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Synthesis and Properties of 5-Mercaptomethyluracil and Related Derivatives[1]

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Reaction of 5-hydroxymethyluracil with the appropriate hydrogen halide afforded 5-chloro-, -bromo-, or -iodomethyluracil. Treatment of 5-chloromethyluracil with thioacetamide in dimethylformamide solution led to 5-acetiminothiomethyluracil hydrochloride from which 5-mercaptomethyl- and 5-acetylthiomethyluracil were obtained by alcoholysis or by hydrolysis. Alkaline solutions of 5-mercaptomethyluracil and its acetyl derivative gave 5-methyl- and 5-ethylthiomethyluracil upon reaction with methyl and ethyl iodide, and 5-benzylthiomethyluracil was obtained with benzyl chloride. 5-Chloromethyluracil reacted with potassium thiocyanate, thiourea, thioglycolic acid, ammonium dithiocarbamate, and benzyl mercaptan to yield 5-thiocyanomethyluracil, 5-(S-thioureido)methyluracil hydrochloride, 5-carboxymethylthio-, 5-(S-dithiocarbamyl)-, and 5-benzylthiomethyluracil, respectively. Condensation of 5-chloro- and 5-mercaptomethyluracil led to bis(thyminyl) sulfide, which was oxidized to its sulfone. Upon oxidation of 5-mercaptomethyluracil and its acetyl derivative, bis(thyminyl) disulfide was obtained. These 5-halogeno- and 5-mercaptomethyluracils were transformed to thymine with tin and hydrochloric acid or with Raney nickel. Some physical and chemical properties of the new compounds are described. 6-Mercaptomethyluracil showed a marked inhibitory activity on mouse Ehrlich carcinoma (fluid form) and complete inhibition of Krebs II (ascitic) tumor.

The occurrence of 5-hydroxymethyluracil and 5-hydroxymethylcytosine in DNA and the hydroxymethylation reactions involved in the biosynthesis of such pyrimidines, as well as thymine,[2] prompted the study of the synthesis of new derivatives of these substances which might affect growth. Analogs of these compounds which manifest powerful inhibitory activity in biological systems, and even exhibit antitumor and antiviral effects, include 5-fluorouracil,[3] 5-trifluoromethyluracil,[4] 5-fluorocytosine[5] and their nucleosides, and 5-iododeoxyuridine.[6] In the course of this study, methods were developed for the synthesis of 5-monohalogeno and 5-mercapto derivatives of 5-hydroxymethyluracil and these are described in this report.

Very few derivatives of mercaptomethylpyrimidines have been described previously.[7] These include 4-amino-5-mercaptomethylpyrimidine[8] and 4-mercaptomethyl-5-phenoxyuracil,[9] which could only be synthesized by laborious procedures. There has been a report of the preparation of thyminylcysteine obtained by the interaction of 5-hydroxymethyluracil (I) and cysteine in HCl.[10]

In an extension of previous studies of the introduction of sulfur into purines,[11,12] 5-chloromethyluracil (II) (Scheme I) was converted into a variety of mercaptomethyl derivatives by reaction with thioacetamide and other thio reagents. Earlier synthesis of II were accomplished in 57% yield, by the chloromethylation of uracil with trioxymethylene in concentrated HCl at 80°, and in 37% yield by treatment of 5-hydroxymethyluracil (I) with hot HCl.[13] The synthesis of II was achieved in greater yield (90%) by simple reaction of I with concentrated HCl at room temperature. 5-Bromo- (III) and 5-iodomethyluracil (IV) were obtained in a similar manner from I with concentrated HBr and HI in quantitative and 82% yield, respectively.[14] Treatment of 5-chloromethyluracil (II) with HI below 0° led also to the iodo derivative IV in 91% yield.[15]

When a solution of 5-chloromethyluracil (II) and thioacetamide[16] in dimethylformamide was heated at

(1) This investigation was supported by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant CA 03190-09) and The Atomic Energy Commission (Contract No. AT[30-1], 910) and aided by Grant T-1285F from the American Cancer Society and the First National City Bank Grant for Research from the American Cancer Society. Presented in part at the 149th National Meeting of the American Chemical Society, Detroit, Mich., April 1965; Abstracts, p. 7N.

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(3) For a review on 5-fluorouracil, *cf.* R. E. Handschumacher and A. D. Welch in "The Nucleic Acids," Vol. III, E. Chargaff and J. N. Davidson, Ed., Academic Press Inc., New York, N. Y., 1960, p. 498.

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(7) *Cf.* D. J. Brown, "The Pyrimidines," Interscience Publishers, Inc. New York, N. Y., 1962, p. 277.

