[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, UNIVERSITY OF NOTRE DAME, AND THE JOHN HARRISON LABORATORY OF CHEMISTRY, UNIVERSITY OF PENNSYLVANIA]

The Synthesis of Some Pyrimidine Metabolite Analogs[1]

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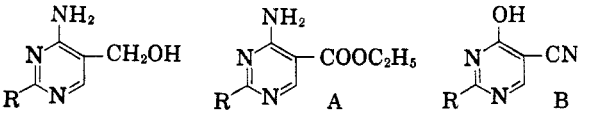
A number of tri- and tetra-substituted pyrimidines have been prepared for microbiological screening as anti-metabolites. One of several new compounds, 4-amino-5-hydroxymethyl-2-methylthiopyrimidine, shows interesting biological activity. The synthesis of hydroxymethylcytosine has been greatly improved.

Considerable interest in 5-hydroxymethylpyrimidines has recently developed. A new pyrimidine base, 5-hydroxymethylcytosine (HMC) (I), has been shown to be present in place of cytosine in the deoxyribonucleic acid (DNA) of the even-membered bacteriophages of *E. coli*[5,6] and its synthesis has been described.[7,8] The closely related "toxopyrimidine," which is the pyrimidine moiety (II) of vitamin B_1, causes convulsions in mice and appears to be a pyridoxine antagonist.[9,10] As important biological activity is associated with this type of pyrimidine structure, it was decided to synthesize an analog as a potential anti-metabolite which might interfere with DNA synthesis or with some other vital metabolic pathway and thus show antitumor activity. 4-Amino-5-hydroxymethyl-2-methylthiopyrimidine (III) immediately suggested itself, for the following reasons: (1) it would be an analog of both I and II (and possibly of pyridoxine); (2) it might be an intermediate in an improved synthesis of hydroxymethylcytosine; and (3) because there is an indication that the 2-methylthio group may confer activity on some pyrimidines.[11]

In condensation reactions to form pyrimidines, it is well known that some compounds (*e.g.*, thiourea, guanidine) react better than others (*e.g.*, urea, alkylisothioureas, alkylamidines). In the condensation reaction with ethyl ethoxymethylenecyanoacetate (IV), a pyrimidine of type A or type B will be formed, depending on whether the cyano or the carbethoxy group condenses. The synthesis of V has recently been twice reported.[7,8] In each case it was prepared from VI, where the latter was obtained in a maximum yield of 14% from ethyl isothiourea and IV, VII (or an open-chain compound which may be cyclized to VII) being by far the main product. As acetamidine, a weak reactant in this condensation, also gives a product of type B (VIII),[12] it seemed possible that thiourea and guanidine[13] might react to give a pyrimidine of type A, in which case pyrimidines of the required structure (A, R = alkylthio) would be best synthesized *via* the 2-mercapto compound. That this is indeed the case we have shown by preparing IX in 78% yield[14] from thiourea and IV, thus confirming the results of earlier workers,[15,16] though their yields were much lower. A small quantity of X is also formed, and can be readily separated and distinguished by its infrared spectrum.



I R = OH
II R = CH_3
III R = CH_3S
V R = C_2H_5S

A

B

VI R = C_2H_5S
XII R = CH_3S
XVI R = SCH_2COOH
IX R = SH
XV R = OH

VII R = C_2H_5S
VIII R = CH_3
X R = SH

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(5) G. R. Wyatt and S. S. Cohen, *Nature*, **170**, 1072 (1952).

(6) G. R. Wyatt and S. S. Cohen, *Biochem. J.*, **55**, 774 (1953).

(7) A. Dornow and G. Petsch, *Ann.*, **588**, 45 (1954).

(8) C. S. Miller, *J. Am. Chem. Soc.*, **77**, 752 (1955).

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(10) K. Makino, T. Kinoshita, Y. Aramaki, and S. Shintani, *Nature*, **174**, 275 (1954).

