tane dihydrochloride (IVc) was dissolved in 50 cc. of methanol and hydrogenated over 6 g. of 10% palladized charcoal. The absorption of hydrogen was essentially complete in 90 seconds. This is a reduction rate comparable with that found with benzyl alcohol and cyclohexene and is the most rapid the senior author has observed in an *N*-debenzylation except for the first step in the debenzylation of IVb to Vb. After removal from the catalyst, the solvent was evaporated *in vacuo* and the residue was recrystallized from methanol.

3-Acetyl-8-ethyl-3,8-diazabicyclo[3.2.1]octane (VIc). One-fiftieth mole (4.3 g.) of Vc dihydrochloride was placed in a flask and covered with 25 cc. of acetic anhydride. Three grams of potassium carbonate was added and the flask was heated, first on a steam bath, then cautiously with a free flame. There was considerable but not violent evolution of gas. As the reaction-mixture became rather thick from deposition of solid, 12 cc. more of acetic anhydride was added, and boiling was continued until the still-head, used as condenser, was thoroughly heated. Five grams more of potassium carbonate was then added and the mixture was refluxed a few minutes longer. The reaction-mixture was cooled, methanol was added to react with the excess anhydride, and volatile material was removed *in vacuo*. The residual material was diluted with water to about 50 cc. and concd. potassium hydroxide solution was added to *p*H 8. The solution was cooled and then made strongly alkaline. The solution (volume now about 80 cc.) was extracted thrice with 1:1 ether-benzene mixture (3 × 50 cc.). The third extract, when evaporated, was found to contain only 0.2 g. of oil.

The combined extracts were dissolved in 20 cc. of hexane and refrigerated, however, no crystals formed. The material was therefore converted to the hydrochloride which was crystallized from ethanol-ether mixture.

Acknowledgment. The authors wish to express their gratitude to Burroughs Wellcome & Co. (U.S.A.), Inc., for the use of the laboratory facilities of The Wellcome Research Laboratories.

BROOKLYN 1, N. Y.

[CONTRIBUTION FROM MIDWEST RESEARCH INSTITUTE]

Pyrimidines. II. Orotic Acid Analogs[1,2]

G. DOYLE DAVES, JR., FRED BAIOCCHI, ROLAND K. ROBINS, AND C. C. CHENG

Received November 4, 1960

The syntheses of all eight structural analogs of orotic acid which contain amino, hydroxy, and thio substituents at positions 2 and 6 are reported. The chlorination of orotic acid with phosphorus oxychloride and *N,N*-dimethylaniline yields 2,6-dichloro-4-pyrimidinecarboxylic acid. Previous structures proposed for this chlorination product have been shown to be in error. A practical, large-scale synthesis of orotic acid has been devised, and a new route to the useful synthetic intermediate, methyl 2,6-dihydroxy-4-pyrimidinecarboxylate is reported. Formamidine has been condensed with the sodium derivative of diethyloxalacetate to give 6-hydroxy-4-pyrimidinecarboxylic acid in good yield. Several novel reactions involving methyl 2,6-dichloro-4-pyrimidinecarboxylate and methyl 6-chloro-2-methylthio-4-pyrimidinecarboxylate have been studied.

The importance of orotic acid in pyrimidine nucleotide synthesis has been well established.[3] Derivatives such as 5-chloro-, 5-bromo-, and 5-fluoroorotic acid[4,5] have been shown to exhibit orotic acid antimetabolite activity in various biological systems. 6-Uracil methyl sulfone and 6-uracilsulfonamide, synthesized[6] as orotic acid antagonists, have exhibited significant antitumor activity[7-10] against several types of tumor growth. Other pyrimidines related to orotic acid have been shown to exhibit interesting antitumor properties.[11]

As part of a general program to investigate pyrimidines as potential anti-neoplastic agents[12] a synthetic study of simple orotic acid derivatives was undertaken in this laboratory. As a result of this effort all the structural isomers of 2,6-disubstituted 4-pyrimidinecarboxylic acid which contain

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(2) Presented in part before the Division of Medicinal Chemistry, 137th Meeting of the American Chemical Society, Cleveland, Ohio, April 1960.

(3) See, for example: (a) F. W. Chattaway, *Nature*, **153**, 250 (1944); (b) H. S. Loring and J. G. Pierce, *J. Biol. Chem.*, **153**, 61 (1944); (c) H. J. Rogers, *Nature*, **153**, 251 (1944); (d) H. K. Mitchell, M. B. Houlahan, and J. F. Nye, *J. Biol. Chem.*, **172**, 525 (1948); (e) H. Arvidson, N. A. Eliasson, E. Hammarsten, P. Reichard, H. von Ubish, and S. Bergstrom, *J. Biol. Chem.*, **179**, 169 (1949); (f) V. R. Potter and C. Heidelberger, *Physiol. Rev.*, **30**, 487 (1950); (g) L. L. Weed and D. W. Wilson, *J. Biol. Chem.*, **193**, 435 (1951); (h) L. D. Wright and C. S. Miller, *Proc. Soc. Exptl. Biol. Med.*, **81**, 131 (1952); (i) U. Logervist and P. Reichard, *Acta Chem. Scand.*, **8**, 361 (1954); (j) A. M. Moore and J. B. Boylen, *Arch. Biochem. Biophys.*, **54**, 312 (1955); and (k) M. Green and S. S. Cohen, *J. Biol. Chem.*, **225**, 387 (1957).

(4) J. E. Stone and V. R. Potter, *Cancer Research*, **16**, 1033 (1956).

(5) J. E. Stone and V. R. Potter, *Cancer Research*, **17**, 800 (1957).

(6) S. H. Greenbaum, *J. Am. Chem. Soc.*, **76**, 6052 (1954).

(7) W. H. Prusoff, *Cancer Research*, **18**, 603 (1958).

(8) J. J. Jaffe and J. R. Cooper, *Cancer Research*, **18**, 1089 (1958).

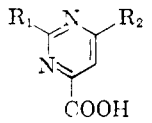
(9) M. T. Hakala, L. W. Law, and A. D. Welch, *Proc. Am. Assoc. Cancer Research*, **2**, 113 (1956).

(10) W. L. Holmes and A. D. Welch, *Cancer Research*, **16**, 251 (1956).

(11) See, for example: (a) C. Heidelberger, N. K. Chadhuri, P. Danneberg, D. Mooren, L. Griesbach, R. Duschinsky, R. J. Schnitzer, E. Pleven, and J. Scheiner, *Nature*, **179**, 663 (1957); (b) C. Heidelberger, D. Mooren, L. Griesbach, and B. J. Montag, *Proc. Am. Assoc. Cancer Research*, **2**, (1957); *Cancer Research*, **18**, 305 (1958); and (c) D. B. McNair Scott, T. L. V. Ulbricht, M. L. Rogers, E. Chu, and C. Rose, *Cancer Research*, **19**, 15 (1959).

(12) H. C. Koppel, R. H. Springer, R. K. Robins, and C. C. Cheng, "Pyrimidines. I." *J. Org. Chem.*, **26**, 792 (1961).

the hydrogen bonding functional groups, hydroxy, thio, and amino at positions 2 and 6 (I–IX) have now been prepared.



I. $R_1, R_2 = OH$
II. $R_1 = NH_2, R_2 = OH$
III. $R_1 = SH, R_2 = OH$
IV. $R_1 = OH, R_2 = NH_2$
V. $R_1, R_2 = NH_2$
VI. $R_1 = SH, R_2 = NH_2$
VII. $R_1 = OH, R_2 = SH$
VIII. $R_1 = NH_2, R_2 = SH$
IX. $R_1, R_2 = SH$

The isolation[13] and various syntheses[14–26] of orotic acid (I) have been reported. Mitchell and Nyc[21,22] and Fox and co-workers[26] have adequately reviewed this earlier work. Two of these eight analogs of orotic acid, 2-amino-6-hydroxy-4-pyrimidinecarboxylic acid[27] (II) and 6-hydroxy-2-thio-4-pyrimidinecarboxylic acid[19,23,28] (III), were known prior to the present study.