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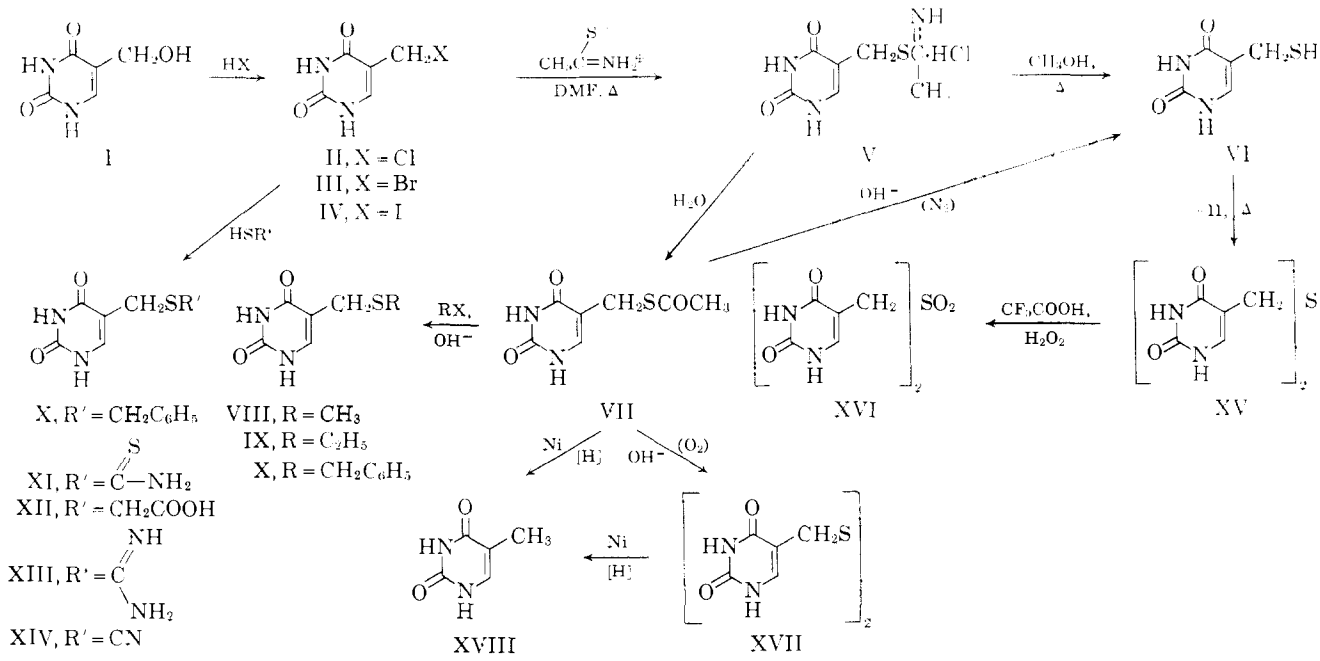
(13) (a) W. A. Skinner, M. G. M. Schelstraete, and R. B. Baker, *J. Org. Chem.*, **25**, 149 (1960); (b) J. A. Carbon, *ibid.*, **25**, 1731 (1960); (c) J. H. Burckhalter, R. J. Seiwald, and H. C. Scarborough, *J. Am. Chem. Soc.*, **82**, 991 (1960).

(14) 5-Bromomethyluracil was previously prepared in 79% yield from I and hydrogen bromide in glacial acetic acid (*cf.* ref. 13b).

(15) *Cf.* (a) E. Fischer, *Ber.*, **31**, 2550 (1898); (b) G. B. Elion and G. H. Hitchings, *J. Am. Chem. Soc.*, **78**, 3510 (1956).

(16) (a) Thioacetamide should be considered predominantly as a zwitterion, $CH_3C(S^{\ominus})=NH_2^{\oplus}$: *cf.* E. Allenstein and P. Quis, *Chem. Ber.*, **97**, 3162 (1964). (b) For a review on thionamides, *cf.* R. N. Hurd and G. DeLaMater, *Chem. Rev.*, **61**, 45 (1961).

SCHEME I



40°, 5-acetiminothiomethyluracil hydrochloride (V) separated in 62% yield. In a similar manner from the bromo derivative III, the corresponding hydrobromide of V was obtained in 84% yield. By heating under reflux with anhydrous methanol or ethanol, V was rapidly transformed to 5-mercaptomethyluracil (VI) in 84% yield; the reaction took place more slowly at room temperature. Compound V was very soluble in cold water; upon standing several minutes, a copious crystalline precipitate separated from its solution. The hydrolytic reaction and precipitation were complete in 1 hr. at 25° to give 5-acetylthiomethyluracil (VII) in 88% yield.[17] Compounds VI and VII were converted to thymine (XVIII) in 72 and 88% yield, respectively, upon desulfuration with Raney nickel.