(11) D. B. McNair-Scott, C. S. Rose, M. L. Rogers, T. L. V. Ulbricht, C. C. Price, and R. Jones, Jr., in preparation.

(12) A. R. Todd and F. Bergel, *J. Chem. Soc.*, 364 (1937).

(13) Guanidine reacts to give 5-carbethoxy-2,4-diaminopyrimidine (T. L. V. Ulbricht, unpublished).

(14) Not 64% as quoted in a brief earlier report on part of this work (T. L. V. Ulbricht and Charles C. Price, *Chemistry & Industry*, 1221 (1955).

(15) T. B. Johnson and J. A. Ambler, *J. Am. Chem. Soc.*, **33**, 978 (1911).

(16) G. W. Anderson, I. F. Halverstadt, W. H. Miller, and R. O. Roblin, Jr., *J. Am. Chem. Soc.*, **67**, 2197 (1945).

TABLE I

PREPARATIONS OF SOME SUBSTITUTED PYRIMIDINES

Compound	Pyrimidine Substituents 2	4	5	6	Reference	Yield, %	Solvent of cryst.	M.P., °C.
XVIII	NH_2	NH_2		OH	20	90	Water	286 dec.
XIX	NHCN	NH_2		OH	21	90	Water	—
XX	NH_2	NH_2	NO	OH	22	95	Aq. alk.-acid	—
XXI	NHCN	NH_2	NO	OH	23	81	Aq. alk.-acid	—
XXIII	SH	NH_2		OH	24	98	Water	—
XXIV	SCH_3	NH_2		OH	25	83	Water	267 dec.
XXV	SCH_3	NH_2		Cl	26	69	Methanol	132
XXVI	SCH_3	NH_2		OCH_3	27	90	Methanol	144
XXVII	SCH_3	NH_2	NO	OH	25	90	Gl. acetic	Dec. from 255
XXX	NH_2	OH		OH	28	85	Water	—
XXXI	NH_2	Cl		Cl	28	32	Benzene	220
XXXII	NH_2	NH_2		Cl				
				from XXXI	29	73	Water	195–197
				from XVIII	30	53	Acetone	190–200
XXXIII	NH_2	NH_2		OC_2H_5	30	63	Ethanol	165–168

(20) W. Traube, German Patent 134,984; *Friedl.*, **6**, 119. (21) W. Traube, *Friedl.*, **7**, 663. (22) W. Traube and H. W. Dudley, *Ber.*, **46**, 3843 (1913). (23) W. Traube, German Patent 206,453; *Friedl.*, **9**, 1005. (24) W. Traube, *Ann.*, **331**, 71 (1904); *Friedl.*, **7**, 684. (25) C. D. Johns and E. J. Baumann, *J. Biol. Chem.*, **14**, 384 (1913). (26) J. Baddiley and A. Topham, *J. Chem. Soc.*, 678 (1944). (27) C. O. Johns and B. M. Hendrix, *J. Biol. Chem.*, **20**, 157 (1914). (28) E. Buttner, *Ber.*, **36**, 2228 (1903). (29) R. Hull, B. J. Lovell, H. T. Openshaw, and A. R. Todd, *J. Chem. Soc.*, 41 (1947). (30) B. Roth, J. M. Smith, and M. E. Hultquist, *J. Am. Chem. Soc.*, **72**, 1914 (1950).

Methylation of IX with dimethyl sulfate gave *4-amino-5-carbethoxy-2-methylthiopyrimidine* (XII) in 79% yield. Under the conditions of the reaction, a little hydrolysis occurs, and some *4-amino-2-methylthiopyrimidine-5-carboxylic acid* (XIII) can be isolated. The structure of this compound was confirmed by showing that it could be prepared by hydrolysis of XII. Reduction of the ester (XII) with lithium aluminum hydride gave 85% of *4-amino-5-hydroxymethyl-2-methylthiopyrimidine* (III).

