stirred at room temperature for 24 hr., the volume reduced to 20 ml. *in vacuo*, and a yellowish semicrystalline compound was collected after standing for 3 hr. at 3°. This compound was crystallized from water (charcoal), and a yield of 0.35 g. (20%) was obtained, m.p. 295° dec.

Anal. Calcd. for $C_7H_7N_3O_5$: C, 39.4; H, 3.32; N, 19.7. Found: C, 39.2; H, 3.49; N, 19.5.

Ultraviolet absorption spectrum; in 0.1 *N* hydrochloric acid, λ_{max} 271 mμ (ε 10,700); λ_{min} 241 mμ (ε 3160); λ_{max} 220 mμ (ε 11,400); in 0.1 *N* sodium hydroxide, λ_{max} 291 mμ (ε 14,600); λ_{min} min 260 mμ (ε 3290); λ_{max} 240 mμ (ε 10,200). Paper chromatography: R_f 0.14 in butanol–water, 86:14; R_f 0.36 in butanol–acetic acid–water, 5:3:2.

Preparation of 5-trifluoromethyluracil-2-C^{14} and 5-Trifluoromethyl-2′-deoxyuridine-2-C^{14}.—Barium carbonate-C^{14} (100 mc.) was diluted with nonradioactive barium carbonate to give a total of 4.5 g. This was converted in two batches into barium cyanamide by heating for hr. at 850° in a slow stream of ammonia gas, and 4.0 g. was obtained.[28] Water (20 ml.) was added to the barium cyanamide, the bigger lumps were crushed, and the suspension was cooled to 5°. Sulfuric acid was added dropwise with stirring until the pH reached 7.0 and the barium sulfate was removed by centrifugation. The supernatant solutions and the washings from the barium sulfate were combined and the aqueous solution was concentrated to a small volume *in vacuo* at 30°. The cyanamide was converted into urea[28] by treatment of 10 ml. of solution with 1.5 ml. of concentrated hydrochloric acid and refluxing for 10 min. The solution was cooled, neutralized with sodium bicarbonate, and evaporated to dryness *in vacuo*. The residue was extracted with five 20-ml. portions of boiling absolute ethanol, and the combined extracts were evaporated to 20 ml. and cooled. The small amount of sodium chloride present was filtered, and the filtrate was evaporated to dryness to give 1.105 g. (81%) of urea. The urea was acetylated with a mixture of 1.85 ml. of acetic anhydride, 0.75 ml. of acetic acid, and 0.02 ml. of concentrated sulfuric acid, which was heated to 130° for 10 min. and allowed to cool. The N-acetylurea crystallized, was dissolved in 30 ml. of water, passed through a 1.5 × 20 cm. column of Dowex-1-formate, and eluted with 80 ml. of water. The solution was evaporated to dryness, and the N-acetylurea was crystallized from water to give 1.615 g. (86%).

The N-acetylurea (0.016 mole) and 2.38 g. (0.011 mole) of IV were dissolved in 15 ml. of redistilled dimethylformamide and heated for 3 hr. at 125°. The solvent was evaporated *in vacuo*, and the residue was crystallized from water (charcoal) to give a yield of 1.02 g. (40%) of labeled VIII. This was then heated under reflux in 15 ml. of 5 *N* hydrochloric acid for 1 hr. and evaporated to dryness *in vacuo*. The residue was crystallized from water (charcoal) to give 290 mg. (38%) of dihydrotrifluoromethyluracil-2-C^{14}. No additional crystalline material could be obtained from the mother liquors. Thus, 290 mg. was dissolved in 10 ml. of glacial acetic acid and brominated with 600 mg. of bromine in 6 ml. of acetic acid at reflux temperature for 90 min. The bromine and acetic acid were evaporated *in vacuo*, and the residue was coevaporated 3 times with absolute ethanol and heated in 10 ml. of dimethylformamide at 130° for 1 hr. After evaporation of the solvent *in vacuo* and coevaporation with water, the residue was passed through a 1.5 × 20 cm. column of Dowex-1-formate, and after elution of impurities with water, the 5-trifluoromethyl-2-C^{14} uracil was eluted with 0.05 *M* formic acid. The solution was evaporated to dryness, coevaporated with water to give 242 mg. (85%) of product that gave only a single radioactive spot in three paper chromatographic systems. The over-all yield, based on barium carbonate, was 5.9%. The specific activity was 4.15 mc./mmole (23 μc./mg.)

The labeled 5-trifluoromethyluracil (234 mg.) was converted to the deoxyribonucleoside with an enzyme obtained from *Lactobacillus Leichmanii*, kindly provided by Dr. Jack Siegel of the Pabst Laboratories, Milwaukee, Wis., and was purified as described above to give 158 mg. (41%) of 5-trifluoromethyl-2′-deoxyuridine-2-C^{14}, which gave only a single radioactive spot in three paper chromatographic systems, and had a specific activity of 4.15 mc./mmole (14 μc./mg.).

Acknowledgment.—We are grateful to Mrs. Nancy W. Remy for skillful and devoted technical assistance.

(28) S. H. Zbarsky and I. Fischer, *Can. J. Res.*, **27B**, 81 (1949).

Pyrimidine Derivatives. V. Synthesis of Substituted Pyrimidines from 4-Amino-6-chloro-2-methylthiopyrimidine[1-3]

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The use of 4-amino-6-chloro-2-methylthiopyrimidine as a versatile intermediate for the synthesis of a number of substituted pyrimidines is described. In the course of this work several previously unreported pyrimidines have been prepared. Feigl's iodine–azide reagent has been employed for the rapid detection of mercaptopyrimidines and a sponge nickel catalyst has been found to be quite satisfactory for the facile dethiation of these derivatives. Quantitative ultraviolet absorption spectra are given for all compounds, as well as a summary of growth-inhibitory properties in several *in vitro* and *in vivo* bioassay systems.