Alkaline solutions of 5-acetylthiomethyluracil (VII) or 5-mercaptomethyluracil (VI) afforded the corresponding 5-methyl- (VIII, 64%), 5-ethyl- (IX, 74%), and 5-benzylthiomethyluracil (X, 98%), upon reaction at 5° with methyl or ethyl iodide or with benzyl chloride.

5-Chloromethyluracil (II) with a variety of reagents (with or without dimethylformamide), such as benzyl mercaptan, ammonium dithiocarbamate, thioglycolic acid, thiourea, and potassium thiocyanate afforded 5-benzylthiomethyluracil (X), 5-(S-dithiocarbamyl)methyluracil (XI), 5-carboxymethylthiomethyluracil (XII), 5-(S-thioureido)methyluracil (XIII), and 5-thiocyanomethyluracil (XIV), respectively. The yields ranged from 66 to 98%. When equimolar amounts of 5-chloromethyluracil (II) and 5-mercaptomethyluracil (VI) were heated at 100° in dimethylformamide, bis-(thyminyl) sulfide (XV) was obtained in quantitative yield.

Oxidation of XV with hydrogen peroxide in trifluoroacetic acid gave bis(thyminyl) sulfone (XVI) in 46% yield. Solutions of 5-mercaptomethyluracil (VI) or its acetyl derivative VII in concentrated aqueous ammonia were easily oxidized with air to the disulfide XVII in 78 and 84% yield, respectively. Compound XVII was also isolated in 95% yield from solutions of 5-dithiocarbamylmethyluracil (XI) in concentrated aqueous ammonia after oxidation with air.[18] In a nitrogen atmosphere, solutions of 5-acetylthiomethyluracil (VII) in concentrated aqueous ammonia were hydrolyzed to 5-mercaptomethyluracil (VI) in 51% yield.

The ultraviolet spectra of the 5-mercaptomethyl derivatives are shown in Table I.

Infrared spectral studies showed that deacetylation of 5-acetylthiomethyluracil (VII) to 5-mercaptomethyluracil (VI) was accompanied by the appearance of the band at 2620 cm.$^{-1}$ which is characteristic of the -SH stretching frequency of VI.[19]

Biological Activity.—All the new 5-mercaptomethyluracil derivatives have been examined in the Division of Experimental Chemotherapy against Sarcoma 180 in mice, and were found to be generally toxic at the 125-mg./kg. level of administration. Only 5-mercaptomethyluracil (VI) showed some inhibitory activity. The results of the screening tests are shown in Table II.

Experimental Section

Methods.—Ultraviolet absorption spectra were determined with a Cary recording spectrophotometer, Model 11. Ascending paper chromatography was run on Whatman No. 1 paper in the following solvent systems[20]: concentrated aqueous NH_3–water–isopropyl alcohol (10:20:70); 1-butanol–water–acetic acid (50:25:25); and 1 *M* ammonium acetate–ethanol (35:70).

(17) Thiolimidic esters (or "isothionamides") are strong bases which are hydrolyzed in warm or boiling water to acetylthio derivatives. *Cf.* (a) S. Gabriel and P. Heymann, *Ber.*, **24**, 788 (1891); (b) W. Steinkopf and S. Müller, *ibid.*, **56**, 1931 (1923).

(18) It is well known that oxidation of thiophenols to disulfides occurs smoothly upon solution in concentrated aqueous NH_3 [R. Leuckart, *J. prakt. Chem.*, [2] **41**, 179 (1890)]. *Cf.* (a) C. M. Suter, "The Organic Chemistry of Sulfur," John Wiley and Sons, Inc., New York, N. Y., 1944, p. 660; (b) E. E. Reid, "The Organic Chemistry of Bivalent Sulfur," Vol. III, Chemical Publishing Co., New York, N. Y., 1960, p. 362.

(19) (a) We are indebted to Dr. D. Hoffmann and Mr. J. Rubin for the determination of infrared spectra. (b) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd Ed., Methuen and Co. Ltd., London, 1962, p. 350.

(20) C. F. Carter, *J. Biol. Chem.*, **233**, 138 (1956).