This represents a four-step synthesis from ethyl cyanoacetate. A possible alternative method of synthesis would be the condensation of thiourea (methyl isothiourea would probably give a lower yield) with a derivative of β-ethoxy-α-formylpropionitrile, as has been used in a synthesis of vitamin B_1.[17] This would be a five-step synthesis from β-hydroxypropionitrile, and consequently hardly advantageous for the preparation of III, although the lithium aluminum hydride reduction would be avoided. Actually this synthesis would yield the ethoxymethyl compound (XIV), which is considerably less active (see below) than III. However, this might be the best synthesis of the 2-mercapto analog. The corresponding ester (IX) is insoluble in ether, tetrahydrofuran, and N-ethylmorpholine; an attempt to reduce it in ether suspension with lithium aluminum hydride was unsuccessful.

A few attempts were made to prepare 5-mercaptomethylpyrimidines. Compound III was converted to the bromide and treated with thiourea and hydrolyzed, but the only product isolated, obtained when ethanol was used as the solvent, was *4-amino-5-ethoxymethyl-2-methylthiopyrimidine* (XIV). Vitamin B_1 did not react with thiourea to give the mercaptomethyl analog of "toxopyrimidine."

HMC may be prepared in 60% yield by the hydrolysis of III with hydrochloric acid, and this method therefore gives a much better over-all yield than that proceeding *via* ethyl isothiourea. HMC has also been prepared[8] from 4-amino-5-carbethoxy-2-hydroxypyrimidine[18] (XV); this latter compound is obtained by hydrolysis of IX with chloroacetic acid and hydrochloric acid. Depending on the conditions employed, one may isolate 4-amino-5-carbethoxypyrimidine-2-thioglycolic acid[15] (XVI), or IV, or 4-amino-2-hydroxypyrimidine-5-carboxylic acid[19] (XVII).

Of twenty-nine pyrimidines prepared in all, thirteen compounds, most of which were required as intermediates, were obtained by the methods summarized in Table I.

Attempts to prepare 2-acetamido-4-amino-6-hydroxypyrimidine from acetylguanidine[31,32] were unsuccessful. Even when the condensation was carried out in absolute ethanol in the absence of sodium, only the 2-aminopyrimidine (XVIII) and ethyl acetate were formed. In the preparation of XXI from ethyl isonitrosocyanoacetate,[33] there was no reaction using sodium in ethanol; potassium in methanol had to be used as the condensing agent.[23] *5-Bromo-2,4-diamino-6-hydroxypyrimidine* (XXII) was obtained by the bromination of XVIII,

(17) G. V. Chevlintsev and Z. V. Benevolenskaya, *J. Gen. Chem. USSR*, **14**, 1142 (1944). [*Chem. Abstr.*, **40**, 4069 (1946)].

(18) H. L. Wheeler and C. O. Johns, *Am. Chem. J.*, **38**, 601 (1907).

(19) H. L. Wheeler and C. O. Johns, *Am. Chem. J.*, **38**, 599; **40**, 243 (1908).

(31) A. Korndorfer, *Archiv. Pharm.*, **241**, 467 (1903).

(32) W. Traube, *Ber.*, **43**, 3588 (1910).

(33) M. Conrad and A. Schulze, *Ber.*, **42**, 735 (1909).

and *4-amino-5-bromo-6-methoxy-2-methylthiopyrimidine* (XXVIII) similarly from XXVI. However, this latter compound could not be nitrosated, confirming the general view that a pyrimidine must possess two tautomeric groups (*e.g.*, OH, NH_2) before the 5-position is sufficiently activated for nitrosation to occur.[34] The reaction of XXVI with acetyl nitrate led to destruction of the pyrimidine ring, presumably *via* the oxidation of the 2-methylthio group. With XVIII, acetyl nitrate gave, not a nitroderivative, but 2,4-diacetamido-6-hydroxypyrimidine[34a] (XXIX). 2,4-Diamino-6-ethoxy-5-nitrosopyrimidine (XXXIV) was obtained in very good yield from XXXIII by a modification of the procedure of Roth, *et al.*[30]

The infrared spectra of the nitrosopyrimidines XX, XXI, XXVII, and XXXIV, as well as the difficulties encountered in condensing them with methylene compounds,[35] indicated that the nitroso group is predominantly in the isonitroso form. The synthesis of 4,6-dimethyl-5-nitrosopyrimidines was therefore investigated, as in such compounds the nitroso group cannot tautomerize with one of the groups adjacent to it.