The chlorination of orotic acid (I) with phosphorus oxychloride has been reported by Biscaro and Belloni.[29] Later Bachstez[17] employed a mixture of phosphorus oxychloride, *N,N*-dimethylaniline and phosphorus pentachloride as a chlorination medium. Neither of these investigators definitely established the structure of their chlorination products. Since at the time of their work the structure of orotic acid (I) had not been clearly established, Biscaro and Belloni did not attempt to assign a definite structure to their compound but reported it to have the empirical formula $C_6H_4N_2O_3Cl_2 \cdot H_2O$. However, Bachstez, on the basis of elementary analyses, postulated the structure of his chlorination product to be a dihydropyrimidine (XI). Experiments performed in this laboratory prove conclusively that the correct structure of the chlorination product is the expected, 2,6-dichloro-4-pyrimidinecarboxylic acid (XII). The potassium salt of orotic acid[30] and phosphorus oxychloride in the presence of *N,N*-dimethylaniline resulted in the preparation of XII in 50% yield. This compound exhibited identical ultraviolet and infrared spectra and possessed identical paper chromatographic behavior when compared with the products obtained according to the directions of Biscaro and Belloni[29] and Bachstez.[17]

The analytical results (see Table I) reported by Biscaro and Belloni[29] and Bachstez[17] strongly suggest that these investigators actually had isolated the mono- and dihydrates of XII. It has been demonstrated in our laboratory that 2,6-dichloro-4-pyrimidinecarboxylic acid (XII) gradually absorbs moisture from the air.

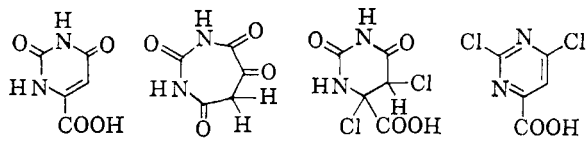
Compound XII reacted smoothly with thiourea in ethanolic solution to yield 2,6-dithio-4-pyrimidinecarboxylic acid (IX). Also XII reacted with ethanolic ammonia and methylamine to form 2,6-diamino-4-pyrimidinecarboxylic acid (V) and 2,6-bis(methylamino)-4-pyrimidinecarboxylic acid, respectively. When XII was allowed to react with ethanolic ammonia at lower temperature, a mixture of isomers, which could not be separated, was produced.

When 6-hydroxy-2-methylthio-4-pyrimidinecarboxylic acid[23] was heated in methanol saturated with dry hydrogen chloride, a readily separable mixture of two esters, XIV and XV, was obtained. The structure of the major product, XIV, was easily identified as the expected methyl 6-hydroxy-2-methylthio-4-pyrimidinecarboxylate. The structure of the other product, methyl 6-methoxy-2 methylthio-4-pyrimidinecarboxylate (XV), was established as follows: Acid hydrolysis of XV gave orotic acid in excellent yield. When XV was hydrolyzed in base, it was converted almost quantitatively to the starting material, 6-hydroxy-2-methylthio-4-pyrimidinecarboxylic acid (XIII). Sodium methoxide, in methanol, containing a trace of water, hydrolyzed the ester group of XV to form 6 - methoxy - 2 - methylthio - 4 - pyrimidinecarboxylic acid (XVIII). This acid (XVIII) was also prepared from methyl 6-chloro-2-methylthio-4-pyrimidinecarboxylate (XVII), sodium methoxide, and a trace of water. Compound XVII, in turn, was obtained in excellent yield from methyl 6-hydroxy - 2 - methylthio - 4 - pyrimidinecarboxylate (XIV) and phosphorus oxychloride. The minor product resulting from the esterification of XIII, which possesses an "extra" methyl group, is the 6-methoxy derivative, XV, rather than either of the theoretically possible *N*-methyl derivatives. When XIII was esterified in methanol containing a trace of water, a good yield of the methyl ester of orotic acid (XVI)[15,17,23,26] was obtained. Since no previously reported synthesis of orotic acid ester could be satisfactorily adapted to large-scale preparations

(13) G. Biscaro and E. Belloni, *Ann. Soc. Chim. Milano*, **11**, 18 (1905).
(14) R. Müller, *J. prakt. Chem.*, **56**, 475 (1897).
(15) H. L. Wheeler, *Am. Chem. J.*, **38**, 358 (1907).
(16) R. Behrend and K. Struve, *Ann.*, **378**, 153 (1910).
(17) M. Bachstez, *Ber.*, **63A**, 1000 (1930).
(18) G. E. Hilbert, *J. Am. Chem. Soc.*, **54**, 2076 (1932).
(19) T. B. Johnson and E. F. Schroeder, *J. Am. Chem. Soc.*, **53**, 1989 (1931); **54**, 2941 (1932).
(20) R. Kitamura, *J. Pharm. Soc. Japan*, **57**, 209 (1937).
(21) H. K. Mitchell and J. F. Nyc, *J. Am. Chem. Soc.*, **69**, 674 (1947).
(22) J. F. Nyc and H. K. Mitchell, *J. Am. Chem. Soc.*, **69**, 1382 (1947).
(23) H. Vanderhaeghe, *Bull. Soc. Chim. Belges*, **62**, 611 (1953).
(24) B. W. Langley, *J. Am. Chem. Soc.*, **78**, 2136 (1956).
(25) British Patent **800,709**, Sept. 3, 1958.
(26) J. J. Fox, N. Yung, and I. Wempen, *Biochem. Biophys. Acta.*, **23**, 295 (1957).
(27) S. Ruhemann and H. E. Stapleton, *J. Chem. Soc.*, **77**, 804 (1900); *J. Chem. Soc. Proc.*, **16**, 121 (1900).
(28) M. Bachstez, *Ber.*, **64**, 322 (1931).
(29) G. Biscaro and E. Belloni, *Ann. Soc. Chim. Milano*, **11**, 71 (1905).
(30) The potassium salt of orotic acid rather than orotic acid itself was used for the chlorination, since the latter contains one mole of water of crystallization which can only be removed with great difficulty.

TABLE I

CHLORINATION PRODUCT OF OROTIC ACID (I)