For some time a program of synthesis of pyrimidine derivatives for biological evaluation has been in progress in these laboratories[3] and, in connection with this program, the preparation of various pyrimidines as potential cancer chemotherapeutic agents and as precursors of condensed pyrimidine systems has been undertaken. This communication describes the synthesis of a number of substituted pyrimidines starting from the versatile intermediate 4-amino-6-chloro-2-methylthiopyrimidine (III), which was prepared following the method of Baker, *et al.*[4]

4-Amino-6-hydroxy-2-methylthiopyrimidine (II) was obtained by reaction of thiourea and ethyl cyanoacetate, with methylation *in situ* of the anion of 4-amino-6-hydroxy-2-mercaptopyrimidine (I) by means of freshly distilled dimethyl sulfate. The use of aged dimethyl sulfate led to the isolation of I[5] as a by-prod-

(1) This investigation was supported in part by research grants (CY3335 and C6516) from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) A preliminary report of part of this work has been presented: E. J. Modest and H. N. Schlein, Résumés des Communications, 3me Congrès International de Biochimie, Bruxelles, August 3, 1955, p. 33.

(3) For paper IV in this series see E. J. Modest, S. Chatterjee, G. E. Foley, and S. Farber, *Acta, Unio Intern. Contra Cancrum*, in press.

(4) B. R. Baker, J. P. Joseph, and R. E. Schaub, *J. Org. Chem.*, **19**, 631 (1954).

(5) W. Traube, *Ann.*, **331**, 64 (1904).

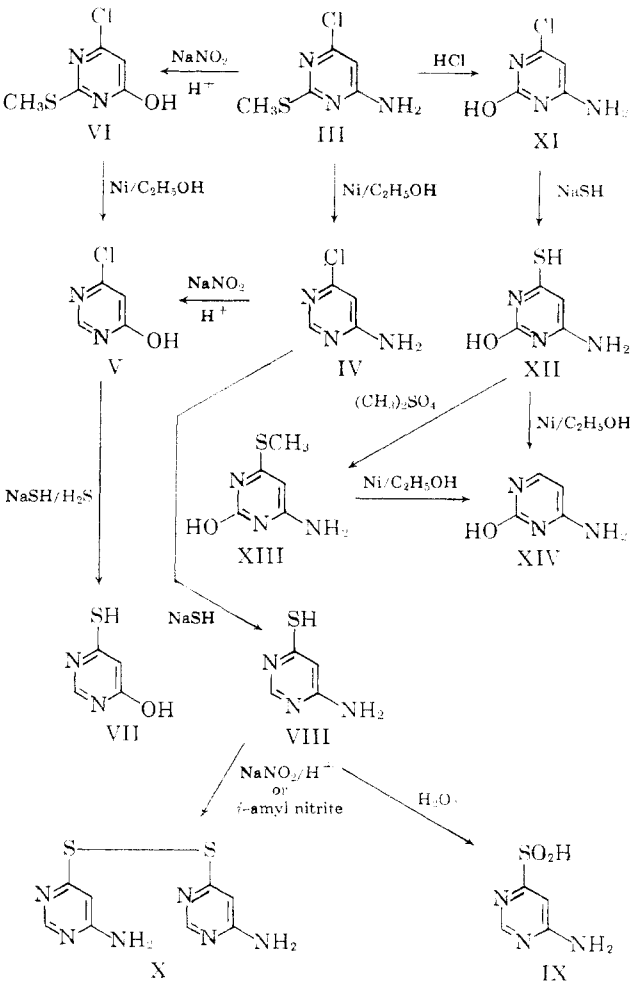
uct. Chlorination of II gave III.

Dethiation of III afforded 4-amino-6-ch oropyrimidine (IV), whose preparation has subsequently been described by chlorination of 4-amino-6-hydroxypyrimidine[6] or by monoamination of 4,6-dichloropyrimidine.[7] The reaction was accomplished in ethanol by means of a commercially available sponge nickel catalyst,[8] which is much less pyrophoric and retains its activity on prolonged storage in contrast to the usual W-2 Raney modification.[9] A ratio of 8 g. of nickel (apparent bulk density 0.8–1.0)[8] per gram of III was found to be optimum for the dethiation.

Treatment of IV with a 50% excess of sodium nitrite in warm dilute acetic acid solution gave 6-chloro-4-hydroxypyrimidine (V). The 5-nitroso derivative of IV did not form, in agreement with the generalization of Lythgoe and Todd[10,11] that 5-unsubstituted pyrimidines with only one electron-releasing group in position 4 or 6 cannot be successfully nitrosated. Compound V was also prepared by an alternate route. When III was treated with sodium nitrite under similar conditions, 6-chloro-4-hydroxy-2-methylthiopyrimidine (VI) was obtained. Dethiation of VI with Davison sponge nickel then gave V, identical with the previous sample in all respects, including melting point and mixture melting point and ultraviolet and infrared absorption spectra.

Isbecque and co-workers[12] have described the synthesis of V by acid hydrolysis of 6-chloro-4-methoxypyrimidine and the conversion of V into 4-hydroxy-6-mercaptopyrimidine (VII) with sodium hydrosulfide in ethylene glycol. They were unable to thiate V in aqueous solution. Prior to their report, we had prepared V and had successfully thiated the compound in aqueous solution at steam-bath temperature with a 5-fold excess of sodium hydrosulfide, while hydrogen sulfide was continuously bubbled through the reaction mixture.

