was collected, washed with cold water, and recrystallized from boiling water.

Compound III, 3.66 g. (65%) of light brown needles, had m.p. 299–300° dec.

Anal. Calcd. for $C_6H_9N_3O_2S$: N, 22.21; S, 16.95. Found: N, 22.30; S, 17.50.

Compound II, 3.20 g. (53%) of pale yellow prisms, had m.p. 262° dec.

Anal. Calcd. for $C_7H_{11}N_3O_2S$: N, 20.88; S, 15.93. Found: N, 20.70; S, 15.77.

6-Amino-2,4-dihydroxy-5-(2-hydroxy-*n*-propyl)pyrimidine (IV).—A solution of 5.75 g. (250 mg.-atoms) of sodium in 400 ml. of dry

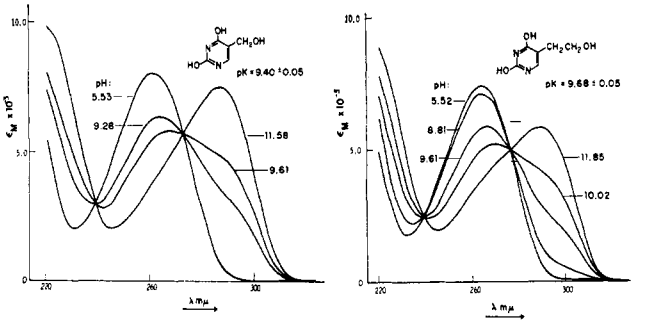


Fig. 1.—Spectra of 5-hydroxymethyl- and 5-(2-hydroxyethyl)uracils. W. E. Cohn [*J. Biol. Chem.*, **235**, 1488 (1960)] records pK 9.8 for 5-hydroxymethyluracil.

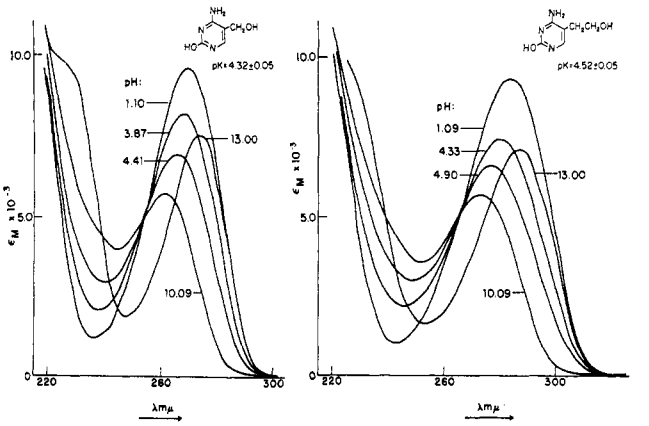


Fig. 2.—Spectra of 5-hydroxymethyl- and 5-(2-hydroxyethyl)-cytosines. The difference, 0.20 units, between pK values observed for these cytosine derivatives is similar to that, 0.28, observed for the corresponding uracils.

(15) Y. Mizuno, M. Ikehara, and K. A. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **10**, 647 (1962).

(16) (a) R. W. Chambers, V. Kurkov, and R. Shapiro, *Biochemistry*, **2**, 1192 (1963). (b) The A_{280}–A_{260} ratio at pH 12 of >1.8 was the criterion of the presence of an H-bond. The authors thank Dr. R. W. Chambers for drawing our attention to this point prior to appearance to their publication, and for discussion of its significance.

(17) V. V. Feofilactov and A. S. Onischchenko[11] report yellow oil, 30.4% yield, b.p. 176–178° (8 mm.).