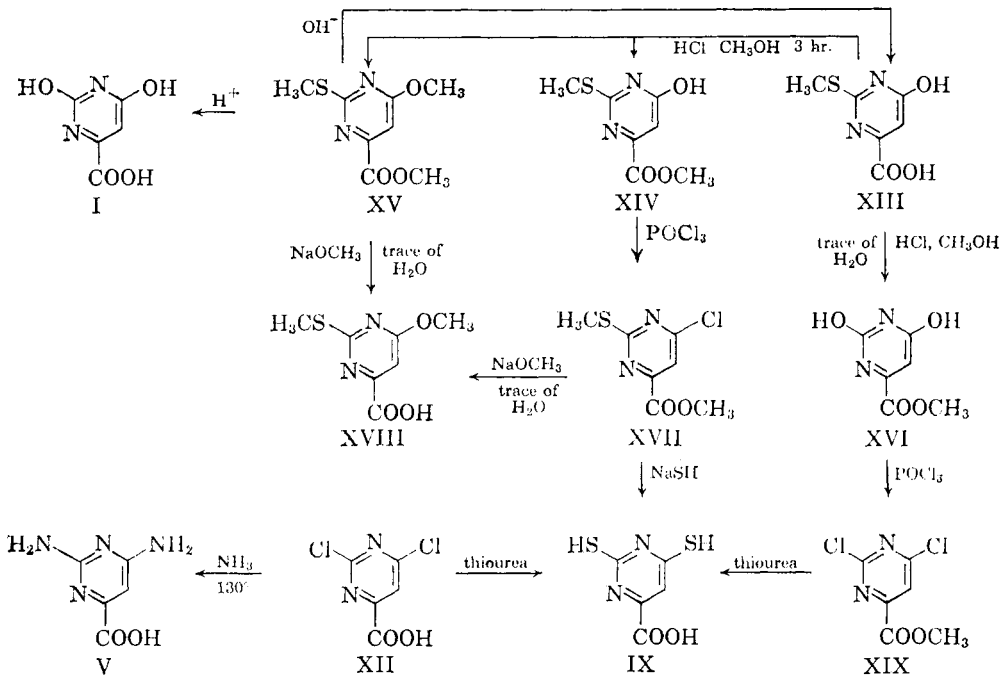


I X[a] XI[b] XII

Calcd.	$C_5H_4N_2O_3Cl_2 \cdot H_2O$	XI	XII	$XII \cdot H_2O$	$XII \cdot 2H_2O$
C	26.2	26.4	31.1	28.5	26.2
H	2.62	1.76	1.04	1.90	2.62
N	12.2	12.3	14.5	13.3	12.2
Cl	31.0	31.3	36.8	33.6	31.0
H_2O	7.86	....	....	8.53	15.7
Mol. wt.	229	227	193	211	229

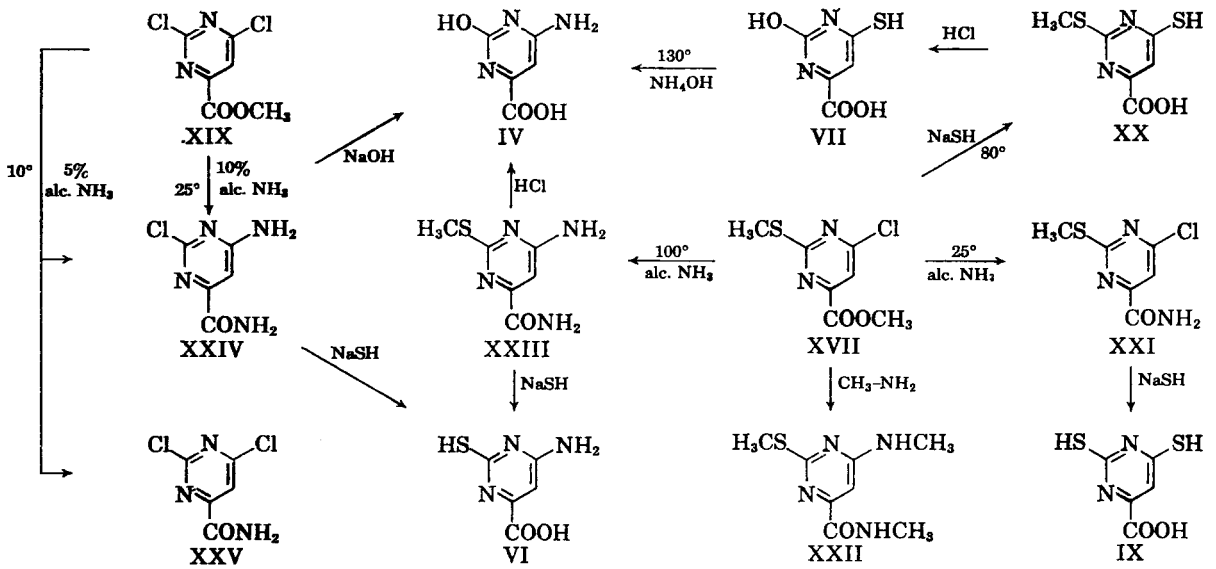
			Our Chlorination Product	
Found	Biscaro and Belloni[29]	Bachstez[17]	Dried at 80° (12 hr.)	Dried at room temp. (3 weeks)
C	...	...	31.2	28.7
H	...	...	1.34	1.95
N	13.16, 13.10, 12.04	12.13	14.6	13.1
Cl	31.39	31.9	...	...
H_2O	8.26	...	...	...
Mol. wt.	...	235	...	...
M.p.	115°C	115°C	115–117°C	115–117°C

[a] Structure assigned by Biscaro and Belloni[29] for orotic acid. [b] Structure assigned by Bachstez[17] for the chlorinated product of orotic acid.



in this laboratory, this new synthesis proved to be highly useful.

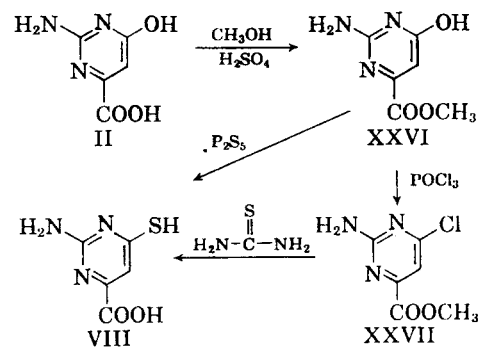
The chlorination of the methyl ester of orotic acid (XVI) in phosphorus oxychloride produced a 72% yield of methyl 2,6-dichloro-4-pyrimidinecarboxylate (XIX). This compound was readily converted to IX by thiourea in ethanol. IX was also prepared from methyl 6-chloro-2-methylthio-4-pyrimidinecarboxylate (XVII) and sodium hydrosulfide in ethylene glycol at 150°. 2-Methylthio-6-thio-4-pyrimidinecarboxylic acid (XX) was obtained from XVII and sodium hydrosulfide when ethanol rather than ethylene glycol was used as the reaction solvent. When XX was refluxed in 2*N* hydrochloric acid an almost quantitative yield of 2-hydroxy-6-thio-4-pyrimidinecarboxylic acid (VII) was obtained. The structure of VII was established since the isomeric 6-hydroxy-2-thio-4-pyrimidinecarbox-



ylic acid (III)[23] is known. Aqueous ammonia and VII heated at 130° gave 6-amino-2-hydroxy-4-pyrimidinecarboxylic acid (IV).

When methyl 6-chloro-2-methylthio-4-pyrimidinecarboxylate (XVII) was allowed to react with ethanolic ammonia at low temperature, the 6-chloro group was retained and the ester was converted to an amide to yield 6-chloro-2-methylthio-4-pyrimidinecarboxamide (XXI). In a similar manner 2,6-dichloro-4-pyrimidinecarboxamide (XXV) was obtained when methyl 2,6-dichloro-4-pyrimidinecarboxylate (XIX) was treated with 5% ethanolic ammonia at 10°, although an equal amount of 6-amino-2-chloro-4-pyrimidinecarboxamide (XXIV) was found to be present. When 10% ethanolic ammonia was employed at room temperature XXIV was produced exclusively. The chloro and the ester groups of XVII were both replaced at a higher temperature or when methylamine was employed. Thus, while the preparation of 6-amino-2-methylthio-4-pyrimidinecarboxamide (XXIII) resulted from XVII and ethanolic ammonia at 100° in a sealed vessel, methylamine and XVII reacted readily at 15–20° to give 6-methylamino-2-methylthio-4-pyrimidine-*N*-methylcarboxamide (XXII) in excellent yield. Hence, the site on chloro-4-pyrimidinecarboxylates most susceptibile to nucleophilic attack is the carbonyl carbon atom at position 4 rather than the chloro-substituted ring carbon atoms at positions 2 and 6.

The structure of XXIV was established as follows: When XXIV was subjected to paper chromatographic analysis in various solvent systems only one spot was observed. Sodium hydrosulfide in ethylene glycol at 150° converted XXIV to 6-amino-2-thio-4-pyrimidinecarboxylic acid (VI) in good yield. This product was identical in every respect with a sample of VI prepared from sodium hydrosulfide and 6-amino-2-methylthio-4-pyrimidinecarboxamide (XXIII). Furthermore, 6-amino-2-hydroxy-4-pyrimidinecarboxylic acid (IV) was readily obtained by acidic hydrolysis from XXIII or by either acidic or basic hydrolysis from XXIV. The instability of the 4-carboxamide group of compounds XXIII and XXIV in aqueous media is to be noted since hydrolysis occurred very readily in either acid or base.