Refluxing IV with sodium hydrosulfide in ethanol for 1 week produced 4-amino-6-mercaptopyrimidine (VIII), subsequently obtained[13] by reaction of IV and sodium hydrosulfide in ethylene glycol at an elevated temperature for a brief period of time. This compound was of interest as a possible alternate starting material for the synthesis of 4,5-diamino-6-mercaptopyrimidine, which has been prepared by the simultaneous reduction and thiation of 4-amino-6-chloro-5-nitropyrimidine with sodium hydrosulfide and hydrogen sulfide,[14] and



more recently by the direct thiation of 4,5-diamino-6-hydroxypyrimidine with phosphorus pentasulfide in refluxing β-picoline.[15]

In accordance with the general rule[10,11] that 5-unsubstituted pyrimidines containing electron-releasing groups at both the 4- and 6-positions readily undergo nitrosation at the 5-position in the presence of mineral acid, it was anticipated that VIII would undergo facile nitrosation and that the nitroso derivative could then be reduced to the diamine. However, when a solution of VIII and sodium nitrite in dilute sodium hydroxide was acidified, the white solid that precipitated immediately (X) did not exhibit the expected color or other properties characteristic of the 5-nitroso compound. The product, which showed a single absorbing spot on paper chromatography, was totally insoluble at pH 10 and exhibited a hypsochromic shift in the ultraviolet at pH 1 when compared with VIII, consistent with a disulfide. This compound was characterized as bis(4-amino-6-pyrimidyl) disulfide, a structure supported by microchemical analysis.

The absence of the mercapto group in X and the possible presence of a disulfide linkage were further indicated by the slow reaction of X with Feigl's iodine–azide reagent.[16] This reagent has been used routinely in this laboratory as a rapid test for mercaptopyrimidines. Thioketones and thiols catalytically accelerate

(6) C. W. Whitehead and J. J. Traverso, *J. Am. Chem. Soc.*, **80**, 2185 (1958).

(7) H. Goldner, *Chem. Tech.* (Berlin), **12**, 495 (1960); *Chem. Abstr.*, **55**, 5517*b* (1961).

(8) Available under water in 32-kg. pails, 50% solids, as sponge nickel hydrogenation catalyst, grade 986, from the Davison Chemical Division, W. R. Grace and Co., Baltimore, Md. The apparent bulk density is calculated by measuring the dry (110°) weight per unit volume of a settled aqueous suspension of the catalyst. A more general report of the use of this catalyst for dethiation reactions will be the subject of another communication. A referee has pointed out that Raney Active Nickel Catalyst, Raney Catalyst Co., Chattanooga, Tenn., is equally effective and also keeps well.

(9) R. Mozingo, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p. 181.

(10) B. Lythgoe, A. R. Todd, and A. Topham, *J. Chem. Soc.*, 315 (1944).

(11) B. Lythgoe, *Quart. Rev.*, **3**, 205 (1949).

(12) D. Isbecque, R. Promel, R. C. Quinaux, and R. H. Martin, *Helv. Chim. Acta*, **42**, 1317 (1959).

(13) H. C. Koppel, R. H. Springer, R. K. Robins, and C. C. Cheng, *J. Org. Chem.*, **26**, 792 (1961). We had completed the preparation of VIII, XII, and XIII prior to the appearance of this article.

(14) G. B. Elion and G. H. Hitchings, *J. Am. Chem. Soc.*, **76**, 4027 (1954).

(15) A. G. Beaman and R. K. Robins, *ibid.*, **83**, 4038 (1961).

(16) F. Feigl, "Spot Tests in Organic Analysis," 5th English Ed., Elsevier Publishing Co., Amsterdam, 1956, p. 228.

the reaction of iodine and sodium azide, producing an almost instantaneous discharge of color of the yellow-brown test solution and the evolution of nitrogen gas. Complete lack of reaction is a reliable indication of the absence of such groups. Disulfides, particularly those with low solubility in water (*e.g.*, cystine), give a distinct iodine–azide reaction only after they have been in contact with the reagent solution for some time. X was in contact with Feigl's reagent for 30–40 min. before the color was completely discharged.

Attempted nitrosation of VIII with isoamyl nitrite in ethanol at room temperature or at reflux, or in ethylene glycol, cellosolve, or carbitol at steam-bath temperatures, also gave X. The failure to obtain 4-amino-6-mercapto-5-nitrosopyrimidine not only violates the well accepted rule of Lythgoe and Todd but also illustrates the rather unusual use of nitrous acid for disulfide formation. Obviously, in the presence of nitrous acid, which is an oxidizing agent as well as a nitrosating agent, oxidation of the mercapto group occurs in preference to the introduction of the nitroso group.

The ease of oxidation of the mercapto group of VIII was further indicated by the formation of 4-amino-pyrimidine-6-sulfinic acid (IX) from VIII and hydrogen peroxide (30%) at room temperature. Compound IX showed the behavior of a zwitterion, being soluble at pH 1 and pH 10 but only sparingly soluble at pH 7, and gradually darkening above 180° without melting below 360°. The infrared spectrum showed strong absorption bands at 9.18 and 9.83 μ, characteristic of sulfinic acids.[17]

4-Amino-6-chloro-2-hydroxypyrimidine (XI) was obtained in 72% yield by hydrolysis of the methylthio group of III with concentrated hydrochloric acid. Wheeler and Liddle[18] have prepared a number of hydroxypyrimidines by acid hydrolysis of the corresponding alkylthiopyrimidines. The preparation of XI in this manner was originally reported by Wheeler and Jamieson[19] and later by Koppel, *et al.*,[13] but adequate characterization and yields were not given. It was observed that even after several recrystallizations from water, XI had a tendency to retain some hydrochloric acid, which could be removed only after a neutralization step was included in the purification. XI was converted into 4-amino-2-hydroxy-6-mercaptopyrimidine (XII) by thiation in refluxing ethanolic sodium hydrosulfide. The synthesis of XII by treatment of XI with aqueous sodium hydrosulfide in a stainless steel bomb has recently been described.[13]

Methylation of XII by means of dimethyl sulfate in dilute sodium hydroxide gave 4-amino-2-hydroxy-6-methylthiopyrimidine (XIII), recently reported by Koppel, *et al.*[13] Feigl's reagent was again used to show that S-methylation had occurred rather than O-methylation. Methylation was shown to have taken place at the sulfur atom by the observation that, in contrast to XII, which possesses a mercapto group and reacts almost instantaneously with the iodine–azide reagent, XIII failed to give a reaction with the test solution. Furthermore, both XII and XIII gave the same product, cytosine (XIV), on dethiation with Davison sponge nickel. The identity of both samples of XIV with an authentic sample of cytosine was established by comparison of ultraviolet and infrared spectra, microchemical analyses, mixture melting points, and ascending paper chromatography in 2:1 1-propanol–ammonia (4.2% aqueous) and in 5% acetic acid.