TABLE I

ULTRAVIOLET SPECTRAL PROPERTIES OF SUBSTITUTED URACILS[a]

Compd.	λ_{max}, mμ (ε × 10³)			λ_{min}, mμ (ε × 10³)		
		pH 7.2[b]	pH 10.5		pH 7.2	pH 10.5
Uracil[c]	...	259 (8.20)	284 (5.40)	...	228 (1.8)	241 (2.2)
Thymine[c]	...	264 (7.89)	291 (5.44)[d]	...	234 (1.9)	247 (2.3)
	pH 1		pH 13			
5-Mercaptouracil[e]	274 (7.30)	...	291 (8.80)	...	...	...
5-Uracilyl disulfide[e]	272 (15.00)	...	290 (19.0)	...	...	...
4-Mercaptouracil[f]	306 (19.60)	...	302 (25.2)	...	...	...
5-Substituent	pH 1	pH 6.9[g]	pH 12	pH 1	pH 6.9	pH 12
$-CH_2OH$[h] (I)	261 (8.04)	261 (8.08)	286 (7.40)	230 (1.91)	230 (2.00)	243 (2.17)
$-CH_2SH$ (VI)	266 (8.29)	267 (7.93)	287 (5.65)	237 (3.37)	237 (3.20)	268 (4.84)
$-CH_2SCOCH_3$ (VII)	265 (8.07)	265 (8.05)	291 (5.98)	246 (5.77)	246 (5.77)	267 (4.70)
$-CH_2SCH_3$ (VIII)	265 (7.74)	265 (7.86)	283 (7.51)	237 (3.06)	238 (2.82)	255 (3.22)
$-CH_2SC_2H_5$ (IX)	265 (8.02)	265 (7.68)	284 (7.33)	237 (3.59)	238 (3.26)	256 (3.36)
	pH 1	pH 6.9	pH 11	pH 1	pH 6.9	pH 11
$-CH_2SCH_2C_6H_5$ (X)	264 (6.71)	264 (6.71)	283 (7.05)	242 (4.02)	242 (4.00)	257 (3.86)
$-CH_2SC(=NH)NH_2$ (XI)	268 (14.4) 247 (1.06) (sh)	267 (13.6) 248 (10.3)	...	235 (8.3)	238 (8.25)	...
$-CH_2SCH_2COOH$ (XII)	265 (10.6)	265 (7.71)	283 (7.55)	237 (3.68)	238 (3.16)	255 (3.39)
$-CH_2SC(=NH)NH_2 \cdot HCl$ (XIII)	265 (9.43) (sh)	261 (9.51)	...	243 (6.51)	...	...
$-CH_2SCN$ (XIV)	260 (9.01)	260 (8.86)	278 (7.87)	232 (3.72)	233 (3.85)	249 (2.98)
$-CH_2]_2S$ (XV)[i]	264 (7.24)	...	283 (15.0)	238 (2.95)	...	257 (7.61)
$-CH_2]_2SO_2$ (XVI)[i]	...	...	282 (14.8)	...	...	258 (7.37)
$-CH_2S]_2$ (XVII)[i]	...	...	282 (14.2)	...	...	259 (8.37)

[a] All of these derivatives were sufficiently stable in alkali, with the exception of XI and XIII, to permit spectroscopic examination. [b] Sodium acetate buffer. [c] D. Shugar and J. J. Fox, *Biochim. Biophys. Acta*, **9**, 199 (1952). [d] pH 12–13. [e] Data of T. J. Bardos, R. H. Herr, and T. Enkoji, *J. Am. Chem. Soc.*, **77**, 960 (1955). [f] Data of H. C. Koppel, R. H. Springer, R. K. Robins, and C. C. Cheng, *J. Org. Chem.*, **26**, 792 (1961). [g] Phosphate buffer. [h] In 10 *N* HCl, λ_{max} 264 mμ (ε 8.51 × 10³), λ_{min} 236 mμ (ε 2.80 × 10³); in 5 *N* HCl, λ_{max} 262 mμ (ε 8.18 × 10³), λ_{min} 231 mμ (ε 2.28 × 10³); these data may correspond to the spectrum of 5-chloromethyluracil (II), as this compound was synthesized under these conditions (*cf.* also ref. 10). [i] Insolubility in neutral and/or acid solution did not permit a complete determination.

TABLE II

EFFECT OF 5-MERCAPTOMETHYLURACIL (VI) ON VARIOUS MOUSE TUMORS

Tumor	Dose,[a] mg./kg./day	
	62.5	50
Sarcoma 180	T	−
Sarcoma 180 (ascitic)	+	+
Ehrlich carcinoma (fluid form)	+++	±
Ridgeway osteogenic sarcoma	+	±
Taper hepatoma (ascitic)		+
Krebs II (ascitic)		+++

[a] T, toxic; −, no effect; ±, slight inhibition; +, moderate inhibition; +++, complete inhibition or destruction of tumors. Data courtesy of Dr. K. Sugiura.