2-Amino-6-hydroxy-4-pyrimidinecarboxylic acid (II) was esterified and the ester (XXVI) was smoothly chlorinated with phosphorus oxychloride to yield methyl 2-amino-6-chloro-4-pyrimidinecarboxylate (XXVII) which was converted in 54% yield to the desired compound, VIII, by reaction with thiourea in ethanolic solution. 2-Amino-6-thio-4-pyrimidinecarboxylic acid (VIII) was also prepared in lower yield (35%) by the direct thiation of XXVI. The ultraviolet absorption data of orotic acid analogs are listed in Table II.

A new method of preparation of 6-hydroxy-4-pyrimidinecarboxylic acid (XXVIII)[31–33] was ac-

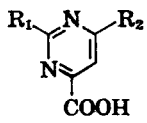
(31) H. Bredereck, H. Ulmer, and H. Waldman, *Ber.*, **89**, 12 (1956).

(32) E. Cherbuliez and K. N. Stavritch, *Helv. Chim. Acta*, **5**, 267 (1922).

(33) M. Claesen and H. Vanderhaeghe, *Bull. Soc. Chim. Belges*, **66**, 292 (1957).

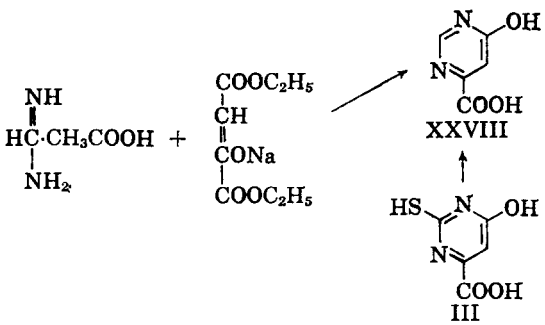
TABLE II

ULTRAVIOLET ABSORPTION OF OROTIC ACID ANALOGS



Compound	R_1	R_2	pH 1 λ_{max} (mμ)	pH 1 ε	pH 11 λ_{max} (mμ)	pH 11 ε
I	OH	OH	282	8,000	284	6,300
II	NH_2	OH	276	5,600	293	4,200
III	SH	OH	270	22,800	234	11,100
					262	17,600
					320	4,150
IV	OH	NH_2	294	9,000	285	6,550
V	NH_2	NH_2	287	6,150	300	5,000
VI	SH	NH_2	226	12,300	270	16,900
			277	32,000		
			325	3,200		
VII	OH	SH	250	5,100	342	11,000
			346	11,500		
VIII	NH_2	SH	263	7,000	328	8,300
			347	10,400		
IX	SH	SH	278	30,600	262	19,200
			359	9,800	279	19,500
					370	8,400

complished by the condensation of formamidine acetate[34] with the sodium salt of diethyloxalacetate in aqueous solution. Contrary to the report by Claesen and Vanderhaeghe[33] that 6-hydroxy-2-thio-4-pyrimidinecarboxylic acid (III) could not be successfully dethiated by Raney nickel, it was found in this laboratory that XXVIII was produced in 48% yield by this method, thus providing a second practical route to 6-hydroxy-4-pyrimidinecarboxylic acid (XXVIII).



Several alkylthio derivatives (see Table III) were prepared by direct alkylation of the appropriate thio-orotic acid derivative.

EXPERIMENTAL[35]

2,6-Dichloro-4-pyrimidinecarboxylic acid (XII). To a suspension of 100 g. of finely powdered potassium salt of orotic acid in 1.5 l. of phosphorus oxychloride was added 100 ml. of C.P. *N,N*-dimethylaniline. The resulting reaction mixture was heated under reflux for 3 hr. The excess phosphorus oxychloride was then distilled from the reaction mixture under reduced pressure using a steam bath as a source of heat. The dark, sirupy residue was poured onto 2–3 kg. of crushed ice accompanied by vigorous stirring. This ice-cold aqueous solution was extracted with 750–1000-ml. portions of ether until a total of 6 l. of ether had been used or until evaporation of a small volume of the ether extract showed no product to be present. The combined etheral extract was washed with ice water until the *p*H of the water was 4. The ether was then shaken with 25–50 g. of charcoal, filtered, and dried over sodium sulfate. Upon distillation of the ether 57 g. of crude product, m.p. 108–110°, was obtained. This yellow product was recrystallized from heptane to yield 50 g. (50%) of pure, white 2,6-dichloro-4-pyrimidinecarboxylic acid (XII), m.p. 115–117°, $\lambda_{max}^{C_2H_5OH}$ 273 mμ (ε 5000). For analysis a sample was dried at 80° for 12 hr.

Anal. Calcd. for $C_5H_2N_2O_2Cl_2$: C, 31.1; H, 1.0; N, 1.45. Found: C, 31.2; H, 1.34; N, 14.6.

A sample allowed to stand for three weeks at room temperature had an analysis corresponding to the monohydrate.

Anal. Calcd. for $C_5H_2N_2O_2Cl_2 \cdot H_2O$: C, 28.5; H, 1.9; N, 13.3. Found: C, 28.7: H, 1.95; N, 13.1.

2,6-Dithio-4-pyrimidinecarboxylic acid (IX). *Method A.* A mixture of 6.5 g. of methyl 6-chloro-2-methylthio-4-pyrimidinecarboxylate (XVII) and 10 g. of sodium hydrosulfide was suspended in 25 ml. of ethylene glycol and heated at 130° for 30 min. The deep red sclution was then poured into 300 ml. of water, boiled with charcoal, and filtered. The hot filtrate was acidified with hydrochloric acid and the resulting precipitate filtered. The dry product (4.8 g.) was recrystallized from water to yield 3.9 g. (69%) of IX as yellow crystals, m.p. 281–282°.

Anal. Calcd. for $C_5H_4N_2O_2S_2$: C, 31.9; H, 2.1; N, 14.9. Found: C, 32.1; H, 1.8; N, 15.2.

Method B. A mixture of 25 g. of thiourea and 25 g. of 2,6-dichloro-4-pyrimidinecarboxylic acid (XII) was suspended in 400 ml. of ethanol and heated under reflux for 3 hr. The solution was then diluted with 500 ml. of water, boiled with charcoal, and filtered. The filtrate was acidified with hydrochloric acid and the resulting yellow precipitate was filtered, washed with water, and dried. The crude product (12.2 g.) was recrystallized from water to yield 9.7 g. (39.5%) of product which exhibited identical ultraviolet and infrared spectra with a sample prepared by Method *A*.

Method C. A mixture of 10 g. of thiourea and 10 g. of methyl 2,6-dichloro-4-pyrimidinecarboxylate (XIX) was suspended in 200 ml. of ethanol and heated under reflux for 1–2 hr. during which time the solution became clear and finally a yellow precipitate formed. This precipitate was filtered from the cold solution and dissolved in dilute sodium hydroxide. This solution was boiled with charcoal for 15 min., filtered, and acidified with hydrochloric acid. The resulting yellow precipitate was filtered and dried. This crude product (6.8 g.) was recrystallized from water to yield 5.1 g. (56%) of IX identical in every respect with samples prepared by methods *A* and *B*.

Method D. A mixture of 3 g. of XXI and 3 g. of sodium hydrosulfide in 25 ml. of ethylene glycol was heated at 130° for 45 min. The reaction mixture was then poured into 150 ml. of water and the resulting solution was boiled with charcoal for 15 min., filtered, and the filtrate was acidified to yield 1.9 g. of 2,6-dithio-4-pyrimidinecarboxylic acid (IX).