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA

Compound	pH 1 λ_{max}, mμ	pH 1 $\epsilon \times 10^{-3}$	pH 10 λ_{max}, mμ	pH 10 $\epsilon \times 10^{-3}$
I	246	7.69	243	17.45
	277	18.98	259[a]	8.89
			289	13.16
II	236	17.74	264.5	8.30
	270	9.51		
III	244.5	17.06	230	19.58
	280	5.76	253	10.27
			287	6.06
IV	239	8.31	237	12.54
	251[a]	6.59	272	3.30
V	226	5.63	232	10.35
	272	4.10	267	3.52
VI	244	7.09	221	18.16
	293	9.63	249	8.68
			279	6.75
VII	244	9.08	228[a]	13.92
	305	16.54	238	15.50
			294	15.84
VIII[b]	240	16.82	226	18.55
	304	21.25	243	17.30
			291	14.47
IX	249	13.27	235	10.00
			280	3.71
X	233	30.61	[c]	
	281	14.28		
XI	279	13.02	281.5	9.01
XII	244	7.86	222	16.79
	318[d]	37.81[d]	305	25.42
XIII	235[a]	6.60	287	12.63
	295[e]	22.11[e]		
XIV	275	10.81	267.5	6.46

[a] Inflection. [b] Lit.[13] pH 1, 237 (6.60), 303 (28.70); pH 11, 244 (7.00), 289 (16.30). [c] Insoluble. [d] Lit.[13] pH 1, 317 (40.50). [e] Lit.[13] pH 1, 295 (13.3).

Quantitative ultraviolet absorption spectra of compounds prepared during the course of this investigation are given in Table I. Our spectrophotometric results are in agreement with the generalizations of Koppel, *et al.*,[13] that the major absorption peak of 4-mercaptopyrimidines at pH 1 occurs in the range of 300–320 mμ and that the major absorption maximum of mercaptopyrimidines undergoes a hypsochromic shift from pH 1 to pH 10.

Biological Activity.—The fourteen pyrimidine derivatives discussed herein have been examined in various *in vitro* and *in vivo* bioassay systems at the Children's Cancer Research Foundation. We are indebted to Dr. George E. Foley for the determination of the biological activity of these compounds in selected bacterial and mammalian cell culture systems, and to Dr. Charlotte L. Maddock and Dr. Sidney Farber for the biological data against transplantable mouse tumors. In the *Streptococcus faecalis* No. 8043–folic acid bioassay system,[20] only two compounds were found to be active at

(17) L. J. Bellamy, "The Infra-red Spectra of Complex Molecules," 2nd Ed., Methuen and Company, London, 1958, p. 350.

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

(19) H. L. Wheeler and G. S. Jamieson, *ibid.*, **32**, 342 (1904).

(20) G. E. Foley, R. E. McCarthy, V. M. Binns, E. E. Snell, B. M. Guirard, G. W. Kidder, V. C. Dewey, and P. S. Thayer, *Ann. N. Y. Acad. Sci.* **76**, 413 (1958).

a 50% inhibiting dose (ID_{50}) less than 100 μg/ml.: VIII (ID_{50} = 30 μg/ml.) and IX (ID_{50} = 40 μg/ml.). Of three compounds (V, IX, and X) investigated for activity against KB cells (human epidermoid carcinoma) in culture,[21,22] the disulfide (X) was active at an ID_{50} of 55 μg/ml. All compounds were examined for antitumor activity according to the standard assay procedures employed in this Foundation.[23] These include four transplantable mouse tumors: L1210 ascitic lymphatic leukemia in the BDF/1 hybrid, P1534 lymphatic leukemia in the DBA/2 inbred, C1498 myelogenous leukemia in the C57Bl/6 inbred, and DBRB mammary carcinoma in the DBA/1 inbred strain. Compounds I, VII, XI, and XIII, when administered intraperitoneally, exhibited slight to moderate tumor inhibition in the C1498 tumor system.

Experimental[24]

The ultraviolet absorption spectra reported were measured with a Cary Model 11 spectrophotometer. Spectra at pH 1 were taken in 0.1 *N* hydrochloric acid and at pH 10 in 0.05 *M* sodium carbonate–sodium borate buffer. Infrared spectra were determined in potassium bromide disks with a Perkin-Elmer Model 137B spectrophotometer. Paper chromatography was done by the ascending technique on Whatman No. 1 paper and spots were located by visual examination under ultraviolet light.[25] Melting points are corrected and were taken at 2°/min. by the capillary method in a modified Wagner–Meyer melting point apparatus.[26] Decomposition points are not reproducible unless conditions are rigidly controlled. If not specified, drying of analytical samples was carried out at 70–100° for 17 hr. *in vacuo* over phosphorus pentoxide.

4-Amino-6-chloro-2-methylthiopyrimidine (III).—This compound was prepared in two steps according to Baker, *et al.*[4] Condensation of thiourea and ethyl cyanoacetate in alcoholic sodium methoxide, with methylation *in situ* by freshly distilled dimethyl sulfate, gave II in 85–90% yield, m.p. 267° dec. (lit. m.p. 261–262° dec.[4]; 267° dec.[27]). Freshly distilled dimethyl sulfate or undistilled material having a pH greater than 4 in 1:1000 aqueous dilution is required for this methylation. The use of aged dimethyl sulfate having a pH below 3 in 1:1000 aqueous dilution led to formation of I[5] (no m.p. below 360°) as a by-product in 5–10% yield.

