b.p. 150° (1.5 mm.) $n^{25}D$ 1.4580. The infrared spectrum of this material showed bands at 2.89 and 4.41 μ.

Anal. Calcd. for $C_6H_8N_2O$: C, 58.05; H, 6.50; N, 22.57. Found: C, 58.0; H, 6.5; N, 22.2.

From the reaction mixture a lower boiling fraction (4.3 g.), b.p. 79–82° (0.6 mm.), was obtained. Redistillation gave the pure material (2.9 g.), b.p. 68–70° (0.3 mm.), $n^{25}D$ 1.4620. This substance exhibits bands in the infrared at 2.91, 4.46, and 6.10 μ and appears to be 4-hydroxy-2-pentenecarbonitrile.

Anal. Calcd. for C_5H_7NO: C, 61.9; H, 7.2; N, 14.4. Found: C, 61.8; H, 7.4; N, 14.4.

2-Amino-6-bromo-3-methylpyridine and 2-Amino-6-bromo - 5 - methylpyridine.—3 - Hydroxy - 2 - methylglutaronitrile (2.9 g.) was dissolved in methylene chloride (25 ml.) and hydrogen bromide bubbled through the solution for 1 hr. The methylene chloride was removed under reduced pressure and the oily residue treated with saturated sodium hydrogen carbonate solution (100 ml.) The tan colored crystals which deposited were removed by filtration and air-dried (3.2 g.) m.p. 63–65°. Further crystallization of this material from ether–petroleum ether (b.p. 30–35°), while serving to give a colorless product, scarcely affected the melting point. A sample of this material (1.2 g., m.p. 67–68.5°) was dissolved in ether and chromatographed over neutral alumina (20 g.). The first two fractions eluted by ether (100 ml.) contained 0.63 g. of material which, after three crystallizations from ether–petroleum ether, afforded pure 2-amino-6-bromo-3-methylpyridine (0.25 g. as long white needles, m.p. 114–114.5°).

Anal. Calcd. for $C_6H_7N_2Br$: C, 38.5; H, 3.74; N, 14.97; Br, 42.78. Found: C, 38.4; H, 3.8; N, 14.7; Br, 42.5.

Subsequent elution of the column with ether (7 × 50 ml.) also gave crystalline material which was recrystallized from ether–petroleum ether to give white feathery crystals (0.25 g.) m.p. 90–95°. The infrared spectrum of this material indicated the presence of approximately 5% of the higher melting isomer. However, three further crystallizations from the same solvent mixture led to pure 2-amino-6-bromo-5-methylpyridine (0.1 g.), m.p. 97.5–98°. Found: C, 38.7; H, 3.6; N, 14.8; Br, 42.7.

The Phenylurethane of 2-Amino-3-methylpyridine.—2-Amino-6-bromo-3-methylpyridine (0.1 g.) was dissolved in dry ethanol (20 ml.) containing potassium hydroxide (0.05 g.). This solution was then stirred with hydrogen and a palladium catalyst (50 mg., 10% palladium-on-charcoal) until the calculated amount of hydrogen had been adsorbed (10 min.). Removal of the catalyst and the alcohol by the usual methods, followed by dilution with water and isolation of the product by ether extraction, led to a small quantity of a brown oil. This was dissolved in ether (2 ml.) and 2 drops of phenyl isocyanate added. Almost immediately, white crystals began to separate and after 30 min. these were removed by filtration and recrystallized from acetone–ether, m.p. 176–177°. A mixed melting point of this material with a sample of the phenylurethane (m.p. 178°) prepared from an authentic specimen of 2-amino-3-methylpyridine showed no depression.

Anal. Calcd. for $C_{13}H_{13}N_3O$: C, 68.70; H, 5.77; N, 18.49. Found: C, 68.7; H, 5.8; N, 18.3.

The Phenylurethane of 2-Amino-5-methylpyridine.—2-Amino-6-bromo-3-methylpyridine (42 mg.) was hydrogenated and the product isolated as in the above experiment. The white crystalline solid obtained had m.p. 73–75° and did not depress the melting point of authentic 2-amino-5-methylpyridine (m.p. 73–76°). The infrared spectra of the two materials were also identical. The phenylurethan prepared as in the above experiment crystallized from methanol in white needles, m.p. 197.5–198.5°, and did not depress the melting point of authentic 2-amino-5-methylpyridine phenylurethan, m.p. 196.5–197.5°. Found: C, 69.0; H, 5.8; N, 18.6.

Diethyl 3-Hydroxyglutarate.—3-Hydroxyglutaronitrile 11 g., 0.1 mole) was dissolved in dry ethanol (13.8 g., 0.3 mole) and dry ether (50 ml.). The mixture was cooled to −15° and dry hydrogen chloride gas passed through slowly for 6 hr. The excess hydrogen chloride, ether, and alcohol were removed under reduced pressure, and the remaining solid dissolved in water (40 ml.) and warmed at 40–50° for 30 min. The oil which separated out of solution was isolated by ether extraction and distilled under reduced pressure. Diethyl 3-hydroxyglutarate (13.3 g., yield 65%) was collected at 91–93° at 0.25 mm. [reported[29] b.p. 105–107° (2 mm.)].

Acknowledgment.—We would like to thank Dr. C. K. Fitz, who carried out all elemental analyses.

(29) H. L. Lochte and P. L. Pickard, *J. Am. Chem., Soc.*, **68**, 721 (1946).

Condensed Pyrimidine Systems. XXII. *N*-Methylpurines

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A group of 1- and 3-monomethylpurines has been prepared by complete synthesis. Among the new derivatives are 3-methyladenine, 3-methylguanine, and the 1- and 3-methyl derivatives of 6-mercaptopurine. A number of 7- and 9-methyl derivatives have been obtained by direct methylation of 6-chloropurine, conversion to the mercapto derivatives, and subsequent separation of the 7- and 9-methylpurine-6-thiols. Several ring openings and rearrangements have been observed in the course of attempts to prepare 1-methyladenine.

Studies on *N*-methylpurines were undertaken in this laboratory a number of years ago in connection with investigations of the effect of such substitution upon biological activity, e.g. the specificity of the purine requirement of microorganisms,[1,2] the specificity of enzymes such as guanase[3] and xanthine oxidase,[4] and the effects of structural modifications upon the activity of purine antagonists such as 6-mercaptopurine (purine-6-thiol)

(1) G. H. Hitchings, G. B. Elion, and E. A. Falco, *J. Biol. Chem.*, **185**, 643 (1950).

(2) G. H. Hitchings and G. B. Elion, *Proc. Intern. Congr. Biochem., Third, Brussels*, **1955** (1956), Academic Press, New York, p. 55.

(3) G. H. Hitchings and E. A. Falco, *Proc. Nat. Acad. Sci. U.S.*, **30**, 294 (1944).

(4) D. C. Lorz and G. H. Hitchings, *Federation Proc.*, **9**, 197 (1950).

(6-MP).[5-7] When this work was begun, a number of *N*-methyl isomers of the natural purines were as yet unknown—*e.g.* 3-methyladenine and 3-methylguanine—and the only *N*-methyl derivative of 6-MP which had been prepared was the 7-methyl.[8] 1-Methyladenine[9] and 1-methylhypoxanthine[10] had been reported by Brederick to be formed by the hydrolysis of the methylation products of adenosine and inosine, but the assignment of structure appeared to be arbitrary. Moreover, the syntheses of a number of the *N*-methylpurines in the literature were cumbersome, gave poor yields, and offered poor criteria for the identification of the specific isomer. It was hoped that with the newer techniques such as chromatography and ultraviolet absorption spectra, the isomers could be well characterized and information could be obtained about their structural configurations. The recent discovery of a number of *N*-methylpurines in RNA[11-13] and DNA[14,15] has added increased interest and importance to these compounds.

The work to be described here deals mainly with the synthesis of 1- and 3-methyl isomers of the natural purines and 6-MP and of a number of other purines which have served as intermediates for these. In addition, simplified procedures for the preparation of the 7- and 9-methyl isomers of the 6-substituted purines are reported. A comparison of the ultraviolet absorption spectra of the various isomers is likewise included. The first report of these studies was presented several years ago.[16]

The exhaustive studies of Emil Fischer had established the interrelationships among the various methylpurines but the assignment of structure rests ultimately on the identification of paraxanthine as 1,7-dimethylxanthine. The unequivocal synthesis of this compound from 1-methyl-4-nitro-5-carboxyimidazole by Mann and Porter[17] established the structure and corroborated Fischer's original deductions.[18] Methods for the synthesis of all of the *N*-methylxanthines have been reported.[18-21] 1-Methylxanthine[19] and 3-methylxanthine[20] have been employed as reference compounds for the identification of isomers obtained by nondefinitive syntheses.

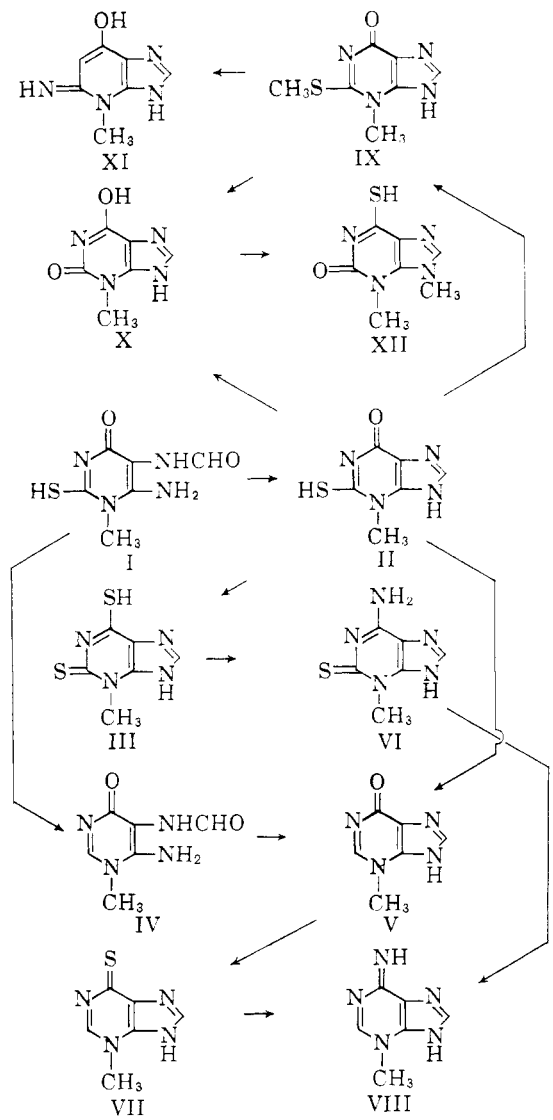

Figure 1

Results and Discussion

The reactions utilized for the synthesis of the 3-methylpurines are depicted in Fig. 1. Traube and Winter[22] had reported that the condensation of *N*-methylthiourea and cyanoacetic ester gave 4 - amino - 2 - mercapto - 3 - methylpyrimidine-6-one which was eventually converted to the 3-methylxanthine (X). Reexamination, with the aid of chromatography, of this condensation, which could theoretically go in either direction, revealed that a single isomer was indeed formed and that it

(5) For purposes of ready understanding and easy reference to the older literature, trivial names have been employed wherever these seemed appropriate. In only a few instances does this deviate from current *Chem. Abstr.* practice. In other cases systematic nomenclature has been employed.

(6) G. B. Elion, G. H. Hitchings, and H. VanderWerff, *J. Biol. Chem.*, **192**, 505 (1951).

(7) D. A. Clarke, G. B. Elion, G. H. Hitchings, and C. C. Stock, *Cancer Res.*, **18**, 445 (1958).

(8) E. Fischer, *Ber.*, **31**, 431 (1898).

(9) H. Bredereck, H. Haas, and A. Martini, *ibid.*, **81**, 307 (1948).

(10) H. Bredereck and A. Martini, *ibid.*, **80**, 401 (1947).