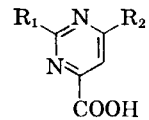
2,6-Diamino-4-pyrimidinecarboxylic acid (V). A mixture of 15 g. of 2,6-dichloro-4-pyrimidinecarboxylic acid (XII) and 650 ml. of 10% ethanolic ammonia was heated in a sealed vessel at 130° for 7 hr. After the reaction mixture had cooled, the product was filtered and dissolved in boiling dilute aqueous ammonia, treated with charcoal, and filtered. The boiling filtrate was acidified with hydrochloric acid to *p*H 3 and the product was filtered, washed with water, and dried. The yield of V (sublimed at 345°) was 9.2 g. (77%).

(34) E. C. Taylor and W. A. Ehrhart, *J. Am. Chem. Soc.*, 82, 3138 (1960).

(35) All melting points were taken on a Thomas-Hoover melting point apparatus. The infrared spectra were taken with a Perkin-Elmer infracord and the ultraviolet absorption were determined with a Beckman DK-2.

TABLE III

ALKYLTHIO-4-PYRIMIDINECARBOXYLIC ACIDS



R_1	R_2	Yield, %	M.P.	Recrystallization Solvent	Calculated			Found			U.V. Absorption			
											*p*H 1		*p*H 11	
					C	H	N	C	H	N	λ_{max}	ϵ	λ_{max}	ϵ
OH	CH_3S	46	228–230	Water	38.7	3.2	15.0	38.8	3.7	15.1	255	10,800	248	13,000
											321	11,500	327	8,500
NH_2	CH_3S	69	255	Water-DMF	38.9	3.8	22.7	39.0	4.0	23.0	318	11,100	317	7,000
NH_2	$C_6H_5CH_2S$	69	234–235	Water-DMF	55.2	4.2	16.1	55.7	4.3	16.3	322	13,000	318	8,100
NH_2	*o*-$ClC_6H_4CH_2S$	68	231–232	Water-DMF	48.8	3.4	14.2	48.9	3.3	13.9	323	8,400	317	8,500
CH_3S	CH_3S	49	145–146	Water-ethanol	35.9[a]	4.3	12.0	36.2	4.4	11.9	245	16,600	250	20,200
											325	8,400	319	6,100
C_2H_5S	OH	79	247–248	Water	42.0	4.0	14.0	41.6	4.0	14.0	241	13,100	295	6,000
											311	7,600		
$CH_3(CH_2)_2S$	OH	42	236–237	Water	44.8	4.7	13.1	44.4	4.4	13.5	242	10,200	295	4,600
											312	6,000		
$CH_3(CH_2)_3S$	OH	53	227–228	Water-ethanol	47.4	5.3	12.3	47.1	5.0	12.2	243	9,500	295	4,300
											313	5,700		
$C_6H_5CH_2S$	OH	62	273–274	Water	55.0	3.8	10.7	54.9	3.6	10.6	242	8,400	291	5,000
											311	6,000		
2,4-$Cl_2C_6H_3CH_2S$	OH	72	270–272	Ethanol	43.5	2.4	8.4	43.7	2.1	8.4	326	8,500	293	5,000

[a] Monohydrate.

Anal. Calcd. for $C_6H_6N_4O_2$: C, 38.9; H, 3.9; N, 36.3. Found: C, 38.8; H, 4.1; N, 36.2.

2,6-Bis(methylamino)-4-pyrimidinecarboxylic acid. A solution of 10 g. of 2,6-dichloro-4-pyrimidinecarboxylic acid (XII) in 100 ml. of 40% aqueous methylamine was stirred in an ice bath for 3 hr. The solution was then diluted with 100 ml. of water and the *p*H was adjusted to 3 with hydrochloric acid. The volume was concentrated to 100 ml. and the product was filtered from the cool solution. After recrystallization from water 4.3 g. (45%) of 2,6-bis(methylamino)-4-pyrimidinecarboxylic acid monohydrate, m.p. 314° dec., was obtained. $\lambda_{max}^{pH\ 1}$ 290 mμ (ε 7400), $\lambda_{max}^{pH\ 11}$ 233 mμ (ε 12,600), 310 mμ (ε 6000).

Anal. Calcd. for $C_7H_{10}N_4O_2 \cdot H_2O$: C, 41.9; H, 6.0; N, 28.0. Found: C, 41.7; H, 5.9; N, 28.0.

6-Hydroxy-2-methylthio-4-pyrimidinecarboxylic acid[23] (XIII). To 6 l. of water containing 400 g. of sodium hydroxide was added 1050 g. of the sodium salt of diethyloxalacetate and 660 g. of *S*-methylthiouronium sulfate. The resulting solution was stirred at room temperature for 8 hr., then boiled with charcoal, and filtered. The hot filtrate, while being stirred vigorously, was acidified to pH 1 with hydrochloric acid. After 30 min. the product was filtered, washed with water, and suspended in a large volume of acetone. After the acetone mixture was stirred for 1 hr. the product was again filtered and dried. The yield of 6-hydroxy-2-methylthio-4-pyrimidinecarboxylic acid (XIII), m.p. 252–254° was 563 g. (63%) which was sufficiently pure for synthetic purposes. A sample recrystallized from water melted at 253–254° (lit.[23] m.p. 250–252°). $\lambda_{max}^{pH\ 1}$ 240 mμ (ε 8700), 310 mμ (ε 5100); $\lambda_{max}^{pH\ 11}$ 250 mμ (ε 3900).

Anal. Calcd. for $C_6H_6N_2O_3S$: N, 15.0. Found: N, 15.1.

A convenient large-scale preparation of orotic acid (I). Since relatively large amounts of orotic acid were required for this investigation, a study of the various synthetic routes[15–26] was made. The method best adapted to the preparation of orotic acid in practical scale involves the acid hydrolysis of the readily available 6-hydroxy-2-methylthio-4 pyrimidinecarboxylic acid (XIII) which produced orotic acid of high purity.

Six hundred grams of 6-hydroxy-2-methylthio-4-pyrimidinecarboxylic acid (XIII) was suspended in 9 l. of 2*N* hydrochloric acid and heated under reflux with stirring for 3 hr. The product was then filtered and suspended in 6 l. of hot water. Solid potassium hydroxide was carefully added until complete solution was attained. Charcoal was added and the solution was boiled for 15 min. and then filtered. The hot filtrate was then acidified to *p*H 1 with hydrochloric acid. The precipitated product was filtered, washed well with water and acetone, and dried. The yield of 447 g. (79%) of I, m.p. 336–338° dec., was sufficiently pure for all synthetic purposes described in this paper.

Action of alcoholic hydrogen chloride on 6-hydroxy-2-methylthio-4-pyrimidinecarboxylic acid (XIII). A suspension of 175 g. of 6-hydroxy-2-methylthio-4-pyrimidinecarboxylic acid (XIII) in 4 l. of methanol was refluxed and stirred vigorously while a stream of gaseous hydrogen chloride was passed through continuously until solution was complete (approximately 1–3 hr.). After 15 additional min. of refluxing the volume of the solution was concentrated under reduced pressure to 500 ml. The resulting white precipitate was filtered, washed well with cold methanol, and dried. The dry product (138 g., m.p. 210–216°) was recrystallized from water to yield 123 g. (61%) of methyl 6-hydroxy-2-methylthio-4-pyrimidinecarboxylate (XIV) ,m.p. 218–219°. $\lambda_{max}^{pH\ 1}$ 240 mμ (ε 10,000), 310 mμ (ε 5700); $\lambda_{max}^{pH\ 11}$ 295 mμ (ε 4600).

Anal. Calcd. for $C_7H_8N_2O_3S$: C, 42.0; H, 4.0; N, 14.0. Found: C, 41.9; H, 4.1; N, 13.9.