Acetylacetone has been successfully condensed with urea and thiourea, using concentrated hydrochloric acid in ethanol,[36] with acetamidine,[37,38] and methoxyacetylacetone with guanidine carbonate by heating the reactants.[39] Isonitrosoacetylacetone[40] (XXXV) under these conditions did not condense with thiourea or guanidine. There was no reaction in the presence of hydrochloric acid or under neutral conditions, such as heating XXXV with thiourea in ethanol.

Presumably one keto group is deactivated by conjugation and hydrogen-bonding in a six-membered ring (Fig. I). If the second keto group reacts, no further reaction can occur unless the isonitroso group now conjugates with the newly formed carbon-nitrogen double bond, to leave the first keto group free; but if this does happen, another six-membered ring may be formed. (Fig. II), which may make it sterically impossible for the free amino and keto groups (indicated by*) to approach one another.

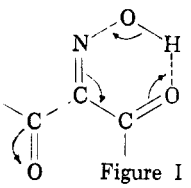

Figure I

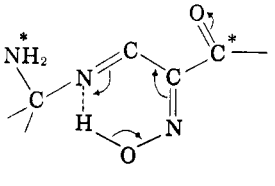

Figure II

In an alkaline medium, where such hydrogen-bonded structures would be labile (and in which the closely analogous ethyl isonitrosocyanoacetate does react), isonitrosoacetylacetone would be expected to decompose, in view of its structure as a derivative of an α,β,γ-triketone. This was shown to be the case, the decomposition sometimes being quite violent, with the formation of black polymeric material. This also explains the failure of the reaction with guanidine carbonate, for XXXV is sufficiently acidic to liberate carbon dioxide and free guanidine from this compound as the temperature is raised.

We were unsuccessful in attempts to prepare 2,4-diamino-6-mercaptopyrimidine from the reaction of the chloro compound XXXII with thiourea in ethanol.[41] A very similar compound, 2-amino-4-methyl-6-chloropyrimidine, has been found to give the dipyrimidyl sulfide instead of the expected mercapto compound under similar conditions,[42] indicating that reaction occurs but that the mercapto compound may be more reactive than thiourea. In our case, XXXII was recovered unchanged.

6-Hydroxy-2,4,5-triaminopyrimidine (XXXVI) and its sulfate were prepared by the reduction of XX with sodium hydrosulfite; this is probably a cleaner and more convenient method than using ammonium sulfide,[22] which gives variable yields.[43] Reaction of XXXVI with chloroacetic acid gave 5-chloracetamido-2,4-diamino-6-hydroxypyrimidine (XXXVII) which unlike the product reported previously,[44] analyzed correctly as the monohydrate. Under mild conditions this compound may be cyclized to a dihydroxanthopterin;[44] the dichloroacetyl derivative similarly gives xanthopterin,[45] whereas the acetamino compound gives 8-methylguanine.[46] Attempts to cyclize XXXVII to the corresponding purine, required for conversion to the 8-mercaptomethyl compound, such as heating it with chloroacetamide alone or in ethyl chloroacetate, or by heating its sodium salt, or by heating the sulfate of XXXVI with chloroacetamide, were unsuccessful.