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(12) P. L. Berquist and R. E. F. Matthews, *Biochim. Biophys. Acta*, **34**, 567 (1959).

(13) J. W. Littlefield and D. B. Dunn, *Biochem. J.*, **70**, 642 (1958).

(14) D. B. Dunn and J. D. Smith, *ibid.*, **68**, 627 (1958).

(15) D. B. Dunn, *Biochim. Biophys. Acta*, **46**, 198 (1961).

(16) G. B. Elion, "The Chemistry and Biology of Purines," Ciba Foundation Symposium, G. E. W. Wolstenholme and C. M. O'Connor, ed., Little, Brown and Co., Boston, 1957, p. 39.

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(18) E. Fischer, *Ber.*, **30**, 2400 (1897).

(19) M. Engelmann, *ibid.*, **42**, 177 (1909).

(20) W. Traube, *ibid.*, **33**, 3035 (1900).

(21) H. Biltz, K. Strufe, E. Topp, M. Heyne, and R. Robl, *Ann.*, **423**, 200 (1921).

(22) W. Traube and F. Winter, *Arch. Pharm.*, **244**, 11 (1906).

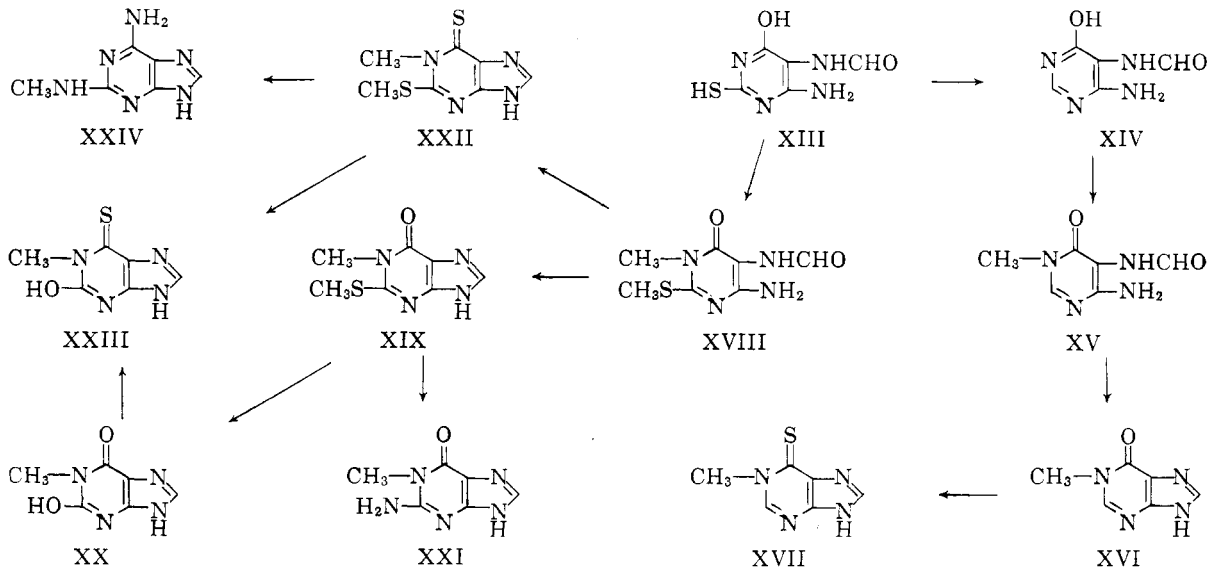


Figure 2

was the 3-methyl isomer. This pyrimidine was therefore used as the starting material for the entire series of 3-methylpurines. The 5-formamido derivative (I) was prepared essentially by the method in the literature[22] but the ring closure to II was accomplished by heating with formamide instead of by Traube's method of heating the sodium salt of I. Dethiolation with Raney nickel transformed II into 3-methylhypoxanthine (V). This reaction is much more satisfactory than in the case of 2-mercaptoadenine[23] and proved to be preferable to Traube's conversion of II to V with nitric acid. An alternative route to V was the dethiolation of I to give IV, followed by ring closure to V. Difficulties were encountered in obtaining IV in a pure state since some deformylation as well as ring closure occurred under the alkaline conditions of the dethiolation. The thiation of V to 3-methylpurine-6-thione (VII) proceeded smoothly with phosphorus pentasulfide in pyridine. Although VII would be converted to 3-methyladenine (VIII) with aqueous ammonia at 140° for 16 hours, a better synthesis of VIII proved to be the thiation of II to the dithio derivative III, followed by replacement of the 6-thiol group by an amino group (*cf.* ref. 24) to give VI, and subsequent dethiolation with Raney nickel.

The methylation of II with dimethyl sulfate gave a methyl derivative which was shown to be IX by its conversion to 3-methylxanthine (X) by acid hydrolysis. Direct hydrolysis of II likewise produced X. Attempts to prepare 3-methylguanine (XI) from IX with either aqueous or alcoholic ammonia were unsuccessful; the products isolated were either unchanged IX or 3-methylxanthine. However, when IX was heated with formamide at 200° for thirty minutes, 3-methylguanine was formed in reasonable yield.[25]

The thiation of 3-methylxanthine produced not the 2,6-dithio derivative (III) but the 6-thio derivative (XII). This was not unexpected, since similar monothiation has been observed in the case of 1,3-dimethyluracil,[26] 1-methyluracil,[27] xanthine,[28] and uric acid[29,30] under similar conditions.

In the 1-methyl series (Fig. 2) the starting material was the formamidopyrimidine XIII[31] which, upon treatment with Raney nickel, was dethiolated to XIV and then methylated to XV. This methyl derivative (XV) was distinct from the 3-methyl isomer (IV). It was readily converted with formic acid to a methylhypoxanthine which was identified as 1-methylhypoxanthine (XVI) since it was distinguishable chromatographically from the 3-methyl isomer (V), as well as from the 7- and 9-methyl isomers and 6-methoxypurine.[32] The treatment of XVI with phosphorus pentasulfide gave 1-methylpurine-6-thione (XVIII), readily distinguishable from the 3-methyl isomer (VII).

Methylation of XIII with two equivalents of dimethyl sulfate gave as the main product the dimethyl derivative XVIII. Confirmation of the structure of XVIII was obtained by ring closure to XIX followed by hydrolyis to 1-methylxanthine

(23) A. Bendich, J. F. Tinker, and G. B. Brown, *J. Am. Chem. Soc.*, **70**, 3109 (1948).

(24) P. B. Russell, G. B. Elion, E. A. Falco, and G. H. Hitchings, *J. Am. Chem. Soc.*, **71**, 2279 (1949).

(25) Subsequently the synthesis of 3-methylguanine by another method was reported: I. J. Borowitz, S. M. Bloom, J. Rothschild, and D. B. Sprinson, *Federation Proc.*, **18**, 195 (1959).

(26) G. B. Elion and G. H. Hitchings, *J. Am. Chem. Soc.*, **69**, 2138 (1947).

(27) J. J. Fox *et al.*, *ibid.*, **81**, 178 (1959).

(28) A. G. Beaman, *J. Am. Chem. Soc.*, **76**, 5633 (1954).

(29) G. B. Elion, S. Mueller, and G. H. Hitchings, *J. Am. Chem. Soc.*, **81**, 3042 (1959).

(30) T. L. Loo, M. E. Michael, A. J. Garceau, and J. C. Reid, *J. Am. Chem. Soc.*, **81**, 3039 (1959).

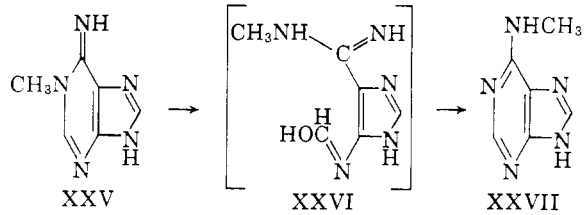
(31) W. Traube, *Ann.*, **64**, 331 (1904).

(32) G. H. Hitchings and G. B. Elion, U.S. Patent 2,746,961, May 22, 1956.

(XX). After the ring closure of XVIII to XIX with formamide, a second product found in the mother liquors was 1-methylguanine (XXI). This was apparently formed by the reaction of the 2-methylmercapto group with the ammonia generated during the heating of formamide. Heating of XIX with ammonium hydroxide likewise gave XXI. The 1-methylguanine formed in this way was found to be free of guanine whereas the 1-methylguanine prepared by the method of Traube[33] from 2,4-diamino-5-formamidopyrimidine-6-ol by methylation and ring closure has, in our experience, often been contaminated with guanine (as determined by chromatography in 86% butanol in an ammonia atmosphere).

Treatment of XVIII with phosphorus pentasulfide resulted in simultaneous ring closure and thiation to give XXII. Hydrolysis of XXII with acid led to the preferential removal of the 2-methylthio group to give 1-methyl-6-thioxanthine (XXIII), which also was obtained by thiation of 1-methylxanthine (XX). The reaction of XXII with ammonium hydroxide at 150° gave a product which was at first identified as 2-amino-6-imino-1-methylpurine on the basis of its analysis and ultraviolet absorption spectrum. It was distinct not only from the 3-methyl isomer XI but also from a possible rearrangement product, 2-amino-6-methylaminopurine.[34] However, further examination revealed that the compound was chromatographically and spectrophotometrically identical with 6-amino-2-methylaminopurine (XXIV).[34] Moreover, it could be deaminated by nitrous acid[35] to 2-methylaminopurine-6-ol. This rearrangement of the 1-methyl derivative of 2,6-diaminopurine to 6-amino-2-methylaminopurine probably involves a ring-splitting at the 1-6 bond followed by ring closure, such as has been described for the rearrangement of 2-imino-1-methylpyrimidine to 2-methylaminopyrimidine.[36,37] This is also similar to the ring opening and closure postulated by Fischer[38] for the rearrangement of 6-amino-2-chloro-7-methylpurine to 7-methylguanine.

An attempt to prepare 1-methyladenine (XXV) by the heating of the 1-methylpurine-6-thione XVII with aqueous ammonium hydroxide resulted in the cleavage of the pyrimidine ring, with the consequent formation of 4-amino-5-imidazolecarboxamide. This type of ring opening occurs with the 4-substituted pteridines,[39,40] particularly when they are *N*-methylated.[41] With alcoholic ammonia at 155°, XVII was converted to 6-methylaminopurine (XXVII). It appeared probable, therefore, that 1-methyladenine might have been formed but also rearranged under these conditions. This view was confirmed by the work of Brookes and Lawley,[42] who recently reported the isolation of 1-methyladenine from the products of methylation and hydrolysis of adenosine and found that the substance does indeed rearrange to 6-methylaminopurine under the influence of aqueous ammonium hydroxide at 100°. This rearrangement is of interest because it appears to differ from that of the corresponding diaminopurine derivative described above in the position of ring opening. Whereas the rearrangement of the diaminopurine appears to involve the 1–6 bond, that of the adenine derivative seems to involve opening of the 1–2 bond and subsequent reclosure:



When XVII was heated with alcoholic ammonia at 165° for three days, the product was adenine rather than 6-methylaminopurine. This may have resulted from an ammonolysis of the intermediate imidazole formed after ring opening (XXVI) or by the displacement of the 6-methylamino group after ring closure.