To the filtrate from the above preparation was added 1 l. of water and the resulting copious, white precipitate was filtered, washed well with water, and dried. After recrystallization from heptane, 35 g. (17.5%) of methyl 6-methoxy-2-methylthio-4-pyrimidinecarboxylate (XV), m.p. 100–102°, was obtained. $\lambda_{max}^{pH\ 1}$ 257 mμ (ε 14,100); $\lambda_{max}^{pH\ 11}$ 252 mμ (ε 14,000).

Anal. Calcd. for $C_8H_{10}N_2O_3S$: C, 44.8; H, 4.7; N, 13.1. Found: C, 44.4; H, 4.6; N, 13.0.

Methyl 2,6-dihydroxy-4-pyrimidinecarboxylate[15,17,23,26] (XVI). A suspension of 175 g. of 6-hydroxy-2-methylthio-4-pyrimidinecarboxylic acid (XIII) in 3 l. of methanol was refluxed, accompanied by mechanical stirring while a stream of gaseous hydrogen chloride was passed through continuously. When solution was complete 150 ml. of concd. hydrochloric acid was carefully added. Reaction conditions were maintained for an additional 10–12 hr. during which time a white precipitate was formed. After the solution was allowed to cool, the product was filtered and washed well with methanol and then water. After drying, 98 g. (71%) of XVI, m.p. 240–241°, was obtained. The melting point was not altered by recrystallization from water. $\lambda_{max}^{pH\ 1}$ 282 mμ (ε 7300); $\lambda_{max}^{pH\ 11}$ 284 mμ (ε 5550).

Anal. Calcd. for $C_6H_6N_2O_4$: N, 16.5. Found: N, 16.5.

Methyl 6-chloro-2-methylthio-4-pyrimidinecarboxylate (XVII). A mixture of 177 g. of methyl 6-hydroxy-2-methylthio-4-pyrimidinecarboxylate (XIV) and 1300 ml. of phosphorus oxychloride was heated under reflux until solution was complete and continued for an additional 10 min. (a total reflux time of approximately 30 min.). The excess phosphorus oxychloride was distilled under reduced pressure and the sirupy residue was poured, accompanied by vigorous stirring, onto 2–3 kg. of crushed ice. The copious, white precipitate which appeared was stirred in the ice-water suspension for about 30 min. and filtered, washed well with 5% sodium bicarbonate, and then with water. After drying overnight in the air, the product (193 g., m.p. 80–90°) was recrystallized from heptane to yield 162 g. (84%) of XVII, m.p. 118–119°. $\lambda_{max}^{C_6H_{14}OH}$ 263 mμ (ε 12,800), 320 mμ (ε 1700)

Anal. Calcd. for $C_7H_7N_2O_2SCl$: C, 38.5; H, 3.2; N, 12.8. Found: C, 39.0; H, 3.3; N, 12.7.

6-Methoxy-2-methylthio-4-pyrimidinecarboxylic acid (XVIII). *Method A.* To a solution of 4 g. of sodium methoxide in 150 ml. of methanol was added 10 g. of methyl 6-chloro-2-methylthio-4-pyrimidinecarboxylate (XVII). The resulting mixture was heated under reflux conditions for 2 hr., then cooled, and the white precipitate filtered. This precipitate was dissolved in 100 ml. of water and the *p*H of the resulting solution adjusted to 2 with hydrochloric acid. The precipitated product was filtered, washed with water, and dried. The crude product was recrystallized from ethanol to yield 5 g. (51%) of XVIII, m.p. 233–234°. $\lambda_{max}^{pH\ 1}$ 257 mμ (ε 14,600); $\lambda_{max}^{pH\ 11}$ 251 mμ (ε 13,200), 290 mμ (ε 3400).

Anal. Calcd. for $C_7H_8N_2O_3S$: C, 42.0; H, 4.0; N, 14.0. Found: C, 42.2; H, 4.1; N, 14.1.

Method B. Ten grams of methyl 6-methoxy-2-methylthio-4-pyrimidinecarboxylate (XV) was treated with 4 g. of sodium methoxide in 150 ml. of refluxing methanol containing several drops of water. The product was isolated and purified as described in Method *A*, to give 5.4 g. (57%) of XVIII, identical in every respect with a sample prepared by Method *A*.

2-Methylthio-6-thio-4-pyrimidinecarboxylic acid (XX). A mixture consisting of 50 g. of methyl 6-chloro-2-methylthio-4-pyrimidinecarboxylate (XVII) and 50 g. of sodium hydrosulfide was suspended in 750 ml. of ethanol and heated under reflux for 3 hr. The orange solution which contained a small amount of solid material was poured into 1 l. of hot water. The resulting solution was boiled for 30 min., treated with charcoal, and filtered. The hot filtrate while being vigorously stirred was acidified to *p*H 2 with hydrochloric acid. After the solution was allowed to cool, the product was filtered, washed with water, and dried. The yield of XX, m.p. 231–233° was 35 g. (75%). A sample recrystallized from ethanol-water melted at 235°. $\lambda_{max}^{pH\ 1}$ 246 mμ (ε 13,400), 279 mμ (ε 11,000), 372 mμ (ε 7300); $\lambda_{max}^{pH\ 11}$ 250 mμ (ε 15,500), 332 mμ (ε 6900).

Anal. Calcd. for $C_6H_6N_2O_2S_2$: C, 35.6; H, 3.0; N, 13.8 Found: C, 35.7; H, 3.4; N, 13.9.

2-Hydroxy-6-thio-4-pyrimidinecarboxylic acid (VII). To 1 l. of 2*N* hydrochloric acid was added 15 g. of 2-methylthio-6-thio-4-pyrimidinecarboxylic acid (XX) and the resulting mixture was heated under reflux for 3 hr. during which time solution became complete. The orange solution was then cooled and the crystallized product was filtered, washed with water, and dried. The product (12 g.) was recrystallized from an ethanol-dimethylformamide mixture to yield 9.7 g. (76%) of VII as deep orange needles, m.p. 307–308°.

Anal. Calcd. for $C_5H_4N_2O_2S$: C, 34.9; H, 2.3; N, 16.3. Found: C, 34.9; H, 2.2; N, 16.1.

6-Chloro-2-methylthio-4-pyrimidinecarboxamide (XXI). A suspension of 10 g. of finely powdered methyl 6-chloro-2-methylthio-4-pyrimidinecarboxylate (XVII) in 300 ml. of 10% ethanolic ammonia was stirred in an ice bath for 2 hr. The crude product was filtered, washed well with ethanol, and dried. After recrystallization from a mixture of water and dimethylformamide 6 g. (66%) of XXI, m.p. 204–205°, was obtained. $\lambda_{max}^{pH\ 1}$ 240 mμ (ε 6500), 312 mμ (ε 4000); $\lambda_{max}^{pH\ 11}$ 301 mμ (ε 2900).

Anal. Calcd. for $C_6H_6N_3OSCl$: C, 35.5; H, 3.0; N, 20.7. Found: C, 35.2; H, 2.9; N, 20.7.

6-Amino-2-methylthio-4-pyrimidinecarboxamide (XXIII). A mixture of 15 g. of methyl 6-chloro-2-methylthio-4-pyrimidinecarboxylate (XVII) and 400 ml. of 28% aqueous ammonia was heated in a sealed vessel at 100° for 8 hr. After the reaction mixture was allowed to cool the product was filtered and dried. Recrystallization from water and dimethylformamide gave 7.5 g. (61%) of XXIII, m.p. 294°. $\lambda_{max}^{pH\ 1}$ 243 mμ (ε 17,900), 297 mμ (ε 4000); $\lambda_{max}^{pH\ 11}$ 308 mμ (ε 3900).

Anal. Calcd. for $C_6H_8N_4OS$: C, 39.1; H, 4.3; N, 30.6. Found: C, 39.4; H, 4.2; N, 30.3.