Anal. Calcd. for $C_4H_5N_3OS$: C, 33.55; H, 3.52; N, 29.35. Found: C, 33.6; H, 4.0; N, 29.0.

Chlorination of II with phosphorus oxychloride and N,N-dimethylaniline afforded III in 55–60% yield. Removal of unchanged starting material by trituration of the crude solid with *N* sodium hydroxide was an important step in the purification of III.[4] After crystallization from 95% ethanol the product melted at 127–128° (lit. m.p. 125–128°[4]; 127–128°[19]; 132°[27]).

4-Amino-6-chloropyrimidine (IV).—Sponge nickel catalyst (Davison Chemical Co.) equivalent to 800 g. (1000 ml., apparent bulk density 0.8)[8] was transferred to a 5-l. flask with water and the excess water was decanted. A solution of 100 g. (0.57 mole) of 4-amino-6-chloro-2-methylthiopyrimidine in 1 l. of 95% ethanol was added, followed by an additional liter of ethanol. The mixture was refluxed for 2.5 hr. with vigorous mechanical stirring, the course of the reaction being followed by ultraviolet absorption spectral measurements. The ethanol solution was decanted through a suction funnel packed with Celite. The nickel residue was washed with three 500-ml. portions of hot 95% ethanol and a 500-ml. portion of hot water. These washes were decanted through the same Celite filter pad. The combined filtrates were taken to dryness *in vacuo* (bath temp. below 50°)[28] and the residue was crystallized from water; yield, 46.3 g. (63%) of IV. Two further crystallizations from 95% ethanol, the first with the aid of Darco,[29] produced analytically pure colorless prismatic rods, which decompose at 208–210° (lit.[5] m.p. 215°). The analytical sample was dried for 17 hr. at 45° *in vacuo* over phosphorus pentoxide.

Anal. Calcd. for $C_4H_4ClN_3$: C, 37.08; H, 3.11; Cl, 27.37; N, 32.44. Found: C, 37.01, 37.23; H, 3.19, 3.36; Cl, 27.4; N, 32.6.

This compound can also be purified by high vacuum sublimation.

6-Chloro-4-hydroxy-2-methylthiopyrimidine (VI).—4-Amino-6-chloro-2-methylthiopyrimidine (10.0 g., 0.057 mole) was partially dissolved in 200 ml. of 25% acetic acid by warming on the steam bath for 20 min. The mixture was cooled to 40° and a solution of 4.8 g. (0.07 mole) of sodium nitrite in water was added with stirring. The reaction mixture was then heated on the steam bath for 2.5 hr., during which time the solution became orange and began to deposit yellow solid. After overnight refrigeration, the solid was removed by suction filtration and air-dried: crude weight 9.03 g. (90%). Recrystallization from 110 ml. of acetonitrile returned 5.77 g. (64% recovery) of white needles melting at 216–217°.

Anal. Calcd. for $C_5H_5ClN_2OS$: C, 34.00; H, 2.85; Cl, 20.07; N, 15.86; S, 18.15. Found: C, 34.05; H, 2.98; Cl, 19.87; N, 15.55; S, 18.18.

6-Chloro-4-hydroxypyrimidine (V). A. From 4-Amino-6-chloropyrimidine (IV).—4-Amino-6-chloropyrimidine (30.0 g., 0.23 mole) was dissolved in 1800 ml. of 25% acetic acid and cooled to 10°. One-half of a solution of 23.7 g. (0.34 mole) of sodium nitrite in 118 ml. of water at 10° was added to the pyrimidine solution below the surface. The resulting dark yellow solution was heated at 50–60° (internal temperature) for 3 hr., during which time the rest of the nitrite solution was added at a rate to maintain an excess of nitrous acid. The reaction solution was allowed to stand at room temperature overnight, at the end of which time the ultraviolet absorption spectrum indicated that the reaction had gone to completion. The solution was then reduced to dryness on a rotary evaporator.[28] The residue was crystallized from 170 ml. of water with the aid of Darco; yield, 18.9 g. (63%) of material free of inorganic salts; m.p. 196–202°. For analysis a sample was recrystallized from water and dried for 24 hr. at room temperature over phosphorus pentoxide *in vacuo*; colorless prismatic plates, m.p. 196–197° (lit.[12] m.p. 192–193°).

Anal. Calcd. for $C_4H_3ClN_2O$: C, 36.80; H, 2.32; Cl, 27.16; N, 21.46. Found: C, 36.81; H, 2.36; Cl, 27.11; N, 21.00.

B. From 6-Chloro-4-hydroxy-2-methylthiopyrimidine (VI).—To a solution of 6-chloro-4-hydroxy-2-methylthiopyrimidine (0.53 g., 0.003 mole) in 50 ml. of 95% ethanol was added 5 ml. of Davison sponge nickel catalyst (apparent bulk density 0.8)[8] and the mixture was stirred magnetically under reflux for 2 hr. The ultraviolet absorption spectrum of a sample of the reaction liquid, measured after 1 hr. of reflux, indicated that complete dethiation to V had taken place. The reaction mixture was decanted through a small Soxhlet thimble and the nickel was extracted with 80 ml. of 95% ethanol for 19 hr. in a Soxhlet extraction apparatus. The extract was combined with the reaction mother liquor and the clear colorless solution was distilled to dryness *in vacuo* at a bath temperature not exceeding 42°.[28] The residue (200 mg., 51%) was crystallized from a minimal volume of boiling water. After overnight refrigeration the solid was collected, washed with ether, and air-dried. The colorless elongated prismatic plates weighed 132 mg. (66% recovery), m.p. 193–195°. For analysis this solid was crystallized once more from water and dried for 23 hr. at 35° *in vacuo*; colorless needles, m.p. 196–197°.