Before the marked lability of 1-methyladenine to alkaline conditions was appreciated, an alternative synthesis of this compound from 1-methyl-2,6-dithioxanthine (XXXII) (Fig. 3) was attempted in a manner analogous to that which had succeeded for 3-methyladenine. Since the direct thiation of 1-methylxanthine produces 1-methyl-6-thioxanthine (XXIII), an attempt was made to prepare XXXII by the demethylation of XXII with gaseous hydrogen iodide in glacial acetic acid, a method successfully employed in the demethylation of some methylthiopyrimidines.[43] When this failed, the series of reactions shown in Fig. 3 was carried out. Benzylation of XIII with one molecular equivalent of benzyl chloride gave XXVIII, which was then methylated in the 1-position to give XXIX. Treatment of XXIX with phosphorus pentasulfide in pyridine gave simultaneous thiation and ring closure to XXXIV. Ring closure of XXIX with formic acid gave XXX, which was debenzylated with sodium in liquid ammonia to 1-methyl-2-thioxanthine (XXXI). On thiation,

(33) W. Traube and H. W. Dudley, *Ber.*, **46**, 3839 (1913).
(34) J. A. Montgomery and L. B. Holum, *J. Am. Chem. Soc.*, **80**, 404 (1958).
(35) C. N. Remy and M. S. Smith, *J. Biol. Chem.*, **228**, 325 (1957).
(36) D. J. Brown, E. Hoerger, and S. F. Mason, *J. Chem. Soc.*, 4035 (1955).
(37) D. J. Brown, *Nature*, **189**, 828 (1961).
(38) E. Fischer, *Ber.*, **31**, 542 (1898).
(39) E. C. Taylor, Jr., "Chemistry and Biology of Pteridines," Ciba Foundation Symposium, G. E. W. Wolstenholme and M. P. Cameron, ed., J. and A. Churchill Ltd., London, 1954, p. 2.
(40) A. Albert, *J. Chem. Soc.*, 2690 (1955).
(41) H. C. S. Wood, "Chemistry and Biology of Pteridines," Ciba Foundation Symposium, G. E. W. Wolstenholme and M. P. Cameron, ed., J. and A. Churchill Ltd., London, 1954, p. 35.
(42) P. Brookes and P. D. Lawley, *J. Chem. Soc.*, 539 (1960).
(43) H. W. Barrett, I. Goodman, and K. Dittmer, *J. Am. Chem. Soc.*, **70**, 1753 (1948).

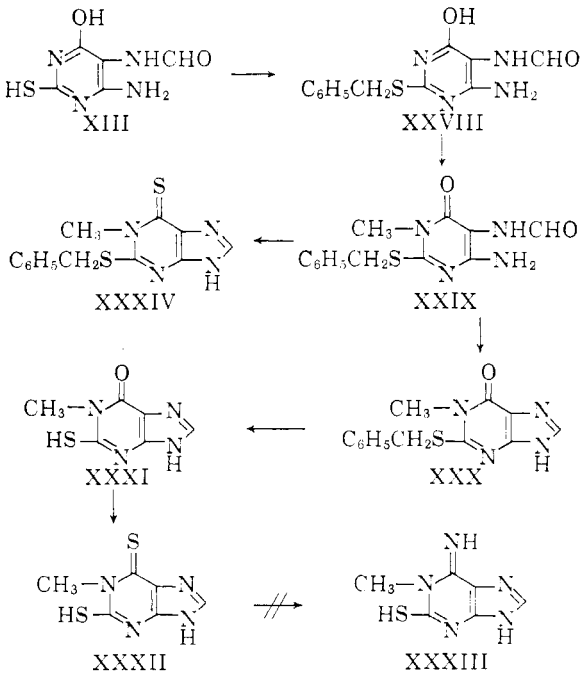


Figure 3

the desired intermediate XXXII was obtained. Reaction of XXXII with aqueous ammonium hydroxide at 140° led to extensive rearrangement and degradation while alcoholic ammonia at 140° gave a monoamino derivative which proved to be not the desired XXXIII but 6-aminopurine-2-thiol. Thus the instability of XXXII to ammonia appeared to be similar to that of 1-methylpurine-6-thione (XVII). At this point attempts to prepare 1-methyladenine were abandoned, since it was concluded that the compound was too unstable to exist under the conditions which were required for its synthesis by the proposed routes.

In order to obtain the 7-methyl and 9-methyl isomers of some of the 6-substituted purines for chromatographic and spectrophotometric comparison with the 1- and 3-methyl isomers, a method simpler than that of complete synthesis was sought. It was found that 6-chloropurine XXXV could be methylated readily with dimethyl sulfate to a mixture of the 7- and 9-methyl isomers (XXXVI, XXXVII) (Fig. 4).[16,44] No attempt was made to separate these isomers; instead, the mixed product was converted to the mixture of 7- and 9-methylpurine-6-thiols. These were easily separable by the differences in their solubilities in water and could be obtained chromatographically pure in this way. The 7-methyl isomer (XXXVIII) was identified by comparison with the 7-methylpurine-6-thiol obtained by the method of Fischer[8] from theobromine *via* 2,6-dichloro-7-methylpurine. The other isomer could then be assigned the 9-methyl configuration (XXXIX). The 9-methyladenine

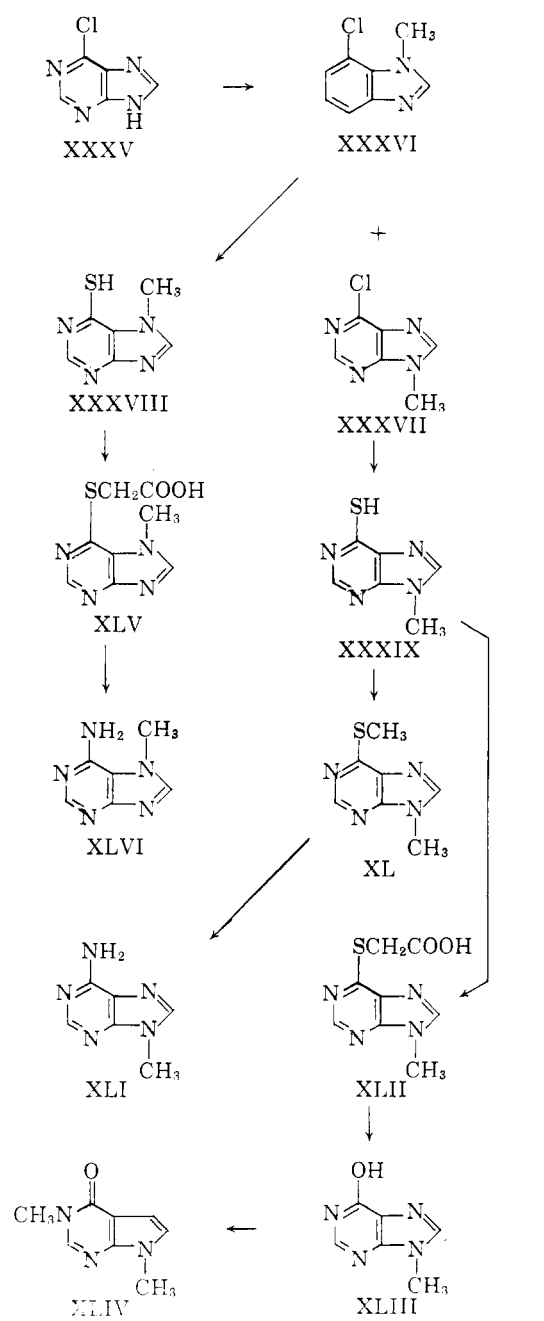


Figure 4

(XLI) prepared from XXXIX *via* the methylthio derivative (XL) was identical with that prepared from 6-amino-9-methyl-2-methylthiopurine.[45] Deamination of 9-methyladenine gave 9-methylhypoxanthine (XLIII), which was also obtained by treatment of XXXIX with chloroacetic acid, to give XLII, followed by acid hydrolysis. Methylation of 9-methylhypoxanthine gave a single compound which presumably is the 1,9-dimethyl derivative (XLIV). The 7-methylhypoxanthine used in the spectrophotometric and chromatographic studies was prepared by the method of

(44) A report recently appeared on the alkylation of 6-chloropurine with higher alkyl halides: J. A. Montgomery and C. Temple, Jr., *J. Am. Chem. Soc.*, **83**, 630 (1961)

(45) G. A. Howard, B. Lythgoe and A. R. Todd, *J. Chem. Soc.*, 556 (1945).

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA OF DERIVATIVES OF HYPOXANTHINE AND 6-MP

Compound	pH	λ_{max}	$E_m \times 10^{-3}$	pK_a[a]	$R_{adenine}$ Solvent A	R_f in Solvents B	C
Hypoxanthine	0	248	11.1	8.79 ± 0.04[b]	0.66	0.58	0.56
	5.12	249	11.1				
	11.08	258	11.3				
	14.0	263	11.7				
1-Methylhypoxanthine	0	249	9.4	8.85 ± 0.04	0.83	0.66	0.62
	5.12	251	9.4				
	11.08	260	9.7				
3-Methylhypoxanthine	0	253	11.0	2.61 ± 0.03	0.56	0.66	0.63
	5.12	264	14.0	8.34 ± 0.04			
	11.08	265	10.9				
	14.0	226; 264	6.6 ; 11.1				
7-Methylhypoxanthine	0	250	10.2	2.12 ± 0.05	0.81	0.61	0.55
	5.12	255.5	9.5	9.00 ± 0.03[c]			
	11.08	262	10.6				
9-Methylhypoxanthine	0	250	10.6[d]	9.15 ± 0.12[e]	0.73	0.64	0.62
	5.12	250	11.8[f]				
	11.08	254	12.4				
6-Methoxypurine	0	254	10.1	9.00 ± 0.02[g]	1.70	0.60	0.47
	5.12	252; 258[h]	9.9 ; 8.55				
	11.08	262	9.9				
Purine-6-thiol	0	326	19.0	<3	0.84	0.40	0.41
	1.02	325[i]	20.5	7.57 ± 0.04			
	5.12	229; 322	7.6 ; 22.2	11.01 ± 0.04[j]			
	8.73	231; 313	11.7 ; 19.3				
	12.89	231; 310	13.9 ; 20.3				
1-Methylpurine-6-thione	1.02	229; 321	11.2 ; 18.7	<3	0.95	0.47	0.44
	5.12	234; 320	9.4 ; 20.7	8.64 ± 0.07			
	11.08	237; 321	10.7 ; 24.0				
3-Methylpurine-6-thione	0	244; 334	5.5 ; 29.2	1.69 ± 0.05	0.54	0.44	0.51
	5.12	245; 338	9.15; 32.2	7.51 ± 0.02			
	11.08	245; 332	11.3 ; 29.9				
7-Methylpurine-6-thiol	0	327.5	17.7	7.92 ± 0.02	0.89	0.45	0.47
	5.12	329	20.4				
	11.08	232; 316	8.8 ; 17.5				
9-Methylpurine-6-thiol	0	326	18.5[k]	1.42 ± 0.04	0.60	..	0.50
	5.12	229; 321	12.7 ; 26.1	7.96 ± 0.02			
	11.08	234; 309	13.0 ; 21.4[k]				
6-Methylthiopurine	0	300	16.3	<1			
	1.02	294	15.4	8.56 ± 0.03[l]	0.94	0.45	0.29
	5.12	290	17.6				
	11.08	290	16.0[m]				

[a] Determined by the method described by W. Stenström and N. Goldsmith, *J. Phys. Chem.*, **30**, 1683 (1928). [b] Ref. 57 reports pK_a = 1.98, 8.94, 12.10. [c] A. G. Ogston, *J. Chem. Soc.*, 1713 (1936) reports pK_a = 8.8. [d] Ref. 55 reports a λ_{max} = 251 (E_m = 6300) at pH 1. [e] Ref. *c* above reports pK_a = 8.9; ref. 49 reports pK_a = 1.86, 9.32. [f] Ref. 49 reports λ_{max} = 250 mμ (E_m = 12,000) at pH 5.5. [g] Ref. 57 reports pK_a = 2.21, 9.16. [h] Inflection. [i] Ref. 52 erroneously reported λ_{max} = 327 mμ at pH 1. [j] Ref. 57 reports pK_a = <2.5, 7.77, 10.84. [k] Ref. 55 reports λ_{max} = 324 mμ (E_m = 19,600) at pH 1 and λ_{max} = 310 mμ (E_m = 16,800) at pH 11. [l] Ref. 57 reports pK_a = 0, 8.74. [m] Ref. 52 erroneously reported λ_{max} = 293 mμ at pH 11. Ref. 57 reports an E_m = 20,400 at 290 mμ at pH 11.1.