TABLE I

Compd.	Positions of substituents on pyrimidines: 2	4	5	6	Spectral data: 0.1 N HCl λ, mμ	0.1 N HCl ε × 10⁻³	0.1 N NaOH λ, mμ	0.1 N NaOH ε × 10⁻³	R_f in solvents: A	B	C
II	SH	OH	CH_2CHCH_3 (OH)	NH_2	max. 282	20.0	294	11.1	0.70	0.54	0.50
					242	7.8	264	11.2			
							240.5	15.4			
					min. 252.5	5.8	273.5	9.4			
					229	6.6	254	10.0			
III	SH	OH	CH_2CH_2OH	NH_2	max. 282	20.0	280	10.7	0.53	...	0.46
					241	7.7	247.5	12.5			
					min. 252	5.9	263	7.7			
							237	10.8			
IV	OH	OH	CH_2CHCH_3 (OH)	NH_2	max. 272	20.4	273	17.4	0.53	0.31	0.43
					min. 243	3.3	247	2.7			
V	OH	OH	CH_2CH_2OH	NH_2	max. 271	18.9	274	15.9	0.41	0.17	0.34
					min. 243.5	3.1	246.5	2.4			
VI	OH	OH	CH_2CH_2OAc	NHAc	max. 276	12.4	274.5	15.1	0.70	0.53	0.53
					min. 240	2.2	248	2.9			
X	NH_2	OH	CH_2CH_2OH	H	max. 261.5	7.3	278	6.9	0.49	0.24	0.48
							233	7.4			
					min. 242	4.7	253	2.5			
XI	SH	OH	CH_2CH_2OH	H	max. 275.5	16.1	259.5	12.6	0.63	0.43	0.58
					min. 241	3.1	243.5	9.9			
XII	OH	OH	CH_2CH_2OH	H	max. 264	7.4	289	5.9	0.46	0.28	0.48
							228.5	4.2			
					min. 233.5	1.7	245.5	1.9			
XIII	SCH_3	OH	CH_2CH_2OH	H	max. 273	9.6	280	8.0	0.82	0.73	0.66
					250	9.4	247.5	9.3			
					min. 260.5	8.9	263	5.7			
					240.5	8.7	237	8.1			
XIV	OH	OH	CH_2CH_2OAc	H	max. 263.5	7.8	289	5.9	0.71	0.55	0.65
					min. 233	1.8	245.5	2.0			
XV	OH	SH	CH_2CH_2OAc	H	max. 333.5	15.9	Hydrolyzes in basic soln.		...	...	0.82
					243	3.8					
					min. 279.5	2.8					
XVI	OH	NH_2	CH_2CH_2OH	H	max. 284	9.3	287	7.2	0.39	0.12	0.53
					min. 243	0.8	254	1.6			

ethanol, to which had been added 9.0 g. (150 mmoles) of urea and 13.5 ml. (125 mmoles) of α-cyano-γ-valerolactone, was refluxed for a week with stirring. It was taken to dryness under vacuum, and the residue was dissolved in a liter of water and extracted twice with 50 ml. of ether. The aqueous phase was neutralized with Amberlite CG-50 (H^+ form). A partial separation of the product resulted. The aqueous phase was decanted carefully, the resin was washed well with water, and the combined aqueous phases when filtered yielded 3.0 g. of crude product. The filtrate was concentrated to 125 ml. under vacuum, treated once more with a small volume of Amberlite CG-50, filtered, and continuously extracted with ether for 12 hr. Further concentration and cooling of the aqueous phase yielded another 1.5 g. of product. The combined crude samples recrystallized from boiling water, to yield 3.0 g. (13%) of a white solid, m.p. 252–253°.

Anal. Calcd. for $C_7H_{11}N_3O_3$: C, 45.40; H, 5.99; N, 22.69. Found: C, 45.57; H, 6.20; N, 22.64.

6-Amino-2,4-dihydroxy-5-(2-hydroxyethyl)pyrimidine (V).—Urea (15 g., 0.25 mole) was added to a solution of 11.5 g. (0.50 mole) of sodium in 300 ml. of absolute ethanol followed by 24 g. (0.2 mole) of α-cyano-γ-butyrolactone, and the mixture was refluxed with stirring. After 24 hr., an additional 7.5-g. portion of urea was added and the refluxing was continued for a total of 4 days. The flask was then allowed to stand in the cold for a few days, and the solid that separated was collected, washed with ether, and dried under vacuum; yield 35.5 g. This material was dissolved in 300 ml. of water, filtered, and the filtrate was neutralized with Amberlite IRC-50 (H^+, 20–50 mesh). The product that separated from the solution was recovered by shaking the resin with water, letting the resin settle for a few seconds, and then decanting the supernatant which contained the product in solution and as a fine suspension. The several liters of water thus collected were concentrated under vacuum to a small volume, cooled, and filtered. The solid (13.8 g.) was dissolved in 2.3 l. of boiling water, and the solution was treated with Norit, filtered, and cooled. The small white needles were collected, washed with water and methanol, and dried under vacuum; yield 11.25 g. (33%), m.p. 308–310°. After recrystallization once more from water the melting point was unchanged.

Anal. Calcd. for $C_6H_9N_3O_3$: C, 42.10; H, 5.30; N, 24.55. Found: C, 42.00; H, 5.41; N, 24.52.