Anal. Found: C, 36.60; H, 2.73; N, 21.30.

The mixture melting point of this material with a sample of V prepared by the action of nitrous acid on IV (above procedure **A**) was undepressed and the ultraviolet and infrared absorption spectra of the two samples were identical.

(21) G. E. Foley and H. Eagle, *Cancer Res.*, **18**, 1012 (1958).

(22) H. Eagle and G. E. Foley, *ibid.*, **18**, 1017 (1958).

(23) C. L. Maddock, G. J. D'Angio, S. Farber, and A. H. Handler, *Ann. N. Y. Acad. Sci.*, **89**, 386 (1960).

(24) Analyses were performed by the Scandinavian Microanalytical Laboratory, Herlev, Denmark; Drs. Weiler and Strauss, Oxford, England; and Dr. Carol K. Fitz, Needham Heights, Mass.

(25) The chromatograms were examined on a viewing box equipped with a 15-w. General Electric germicidal lamp and a Corning filter, No. 9863.

(26) E. C. Wagner and J. F. Meyer, *Ind. Eng. Chem., Anal. Ed.*, **10**, 584 (1939).

(27) T. B. Johnson and C. O. Johns, *Am. Chem. J.*, **34**, 175 (1905).

(28) This compound is volatile and codistils with the solvent if the bath temperature is too high.

(29) Darco G-60 activated carbon, Atlas Chemical Industries, Inc., Wilmington, Del.

4-Hydroxy-6-mercaptopyrimidine (VII).—6-Chloro-4-hydroxypyrimidine was prepared on a 0.46 molar scale according to **A** above. Without isolation of the product, the clear yellow reaction solution was brought to pH 8 by the addition of 560 g. of sodium hydroxide pellets. After addition of 255 g. (2.32 moles) of sodium hydrosulfide trihydrate, the solution was heated on a steam bath at 70–80° (internal temperature) with continuous introduction of hydrogen sulfide gas below the surface. After 2 hr. the reaction was complete as evidenced by the ultraviolet absorption spectrum. The solution was then cooled in an ice bath and the pH adjusted to 5 by the careful addition of 300 ml. of concentrated sulfuric acid. After overnight refrigeration, the precipitate that had formed was collected and extracted directly with 500 ml. of boiling water. After refrigeration for several days, the aqueous solution deposited 6.3 g. (11%) of a yellow crystalline solid. The pH of the original filtrate was lowered to approximately 3 with concentrated sulfuric acid and, after overnight refrigeration, the solid was separated, washed with cold water, and air-dried. The yield was 28 g. (47%); total crude yield 34.3 g. (58%).

Two crystallizations of the crude material from water (100 ml./g.), the first with Darco, gave yellow prismatic rods, which decompose at 227–228° (lit. m.p. 240–242° dec.[12]; 247° dec.[13]).

Anal. Calcd. for $C_4H_4N_2OS$: C, 37.48; H, 3.15; N, 21.86; S, 25.02. Found: C, 37.18, 37.40; H, 3.16, 2.99; N, 21.78; S, 25.15.

4-Amino-6-mercaptopyrimidine (VIII).—To a solution of 169 g. (1.53 moles) of sodium hydrosulfide trihydrate in 750 ml. of 95% ethanol was added 20 g. (0.154 mole) of 4-amino-6-chloropyrimidine. The solution was stirred and refluxed for 7 days and then filtered and evaporated to dryness *in vacuo*. The residue was dissolved in 500 ml. of warm *N* sodium hydroxide and this solution was clarified with Darco, filtered, and brought to pH 8 with sulfuric acid. After overnight refrigeration the solid was collected and redissolved in 300 ml. of *N* sodium hydroxide. Reprecipitation at pH 8 gave 11.1 g. (57%) of VIII. One crystallization from 50% ethanol afforded colorless rhombic plates melting at 307° dec. (lit.[13] m.p. 306°).

Anal. Calcd. for $C_4H_5N_3S$: C, 37.78; H, 3.96; N, 33.04; S, 25.22. Found: C, 37.81; H, 4.27; N, 32.65, 32.64; S, 25.12.

4-Aminopyrimidine-6-sulfinic Acid (IX).—To a solution of 1.0 g. (0.008 mole) of 4-amino-6-mercaptopyrimidine in 11 ml. of *N* sodium hydroxide, cooled in an ice bath, was added dropwise, with stirring, 2 ml. of 30% hydrogen peroxide. The mixture was stirred for 30 min. and then acidified with 6 *N* hydrochloric acid to pH 4.0. A white precipitate formed on acidification and the resulting suspension was stirred at ice-bath temperature for 1.5 hr. On filtration, a white powder was obtained (628 mg., 49%) which, after recrystallization from water, gradually darkens above 180° but does not melt below 360°.

Anal. Calcd. for $C_4H_5N_3O_2S$: C, 30.18; H, 3.17; N, 26.40; S, 20.15. Found: C, 30.3; H, 3.1; N, 26.5; S, 20.2.

The product is only slightly soluble in water but is appreciably soluble at pH 1 and pH 10. The infrared spectrum showed, in addition to a broad peak in the associated N–H stretch region, two strong absorption bands at 9.18 and 9.83 μ, characteristic of sulfinic acids.[17] IX gives a negative reaction for the mercapto group with the iodine–azide reagent.[16]

Bis(4-amino-6-pyrimidyl) Disulfide (X).—A solution of 4.0 g. (0.031 mole) of 4-amino-6-mercaptopyrimidine in 50 ml. of *N* sodium hydroxide containing 2.4 g. (0.035 mole) of sodium nitrite was acidified by dropwise addition of concentrated hydrochloric acid with stirring and ice-bath cooling. The solution turned pale green and a white precipitate settled out almost immediately. Stirring was continued and the mixture was allowed to warm to room temperature. The precipitate was separated by suction filtration and air-dried; yield 2.51 g. (64%).