Fischer.[46] Treatment of 7-methylpurine-6-thiol with chloroacetic acid followed by ammonium hydroxide produced 7-methyladenine (XLVI).

For purposes of establishing the identity and purity of the various isomers described here the compounds were chromatographed in three different solvent systems (Tables I and II). In general, butanol (saturated with water) in an ammonia atmosphere[47] was the most satisfactory for distinguishing between 1- and 3-methyl isomers and between 7- and 9-methyl derivatives. However, because R_f values in this system proved to be very variable, probably because of variations in the concentration of ammonia vapor, adenine was run as a standard and the $R_{adenine}$ (R_f of compound / R_f of adenine) is reported.

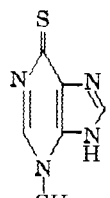
For the derivatives of hypoxanthine and 6-MP, spectra were run at a large number of pH values (Table I) to permit the calculation of pK values. For all the other purines spectral data are given at pH 1 and pH 11 only (Table II). It will be noted that hypoxanthine and 1-methylhypoxanthine have essentially the same pK_a for the dissociation of the first hydrogen, as have 7-methylhypoxanthine and 6-methoxypurine. The pK_a for the 3-methyl derivative is the lowest and that of 9-methylhypoxanthine the highest of the group. It is therefore difficult to assign the first ionization to a position on either the pyrimidine or imidazole ring or the hydroxyl group. Thus a hydrogen dissociates as

(46) E. Fischer, *Ber.*, **31**, 104 (1898).
(47) R. D. Hotchkiss, *J. Biol. Chem.*, **175**, 315 (1948).

easily from the imidazole ring (6-methoxypurine) as from the pyrimidine ring (or hydroxyl group) in 7-methylhypoxanthine. It is perhaps more accurate to consider the charges to be so distributed that one must think of the ionization of the purine ring system as a whole.

Not only does substitution of a methyl group at position 3 of hypoxanthine lower the pK noticeably, but this type of substitution likewise produces the largest bathochromic shift of any of the monomethylations. Methylation at 1 or 7 has little effect on the spectrum. In 9-methylhypoxanthine there is a 4-mμ shift in the maxima of both the neutral and the anionic forms compared to those of hypoxanthine. The resemblance between the ultraviolet and infrared spectra of hypoxanthine and 1-methylhypoxanthine has led to the conclusion that the neutral molecule of hypoxanthine exists mainly in the lactam form.[48]

In the 6-MP series, the pK_a is markedly increased by substitution either in the 1- position or on the sulfur, whereas substitution at 3, 7, or 9 has very little effect. Since in 1-methylpurine-6-thione and in 6-methylthiopurine the ionizable hydrogen must be on the imidazole ring, it is tempting to postulate that in each of the other molecules the proton dissociates from either the N-1 position or the sulfur. If the latter, the structure for 3-methylpurine-6-thione may not be the p-quinoid structure (VIIA) (analogous to the one suggested for 3-methylhypoxanthine)[49] but may have a structure like VII B.



VIIA

VIIB

In any event, the fact that the 3-methyl derivative has an ultraviolet absorption maximum at a higher wave length than any of the other derivatives suggests an arrangement of the double bonds in this molecule different from that in the others.

The absorption maximum of 1-methylpurine-6-thione is almost identical with that of the neutral form of 6-MP. This resemblance between the 1-methyl and the unmethylated compound is even more striking here than in the hypoxanthine series. The existence of unionized 6-MP in the 6-thione form is supported also by the infrared data[49] and the pronounced difference between the spectra of 6-methylthiopurine and 6-MP. Ionization of 1-methylpurine-6-thione has essentially no effect on the absorption maximum in the ultraviolet but a very pronounced hyperchromic effect on the extinction. The effect of methylation at position-7 is to shift the spectrum of 6-MP to slightly longer wave lengths, whereas the 9-substitution has a slightly hypsochromic effect.

No attempt has been made in these studies to fix the basic pK_a of these derivatives exactly. However, it is apparent from an inspection of the ultraviolet absorption maxima that protonation of 3-methylhypoxanthine occurs at a considerably higher pH (pK_a = 2.61) than for any of the other hypoxanthine derivatives. Among the derivatives of 6-MP, protonation likewise occurs most readily on the 3-methyl derivative.

In the methylated adenine derivatives there is very little change in the ultraviolet absorption spectra between pH 1 and pH 11, making spectrophotometric determination of the pK values difficult. As in the other series mentioned above, methylation at 3 produces a bathochromic shift, as does methylation at 7 or on the amino group. The spectrum of 9-methyladenine at pH 1 or pH 11 resembles the spectrum of adenine at pH 1.

In general, 3-methyl substitution of the purines shown in Table II has much more effect on the ultraviolet absorption spectrum than substitution at N-1. This is particularly noticeable in the 3-methyl derivative of guanine which, unlike any of the other guanine derivatives, has its principal absorption maximum at 265 mμ at pH 1 (with only an inflection at 245 mμ) and has only a single absorption maximum at pH 11.

Experimental

Ultraviolet Absorption Spectra.—The spectra were determined on either a model DU or DK-2 Beckman spectrophotometer with solutions containing 5 to 10 mg. per liter. The pH values listed in Tables I and II and used for the determination of pK_a values were obtained after 1 ml. of the stock solution of the compound was diluted to 10 ml. with the following: pH 0, 1 N hydrochloric acid; pH 1.02, 0.1 N hydrochloric acid; pH 2.06, 0.01 N hydrochloric acid; pH 3.06, 0.001 N hydrochloric acid; pH 5.12, acetate buffer; 6.86–8.92, phosphate or Tris buffer, pH 9–11.1, Sørensen glycine–sodium hydroxide buffer; pH 12.9, 0.1 N sodium hydroxide; pH 14.0, 1 N sodium hydroxide.

Paper Chromatography.—Chromatograms were run in ascending fashion on S and S #597 paper in the following solvent systems: solvent A, n-butyl alcohol saturated with water, ammonia atmosphere; solvent B, water containing 5% ammonium sulfate and 5% isopropyl alcohol; solvent C, 5% disodium phosphate–isoamyl alcohol; solvent D, 86% butanol; solvent E, n-butyl alcohol–water–acetic acid (5:4:1) (upper layer used).

3-Methyl-2-thioxanthine (II).—A mixture of 11.2 g. of 4-amino-5-formamido-2-mercapto-3-methylpyrimidine-2-one (I)[22] and 100 ml. of formamide was heated at 185° for 1.5 hr. After chilling, the precipitate of crude purine was collected, washed with water and acetone, and dried at room temperature (8.75 g., 85%). A portion was recrystallized from 500 parts of boiling water and dried at 100° for analysis.

Anal. Calcd. for $C_6H_6N_4OS$: C, 39.6; H, 3.30; N, 30.8. Found: C, 39.9; H, 3.63; N, 31.1.

(48) S. F. Mason, "The Chemistry and Biology of Purines," Ciba Foundation Symposium, G. E. W. Wolstenholme and C. M. O'Connor, eds., Little, Brown and Co., Boston, 1957, p. 60.

(49) D. J. Brown and S. F. Mason, *J. Chem. Soc.*, 682 (1957).

TABLE II

SPECTROPHOTOMETRIC AND CHROMATOGRAPHIC DATA

Purine Substituents					pH 1		pH 11		$R_{adenine}$	R_f in Solvent	
1	2	3	6	Other	λ_{max}	$E_m \times 10^{-3}$	λ_{max}	$E_m \times 10^{-3}$	Solvent A	B	C
			NH_2		263	13.1	267	11.7	1.00	0.40	0.36
		CH_3	NH		274	17.0	273	13.3	1.50	.69	.44
			NH_2	7-CH_3	273	14.0	268	10.6	1.36	.54	.50
			NH_2	9-CH_3[a]	260	13.7	261	14.0	1.95	.42	.40
			$NHCH_3$[b]		267	14.9	272	15.3	1.60	.45	.39
CH_3			O	9-CH_3	251.5	9.07	251.5	9.63	1.70	.71	..
	OH		OH		265	9.55[c]	240; 277	8.25; 9.10	0.02	.44	.50
CH_3	OH		O		265	9.80	242; 277	7.40; 8.70	0.45	.55	.55
	OH	CH_3	O		271	10.2	275	12.1	0.51	.55	.50
	SH		OH[d]		285	21.6	278	16.8	0.17	..	..
CH_3	SH		O		234; 283	15.1 ; 19.2	286	16.2	0.77	.32	.38
	SH	CH_3	O		285	19.4	240; 292	17.7 ; 18.3	1.38	.37	.35
	OH		SH[e]		263; 340	5.52; 23.5	248; 340	8.70; 21.5	0.19	..	.41
CH_3	OH		S		269; 331; 338	7.15; 17.4 ; 17.6	249; 340	7.75; 16.2	0.57	.31	.41
	OH	CH_3	S		245—265; 345	4.75; 27.7	253; 273; 336	6.15; 6.00; 24.3	0.92	.37	.39
	SH		SH[e]		255; 298; 350	11.7; 19.9 ; 14.2	253; 270–275[g]; 347	21.3 ; 15.1 ; 13.5	0.27	..	..
CH_3	SH		S		255; 298; 350	13.3; 22.8 ; 12.6	254; 280[g]; 347	18.8 ; 12.6 ; 11.5	0.78	..	.26
	SH	CH_3	S		254; 298; 352	13.2 ; 18.8 ; 16.0	257; 302; 342	13.1 ; 19.8 ; 19.0	1.15	..	.26
	CH_3S		OH[d]		265	16.4	270	14.1	0.51	.48	.40
CH_3	CH_3S		O		266	13.8	273	11.4	2.35	.50	.34
	CH_3S	CH_3	O		238; 278	15.7 ; 13.9	235; 282	20.3 ; 13.5	1.45	.52	.45
CH_3	CH_3S		S		238[g]; 266; 335; 340	12.5; 12.9; 12.7; 16.5	247; 331	21.6 ; 19.5	0.04	0	0
CH_3	$C_6H_5CH_2S$		O		270	14.3	275	12.2	..	..	..
CH_3	$C_6H_5CH_2S$		S		238[g]; 267; 335	1.27 ; 12.8 ; 15.8	247; 332	20.8 ; 18.0	3.61	..	..
	SH		NH_2[h]		240; 285	17.5 ; 19.5	244; 276; 298	20.9; 10.2 ; 10.7	0.57	..	0.26
	S	CH_3	NH_2		240; 284	18.8 ; 19.2	250; 305	29.0 ; 15.8	0.93	0.45	.26
	NH_2		OH		248; 273[g]	11.5 ; 7.40	245; 273	6.80; 7.40	0.23	.45	.42
CH_3	NH_2		O		251; 272	9.95; 6.80	256; 275	7.50; 8.60	0.80	.49	.45
	NH_2	CH_3	O		245[g]; 265	8.06; 10.9	274	13.0	0.43	.50	.48
	$NHCH_3$		OH[d]		250; 280	12.3 ; 6.90	245; 279	8.00; 7.25	0.82	.51	.53
	NH_2		NH_2		241; 282	10.1 ; 9.75	245; 282	6.30; 9.05	0.73	.37	.25
	CH_3NH		NH_2[i]		242; 289	12.3; 8.90	247; 287	7.20; 6.92	1.60	.45	.23
	NH_2		$NHCH_3$[j]		246; 280	11.3 ; 12.7	256; 284	6.80; 12.0	1.85	.50	.31

[a] Ref. 55 reports λ_{max} = 261 mμ (E_m = 14,600) at pH 1, λ_{max} = 262 mμ (E_m = 11,900) at pH 11. [b] Ref. 52. [c] Cf. L. F. Cavalieri, J. J. Fox, A. Stone, and N. Chang, *J. Am. Chem. Soc.*, **76**, 1119 (1954). [d] G. B. Elion, W. H. Lange, and G. H. Hitchings, *J. Am. Chem. Soc.*, **78**, 217 (1956). [e] Ref. 28. [f] Shoulder. [g] Inflection. [h] Ref. 23. [i] Ref. 34 reports λ_{max} = 240 (inflection), 288 mμ (E_m = 10,900, 7800) at pH 1, λ_{max} = 289.5 (E_m = 7190) in 0.1 *N* sodium hydroxide. Ref. 35 reports λ_{max} = 215, 240, 288 mμ at pH 2 and λ_{max} = 221, 245, 288 mμ at pH 11 for the compound eluted from a chromatogram. [j] Ref. 34 reports λ_{max} = 246, 277 mμ (E_m = 12,500; 13,700) at pH 1 and λ_{max} = 282 mμ (E_m = 13,700 in 0.1 *N* sodium hydroxide).