6-Methylamino-2-methylthio-4-pyrimidine-N-methylcarboxamide (XXII). To 200 ml. of 20% ethanolic methylamine stirring at 5° was added in small portions 30 g. of methyl 6-chloro-2-methylthio-4-pyrimidinecarboxylate (XVII). After the reaction mixture had stirred for 2 hr. at 5–15° the product was filtered, washed with methanol, and dried. Recrystallization of this crude product from water and dimethylformamide yielded 19 g. (65%) of XXII, m.p. 191–192°. $\lambda_{max}^{pH\ 1}$ 251 mμ (ε 27,800), 300 mμ (s)(ε 5000); $\lambda_{max}^{pH\ 11}$ 235 mμ (ε 25,400), 321 mμ (ε 4900).

Anal. Calcd. for $C_8H_{12}N_4OS$: C, 45.3; H, 5.7; N, 26.4. Found: C, 44.9; H, 5.6; N, 26.3.

Methyl 2,6-dichloro-4-pyrimidinecarboxylate (XIX). A mixture of 23 g. of methyl 2,6-dihydroxy-4-pyrimidinecarboxylate (XVI) and 300 ml. of phosphorus oxychloride was heated under reflux until complete solution was attained (about 5 hr.). The excess phosphorus oxychloride was distilled under reduced pressure and the sirupy residue was poured, accompanied by vigorous stirring, onto 500 g. of crushed ice. The resulting white precipitate was filtered and dissolved in a small volume of ether. The filtrate was extracted twice with 250-ml. portions of ether and discarded. The combined ether extract was washed well with water, treated with charcoal, and filtered. After being dried over sodium sulfate, the ether was evaporated, yielding 24 g. of product, m.p. 50–54°. After recrystallization from heptane-benzene 20 g. (72%) of XIX, m.p 55–56°, was obtained. λ_{max}^{EtOH} 274 mμ (ε 4100).

Anal. Calcd. for $C_6H_4N_2O_2Cl_2$: C, 34.7; H, 1.9; N, 13.5. Found: C, 34.9; H, 2.3; N, 13.5.

The action of ethanolic ammonia on methyl 2,6-dichloro-4-pyrimidinecarboxylate (XIX). A solution of 20 g. of methyl 2,6-dichloro-4-pyrimidinecarboxylate (XIX) in 150 ml. of ethanol was added slowly to 150 ml. of 10% ethanolic ammonia which was stirred at 10° in an ice bath. After 2 hr. 14 g. of crude product was filtered and the filtrate was concentrated to yield an additional 2 g. This combined crude product was extracted with boiling ethyl acetate and yielded 8.0 g. (43%) of 2,6-dichloro-4-pyrimidinecarboxamide (XXV), m.p. 170°. $\lambda_{max}^{pH\ 1}$ 287 mμ (ε 3900); $\lambda_{max}^{pH\ 11}$ 292 mμ (ε 3200).

Anal. Calcd. for $C_5H_3N_3OCl_2$: C, 31.3; H, 1.6; N, 21.9. Found: C, 31.1; H, 1.6; N, 21.6.

The ethyl acetate insoluble portion was recrystallized from water-dimethylformamide and yielded 6.3 g. (38%) of 6-amino-2-chloro-4-pyrimidinecarboxamide (XXIV), m.p. 333–335° (block preheated to 325°). $\lambda_{max}^{pH\ 1}$ 236 mμ (ε 6900), 300 mμ (ε 2400); $\lambda_{max}^{pH\ 11}$ 237 mμ (ε 6700), 300 mμ (ε 2400).

Anal. Calcd. for $C_5H_5N_4OCl$: C, 34.8; H, 2.9; N, 32.5. Found: C, 35.0; H, 3.0; N, 32.2.

When 25 g. of methyl 2,6-dichloro-4-pyrimidinecarboxylate (XIX) was stirred for 3 hr. in 300 ml. of 10% ethanolic ammonia at room temperature and the precipitate which resulted was filtered and recrystallized from water-dimethylformamide, 17 g. (82%) of 6-amino-2-chloro-4-pyrimidinecarboxamide (XXIV), m.p. 333–335°, was obtained. The product gave only one spot in different paper chromatographic solvent systems and exhibited identical ultraviolet and infrared absorption spectra with the previously mentioned ethyl acetate insoluble product.

6-Amino-2-thio-4-pyrimidinecarboxylic acid (VI). *Method A.* A mixture of 10 g. of 6-amino-2-chloro-4-pyrimidinecarboxamide (XXIV) and 10 g. of sodium hydrosulfide was suspended in 100 ml. of ethylene glycol and heated in an oil bath to 130–140°. After 1 hr. the reaction mixture was poured into 300 ml. of 2*N* sodium hydroxide and boiled for 30 min. The solution was then treated with charcoal and filtered. The *p*H of the hot filtrate was adjusted to 2 with hydrochloric acid and the resulting yellow precipitate was filtered, washed with water, and dried. After recrystallization from water-dimethylformamide, 6.2 g. (56%) of VI, m.p. 272–274° dec., was obtained.

Method B. A mixture of 7.5 g. of 6-amino-2-methylthio-4-pyrimidinecarboxamide (XXIII) and 7.5 g. of sodium hydrosulfide was suspended in 30 ml. of ethylene glycol and heated in an oil bath at 150° for 1 hr. The reaction mixture was then poured into 200 ml. of 2*N* sodium hydroxide and boiled for 30 min. The solution was then treated with charcoal, and filtered. The hot filtrate was acidified to *p*H 2 with hydrochloric acid and the resulting precipitate was filtered, washed with water, and dried. After recrystallization from water-dimethylformamide, 3.2 g. (41%) of VI, identical with that prepared by Method *A*, was obtained.

6-Amino-2-hydroxy-4-pyrimidinecarboxylic acid (IV). *Method A.* A suspension of 10 g. of 6-amino-2-methylthio-4-pyrimidinecarboxamide (XXIII) in 250 ml. of 2*N* hydrochloric acid was refluxed for 3 hr. The reaction mixture was cooled and the product was filtered, washed with water, and dried. This crude product was recrystallized from water and 7.3 g. (81%) of 6-amino-2-hydroxy-4-pyrimidinecarboxylic acid hemihydrate (IV), m.p. 293–294° dec., was obtained.

Anal. Calcd. for $C_5H_5N_3O_3 \cdot {}^1/_2H_2O$: C, 36.6; H, 3.6; N, 25.6. Found: C, 36.6; H, 3.5; N, 25.7.

Method B. A solution of 5 g. of 2-hydroxy-6-thio-4-pyrimidinecarboxylic acid (VII) in 200 ml. of 28% aqueous ammonia was heated at 130° in a sealed vessel for 8 hr. The cooled solution was boiled with charcoal and filtered. The hot filtrate was acidified to *p*H 2 with hydrochloric acid and the precipitated product was filtered, washed with water, and dried. After recrystallization from water 2 g. (42%) of IV identical with that prepared by Method *A*, was obtained.

Method C. A solution of 15 g. of 6-amino-2-chloro-4-pyrimidinecarboxamide (XXIV) in 300 ml. of 2*N* sodium hydroxide was heated under reflux conditions for 4 hr. The hot solution was then treated with charcoal and filtered. The filtrate was acidified to *p*H 2 with hydrochloric acid and the resulting white precipitate was filtered, washed with water, and dried. After recrystallization from water 8 g. (56%) of product, identical with IV prepared by Methods *A* and *B*, was obtained.