All the pyrimidines were screened for activity which interferes with the growth of three mutant strains of *E. coli* with specific growth requirements: 113-3 (vitamin B_{12}), B-96 (purines), and 15T$^-$ (thymine). All the nitroso compounds showed activity against B-96 and 15T$^-$; XX and XXIV

(34) B. Lythgoe, *Quart. Revs.*, **3**, 205 (1949).
(34a) A. R. Phillips and J. Mentha, *J. Am. Chem. Soc.*, **76**, 6200 (1954).
(35) T. L. V. Ulbricht and C. C. Price, in preparation.
(36) P. N. Evans, *J. prakt. Chem.*, **48**, 493 (1893).
(37) H. Kondo and M. Yanai, *J. Pharm. Soc. Japan*, **57**, 747 (1937); [*Chem. Abstr.*, **32**, 172 (1938)].
(38) A. Bowman, *J. Chem. Soc.*, 494 (1937).
(39) C. C. Price, N. J. Leonard, and D. Y. Curtin, *J. Org. Chem.*, **10**, 318 (1945).
(40) L. Wolff, *Ann.*, **325**, 139 (1902).
(41) M. P. V. Boarland and J. F. W. McOmie, *J. Chem. Soc.*, 1218 (1951).
(42) M. Polonovski and H. Schmitt, *Bull. soc. chim.*, **5**, 616 (1950).
(43) Z. Zakrzewski, personal communication to T.L.V.U.
(44) G. H. Hitchings and G. B. Elion, *J. Am. Chem. Soc.*, **71**, 467 (1949).
(45) R. Purrmann, *Ann.*, **546**, 98 (1940).
(46) W. Traube, *Ann.*, **432**, 266 (1923).

could be reversed in B-96 by either adenine or guanine. XXI, XXIV and XXVII showed activity against 113–3, which could be reversed by vitamin B_{12} or methionine. These compounds are being tested for antitumor activity in L 1210 leukemia.

4-Amino-5-hydroxymethyl-2-methylthiopyrimidine (III) is inactive against the above organisms, and also against *S. faecalis* (wild strain), and it does not interfere with the synthesis of T_2 bacteriophage in its bacterial host, *E. coli*, B. (It is in the DNA of this virus that HMC is found.) However, III is a very strong inhibitor (and the 5-ethoxymethyl compound (XIV) less strong) of the *E. coli* mutant which requires the pyrimidine moiety of vitamin B_1 (II) for growth.[48] The compound is being tested on other systems. Preliminary results indicate that it retards the growth of certain experimental tumors.[49,50]

EXPERIMENTAL*

Ethyl ethoxymethylenecyanoacetate (IV). This was prepared by the method of De Bollemont,[51] the fraction b.p. 181°/16 mm. being recrystallized from ethanol to give a colorless product, m.p. 52–53°.

4-Amino-5-carbethoxy-2-mercaptopyrimidine (IX).[16] To a solution of sodium (9.2 g.) in absolute ethanol (250 ml.) were added thiourea (33.4 g.) and IV (67.5 g.) and the solution was stirred and refluxed for 6 hours. Water (750 ml.) was added, and the solution was acidified with acetic acid and heated to boiling with vigorous stirring. After ensuring that the solution was acid, it was cooled to 30° and filtered, to give 62.9 g. (78%) of IX. This may be recrystallized from a large volume of 50% acetic acid to give colorless needles, m.p. 260–262°.

5-Cyano-4-hydroxy-2-mercaptopyrimidine (X).[16] The filtrate from the above experiment was cooled overnight at 0°, and the product was filtered and recrystallized from 10% acetic acid (charcoal) to give 3.3 g. (4.4%) of X, m.p. 265°, dec., with previous darkening. In the infrared, this compound has a strong peak at 4.44 μ, and no absorption in the 5.8 μ range, whereas IX has a strong peak at 5.88 μ, and no peak in the 4.4 μ range.