3-Methyl-2,6-dithioxanthine (III).—A mixture of 5.6 g. of 3-methyl-2-thioxanthine (II), 20 g. of powdered phosphorus pentasulfide, and 250 ml. of dry pyridine was heated under reflux conditions for 3 hr. The pyridine was removed under reduced pressure and the residue was heated for 15 min. with 200 ml. of water. After chilling, the precipitate was collected and the product was purified by solution in 300 ml. of water containing 20 ml. concentrated ammonium hydroxide, filtration, and acidification to a pH value of 4 with hydrochloric acid. The compound did not melt below 340° (3.2 g., 52.5%).

Anal. Calcd. for $C_6H_6N_4S_2$: C, 36.4; H, 3.03; N, 28.3; S, 32.3. Found: C, 36.3; H, 3.30; N, 28.2; S, 32.3.

4-Amino-5-formamido-3-methylpyrimidine-6-one (IV).—A mixture of 6 g. of 4-amino-5-formamido-2-mercapto-3-methylpyrimidine-6-one (I), 200 ml. of water, 5 ml. of concentrated ammonium hydroxide, and 10 ml. of a settled suspension of W-6 Raney nickel catalyst was heated under reflux conditions for 2 hr. The Raney nickel was removed by filtration and was washed with five 100 ml. portions of boiling water. The combined filtrate and washings were evaporated to dryness under reduced pressure. The residue was taken up in 40 ml. of warm water; upon chilling, colorless white needles of 3-methylhypoxanthine separated and were collected (0.9 g., m.p. 301–302° dec.). Since the ultraviolet absorption spectrum of the filtrate (λ_{max} = 258 mμ at pH 1 and λ_{max} = 270 mμ at pH 11) indicated that some deformylation had occurred, the solutionwas evaporated to dryness and the residue was heated under reflux conditions with 50 ml. of 98% formic acid for 2 hr. Upon evaporation to dryness, a residue was obtained which was dissolved in 20 ml. of water. By the addition of 10 parts of acetone to this solution a precipitate (0.9 g.) was obtained which was a mixture of 3-methylhypoxanthine and the desired formamidopyrimidine (IV). A small amount (350 mg.) of IV was eventually isolated from the filtrate by evaporation to a volume of 5 ml. and addition of 20 ml. of acetone. Further purification of IV was accomplished by a repeated solution in 5 ml. of water and reprecipitation with 50 ml. of acetone. The purified product darkened above 200° and melted at 275–280° when put into a hot block. The ultraviolet absorption spectrum showed λ_{max} = 263 mμ (E_m = 7850) at pH 1; λ_{max} = 259 mμ (E_m = 5800) at pH 11. In solvent A, the $R_{adenine}$ was 0.31; in solvent C the R_f = 0.74.

Anal. Calcd. for $C_6H_8N_4O_2$: C, 42.9; H, 4.75; N, 33.3. Found: C, 42.6; H, 4.85; N, 32.9.

3-Methylhypoxanthine (V).—To a suspension of 5 g. (0.0275 mole) of 3-methyl-2-thioxanthine (II) in 200 ml. of water was added 10 ml. of concentrated ammonium hydroxide and 15 ml. of settled W-6 Raney nickel catalyst. The mixture was heated under reflux conditions for 2 hr. The Raney nickel was filtered from the hot solution and washed four times with 150-ml. portions of boiling water. The combined filtrate and washings were taken to dryness under reduced pressure. The crude product (3.6 g., 87%) was recrystallized from 120 ml. of boiling water containing a few drops of acetic acid and decolorized with Darco. The crystalline precipitate (2.7 g.) was collected, washed with water, and dried at 110° for analysis: m.p. 307–309° dec. Another 0.8 g. of product was recovered from the mother liquors by evaporation of the solvent.

Anal. Calcd. for $C_6H_6N_4O$: C, 48.0; H, 4.0; N, 37.3. Found: C, 47.6; H, 4.18; N, 36.8.

6-Amino-3-methylpurine-2-thione (VI).—A mixture of 5 g. (0.025 mole) of 3-methyl-2,6-dithioxanthine (III) and 50 ml. of concentrated ammonium hydroxide was heated in a sealed tube at 140° for 24 hr. After cooling, the mixture was diluted with 20 ml. of water and adjusted to a pH value of 8 by the addition of hydrochloric acid. The precipitate of crude 6-amino-3-methylpurine-2-thione was collected and purified by solution in 200 ml. of 0.05 *N* sodium hydroxide and acidification with acetic acid to a pH value of 6 (2.2 g., 45.5%). The product did not melt below 300°.

Anal. Calcd. for $C_6H_7N_5S$: C, 39.8; H, 3.86; N, 38.7. Found: C, 40.0; H, 4.02; N, 38.5.

3-Methylpurine-6-thione (VII).—A mixture of 4.3 g. of 3-methylhypoxanthine (V), 12 g. of powdered phosphorus pentasulfide, and 100 ml. of dry pyridine was heated under reflux conditions for 2.5 hr. The pyridine was removed under reduced pressure and the residue was heated for 15 min. with 200 ml. of water. The aqueous suspension was chilled and filtered. The crude precipitate of 3-methylpurine-6-thione was recrystallized from 750 ml. of boiling water with the addition of Darco. The yellow needles (2.35 g., 50%) melted at 322–323° dec.

Anal. Calcd. for $C_6H_6N_4S$: C, 43.3; H, 3.61; N, 33.7; S, 19.3. Found: C, 43.4; H, 3.93; N, 24.1; S, 19.2.

3-Methyladenine (VIII). A. From VI.—A mixture of 3.55 g. (0.0196 mole) of 6-amino-3-methylpurine-2-thione (VI), 250 ml. of water, 20 ml. of concentrated ammonium hydroxide, and 10 ml. of settled Raney nickel catalyst was heated under reflux conditions for 2.5 hr. The reaction mixture was then treated as described above for 3-methylhypoxanthine (V). The crude product, after recrystallization from 80 ml. of absolute ethanol, gave colorless crystals (1.45 g.) melting at 291–292° dec. An additional 0.9 g. of 3-methyladenine was recovered by evaporation of the alcoholic filtrate; the total yield was therefore 80%.

Anal. Calcd. for $C_6H_7N_5$: C, 48.3; H, 4.68; N, 47.0. Found: C, 48.3; H, 4.76; N, 47.2.

B. From VII.—A solution of 4 g. of 3-methylpurine-6-thione (VII) in 50 ml. of concentrated ammonium hydroxide was heated in a sealed tube at 140° for 24 hr. After cooling, the reaction mixture was filtered to remove a precipitate (0.5 g.) which proved to be a mixture of starting material and 3-methyladenine on spectrophotometric examination. The filtrate was taken to dryness under reduced pressure leaving a dark sirupy residue. This residue was dissolved in 100 ml. of absolute ethanol and filtered to remove a blue-green residue. To this solution was added 20 ml. of hydrogen chloride–saturated ethanol and 400 ml. of anhydrous ether. The pale green precipitate which formed was collected, washed with ether, and dried in a vacuum desiccator (1.7 g.). The ultraviolet absorption spectrum showed this precipitate to contain 60% 3-methyladenine; chromatography revealed the presence of at least two unknown impurities. Since the synthesis by method A had proved satisfactory, the further isolation of 3-methyladenine from the mixture was not pursued.

3-Methyl-2-methylthiopurine-6-one (IX).—To a solution of 10 g. (0.055 mole) of 3-methyl-2-thioxanthine (II) in 55 ml. of 2 *N* aqueous sodium hydroxide was added dropwise, with stirring, 5.87 ml. (0.0605 mole) of dimethyl sulfate. The temperature of the reaction mixture was kept between 20 and 30° throughout the addition. One hour after all the dimethyl sulfate had been added, the mixture was neutralized with acetic acid, chilled, and filtered. The crude product (7.25 g., 67%) melted at 300–305° (dec.). After two recrystallizations from 200 parts of water, with the addition of Darco, the melting point was raised to 321°.

Anal. Calcd. for $C_7H_8N_4OS$: C, 42.9; H, 4.08; N, 28.6. Found: C, 42.7; H, 4.15; N, 28.7.

3-Methylxanthine (X).—A mixture of 5 g. of 3-methyl-2-methylthiopurine-6-one and 200 ml. of 6*N* hydrochloric acid was heated under reflux conditions for 3 days. The mixture was then evaporated to dryness and the residue, after being leached with 100 ml. of water, was washed with acetone and dried. The product (3.2 g., 76%) was identified as 3-methylxanthine by its ultraviolet absorption spectrum and paper chromatography. It was identical in all respects with an authentic sample prepared by complete synthesis from *N*-methylurea and ethylcyanoacetate by the method of Traube.[20]

3-Methylguanine (XI).—A mixture of 0.5 g. of 3-methyl-2-methylthiopurine-6-one (IX) and 5 ml. of formamide was heated in a test tube in an oil bath at 190–200° for 30 min. Evolution of methyl mercaptan was detected when the

temperature reached 160°; at the end of the reaction time the smell of methyl mercaptan was no longer apparent. The mixture was cooled and the precipitate of crude 3-methylguanine was collected and washed with formamide and acetone. The precipitate was purified by solution in 30 ml. of 0.07 *N* hydrochloric acid, treatment with Darco, and reprecipitation at pH 7.5 by the addition of ammonium hydroxide (0.2 g., 48%). A 100-mg. portion of the product was converted to the sulfate by solution in 2.5 ml. of hot 2 *N* sulfuric acid, treatment with Darco, dilution of the filtrate with 7.5 ml. of ethanol, and chilling. The crystalline sulfate was collected by centrifugation, washed with alcohol, and dried in a vacuum desiccator.

Anal. Calcd. for $C_6H_7N_5O \cdot 1/2\ H_2SO_4 \cdot H_2O$: C, 31.1; H, 4.32; N, 30.1; H_2O, 7.75. Found: C, 31.2; H, 4.18; N, 30.1; H_2O (140°), 7.85, regained 4.4% on exposure to air.

3-Methyl-6-thioxanthine (XII).—A mixture of 3 g. of 3-methylxanthine (X), 5 g. of powdered phosphorus pentasulfide, and 50 ml. of dry pyridine was heated under reflux conditions for 3 hr. and then evaporated to dryness under reduced pressure. The residue was boiled with 100 ml. of water for 15 min. and filtered hot. The filtrate was chilled and the yellow precipitate of 3-methyl-6-thioxanthine (0.66 g., 60%) was collected. A portion of the product was recrystallized from 12 parts of boiling water and dried at 100° for analysis, m.p. 320–322° dec.

Anal. Calcd. for $C_6H_6N_4OS$: C, 39.6; H, 3.30; N, 30.8. Found: C, 39.4; H, 3.40; N, 31.0.