6-Acetamido-5-(2-acetylhydroxyethyl)-2,4-dihydroxypyrimidine (VI).—A mixture of 170 mg. (1 mmole) of XI and 5 ml. of acetic anhydride was refluxed gently for 5 hr. The clear solution was cooled; the resulting crystalline product was collected and washed several times with ether and dried under vacuum over solid potassium hydroxide and phosphorus pentoxide; yield 210 mg. (82%), m.p. 268–269° dec.

Anal. Calcd. for $C_{10}H_{13}N_3O_5$: C, 47.05; H, 5.13; N, 16.46. Found: C, 47.49; H, 5.13; N, 16.65.

Sodium Derivative of α-Hydroxymethylene-γ-butyrolactone (IX).—To a cooled suspension of 54.0 g. (1 mole) of sodium methoxide in a liter of dry ether, a mixture of 80 ml. (1.3 moles) of methylformate and 76 ml. (1 mole) of γ-butyrolactone was added dropwise with stirring over a 2-hr. period; the stirring was continued at room temperature overnight. The reaction mixture was then cooled in an ice bath for 1 hr. to complete the separation of the cream-colored product which was then collected, washed with dry ether, and dried under vacuum; yield 123 g. (90%), sinters at 117°, and melts at 123–126°. It was found to be stable if kept under vacuum over phosphorus pentoxide and was used without further purification.

2-Amino-4-hydroxy-5-(2-hydroxyethyl)pyrimidine (X).[18]—The sodium derivative of α-hydroxymethylene-γ-butyrolactone (IX, 1.36 g., 10 mmoles) was added to a solution of 955 mg. (10 mmoles) of guanidine hydrochloride in 50 ml. of absolute ethanol, and the reaction mixture was boiled under reflux for 5 hr. with stirring. It was taken to dryness under vacuum; the residue was dissolved in 60 ml. of hot water, filtered, and cooled. The crude product was crystallized once more from 25 ml. of hot water to yield short white needles, 300 mg. (19%), m.p. 268–270°.

Anal. Calcd. for $C_6H_9N_3O_2$: C, 46.44; H, 5.85; N, 27.08. Found: C, 46.70; H, 5.87; N, 26.80.

4-Hydroxy-5-(2-hydroxyethyl)-2-mercaptopyrimidine (XI).—The sodium derivative of α-hydroxymethylene-γ-butyrolactone (61 g., 450 mmoles) was dissolved in 400 ml. of a saturated, aqueous solution of thiourea. The reaction mixture, allowed to stand at room temperature overnight, was then heated on the steam bath for 0.5 hr. The warm solution was filtered, cooled to room temperature, and acidified with glacial acetic acid. The white crystalline precipitate was collected, washed with cold water, and dried over phosphorus pentoxide and solid potassium hydroxide under vacuum. When recrystallized from hot water, it yielded white needles, 33.8 g. (44%). For analysis, it was recrystallized once more from water, m.p. 253–256°.

Anal. Calcd. for $C_6H_8N_2O_2S$: N, 16.23; S, 18.62. Found: N, 16.88; S, 18.64.

4-Hydroxy-5-(2-hydroxyethyl)-2-methylmercaptopyrimidine (XIII).—The thiol XI (18.7 g., 110 mmoles) was dissolved in 143 ml. of 1 *N* sodium hydroxide, 14.3 g. (110 mmoles) of methyl iodide was added, and the mixture was shaken for 3 hr. The precipitate was collected, washed three times with cold water, and dried under vacuum over phosphorus pentoxide; yield 14.05 g. (70%). It was used without further purification for the preparation of XVII. A small sample was recrystallized from hot water as white needles melting at 184–185°.

Anal. Calcd. for $C_7H_{10}N_2O_2S$: C, 45.14; H, 5.41; N, 15.04. Found: C, 44.88; H, 5.28; N, 14.61.

5-(2-Hydroxyethyl)uracil (XII). Method A.—To sodium (2.3 g., 0.1 g.-atom) dissolved in 150 ml. of dry ethanol 6.0 g. (0.1 mole) of urea, followed by 13.6 g. (0.1 mole) of the sodium derivative of α-hydroxymethylene-γ-butyrolactone, was added. The mixture was refluxed with stirring for 48 hr., when an additional 6.0 g. of urea was added and reflux was continued for a total of 5 days. After removal of the solvent under vacuum, the residue was dissolved in 250 ml. of water and neutralized with Dowex-50 (H^+, 20–50 mesh). The resin was separated by filtration and washed with water until the O.D. of the washings dropped below 0.2 at 265 mμ; the combined filtrates were then taken to dryness under vacuum. The residue was dissolved in 500 ml. of methanol, filtered, and cooled in a dry ice bath for a day. The solvent was decanted from the precipitated solid, which was transferred to a filter with petroleum ether (b.p. 30–60°) and washed with a small volume of cold methanol. It was recrystallized twice, first from 20 ml. of water and methanol (5:15) and then from 10 ml. of methanol after treatment with Norit. Additional crystals were obtained from the mother liquors by concentration to one-half volume, cooling to −40°, and recrystallizing the solid from 15 ml. of methanol after treatment with Norit. The combined batches were recrystallized once more from methanol as clusters of small prisms, yield 400 mg., sublimes above 200°, and melts at 273–274°.