4-Amino-5-carbethoxy-2-methylthiopyrimidine (XII). To a solution of 10.8 g. of 86% potassium hydroxide in 150 ml. of water was added 30 g. of IX and the mixture was warmed slightly to get most of the mercapto compound in solution. Dimethyl sulfate (20 g.) was added gradually with shaking, the flask being cooled occasionally, so that the temperature was about 60°. When addition was complete, the mixture was allowed to stand for an hour, made alkaline, filtered, washed, and dried, to give 25.2 g. (79%) of *4-amino-5-carbethoxy-2-methylthiopyrimidine*. This may be recrystallized from dilute acetic acid to give tiny, glistening plates, m.p. 130–131°.

Anal. Calc'd for $C_8H_{11}N_3O_2S$: C, 45.1; H, 5.2; N, 19.7; S, 15.0. Found: C, 45.1; H, 5.3; N, 19.7; S, 14.7.

XII is readily soluble in ethanol, and sparingly soluble in ether.

4-Amino-2-methylthiopyrimidine-5-carboxylic acid (XIII). (*a*) *From* IX. The alkaline filtrate from the above methylation is acidified with acetic acid and allowed to stand overnight and filtered, giving 3.3 g. (12%) of *4-amino-2-methylthiopyrimidine-5-carboxylic acid* (XIII). Recrystallizations from aqueous acetic acid or methanol gave a product, m.p. 220–230°, which was substantially pure.

Anal. Calc'd for $C_6H_7N_3O_2S$: C, 39.0; H, 4.0; N, 22.5; S, 17.1. Found: C, 39.4; H, 3.8; N, 22.7; S, 17.3.

However, by dissolving the acid in dilute ammonia, charcoaling, filtering and acidifying, a product was eventually obtained which melted at 251–252°, dec.

(*b*) *From* XII. A 0.5-g. sample of this compound was heated in water (15 ml.) containing potassium hydroxide (1.1 g.) and 60° for one hour, with occasional stirring. Almost all the solid went into solution by the end of this period. The solution was filtered and acidified with acetic acid, giving 0.3 g. of XIII, m.p. 248–249°, dec. After purification as above, its melting point, alone or admixed with the product prepared as under (*a*), was 251–252°, dec.

4-Amino-5-hydroxymethyl-2-methylthiopyrimidine (III). XII (10 g.) was reduced with lithium aluminum hydride (2.8 g.) in anhydrous ether (600 ml.) using the Soxhlet method and stirring and refluxing for three hours. Ethyl acetate and then water, were added, and the solution was filtered. The solid was extracted four times with 100 ml. of acetone by shaking vigorously and filtering each time. The acetone and ether solutions were evaporated to dryness, the residue was extracted with a large volume of benzene, and the solution was concentrated to give colorless needle-like prisms, m.p. 126–127°. The yield of *4-amino-5-hydroxymethyl-2-methylthiopyrimidine* was 6.8 g. (85%).

Anal. Calc'd for $C_6H_9ON_3S$: C, 42.1; H, 5.3; N, 24.6; S, 18.9. Found: C, 42.4; H, 4.9; N, 24.3; S, 18.6.

4-Amino-5-ethoxymethyl-2-methylthiopyrimidine (XIV). Water-free acetic acid (35 ml.) was saturated with anhydrous hydrogen bromide at 0°, a solution of (2.4 g.) of III in 130 ml. of acetic acid was added, and the mixture was heated on a steam-bath for two hours. The solvent was removed under reduced pressure, leaving a colorless residue, presumed to the hydrobromide of 4-amino-5-bromomethyl-2-methylthiopyrimidine. This was washed with anhydrous ether and rapidly filtered. (The hydrobromide is very hygroscopic.) The yield was 4.3 g. (97%). The product was taken up in absolute ethanol, a solution of 300 mg. of sodium in ethanol was added, the solution was filtered from sodium bromide and then was refluxed with thiourea (1.16 g.) for two hours. The solvent was removed and the residue was refluxed with water (20 ml.) and sodium hydroxide (1.2 g.) for 30 minutes. After cooling and neutralizing with acetic acid, the solution was either extracted with ether, or the solid which separated was filtered and extracted with benzene. Both methods gave about 1.1 g. of *4-amino-5-ethoxymethyl-2-methylthiopyrimidine*, which, after recrystallization from petroleum ether, melted at 101–105°.