One gram of product was dissolved in 15 ml. of *N* hydrochloric acid with heating. The solution was then cooled and neutralized with 0.5 *N* ammonia. Suction filtration and air-drying returned 0.92 g. of white solid (92% recovery), which decomposed at 228°, was completely insoluble at pH 10, and reacted very slowly with Feigl's iodine–azide reagent (negative test for the mercapto group and possible indication of a disulfide linkage).[16] This compound showed a single absorbing spot on ascending paper chromatography in two solvent systems: 4:1:1 1-butanol–acetic acid–water (R_f 0.76) and 1-butanol saturated with water (R_f 0.70)

Anal. Calcd. for $C_8H_8N_6S_2$: C, 38.08; H, 3.20; N, 33.31; S, 25.42. Found: C, 38.0; H, 3.20; N, 33.0; S, 25.5.

The product was also formed in almost quantitative yield when a suspension of 4-amino-6-mercaptopyrimidine in absolute ethanol was stirred with excess isoamyl nitrite at room temperature overnight. Yields of X were high but variable when VIII was treated with excess isoamyl nitrite in ethanol at reflux or in ethylene glycol, Cellosolve, or Carbitol at steam bath temperature.

4-Amino-6-chloro-2-hydroxypyrimidine (XI).—To 15.0 g. (0.085 mole) of 4-amino-6-chloro-2-methylthiopyrimidine was added 75 ml. of concentrated hydrochloric acid and the resulting mixture slowly evaporated to dryness on a steam bath over a period of 8 hr. Within 1 hr. a clear deep yellow solution was obtained and after 5 hr. the ultraviolet absorption spectrum of the mixture indicated complete hydrolysis. The pale yellow residue was pulverized, extracted with 300 ml. of absolute ethanol at room temperature to remove any unreacted starting material, and then crystallized directly from 500 ml. of boiling water with Darco. After refrigeration for 2 days, the colorless solid (6.2 g.) was collected and washed with ether. The filtrate was reduced to half volume under vacuum and upon refrigeration gave a second crop of 2.75 g. The over-all crude yield was 8.95 g. (72%). A sample of this solid was crystallized once more from water and submitted for analysis.

Anal. Calcd. for $C_4H_4ClN_3O$: C, 33.01; H, 2.77; Cl, 24.36; N, 28.87. Found: C, 30.98; H, 3.04; Cl, 26.5; N, 25.9.

In view of these analytical results it appeared possible that the compound might be contaminated with a small amount of hydrochloric acid. In support of this conclusion the pH of a saturated aqueous solution of this sample was found to be 3.99. Therefore, the following purification procedure, including a neutralization step, was carried out. Once crystallized material (650 mg.) was dissolved in 33 ml. of 1.6 *N* ammonium hydroxide and precipitated with dilute sulfuric acid at pH 8.5. After overnight refrigeration the crystals were collected, washed with a small volume of water, and air-dried; recovery, 350 mg. One crystallization from water afforded 295 mg. of analytically pure colorless prismatic rods, which shrank and turned orange at 240–245° but did not melt below 360°. The pH of a saturated aqueous solution was 6.52.

Anal. Found: C, 33.4; H, 2.76; Cl, 24.15; N, 28.4.

4-Amino-2-hydroxy-6-mercaptopyrimidine (XII).—One gram (0.0069 mole) of 4-amino-6-chloro-2-hydroxypyrimidine was suspended in a solution of 7.57 g. (0.069 mole) of sodium hydrosulfide trihydrate in 30 ml. of 95% ethanol. The yellowish suspension was refluxed with stirring for 8 hr. Most of the solid dissolved at the reflux point and an almost colorless precipitate began to deposit from the solution soon after. The suspension, showing the ultraviolet absorption spectrum of XII, was cooled to room temperature; the solid was collected and washed with a small volume of absolute ethanol. The crude product was dissolved in 8.5 ml. of 3 *N* ammonium hydroxide and the solution was clarified with Darco and filtered. The pH of the yellow filtrate was adjusted to approximately 6–7 with dilute sulfuric acid. After refrigeration for a few hours, the crystals that had formed were collected, washed with cold water and 95% ethanol, and air-dried. The pale yellow prismatic rods weighed 0.2 g. An additional 70 mg. of crystals, which deposited from the filtrate after the volume had been reduced, was collected and combined with the first crop; total yield, 0.27 g. (27.4%). The analytical sample was prepared by reprecipitation from 3 *N* ammonia with dilute sulfuric acid at pH 8.5. The off-white prismatic rods, collected after overnight refrigeration, gave a positive test for the mercapto group with the iodine–azide reagent[16] and did not melt below 360° (lit.[13] 355° dec.).

Anal. Calcd. for $C_4H_5N_3OS$: C, 33.55; H, 3.52; N, 29.35; S, 22.40. Found: C, 33.84; H, 3.68; N, 29.00; S, 22.22.

4-Amino-2-hydroxy-6-methylthiopyrimidine (XIII).—Redistilled dimethyl sulfate (10 ml.) was added slowly with hand-swirling to a solution of 4-amino-2-hydroxy-6-mercaptopyrimidine (7.24 g., 0.0505 mole) in 400 ml. of dilute sodium hydroxide (23 g. in 400 ml. of water). The reaction mixture was warmed on the steam bath for 1 hr., with occasional shaking, and filtered to remove undissolved impurities. The pH of the filtrate was adjusted to approximately 8 with dilute sulfuric acid and crystallization started immediately. After refrigeration overnight, the crystals were collected, washed with cold water, and air-dried; 7.9 g. (quantitative yield). For analysis, a sample was crystallized first from water with Darco and then from 70% ethanol; colorless shiny prismatic needles, which melted at 278° dec.