The determination of melting points was carried out with a Mel-Temp melting point apparatus and the temperatures were corrected. Analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

5-Chloromethyluracil (II).—5-Hydroxymethyluracil[10,21] (I, 5.68 g., 0.04 mole) was dissolved in concentrated HCl (50 ml.) at 25°. A copious crystalline precipitate appeared after a few minutes. After 30 min. at room temperature, it was collected on a sintered-glass filter, washed with a little concentrated HCl, and dried *in vacuo* (P_2O_5) to yield 5.76 g. (90%) of a colorless crystalline product which sintered at 340° and melted at 350–352° with effervescence. This material had the same melting point as an authentic sample of 5-chloromethyluracil prepared from uracil, trioxymethylene, and HCl.[13a] The mixture melting point of this product with authentic 5-chloromethyluracil showed no depression. Dimethylformamide solutions of II made by this method were uniformly clear, while those of II obtained from uracil contained variable proportions of amorphous insoluble material (in some instances up to 25% of the II employed).[22]

(21) Supplied by Calbiochem and by Cyclo Chemical Co., Los Angeles, Calif.

(22) In contrast to the reported insolubility of II in dimethylformamide,[13b] we were able to prepare 30% solutions of II in that solvent at 40–50°.

5-Bromomethyluracil (III).—5-Hydroxymethyluracil (I, 1.0 g., 6.6 mmoles) was treated with concentrated HBr (7 ml.) in a method similar to that indicated for II; yield, 1.41 g. (quantitative); m.p. >300°.

5-Iodomethyluracil (IV). A.—5-Chloromethyluracil (II, 3.0 g., 19 mmoles) was added slowly, with continuous stirring, to concentrated HI (*d* 1.70) (30 ml.) at temperatures ranging between −5 and −10°. The suspension was kept at this temperature for 3 hr. and then filtered by means of a sintered-glass funnel. The residue was washed first with a little cold HI and then with ice water and finally dried *in vacuo* (P_2O_5) to give 4.20 g. (89%) of a light brown crystalline product, m.p. 252° dec.

5-Iodomethyluracil (IV) was stable in water suspension at 5° but it decomposed at temperatures above 40° or in alkaline solutions. IV was hydrolyzed by boiling water at pH above 7, to 5-hydroxymethyluracil (I). Reduction of IV (0.50 g., 2 mmoles) with tin (3.5 g.) and concentrated HCl (15 ml.) at 60° gave 0.12 g. (48%) of chromatographically pure thymine (XVIII).

Anal. Calcd. for $C_5H_5IN_2O_2$: C, 23.83; H, 2.00; I, 50.36; N, 11.11. Found: C, 23.81; H, 2.08; I, 49.93; N, 11.01.

B.—5-Hydroxymethyluracil (I, 0.586 g., 4 mmoles) was dissolved in concentrated HI (*d* 1.70) (5 ml.) at 25°. After a few minutes, an abundant precipitate appeared, which upon treatment as in method A gave 0.822 g. (79%) of a light brown crystalline material, m.p. 252° dec. The mixture melting point of this product with that obtained by method A showed no depression.

5-Acetiminothiomethyluracil Hydrochloride (V).—5-Chloromethyluracil (II, 6.40 g., 0.040 mole) and thioacetamide (6.0 g., 0.080 mole), dissolved in 30 and 20 ml. of dimethylformamide, respectively, were combined. When the resulting solution was warmed slowly to 40°, a copious crystalline precipitate appeared.[23,24] Heating was continued to 70°, the mixture was kept at this temperature for 30 min., cooled, and filtered, and the residue was thoroughly washed with ether to yield 5.81 g. (62%)

(23) The reagents must be anhydrous; otherwise, V does not precipitate and the yield of 5-mercaptomethyluracil is lowered to 20–30%.

(24) Accelerating effects on reactions with the use of dimethylformamide as a solvent have also been observed by several authors: (a) T. P. Johnston, L. B. Holum, and J. A. Montgomery, *J. Am. Chem. Soc.*, **80**, 6265 (1958); (b) H. E. Zaugg, *ibid.*, **83**, 837 (1961); (c) R. W. Balsiger, A. L. Fikes, T. P. Johnston, and J. A. Montgomery, *J. Org. Chem.*, **26**, 3386 (1961).

of colorless needles, m.p. 290–292° (effervescence). V was unstable in water, methanol, and ethanol. An analytical sample was prepared from II obtained from pure 5-hydroxymethyluracil and concentrated HCl.