Anal. Calc'd for $C_8H_{13}N_3OS$: C, 48.3; H, 6.6; N, 21.1; S, 16.1. Found: C, 48.8; H, 6.7; N, 20.5; S, 16.2.

4-Amino-2-hydroxy-5-hydroxymethylpyrimidine (*HMC*) (I). III (1.0 g.) was refluxed with hydrochloric acid (diluted 10:1, 20 ml.) for four hours, cooled, made alkaline with sodium hydroxide, cooled to 0°, filtered, neutralized, concentrated under reduced pressure and cooled, giving (I), 565 mg. (69%). The product was recrystallized from a small volume of water (charcoal) and dried *in vacuo*, giving 485 mg. (60%). An infrared spectrum was identical with that of an authentic specimen supplied by Dr. J. S. Gots.

4-Amino-5-carbethoxypyrimidine-2-thioglycollic acid (XVI).[15] The 2-mercapto compound (IX, 4.0 g.) was refluxed with 2.0 g. of chloroacetic acid in 30 ml. of water and 10 ml. of acetic acid until solution occurred, and then was evaporated

(48) J. S. Gots and T. L. V. Ulbricht, in preparation.

(49) Unpublished results by R. Jones, Jr., Doris McKenzie, W. F. Dunning, and C. F. Weaver.

(50) J. F. Holland and R. Guthrie, personal communication to Dr. J. S. Gots.

* Melting points are uncorrected. Where no melting point is given, the compound either decomposes on heating, or does not melt below 300°. Analyses are by Microtech. Inc., Skokie, Illinois, and Drs. Weiler and Strauss, Oxford, England.

(51) M. E. G. Bollemont, *Bull. soc. chim.*, [3], **25**, 20 (1901).

to dryness on a steam-bath, giving 6 g. of crude XVI, m.p. 185–191°, dec., which may be recrystallized from water.

4-Amino-5-carbethoxy-2-hydroxypyrimidine (XV). III (4.0 g.) and chloroacetic acid (2.0 g.) were refluxed in 25 ml. of water and 5 ml. of acetic acid until solution occurred, 30 ml. of hot concentrated hydrochloric acid was added, and the solution was refluxed for ten minutes, and then was evaporated to dryness on a steam-bath. The residue was extracted twice with 50 ml. of hot water, and was neutralized with concentrated ammonia, giving 1.4 g. of XV. By extracting the residue a second time and concentrating the mother liquors, a further 380 mg. was obtained. The product may be recrystallized from water (charcoal) giving colorless needles, m.p. 278–282° (Lit.[18] m.p. 260–275°).

4-Amino-2-hydroxypyrimidine-5-carboxylic acid (XVII). The insoluble residue from the above experiment (1.67 g.) is crude XVII. It may be purified by washing with hot dilute acetic acid, dissolving the residue in concentrated ammonia, diluting with water, charcoaling, filtering, and acidifying with acetic acid. The washed, dried product melted at 250°, dec. (Lit.[19] m.p. 256–257°).

2,4-Diamino-5-bromo-6-hydroxypyrimidine (XXII). To a solution of XVIII (4.3 g.) in 75 ml. of glacial acetic acid containing 12.2 g. of sodium acetate hydrate, was slowly added a solution of bromine (4.8 g., 1.5 ml.) in 30 ml. of glacial acetic acid with stirring during one hour. The solution was then heated on a steam-bath for one hour, poured into water (600 ml.), and made slightly alkaline with concentrated ammonia (about 120 ml.). After cooling, the crystals of *2,4-diamino-5-bromo-6-hydroxypyrimidine* (yield 5.4 g., 88%) were filtered. Recrystallization from water gave colorless needles, m.p. 255° dec.