Anal. Calcd. for $C_5H_7N_3OS$: C, 38.20; H, 4.49; N, 26.73; S, 20.40. Found: C, 38.48, 38.57; H, 4.25, 4.42; N, 26.29, 26.42; S, 20.9.

This compound has recently been described by Koppel, *et al.*,[18] who record m.p. 294° dec., but the nitrogen analysis is unsatisfactory.

Cytosine (XIV). A. By Dethiation of 4-Amino-2-hydroxy-6-mercaptopyrimidine (XII).—To a nearly complete solution of 4-amino-2-hydroxy-6-mercaptopyrimidine (0.5 g., 0.0036 mole) in 35 ml. of 95% ethanol was added 5 ml. of Davison sponge nickel catalyst (apparent bulk density 0.85),[8] 20 ml. of 95% ethanol being used to transfer the nickel into the reaction flask. The suspension was refluxed with stirring for 1 hr.; the ultraviolet absorption spectrum indicated that the reaction had gone to completion within that time. The supernatant liquid, while still hot, was decanted and filtered by gravity. The nickel slurry in the flask was extracted three times with small portions of 95% ethanol and the extracts were filtered through the same funnel. After refrigeration overnight, the crystals that had formed were collected, washed with 2–3 ml. of 95% ethanol, and air-dried; colorless, thin, shiny prismatic needles; yield, 0.167 g. (43%). These crystals, which lost solvent of crystallization and became chalky on drying at 70° *in vacuo* over phosphorus pentoxide for 17 hr., were submitted directly for analysis, m.p. 323° dec.

Anal. Calcd. for $C_4H_5N_3O$: C, 43.24; H, 4.54; N, 37.82. Found: C, 43.16; H, 4.53; N, 37.90, 37.99.

An additional 100 mg. of XIV was obtained from the filtrate of the first crop of crystals on evaporation of the filtrate to dryness *in vacuo* and recrystallization of the dry residue (128 mg.) from 35 ml. of 95% ethanol; total yield, 267 mg. (68%).

B. By Dethiation of 4-Amino-2-hydroxy-6-methylthiopyrimidine (XIII).—4-Amino-2-hydroxy-6-methylthiopyrimidine (0.5 g., 0.0032 mole) was suspended in 100 ml. of 95% ethanol and warmed on a steam bath for approximately 0.5 hr. to dissolve most of the solid. The solution was cooled to room temperature and to it was added 5 ml. of Davison sponge nickel catalyst (apparent bulk density 0.85).[8] The suspension was refluxed with stirring for 1.5 hr. and then filtered by decantation from the nickel, as is procedure A, while still hot. The nickel was washed three times with small portions of 95% ethanol and the washings were added to the filtrate. The alcoholic solution was evaporated to dryness under reduced pressure and the colorless residue (200 mg.) was crystallized directly from 55 ml. of 95% ethanol. After overnight refrigeration the crystals were collected, washed with cold 95% ethanol, and air-dried; yield, 53 mg. (15%). Concentration of the mother liquor yielded an additional 74 mg. (21%). The total yield was 127 mg. (36%) of colorless, shiny prismatic plates, which, after recrystallization from 95% ethanol, melted at 320–322° dec.

Anal. Found: C, 43.29, 43.20; H, 4.58, 4.32; N, 37.58, 37.58.

These crystals were shown to be identical with those prepared by procedure A and to a sample of highly pure cytosine obtained from Nutritional Biochemicals Corp., having the same melting point and mixture melting point (321–323° dec.), ultraviolet and infrared absorption spectra, and ascending paper chromatographic behavior in two solvent systems: 5% acetic acid (R_f 0.79) and 2:1 1-propanol–ammonia (4.2% aqueous) (R_f 0.71).

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Derivatives of Purinethiols. Purine Thiolcarbonates and Related Compounds[1]

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The ethyl carbonate and 1-pentyl carbonate derivatives of purine-6-thiol were prepared by the reaction of ethyl and 1-pentyl chloroformates, respectively, with purine-6-thiol. The structures were established by an alternate synthesis from 6-thiocyanatopurine. Analogous ethyl and 1-pentyl carbonate derivatives were obtained from purine-8-thiol, and methyl and ethyl carbonate derivatives from 9-methylpurine-6-thiol. The purine-6-thiol ethyl carbonate has tumor-inhibiting properties. Attempts to prepare thiolcarbamates by the action of isocyanates on purine-6-thiol or on its 9-methyl derivative were unsuccessful. Reaction of 6-methylthiopurine with alkyl chloroformates gave methyl and ethyl 6-(methylthiopurine)-7(or 9)carboxylates. Methyl 8-(methylthiopurine)-7(or 9)carboxylate was obtained similarly. Isocyanates reacted with 6-methylthiopurine to give the 7(or 9) phenyl- 1-naphthyl-, and 1-butylcarbamoyl derivatives.

Because of the inhibitory effect of purine-6-thiol[2] (6-mercaptopurine) on the growth of tumors[3] and on leukemia,[4] compounds that might decompose to give this purine are of possible interest. Such compounds include purine thiolcarbonates, $PSCO_2R$, and purine thiolcarbamates, PSCONHR (where P is the purine nucleus). Simple alkyl thiolcarbonates have shown[5] pharmacological effects similar to those of the thiols. Neither the thiolcarbonate nor thiolcarbamate derivatives of purines have been described, although there has been extensive work[6] on other derivatives of purine-6-thiol.

Purine thiolcarbonates (I–VI, Table I) were prepared

(1) (a) Supported by PHS Grant No. CY-3477 from the National Cancer Institute, Public Health Service; (b) From the Ph.D. thesis of Howard S. Bender, University of Delaware, 1962.

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