4-Amino-5-formamidopyrimidine-6-ol (XIV).[50]—A mixture of 17.5 g. (0.094 mole) of 4-amino-5-formamido-2-mercaptopyrimidine-6-ol (XIII),[22] 5 g. (0.047 mole) sodium carbonate, 50 g. of Raney nickel (W-6) suspension, and 300 ml. of water was heated under reflux conditions for 2 hr. After the Raney nickel had been removed by filtration, the filtrate was adjusted to pH 5 with acetic acid and chilled. The precipitate of 4-amino-5-formamidopyrimidine-6-ol was collected, washed with cold water, and dried in a vacuum desiccator (9.5 g., 65.5%). The Raney nickel was washed repeatedly with a total of 200 ml. of boiling water. These washings and the mother liquor were combined and concentrated, yielding an additional 2.1 g. (14.5%) of product. Recrystallization from 50 parts of boiling water gave an analytical sample, m.p. 287–288° dec., which showed an $R_{adenine} = 0.13$ in solvent A, $R_f = 0.72$ in solvent C. The ultraviolet absorption spectrum shows $\lambda_{max} = 258$ mμ ($E_m = 8700$) at pH 1; $\lambda_{max} = 254$ mμ ($E_m = 5400$) at pH 11.

Anal. Calcd. for $C_5H_6N_4O_2$: N, 36.3. Found: N, 36.1.

4-Amino-5-formamido-1-methylpyrimidine-6-one (XV).—To a solution of 46.2 g. (0.3 mole) of 4-amino-5-formamidopyrimidine-6-ol (XIV) in 300 ml. of 1 *N* aqueous sodium hydroxide was added dropwise, over a 1 hr. period, with stirring, 30 ml. (0.32 mole) of dimethyl sulfate. The mixture was allowed to stand at 25° overnight, and was then chilled and filtered. The precipitate was washed with 100 ml. of cold water and dried at 100° (43.1 g., 86%), m.p. 244° dec. After recrystallization from 10 parts of hot water, the m.p. was raised to 245–247° dec. The ultraviolet absorption spectrum shows $\lambda_{max} = 260$ mμ ($E_m = 6050$) at pH 1; $\lambda_{max} = 261$ mμ ($E_m = 5300$) at pH 11. In solvent A the $R_{adenine} = 0.58$; in solvent C the $R_f = 0.76$.

Anal. Calcd. for $C_6H_8N_4O_2$: C, 42.9; H, 4.75; N, 33.3. Found: C, 42.6; H, 4.72; N, 33.3.

1-Methylhypoxanthine(XVI).—A mixture of 80 g. of 4-amino-5-formamido-1-methylpyrimidine-6-one (XV) and 250 ml. of 98–100% formic acid was heated under reflux conditions for 4 hr. and then evaporated to dryness on the steam bath. The residue was taken up in 150 ml. of water, neutralized with ammonium hydroxide, chilled, and filtered. The precipitate was dried at 110° (60 g., 84.5%). This material was 95% pure as judged by its ultraviolet absorption spectrum. After recrystallization from 30 parts of 50% aqueous methanol with the addition of Darco, the compound melted at 311–312°.

Anal. Calcd. for $C_6H_6N_4O$: C, 48.0; H, 4.0; N, 37.3. Found: C, 47.8; H, 3.57; N, 36.9.

1-Methylpurine-6-thione (XVII).—A mixture of 15 g. of 1-methylhypoxanthine, 60 g. of powdered phosphorus pentasulfide, and 250 ml. of dry pyridine was heated under reflux conditions for 3 hr. The reaction mixture was treated as described above for 3-methylpurine-6-thione (VII) except that, after treatment with hot water, the mixture was adjusted to pH 4 with hydrochloric acid in order to obtain complete precipitation of the product. The crude product, after recrystallization from 1 l. of boiling water, with Darco, formed yellow crystals, m.p. 288–289° dec. (9 g., 54%).

Anal. Calcd. for $C_6H_6N_4S$: C, 43.4; H, 3.61; N, 33.7; S, 19.3. Found: C, 43.4; H, 3.69; N, 33.7; S, 19.2.

Attempted Preparations of 1-Methyladenine from 1-Methylpurine-6-thione. A. Aqueous Ammonium Hydroxide.—A solution of 4 g. of 1-methylpurine-6-thione (XVII) in 80 ml. of concentrated ammonium hydroxide was heated in a sealed tube at 145° for 24 hr. The ultraviolet absorption spectrum of the reaction mixture at pH 1 showed the presence of two maxima at 267 mμ and 320 mμ with a ratio of o.d. 267 mμ/o.d. 320 mμ = 2.9. The absorption band at 320 mμ was due to starting material. The reaction mixture was heated on the steam bath until no more ammonia gas escaped. The solution (pH 7.5) was then diluted to 50 ml. with water and put through a Dowex-50 (H^+) column (22 mm. in diameter × 50 mm. in height). Elution with 0.5 *N* hydrochloric acid removed the material with the $\lambda_{max} = 267$ mμ first; later fractions contained a mixture of this and starting material. The first 400 ml. of the eluate were evaporated to dryness under reduced pressure to give a solid residue at 2.95 g. (The following 300 ml. of eluate gave 0.52 g. of residue; subsequent fractions gave a total of 1.3 g. of material.) Two recrystallizations of this residue (60% pure by spectrophotometric assay) from 100-ml. portions of absolute ethanol gave 0.6 g. of a material with a m.p. = 253–254°. This was identified by melting point (255–256°[51]), analysis, ultraviolet absorption spectrum and paper chromatography as 4-amino-5-imidazolecarboxamide hydrochloride. The ultraviolet absorption spectrum showed $\lambda_{max} = 240, 267$ mμ (E_m 7700, 9750) at pH 1; $\lambda_{max} = 269$ mμ ($E_m = 10{,}800$) at pH 11. It gave the following R_f values, identical with those of an authentic sample of 4-amino-5-imidazolecarboxamide hydrochloride made by the method of Shaw[51]: solvent A, $R_{adenine} = 1.16$, solvent B, $R_f = 0.59$; solvent C, $R_f = 0.59$.

Anal. Calcd. for $C_4H_6N_4O \cdot HCl$: C, 29.5; H, 4.30; N, 34.5; Cl, 21.8. Found: C, 29.6; H, 4.62; N, 35.0; Cl, 21.9.

B. Alcoholic Ammonia.—A mixture of 2 g. of 1-methylpurine-6-thione and 50 ml. of ethanolic ammonia (saturated at 0°) was heated in a sealed tube at 155° for 24 hr. After cooling, the reaction mixture showed an ultraviolet absorption spectrum with two maxima at pH 1: 265 mμ and 320 mμ $\frac{\text{o.d. at 265 mμ}}{\text{o.d. at 320 mμ}} = 0.63$. (This was mainly unchanged 1-methylpurine-6-thione with a small amount of a new product absorbing at 265 mμ). After removal of the excess ammonia in an air stream, the mixture was filtered to remove 0.3 g. of unchanged material. The filtrate was taken to dryness and the residue was leached with 35 ml. of absolute ethanol, which left undissolved another 0.3 g. of starting material. From the filtrate, which was made acidic with alcoholic hydrogen chloride, was eventually isolated by repeated fractional recrystallizations 100 mg. of a product

(50) This compound has also been prepared by the formylation of 4,5-diaminopyrimidine-2-ol: L. F. Cavalieri and A. Bendich, *J. Am. Chem. Soc.*, **72**, 2587 (1950).

(51) E. Shaw and D. W. Woolley, *J. Biol. Chem.*, **181**, 89 (1949).

which, although still not analytically pure, was identified as 6-methylaminopurine hydrochloride. It melted at 281° (dec.); authentic 6-methylaminopurine hydrochloride[52] gives a m.p. = 289°; mixed m.p. = 283–284°. A 50-mg. portion of the hydrochloride was converted to the free base by solution in 3 ml. of water and addition of ammonium hydroxide; the free base melted at 304–305° dec., authentic 6-methylaminopurine, m.p. 312–314°; mixed m.p. = 303–305° dec. The compound had an ultraviolet absorption spectrum identical with that of the authentic specimen and also showed the same R_f values as 6-methylaminopurine in three chromatographic solvents (Table II). In a subsequent experiment, in which 1 g. of 1-methylpurine-6-thione was heated with ethanolic ammonia at 165° for 3 days, the reaction mixture showed a spectrum with $\frac{\text{o.d. } 265\ m\mu}{\text{o.d. } 320\ m\mu} = 4.7$. Removal of the ammonia and addition of 2 ml. of saturated ethanolic hydrogen chloride and 50 ml. of ether gave a brown precipitate (300 mg.), which on spectrophotometric and chromatographic examination showed the presence of adenine and 1-methylpurine-6-thione in the proportions of 7:1. The aminopurine was identified as adenine by its absorption spectrum, chromatographic behavior in four solvent systems, and the mixed m.p. of its picrate with that of adenine picrate (m.p. 281°, mixed m.p. 280–281°).

4-Amino-5-formamido-1-methyl-2-methylthiopyrimidine-6-one (XVIII).—To a solution of 93.2 g. (0.5 mole) of 4-amino-5-formamido-2-mercaptopyrimidine-6-ol (XIII) in 1 l. of 1 *N* aqueous sodium hydroxide was added with stirring over a 3-hr. period 106.5 ml. (1.14 moles) of dimethyl sulfate. The reaction mixture was allowed to stir at room temperature for an additional 4 hr., adjusted to a pH value of 7, chilled, and filtered. The crude precipitate (m.p. 234–236°) was recrystallized from 900 ml. of boiling water to give 30.3 g. (28.6%) of pure product, m.p. 256–257°. An additional 20.7 g. (19%) of slightly impure product, m.p. 245–250°, was obtained by concentration of the mother liquors of the recrystallization to 300 ml. and chilling. The ultraviolet absorption spectrum showed λ_{max} = 231, 275 mμ at both pH 1 and pH 11. (At pH 1: E_m = 21,200, 10,000; at pH 11: E_m = 19,000, 9700). In solvent A, the $R_{adenine}$ = 2.41; in solvent B, R_f = 0.73; in solvent C, R_f = 0.68.

Anal. Calcd. for $C_7H_{10}N_4O_2S$: C, 39.3; H, 4.67; N, 26.2; S, 15.0. Found: C, 39.3; H, 4.45; N, 25.8; S, 15.2.

1-Methyl-2-methylthiopurine-6-one (XIX). A. Formamide Method.—A mixture of 2.5 g. of 4-amino-5-formamido-1-methyl-2-methylthiopyrimidine-6-one (XVIII) and 20 ml. of formamide was heated in an oil bath at 210–230° for 1 hr. After cooling, the solution was diluted with an equal volume of water and chilled. The gray precipitate was collected, washed with water, and dried at 100° (1.65 g.); it melted at 273–275° dec. After recrystallization from 250 ml. of boiling water containing Darco and a few drops of acetic acid, the product (0.9 g., 42%) melted at 275–277° dec. The spectra of mother liquors from the reaction mixture and from the recrystallization indicated the presence of 1-methylguanine.

Anal. Calcd. for $C_7H_8N_4OS$: C, 42.9; H, 4.08; N, 28.6; S, 16.3. Found: C, 42.9; H, 3.92; N, 29.0; S, 15.9.

B. Formic Acid Method.—A mixture of 2 g. of 4-amino-5-formamido-1-methyl-2-methylthiopyrimidine-6-one (XVIII) and 45 ml. of 98–100% formic acid was heated under reflux conditions for 18 hr. and then was evaporated to dryness. No odor of methyl mercaptan could be detected throughout this time. The residue was leached with 50 ml. of cold water and dried at 100°. The product (1.6 g., 87.5%) melted at 276° dec. and was identical with that of method A above.

1-Methylxanthine (XX).—A solution of 1.1 g. of 1-methyl-2-methylthiopurine-6-one (XIX) in 20 ml. of 6*N* hydrochloric acid was heated under reflux conditions for 24 hr. and then evaporated to dryness. The residue was recrystallized from 150 ml. of boiling water containing 0.5 ml. of concentrated hydrochloric acid. This product (0.65 g., 77%) was identical chromatographically and spectrophotometrically with 1-methylxanthine prepared by the deamination of 1-methylguanine.[33]

Anal. Calcd. for $C_6H_6N_4O_2$: N, 33.7. Found: N, 33.3.