Anal. Calc'd for $C_4H_5BrN_4O$: C, 23.4; H, 2.0; N, 27.3; Br, 39.0. Found: C, 23.7; H, 2.2; N, 27.0; Br, 39.2.

4-Amino-5-bromo-6-methoxy-2-methylmercaptopyrimidine (XXVIII). By the above method there was obtained from XXVI a 77% yield of *4-amino-5-bromo-6-methoxy-2-methylmercaptopyrimidine*, colorless needles from methanol, m.p. 135–136°.

Anal. Calc'd for $C_6H_8BrN_3OS$: C, 28.8; H, 3.2; N, 16.8; S, 12.8; Br, 32.1. Found: C, 29.2; H, 3.2; N, 16.2; S, 12.8; Br, 32.1.

2,4-Diacetylamino-6-hydroxypyrimidine (XXIX). To a solution of 2.0 g. of XVIII in 40 ml. of acetic anhydride, 0.98 ml. of nitric acid in 10 ml. of acetic anhydride was slowly added, the solution becoming colored violet, red, and finally brown It was heated to boiling and allowed to stand for five hours, and was filtered to yield 920 mg. of product. A further 480 mg. was obtained by adding water to the filtrate and neutralizing with ammonia. Recrystallization from glacial acetic acid gave 2,4-diacetamido-6-hydroxypyrimidine.[52]

Anal. Calc'd for $C_8H_{10}N_4O_3$: C, 45.7; H, 4.8; N, 26.7. Found: C, 45.3; H, 4.8; N, 27.0.

2,4-Diamino-6-ethoxy-5-nitrosopyrimidine (XXXIV). XXXIII (15.8 g.) was dissolved in 110 ml. of hot water containing 3.2 ml. of acetic acid and was heated to 80°. A solution of 6.0 g. of sodium nitrite in 20 ml. of water was added with stirring during 15 minutes. The solution was reheated to 80° (the temperature had fallen to 60°) and stirred at room temperature for two hours and filtered, giving 15.0 g. (80%) of XXXIV, m.p., after recrystallization from absolute ethanol, 225°, dec.

Anal. Calc'd for $C_6H_9N_5O_2$: C, 39.4; H, 5.0; N, 38.2. Found: C, 39.4; H, 5.1; N, 37.6.

6-Hydroxy-2,4,5-triaminopyrimidine (XXXVI) *and its sulfate.* A solution suspension of XX (17.0 g.) in 175 ml. of water containing 17.0 g. of sodium hydroxide was added in portions to a solution of 66 g. of sodium hydrosulfite in 200 ml. of water at 80–95°. The solution was charcoaled and filtered, the product (XXXVI) separating on cooling. After filtering it may be purified by dissolving in the minimum volume of water, adjusting to *p*H 7 with ammonia, adding hydrosulfite to prevent oxidation, charcoaling, and filtering. On cooling, the free base is obtained (yield, after drying at 140°, 11.3 g.). Alternatively, the solution can be filtered into 50% sulfuric acid, giving 20.4 g. of the sulfate.

5-Chloroacetamido-2,4-diamino-6-hydroxypyrimidine (XXXVII). XXXVI (2.0 g.) was heated with 20 g. of chloroacetic acid in an oil-bath at 125° for 40 minutes. The product was cooled, diluted with 20 ml. of water, neutralized with ammonia, and filtered. Recrystallization from water (charcoal) gave colorless crystals of 5-chloroacetamido-2,4-diamino-6-hydroxypyrimidine; yield, 2.47 g. (80%). A sample for analysis was dried at 140° *in vacuo* but it regained water of crystallization.

Anal. Calc'd for $C_6H_8ClN_5O_2 \cdot H_2O$: C, 30.7; H, 4.3; N, 29.7; Cl, 15.0. Found: C, 31.1; H, 4.2; N, 29.6; Cl, 14.6.

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(52) Phillips and Mentha[34a] state that the compound does not melt below 340°.