Anal. Calcd. for $C_6H_8N_2O_3$: C, 46.15; H, 5.16; N, 17.94. Found: C, 46.42; H, 5.25; N, 18.10.

Method B.—XIII (7 g., 37.6 mmoles) was dissolved in 200 ml. of 6 *N* hydrochloric acid and the solution was refluxed for 24 hr. When it had been cooled to room temperature, a crystalline product separated and was collected. The filtrate was taken to dryness under vacuum, dissolved and re-evaporated five to six times with methanol, and finally dissolved in *ca.* 350 ml. of hot water, and filtered; the filtrate was neutralized with 50 ml. of Amberlite IR-4B resin (20–50 mesh, OH^-). The resin was washed with 1200 ml. of water; the washings were taken to dryness under vacuum. The residue was recrystallized from methanol to yield 2.5 g. of product. The crystalline product that was collected after the initial cooling was dissolved in 200 ml. of 6 *N* hydrochloric acid, refluxed for 24 hr., and taken to dryness under vacuum. The last traces of hydrochloric acid were removed; the residue was dissolved in 250 ml. of hot water, chilled overnight filtered, and then neutralized with Amberlite IR-4B as the foregoing. The combined washings were taken to dryness, and the residue was recrystallized from boiling methanol to yield 1.1 g. of product.

The melting points of the different batches of product varied from 265–275° when samples were placed on a preheated stage, but they resolidified immediately to a material with m.p. >300°. This transition can pass unnoticed on slow heating. The samples were used without further purification for the preparation of XIV.

5-(2-Acetylhydroxyethyl)uracil (XIV).—A mixture of 1.0 g. (6.4 mmoles) of 5-(2-hydroxyethyl)uracil, 5 ml. of acetic anhydride, and *ca.* 30 ml. of dry pyridine was stirred for 1 hr. under anhydrous conditions. The clear solution was treated with water and evaporated under vacuum at 40° to leave a white residue that was recrystallized from water to yield the monoacetate as white needles, yield 1.06 g. (83%), m.p. 204–205°.

Anal. Calcd. for $C_8H_{10}N_2O_4$: C, 48.55; H, 5.09; N, 14.12. Found: C, 48.28; H, 5.26; N, 14.05.

5-(2-Acetylhydroxyethyl)-2-hydroxy-4-mercaptopyrimidine (XV).—A mixture of 2.14 g. (10.8 mmoles) of XIV and 1.3 g. of phosphorus pentasulfide was refluxed in *ca.* 90 ml. of dry pyridine with stirring for 4 hr. The dark brown solution was concentrated under vacuum at 40° to a few milliliters, twice diluted with water, and reconcentrated. Water was added to the yellow-brown solids and the mixture was cooled overnight. The solids were then collected, washed with cold water, redissolved in 350 ml. of boiling water, treated with charcoal (Darco), filtered, and cooled overnight. Yellow needles separated; yield 600 mg. (26%), starts decomposing at 200°, and melts at 216–219°.

Anal. Calcd. for $C_8H_{10}N_2O_3S$: N, 13.08; S, 14.97. Found: N, 12.80; S, 14.79.

5-Hydroxyethylcytosine (XVI).—XV (215 mg., 1 mmole) was treated with 20 ml. of methanolic ammonia, saturated at 0°, and heated in a sealed tube at 100° for 18 hr. The clear reaction mixture was then chilled for several hours and the product that separated as small white needles was collected, washed with a small volume of cold methanol, and dried under vacuum over phosphorus pentoxide; yield 93.5 mg. (60%). This fairly pure material, further purified for analysis by recrystallization from hot methanol containing a few drops of water, starts decomposing above 200° and melts at 275°.

Anal. Calcd. for $C_6H_9N_3O_2$: C, 46.44; H, 5.85; N, 27.08. Found: C, 46.35; H, 6.07; N, 27.20.

(18) The abstract of U. S. S. R. Patent 129,650 (1960) states that X, XI, and XII have been synthesized; *Chem. Abstr.*, **55**, 5548*d* (1961).