2-Amino-6-hydroxy-4-pyrimidinecarboxylic acid[27] (II). To 1 l. of water containing 100 g. of sodium hydroxide was added 240 g. of practical grade guanidine carbonate. A small amount of insoluble residue was filtered and 560 g. of the sodium salt of diethyloxalacetate was added to the clear

filtrate. The resulting solution was stirred at room temperature for 6 hr. and finally the copious precipitate which appeared during the course of the reaction was filtered and dissolved in 1.5 l. of dilute aqueous sodium hydroxide. This solution was boiled with charcoal and filtered. The hot filtrate was acidified to *p*H 2 with hydrochloric acid. The precipitated product was filtered, washed with water and acetone, and dried. The dry 2-amino-6-hydroxy-4-pyrimidinecarboxylic acid (II), 270 g. (66%), melted with decomposition at 340–345°. After a second reprecipitation a sample melted at 343–345° dec.

Anal. Calcd. for $C_5H_5N_3O_3$: N, 27.1. Found: N, 27.3.

Methyl 2-amino-6-hydroxy-4-pyrimidinecarboxylate. To a solution of 4 l. of methanol and 350 ml. of concd. sulfuric acid was added 100 g. of 2-amino-6-hydroxy-4-pyrimidinecarboxylic acid (II). The resulting mixture was heated under reflux, accompanied by mechanical stirring until total solution was achieved, and continued for an additional 5 hr. (total reaction time was about 15–18 hr.). The volume of the solution was then reduced to 500 ml. and enough water was added to double the volume. The *p*H of the solution was carefully adjusted to 5 with aqueous ammonia. (Note: The appearance of a precipitate when the *p*H is 2 to 3 indicates that the reaction was incomplete.) A small amount of unchanged starting material was filtered and the *p*H of the filtrate was then adjusted to 5. The precipitated crude product was filtered, washed well with water, and dried. The dry product (76 g., m.p. 280–285°) was recrystallized from a water-dimethylformamide solution to yield 71 g. (63%) of methyl 2-amino-6-hydroxy-4-pyrimidinecarboxylate (XXVI), m.p. 293–294°. $\lambda_{max}^{pH\ 1}$ 276 mμ (ε 8150); $\lambda_{max}^{pH\ 11}$ 293 mμ (ε 5900).

Anal. Calcd. for $C_6H_7N_3O_3$: C, 42.6; H, 4.1; N, 24.9. Found: C, 42.6; H, 4.1; N, 24.6.

Methyl 2-amino-6-chloro-4-pyrimidinecarboxylate (XXVII). A mixture of 80 g. of methyl 2-amino-6-hydroxy-4-pyrimidinecarboxylate (XXVI) in 600 ml. phosphorus oxychloride was heated under reflux until solution was complete (about 1 hr.). The excess phosphorus oxychloride was then distilled under reduced pressure. The residue was poured, with vigorous stirring, onto 1 kg. of crushed ice. The resulting aqueous solution was adjusted to *p*H 9–10 by the careful addition of 28% aqueous ammonia. During the addition of the ammonia, additional crushed ice was added to the solution to keep the temperature of the mixture below 0°. This strongly basic solution was extracted four times with 300–400 ml. portions of 2-butanone. The combined extracts were washed with water, treated with charcoal and, after drying over sodium sulfate, evaporated to dryness to yield 46 g. of product, m.p. 133–137°. After recrystallization from benzene 38 g. (43%) of XXVII, m.p. 135–137°, was obtained. $\lambda_{max}^{pH\ 1}$ 319 mμ (ε 4700); $\lambda_{max}^{pH\ 11}$ 308 mμ (ε 4300).

Anal. Calcd. for $C_6H_6N_3O_2Cl$: C, 38.5; H, 3.2; N, 22.5. Found: C, 38.1; H, 3.3; N, 22.5.

2-Amino-6-thio-4-pyrimidinecarboxylic acid (CIII). *Method A.* A mixture consisting of 20 g. of methyl 2-amino-6-chloro-4-pyrimidinecarboxylate (XXVII) and 20 g. of thiourea was suspended in 300 ml. of ethanol and heated under reflux for 2 hr. During this time complete solution was attained and finally a yellow-orange precipitate was formed. This precipitate was filtered from the cooled solution and dissolved in 250 ml. of 2*N* potassium hydroxide solution. The solution was boiled with charcoal for 15 min., the charcoal was filtered, and the hot filtrate was acidified to *p*H 2 with hydrochloric acid. The precipitated yellow-orange product was filtered, washed with water, and dried. The yield of VIII, m.p. 291–293°, was 12 g. (56%). The melting point of a sample recrystallized from water and dimethylformamide was unchanged.

Anal. Calcd. for $C_5H_5N_3O_2S \cdot H_2O$: C, 31.7; H, 3.7; N, 22.2. Found: C, 32.1; H, 3.7; N, 22.2.

Method B. A finely powdered mixture consisting of 30 g. of methyl 2-amino-6-hydroxy-4-pyrimidinecarboxylate (XXVI) and 60 g. of phosphorus pentasulfide was added in small portions, with vigorous stirring, to 500 ml. of pyridine preheated to 80–90°. After the addition was complete the reaction conditions were maintained for 45 min. The volume of the pyridine was concentrated to 250 ml. by distillation under reduced pressure and this concentrated reaction mixture was poured slowly into 2 l. of water and allowed to stand for 12 hr. After this time the solution was brought to boiling and enough solid potassium hydroxide was carefully added so that the *p*H of the solution was 9 to 10. After the solution had boiled for 30 min., it was treated with charcoal and filtered. The hot filtrate was acidified with hydrochloric acid to *p*H 2–3. After standing 24 hr. in a refrigerator the product was filtered, washed with water, and dried. This crude product was reprecipitated from a hot dilute potassium hydroxide solution with dilute hydrochloric acid to yield 12.5 g. (35%) of product, m.p. 292–293°, identical in every respect with a sample prepared by Method *A*.

6-Hydroxy-4-pyrimidinecarboxylic acid[31–33] (XXVIII). *Method A.* To 1 l. of 14% aqueous ammonia were added 75 g. of 6-hydroxy-2-thio-4-pyrimidinecarboxylic acid (III)[33] and 225 g. of Raney nickel (wet with water). The reaction mixture was heated under reflux for 2 hr. following which the Raney nickel was filtered and washed with 200 ml. of hot water. The combined filtrate and washings was acidified to *p*H 2 with hydrochloric acid and the resulting blue crystalline product was filtered. This crude product was dissolved in dilute sodium hydroxide, boiled with charcoal, and filtered. The hot filtrate was acidified with hydrochloric acid and cooled. The still pale blue crystalline product was filtered and recrystallized from water yielding 33 g. (48%) of 6-hydroxy-4-pyrimidinecarboxylic acid hydrate (XXVIII) as white needles, m.p. 276°. $\lambda_{max}^{pH\ 1}$ 227 mμ (ε 7400), 280 mμ (ε 3000); $\lambda_{max}^{pH\ 11}$ 232 mμ (s) (ε 8700), 280 mμ (ε 3200).

Anal. Calcd. for $C_5H_4N_2O_3 \cdot H_2O$: N, 17.7. Found: N, 17.5.

Method B. To 1 l. of water containing 20 g. of sodium hydroxide was added 110 g. of the sodium salt of diethyloxalacetate and 52 g. of formamidine acetate.[33] The resulting solution was stirred at room temperature for 12 hr., then acidified to *p*H 2 with hydrochloric acid, and the precipitated product was filtered. The filtrate was refrigerated overnight and a second crop was obtained. The combined crude product was recrystallized from water to yield 20 g. (63%) of product identical in every respect with a sample prepared by Method *A*.

Preparation of alkylthio derivatives (see Table III). A solution consisting of 0.05 mole of the thiopyrimidinecarboxylic acid in 250 ml. of 1*N* sodium hydroxide was stirred at room temperature while 0.06–0.07 mole of the appropriate alkyl halide was added. After stirring for 5 hr. the reaction mixture was acidified to *p*H 3 and after 15 min. the precipitated product was filtered, washed with water, and dried. The product was recrystallized from the solvent or solvents indicated in Table III.

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