1-Methylguanine (XXI).—The mother liquors of the preparation and purification of XIX by the formamide method (see above) upon standing for several days in the cold deposited 0.58 g. of a product with the spectral characteristics of 1-methylguanine. It was converted to the sulfate by solution in 25 ml. of hot 2 *N* sulfuric acid, treatment with Darco, filtration, and chilling. The crystalline precipitate was collected, washed with acetone, and dried in a vacuum desiccator (0.4 g.). It was identical chromatographically and spectrophotometrically with a sample of 1-methylguanine prepared by the method of Traube.[33]

Anal. Calcd. for $C_6H_7N_5O \cdot 1/2H_2SO_4$: C, 33.6; H, 3.74; N, 32.7. Found: C, 33.3; H, 4.01; N, 32.5.

1-Methyl-2-methylthiopurine-6-thione (XXII).—A mixture of 25 g. (0.117 mole) of 4-amino-5-formamido-1-methyl-2-methylthiopyrimidine-6-one (XVIII), 100 g. of phosphorus pentasulfide, and 1 l. of dried pyridine was heated under reflux conditions for 5 hr. The reaction mixture was treated as described above for 3-methylpurine-6-thione (VII). The crude product was purified by solution in 400 ml. of *N* aqueous sodium hydroxide, filtration, and precipitation by acidification to a pH value of 5 with acetic acid (18.4 g., 74%). This material was 96% pure as determined by ultraviolet absorption spectrophotometry. For analysis, a sample was purified by a repetition of the above reprecipitation procedure and dried at 100°, m.p. 310–315° dec.

Anal. Calcd. for $C_7H_8N_4S_2$: C, 39.6; H, 3.77; N, 26.4. Found: C, 40.1; H, 3.85; N, 26.2.

Attempted Demethylation of 1-Methyl-2-methylthiopurine-6-thione (XXII).—A suspension of 2.2 g. of 1-methyl-2-methylthiopurine-6-thione in 250 ml. glacial acetic acid containing 5 ml. of acetic anhydride was heated on the steam bath in a three-necked flask, supplied with a condenser and drying tube. Hydrogen iodide gas, generated in a separate flask by adding 30 ml. of acetic anhydride slowly to 10 ml. of 47% hydriodic acid and heating, was passed into the reaction mixture. The mixture was heated on the steam bath for 1 hr., during which time some of the solid dissolved. After standing overnight at room temperature the precipitate was collected, washed with acetone, and dried in a vacuum desiccator. The precipitate (1.7 g.) proved to be unchanged starting material.

1-Methyl-6-thioxanthine (XXIII).—A suspension of 5 g. of 1-methyl-2-methylthiopurine-6-thione (XXII) in 150 ml. of 6 *N* hydrochloric acid was heated under reflux conditions for 24 hr. The mixture was evaporated to dryness in an open dish on the steam bath. The residue was purified by solution in 100 ml. of water and 10 ml. of concentrated ammonium hydroxide, removal of a small insoluble residue, and acidification of the filtrate to a pH value of 5 with acetic acid. The pale yellow precipitate of 1-methyl-6-thioxanthine (2.7 g., 43%) melted at 323–325° dec. The ultraviolet absorption spectrum of the mother liquors from the recrystallization indicated the presence of some 1-methylxanthine as well as 1-methyl-6-thioxanthine, so that a small amount of the 6-mercapto group was apparently removed by the hydrolysis.

Anal. Calcd. for $C_6H_6N_4OS$: C, 39.6; H, 3.30; N, 30.8. Found: C, 40.1; H, 3.46; N, 30.9.

(52) G. B. Elion, E. Burgi, and G. H. Hitchings, *J. Am. Chem. Soc.*, **74**, 411 (1952).

6-Amino-2-methylaminopurine (XXIV).—A solution of 5 g. of 1-methyl-2-methylthiopurine-6-thione (XXII) in 50 ml. of concentrated ammonium hydroxide was heated in a sealed tube at 150° for 18 hr. The precipitate which formed in the reaction mixture upon cooling was collected (1.3 g.) and found to have a 13% contamination with starting material as judged by the ultraviolet absorption spectrum. Acidification of the reaction mixture filtrate to a pH value of 5 with acetic acid gave a second precipitate (1.75 g.) which contained 6-amino-2-methylaminopurine mixed with 16% starting material. The two precipitates were combined and recrystallized twice from boiling water (70 parts of water in the first recrystallization and 100 parts in the second) with Darco treatment. After these recrystallizations the product (1.2 g., 39%) was essentially free of starting material as judged by chromatography and ultraviolet absorption spectrum but still gave a low nitrogen analysis (Found: N = 48.8; calcd.: N = 51.2). Final purification was achieved on a small sample by a third recrystallization from 150 parts of boiling water followed by the isolation of the sulfate. For conversion to the sulfate, 100 mg. of the base was dissolved in 5.5 ml. of hot 2 *N* sulfuric acid, treated with Darco, filtered, and chilled. The cold solution was diluted with 5 ml. of absolute ethanol, the crystalline sulfate was centrifuged, washed with ethanol and ether, and dried in a vacuum desiccator. Chromatographic and spectrophotometric data (Table II) showed this to be the salt of 6-amino-2-methylaminopurine.[34]

Anal. Calcd. for $C_6H_8N_6 \cdot 1/2\ H_2SO_4 \cdot 1\ 1/2\ H_2O$: C, 30.0; H, 5.0; N, 34.9; H_2O, 11.2. Found: C, 29.43; H, 5.31; N, 34.6; H_2O (140°), 10.6, regains moisture on exposure to air.

Deamination of XXIV.—To establish unequivocally the identity of XXIV as 6-amino-2-methylaminopurine, deamination studies were carried out. Samples of XXIV and of authentic 6-amino-2-methylaminopurine[34] were deaminated concurrently both by the method of Remy,[35] which employs a large excess of barium nitrite in aqueous acetic acid, and by the use of slightly more than a molecular equivalent of sodium nitrite in dilute hydrochloric acid. In the latter procedure, 0.1 mmole of the compound was dissolved in 5 ml. of water and 3 ml. of 0.1 *N* hydrochloric acid, heated to 60°, and 1.2 ml. of a 0.1 *N* sodium nitrite solution was added. The mixture was allowed to cool slowly to room temperature and was kept at 25° overnight. The precipitate which formed was collected and chromatographed in five solvent systems, side by side with samples of 2-methylaminopurine-6-ol and 1-methylguanine. The principal spots of both deamination products were found at the R_f values for 2-methylaminopurine-6-ol in solvents A, B, and C (*cf.* Table II) and at $R_f = 0.38$ in D and $R_f = 0.52$ in E. For 2-methylaminopurine-6-ol in D, $R_f = 0.38$; in E, $R_f = 0.50$; for 1-methylguanine in D, $R_f = 0.24$; in E, $R_f = 0.41$. The identity of the deamination product as 2-methylaminopurine-6-ol was further confirmed by elution from paper with 0.1 *N* hydrochloric acid and determination of the ultraviolet absorption spectrum. In the acetic acid deamination mixture a second spot (possibly due to *N*-nitroso derivative) was seen on the chromatograms ($R_{adenine} = 0.08$ in A; $R_f = 0.82$ in B; $R_f = 0.05$ in D; and $R_f = 0.23$ in E) but was not identified. In the products from the hydrochloric acid deamination procedure, some unchanged 6-amino-2-methylaminopurine was detected on the chromatograms.

4-Amino-2-benzylthio-5-formamidopyrimidine-6-ol (XXVIII).—To a solution of 20 g. (0.108 mole) of 4-amino-5-formamido-2-mercaptopyrimidine-6-ol (XIII) in 230 ml. of *N* sodium hydroxide was added over a 2-hr. period, with stirring, 14.2 ml. (0.123 mole) of benzyl chloride. After an additional 5 hr. of stirring, the mixture was adjusted to a pH value of 5 with acetic acid, chilled, and filtered. The crude product was recrystallized from 3 l. of absolute ethanol with the addition of Darco (11.5 g., 37.5%), m.p. 236–237° dec. An additional 6 g. (19.5%) of product was recovered from the alcoholic mother liquors by evaporation to dryness.

Anal. Calcd. for $C_{12}H_{12}N_4O_2S \cdot 1/2H_2O$: C, 50.5; H, 4.56; N, 19.65; H_2O, 3.17. Found: C, 50.2; H, 5.10; N (Dumas), 19.6; H_2O (120°) = 3.23. The compound has an ultraviolet absorption spectrum with $\lambda_{max} = 277$ mμ (E_m = 10,100) at pH 1; $\lambda_{max} = 265$ mμ (E_m = 9400) at pH 11.

4-Amino-2-benzylthio-5-formamido-1-methylpyrimidine-6-one (XXIX).—To a solution of 2.8 g. (0.013 mole) of 4-amino-2-benzylthio-5-formamidopyrimidine-6-ol (XXVIII) in 13 ml. of *N* sodium hydroxide and 7 ml. of water was added slowly, with stirring, 1.2 ml. (0.013 mole) of dimethyl sulfate. After 3 hr. of stirring the precipitate was collected, washed with water and alcohol and dried in a vacuum desiccator (1.5 g., 40%). After recrystallization from 500 parts of absolute ethanol, the product melted at 263–264°.

Anal. Calcd. for $C_{13}H_{14}N_4O_2S$: C, 53.8; H, 4.83; N, 19.3. Found: C, 53.9; H, 4.74; N, 19.4.

2-Benzylthio-1-methylpurine-6-one (XXX).—A mixture of 10.2 g. of 4-amino-2-benzylthio-5-formamido-1-methylpyrimidine-6-one (XXIX) and 400 ml. of 98–100% formic acid was heated under reflux conditions for 16 hr. and then evaporated to dryness under reduced pressure. The residue was leached with 100 ml. of ethanol, collected, and dried in a vacuum desiccator (6.0 g., 62.5%), sinters at 237°, melts at 244–246° dec. This material was used for the preparation of 1-methyl-2-thioxanthine without further purification. A small sample was recrystallized from aqueous ethanol, whereupon the melting point was raised to 260–261° dec. (after sintering at 250°).

Anal. Calcd. for $C_{13}H_{12}N_4OS$: N, 20.6. Found: N, 20.3.

1-Methyl-2-thioxanthine (XXXI).—To a solution of 6 g. (0.022 mole) of 2-benzylthio-1-methylpurine-6-one (XXX) in 100 ml. of liquid ammonia was added dropwise, with stirring 1.02 g. (0.044 mole) of sodium. The blue color of the solution was discharged by the addition of a small amount of solid ammonium chloride and the solution was allowed to evaporate to dryness spontaneously. The residue was dissolved in 100 ml. of water, filtered, and acidified with acetic acid. The precipitate was collected, washed with water and alcohol, and dried at 110° (3.5 g., 87.5%). The compound did not melt below 300°.

Anal. Calcd. for $C_6H_6N_4OS$: C, 39.6; H, 3.30; N, 30.8. Found: C, 39.1; H, 3.04; N, 30.6.

1-Methyl-2,6-dithioxanthine (XXXII).—A mixture of 5 g. of 1-methyl-2-thioxanthine (XXXI), 20 g. of powdered phosphorus pentasulfide, and 200 ml. of dry pyridine was heated under reflux conditions for 3.5 hr. and then treated as described above for 3-methylpurine-6-thione. The product was purified by solution in dilute sodium hydroxide, filtration, and precipitated by acidification to a pH value of 5 (4.3 g., 81%). A small sample was recrystallized for analysis from 1000 parts of boiling water and dried at 65°, m.p. 292–294° dec.

Anal. Calcd. for $C_6H_6N_4S_2$: C, 36.4; H, 3.03; N, 28.3. Found: C, 36.2; H, 3.04; N, 27.6.