Anal. Calcd. for $C_7H_{10}ClN_3O_2S$: C, 35.82; H, 3.87; Cl, 15.10; N, 17.91; S, 13.66. Found: C, 35.82; H, 4.28; Cl, 15.21; N, 17.68; S, 13.70.

The corresponding hydrobromide of V (5-acetiminothiomethyluracil hydrobromide) was prepared in a similar manner from 5-bromomethyluracil (III, 0.93 g., 4.5 mmoles), dimethylformamide (7 ml.), and thioacetamide (0.93 g., 12.5 mmoles). A yield of 1.06 g. (84%) of a crystalline product, m.p. 243–245° dec., was obtained.

Anal. Calcd. for $C_7H_{10}BrN_3O_2S$: C, 30.01; H, 3.60; Br, 28.52; N, 15.00; S, 11.43. Found: C, 29.90; H, 3.82; Br, 28.10; N, 14.93; S, 10.98.

5-Mercaptomethyluracil (VI). A.—5-Acetiminothiomethyluracil hydrochloride (V, 3.70 g., 15.7 mmoles) was suspended in methanol (75 ml.) and heated under reflux for 2 hr. The resulting precipitate was cooled, filtered, and washed with cold methanol and ether to yield 2.1 g. (84%) of white crystals, m.p. 270° (after melting, it solidified at 280° and decomposed at 312–314°). Three crystallizations from methanol afforded needles, m.p. 272–274°, dec. at 315°.

Anal. Calcd. for $C_5H_6N_2O_2S$: C, 37.95; H, 3.82; N, 17.70; S, 20.26. Found: C, 37.89; H, 3.78; N, 17.66; S, 20.15.

VI gave positive phosphomolybdate[25a] and nitroprusside[25b] tests, indicating the presence of a free thiol function. It was unchanged by prolonged treatment with an aqueous solution of chloroacetic acid; this behavior contrasts with the known reactivity of mercapto groups directly attached to a pyrimidine nucleus.[26]

The infrared spectrum of 5-mercaptomethyluracil (VI) exhibited a characteristic SH band (ν 2620 cm.$^{-1}$).

B.—A solution of 5-acetylthiomethyluracil (VII, 150 mg., 0.75 mmole) in concentrated aqueous NH_3 (7 ml.) was kept under a nitrogen atmosphere at 25° for 1 hr. The solution was evaporated under reduced pressure and the residue was washed with a little cold water and ethanol to yield 55 mg. (51%) of colorless thin needles, m.p. 270–272°, dec. at 310°. The mixture melting point, R_f values, and ultraviolet spectra indicated that this product was identical with that obtained by method A.

A suspension of VI (316 mg., 2 mmoles) in water (10 ml.) with Raney nickel (1.0 g.) was refluxed for 3 hr. After filtration and evaporation under reduced pressure, 180 mg. (72%) of chromatographically pure thymine (XVIII) was isolated.

5-Acetylthiomethyluracil (VII).—5-Acetiminothiomethyluracil hydrochloride (V, 2.80 g., 12 mmoles) was dissolved in water (40 ml.) at 25°. After a few minutes, a precipitate appeared and the pH of the solution changed from neutral to pH 2. The reaction mixture was kept at the same temperature for 1 hr. and the precipitate was collected, washed with a little cold water, and dried to give 2.10 g. (88%) of a white substance, m.p. 240–242°. After repeated recrystallization from methanol, lustrous rhombohedral plates were obtained, m.p. 244–246°.

Anal. Calcd. for $C_7H_8N_2O_3S$: C, 41.99; H, 4.03; N, 13.99; S, 16.02. Found: C, 41.96; H, 3.96; N, 13.85; S, 16.10.

VII gave a positive test with sodium nitroprusside reagent and 1 *N* NaOH, indicating a free thiol function formed by alkaline hydrolysis of the acetyl group. The infrared spectrum of VII did not show the characteristic SH band which was present in the spectrum of 5-mercaptomethyluracil (VI).

VII (150 mg., 0.75 mmole) was treated with Raney nickel (0.6 g.) in the same manner as VI (see above). A yield of 83 mg. (88%) of thymine was obtained.

5-Methylthiomethyluracil (VIII).—Methyl iodide (0.60 ml.) was added to a solution of 5-mercaptomethyluracil (VI, 1.58 g., 10 mmoles) in cold 1% NaOH (50 ml.) and stirred at 5° overnight. The suspension was filtered and the residue was washed with a little cold water and dried to yield 0.57 g. of a crystalline product, m.p. 255–257°. From the filtrate a second crop was obtained (0.53 g., m.p. 255–257°), affording upon neutralization with acetic acid, a total yield of 64%. A sample was repeatedly recrystal-

TABLE III

REACTIONS OF 5-CHLOROMETHYLURACIL (II) WITH THIO DERIVATIVES

Reagent	Molar ratio	Solvent	Temp., °C.	Time, hr.	Isolation process	Recrysln. solvent	Yield, %	M.p., °C.	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Calcd., % S	Found, % C	Found, % H	Found, % N	Found, % S
$C_6H_5CH_2SH$	6	None	150	5	A	EtOH	98	196	$C_{12}H_{12}N_4O_2S$ (X)[a]	58.04	4.87	11.28	12.92	57.89	4.78	11.35	12.86
$\left[S=C\begin{matrix}\diagup S \\ \diagdown NH_2\end{matrix}^{\oplus}NH_4^{\ominus}\right]$	2.4	DMF[b]	25	16	B	EtOAc	78	310–312 dec.	$C_6H_8N_4O_2S_2$ (XI)	33.16	3.25	19.34	29.51	33.21	3.34	19.40	29.44
$HSCH_2COOH$	8	None	150	4	A	MeOH	70	229–230	$C_3H_5NO_4S$ (XII)[c]	38.89	3.73	12.96	14.83	38.87	3.65	13.05	14.80
$S=C(NH_2)_2$	1.1	DMF	50	0.25	A	EtOH–H_2O	66	251 dec.	$C_6H_9ClN_4O_2S$ (XIII)[d]	30.44	3.83	23.67	13.55	30.59	3.89	23.68	13.71
KSCN	1	DMF	80	2	B	H_2O	69	252 dec.	$C_6H_8N_4O_2S$ (XIV)	39.33	2.75	22.94	17.50	39.55	2.93	22.88	17.69
5-Mercaptomethyluracil (VI)	1	DMF	100	3	A	MeOH–H_2O	100	332–334 dec.	$C_{10}H_{10}N_4O_4S$ (XV)	42.54	3.57	19.85	11.36	42.29	3.53	19.36	11.43

[a] X was also prepared from benzyl chloride (1.2 moles) and 5-mercaptomethyluracil (VI) (1 mole) in 1% NaOH at 5° for 24 hr., in 57% yield. [b] DMF = *N,N*-dimethylformamide. [c] Prolonged refluxing of a solution of XII with 6 *N* HCl did not yield 5-hydroxymethyluracil (IX). This behavior contrasts with the ease of hydrolysis of analogous compounds in the purine series.[26] Attempts to prepare XII from VI and chloroacetic acid were unsuccessful. [d] *Anal.* Calcd.: Cl, 15.15. Found: Cl, 14.98.

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lized from methanol giving colorless needles or prisms, m.p. 258–260°.

Anal. Calcd. for $C_6H_8N_2O_2S$: C, 41.85; H, 4.68; N, 16.27; S, 18.62. Found: C, 41.84; H, 4.73; N, 16.10; S, 18.70.

5-Ethylthiomethyluracil (IX).—Ethyl iodide (0.70 ml.) was added to a solution of 5-mercaptomethyluracil (VI, 1.20 g., 7.6 mmoles) in 1% sodium hydroxide (50 ml.) and stirred at 5° for 48 hr. Treatment similar to that for compound VIII yielded 1.06 g. (74%) of a crystalline product, m.p. 232–234°. A sample recrystallized from methanol afforded colorless needles, m.p. 244–246°.

Anal. Calcd. for $C_7H_{10}N_2O_2S$: C, 45.15; H, 5.41; N 15.05; S, 17.22. Found: C, 45.17; H, 5.01; N, 15.08; S, 17.36,

Reactions of 5-chloromethyluracil (II) with thio derivatives are listed in Table III. Isolation procedure A consisted of filtering the precipitate, washing with ether, and drying in a desiccator *in vacuo* over P_2O_5. In procedure B, the reaction product was evaporated under reduced pressure at 50–60° and the residue was washed with ethyl acetate, ether, and water and dried as above.

Bis(thyminyl) Sulfone (XVI).—Hydrogen peroxide (30%, 6 ml.) was added slowly to a solution of bis(thyminyl) sulfide (XV, 2.0 g., 7 mmoles) in trifluoroacetic acid (20 ml.) at 25°. A precipitate appeared after addition of the peroxide; the resulting suspension was heated at 60° for 30 min. and kept at 25° overnight. The suspension was filtered and the residue was washed with cold water, ethanol, and ether to yield 1.05 g. (46%) of a colorless microcrystalline product, m.p. 340°.

XVI was sparingly soluble in boiling water and the usual organic solvents. Elemental analysis was performed on material obtained from a similar preparation using analytically pure bis(thyminyl) sulfide (XV).

Anal. Calcd. for $C_{10}H_{10}N_4O_6S$: C, 38.21; H, 3.21; N, 17.83; S, 10.20. Found: C, 38.13; H, 3.48; N, 17.61; S, 10.32.

Bis(thyminyl) Disulfide (XVII). A.—A stream of air was bubbled through a freshly prepared solution of 5-acetylthiomethyluracil (VII, 11.40 g., 0.057 mole) in concentrated aqueous NH_3 (250 ml.) at 25°. After 5 min. a copious precipitate appeared, and when the precipitation was complete (24 hr.), the product was collected, washed with water, and dried to yield 4.2 g. of white crystals, m.p. >320°. A second crop of the same material (3.4 g.) was obtained upon concentration of the filtrate, giving a total yield of 84%.

5-Mercaptomethyluracil (VI) in concentrated aqueous NH_3 gave a 78% yield of XVII in a similar manner to that described above.

Compound XVII was sparingly soluble in water and in the usual organic solvents at their boiling point. An analytical sample was prepared from recrystallized VII and concentrated aqueous NH_3 and oxidized with air for 16 hr. at 25°. Colorless needles were obtained, m.p. >320°.

Anal. Calcd. for $C_{10}H_{10}N_4O_4S_2$: C, 38.21; H, 3.21; N, 17.83; S, 20.40. Found: C, 38.20; H, 3.57; N, 17.61; S, 20.45.

Compound XVII gave a negative nitroprusside test, indicating the absence of a free thiol group, but after treatment with a solution of 1 *N* NaOH at 70° for 10 min., a strong positive thiol test with nitroprusside was obtained.

B.—5-(S-Dithiocarbamyl)methyluracil (XI, 2.17 g., 10 mmoles) was dissolved in concentrated aqueous NH_3 (20 ml.) and kept at 25° for 15 hr. The resulting crystalline precipitate was collected, washed with cold water, and dried to yield 1.50 g. (95%) of a crystalline product, m.p. >300° dec. This product was identified as XVII by means of ultraviolet spectra at different pH values, and R_f values in several solvent systems. It (0.314 g., 1 mmole) was treated with Raney nickel (0.8 g.) by a similar method to that described for VI. A yield of 0.090 g. (71%) of chromatographically pure thymine (XVIII) was obtained.

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Nucleosides. XXIX. 1-β-D-Arabinofuranosyl-5-fluorocytosine and Related Arabino Nucleosides[1]

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Reaction of the 5′-*O*-trityl derivative of uridine or 5-fluorouridine with thiocarbonyldiimidazole yielded crystalline 2,2′-anhydro-1-(β-D-arabinofuranosyl)uracils (III, R = H or F; R′ = trityl) *directly* in high yields. These derivatives (III) were converted to the 1-β-D-arabinofuranosyluracil (V, R = H) and 1-β-D-arabinofuranosyl-5-fluorouracil (FUA) (V, R = F) in high yield. FUA was acetylated, thiated, and then alkylated to the 4-methylmercapto derivative IX which was converted with liquid ammonia to 1-β-D-arabinofuranosyl-5-fluorocytosine (FCA, X). FUA (V), FCA (X), and 1-β-D-arabinofuranosylcytosine (CA) were active against Sarcoma 180 in mice. FCA was highly active against transplanted mouse leukemias P815 and P388, and FCA was more strongly active on a molar basis than CA against a 5-fluorouracil-resistant line of mouse leukemia P815. FCA and CA were effective against the 5-fluorouracil-resistant L1210 mouse leukemia. FCA, CA, and IUDR showed essentially the same activity in preventing the development of herpes keratitis in rabbits.

Pyrimidine nucleosides containing the 1-β-D-arabinofuranosyl moiety have exhibited interesting biological properties. 1-β-D-Arabinofuranosylcytosine (CA)[2] is effective against several experimental tumors,[3] was shown to inhibit several DNA viruses in cell cultures, and is effective against herpes simplex keratitis in the rabbit.[4] 5-Iodo-2′-deoxyuridine[5] (IUDR) also exerts antitumor[6] and antiviral activity,[4b,d] and recently it was shown that 1-β-D-arabinofuranosyl-5-iodouracil is active against herpes and vaccinia virus in cell cultures.[7]

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