Reactions of 1-Methyl-2,6-dithioxanthine (XXXII) with Ammonia. A. Aqueous Ammonium Hydroxide.—A solution of 3 g. of XXXII in 50 ml. of concentrated ammonium hydroxide was heated at 135° in a sealed tube for 21 hr. The mixture was then boiled until ammonia gas was no longer evolved. The solution (pH 7) was cooled and filtered and the precipitate washed and dried at 100° (1.2 g.). The ultraviolet absorption spectrum of the filtrate showed that it contained mainly starting material (XXXII). The precipitate showed a $\lambda_{max} = 240$, 285 mμ, at pH 1 and gave five spots on paper chromatography in solvent A ($R_{adenine}$ = 0.02, 0.57, 0.70, 0.90, 1.70). The major component ($R_{adenine} = 0.57$) was identical chromatographically and spectrophotometrically with 6-aminopurine-2-thiol.

B. Alcoholic Ammonia.—A mixture of 2 g. of 1-methyl-2,6-dithioxanthine (XXXII) in 100 ml. of ethanolic ammonia

(saturated with ammonia at 0°) was heated in a sealed tube for 48 hr. at 140°. The mixture was then boiled to remove excess ammonia and chilled. The precipitate (880 mg.) showed a λ_{max} = 240, 285 mμ at pH 1, and gave two spots on paper chromatography. The major component was 6-aminopurine-2-thiol, according to chromatographic behavior in solvents A, B, and C and ultraviolet absorption spectrum of the material eluted from paper. The reaction mixture filtrate contained mainly unchanged XXXII, as determined by the ultraviolet absorption spectrum.

2-Benzylthio-1-methylpurine-6-thione (XXXIV).—A mixture of 4.8 g. of 4-amino-2-benzylthio-5-formamido-1-methylpyrimidine-6-one (XXIX), 20 g. of powdered phosphorus pentasulfide, and 250 ml. of dry pyridine was heated under reflux conditions for 3 hr. and then treated as described above for 3-methylpurine-6-thione. The crude product was dissolved in 250 ml. of 0.2*N* aqueous sodium hydroxide, filtered, and precipitated by acidification with acetic acid (4.4 g., 84%). A sample was purified for analysis by solution in hot 95% ethanol and dilution with an equal volume of water, m.p. 257–258° dec.

Anal. Calcd. for $C_{13}H_{12}N_4S_2$: C, 54.2; H, 4.17; N, 19.4. Found: C, 53.9; H, 4.09; N, 19.5.

6-Chloro-7-(and 9-)-methylpurines (XXXVI and XXVII).—To a solution of 15.45 g. (0.1 mole) of 6-chloropurine[53,54] in 200 ml. of 0.55 *N* sodium hydroxide was added dropwise with stirring over a 25-min. period, 10.5 ml. (0.11 mole) of dimethyl sulfate. At the end of that time the pH value of the reaction mixture was 7. After standing for 2 hr., the mixture was extracted six times with 250-ml. portions of chloroform. The chloroform extracts were combined, dried over sodium sulfate, and taken to dryness on the steam bath under reduced pressure. The mixture of 7-methyl- and 9-methyl-6-chloropurine (11.7 g., 70%) showed a λ_{max} = 268 mμ at pH 1, and λ_{max} = 269 mμ at pH 11. The mixture of isomers was converted to the corresponding purine-6-thiols without any attempt at separation of the chloro derivatives.

7- (and 9-)-Methylpurine-6-thiols (XXXVIII and XXXIX).—To 350 ml. of an aqueous solution 2 *N* sodium hydrosulfide (prepared by saturating 2 *N* sodium hydroxide with hydrogen sulfide at 0°) was added 16 g. of crude 6-chloro-7-(and 9-)-methylpurine. The mixture was kept in a sealed vessel at room temperature for 2 days. The yellow precipitate which formed (13 g.) was collected; upon acidification of the filtrate an additional precipitate (1.65 g.) formed. The 7- and 9-methyl isomers of 6-MP were separated by taking advantage of the pronounced difference in their solubilities in hot water. As Fischer[8] reported, 7-methylpurine-6-thiol is soluble in 60 parts of boiling water. The solubility of the 9-methyl isomer in boiling water is negligible. The first crude 13 g. precipitate was therefore boiled with 1 l. of water and filtered hot; the 7-methyl isomer (XXXVIII) precipitated as the monohydrate in the form of yellow needles on cooling (4.3 g.), m.p. 286–288°. After a second recrystallization from 400 ml. of boiling water, it melted at 296–298°. The hot water-insoluble residue, the 9-methyl isomer (XXXIX), was purified by solution in 300 ml. of dilute aqueous sodium hydroxide, filtration and reprecipitation with acetic acid at a pH value of 5 (6.3 g.); it darkens slowly above 250° but does not melt below 300° (cf. ref. 52). The second precipitate (1.65 g.) obtained from the original reaction mixture was almost entirely the 7-methyl isomer; on recrystallization from 150 ml. of boiling water, it gave 1.4 g. of the 7-methyl compound. The 7- and 9-methyl derivatives are distinguishable not only by their solubilities but also by their ultraviolet absorption spectra and chromatographic behavior. Each isomer, purified as described above, was chromatographically pure. The 7-methyl isomer was identical in all respects with a sample prepared from 2-chloro-7-methylpurine-6-thiol by treatment with hydriodic acid by the method of Fischer.[8]

Anal. Calcd. for $C_6H_6N_4S$: C, 43.4; H, 3.6; N, 33.7. Found: (9-methyl isomer): C, 43.8; H, 3.9; N, 33.8. Found: (7-methyl isomer): N, 33.9.

9-Methyladenine (XLI).—A solution of 3.4 g. (0.0187 mole) of 9-methyl-6-methylthiopurine (XL)[55] in 25 ml. of concentrated ammonium hydroxide was heated at 140° in a sealed tube for 18 hr. After cooling, the mixture was filtered and the precipitate of crude 9-methyladenine (m.p. 298–305°) collected (1.85 g., 67.5%). After two recrystallizations from 20 parts of boiling water and treatment with Darco each time, followed by a recrystallization from 200 volumes of 95% ethanol the 9-methyladenine had a m.p. of 300–302° dec. (m.p. 300° according to Howard *et al.*[45] and Daly and Christensen[56]; m.p. 310° according to Robins and Lin.[55] The ultraviolet absorption spectrum of one sample reported[55] showed a very low E_m *cf.* Table I). The mother liquors contained a mixture of starting material and 9-methyladenine.

Anal. Calcd. for $C_6H_7N_5$: C, 48.3; H, 4.70; N, 47.0. Found: C, 48.7; H, 4.33; N, 46.7.

9-Methylhypoxanthine (XLIII). A. From 9-Methyladenine.—A solution of 300 mg. (0.002 mole) of 9-methyladenine (prepared by method of Howard *et al.*[45]) in 15 ml. of 0.8 *N* hydrochloric acid was heated to 85–90° and a solution of 150 mg. (0.0022 mole) of sodium nitrite in 5 ml. of water was added dropwise. After 0.5 hr. at 90°, the mixture was evaporated to dryness on the steam bath. The residue was dissolved in 20 ml. of hot water, neutralized with sodium hydroxide, treated with Darco, and filtered hot. On chilling, colorless needles deposited (140 mg., 47%).

Anal. Calcd. for $C_6H_6N_4O$: N, 37.3. Found: N, 37.2.

B. From 9-Methylpurine-6-thiol.—To a solution of 1.66 g. (0.01 mole) of 9-methylpurine-6-thiol (XXXIX) in 10 ml. of 2 *N* sodium hydroxide was added 1.1 g. (0.0116 mole) of chloroacetic acid. The mixture was heated on the steam bath for 20 min. At that time the ultraviolet absorption spectrum of the reaction mixture showed a λ_{max} = 288 mμ at pH 1, indicating that the 6-mercapto group was alkylated to form XLII. Concentrated hydrochloric acid (10 ml.) was added and the reaction mixture was heated under reflux conditions for 3 hr. and then evaporated to dryness on the steam bath. The residue was taken up in 25 ml. of hot water, treated with Darco, and filtered. The filtrate was diluted with 75 ml. of absolute ethanol and chilled. The colorless, crystalline precipitate of 9-methylhypoxanthine (0.45 g., 30%) was collected and was shown to be identical in chromatographic behavior and ultraviolet absorption spectrum with an authentic specimen of 9-methylhypoxanthine prepared by the deamination of 9-methyladenine.

Anal. Calcd. for $C_6H_6N_4O$: C, 48.0; H, 4.0. Found: C, 47.9; H, 4.30.

1,9-Dimethylhypoxanthine (XLIV).—To 300 mg. (0.002 mole) of 9-methylhypoxanthine (XLIII) in 5 ml. of 0.4 *N* sodium hydroxide was added 0.19 ml. (0.00204 mole) of dimethylsulfate. The mixture was shaken and allowed to stand at room temperature for 30 min. At the end of this time the mixture was essentially neutral (pH 7.5). It was extracted seven times with 12-ml. portions of chloroform. The chloroform extracts were evaporated to dryness and the solid residue (190 mg.) was recrystallized from 25 ml. of absolute ethanol. The purified product (110 mg., 58%) melted at 255–256°.

Anal. Calcd. for $C_7H_8N_4O$: C, 51.3; H, 4.88; N, 34.1. Found: C, 51.3; H, 4.41; N, 33.9.

7-Methyladenine (XLVI).—To 2 g. (0.012 mole) of 7-methylpurine-6-thiol (XXXVIII) and 1.15 g. (0.012 mole)

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of chloroacetic acid was added 5 ml. of water and 1.5 ml. of concentrated ammonium hydroxide. The mixture was heated in a boiling water bath for 5 min. At the end of that time there was a clear solution and the ultraviolet absorption spectrum (λ_{max} = 290 mμ at pH 1) indicated that the 6-mercapto group had been converted to a 6-carboxyethylthio group to give XLV. The solution was chilled, 50 ml. of concentrated ammonium hydroxide was added to the resultant suspension, and the mixture was heated in a sealed tube at 140° for 18 hr. After cooling, the colorless crystalline precipitate was collected, washed with water, and dried at 120° (1.1 g., 67%), m.p. 320° dec. Upon recrystallization from 40 ml. of water and treatment with Darco, the m.p. was raised to 323° dec. (sealed tube m.p. = 336° dec.) (351° [46]). Sublimation did not raise the m.p.

Anal. Calcd. for $C_6H_7N_5$: C, 48.3; H, 4.70; N, 47.0. Found: C, 47.9; H, 4.67; N, 46.6.

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Studies on Fluorinated Pyrimidines. XIV. The Synthesis of Derivatives of 5-Fluoro-2′-deoxyuridine 5′-Phosphate and Related Compounds[1]

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Phosphate derivatives of 5-fluoro-2′-deoxyuridine (I), a tumor inhibitory compound, have been chemically prepared. These derivatives represent a wide variation in (1) the amount of charge on the phosphate moiety [—O—$PO_3^=$, —O—PO_2^-(OR)$^-$, —O—$PO_2O(CH_2)_2NH(C_2H_5)_2^+$, —O—PO(OR)$_2$], (2) in the nature of the functionally substituted phosphate (steroid, amino alcohol, alcohol, amino acid, di- and triphosphates), and (3) in the stereochemical configuration at the nucleoside moiety of 5-fluoro-2′-deoxyuridylyl(5′ → 3′)5-fluoro-2′-deoxyuridine (VIII and XIII).

5-Fluorouracil[3] (5-FU), and its nucleosides, 5-fluoro-2′-deoxyuridine[4,5] (5-FUDR, I) and 5-fluorouridine[4,6] (5-FUR), have been shown to possess significant tumor inhibitory activity in certain solid human tumors[7] and in transplanted[8] mouse and rat tumors. One of the mechanisms by which these fluorinated pyrimidines exert their carcinostatic effect is the inhibition of the *de novo* biochemical pathway of thymidylic acid biosynthesis.[9] The recent work of Cohen, *et al.*,[10] with bacterial systems, and of Harbers and Heidelberger[11] and of Hartmann and Heidelberger[12] with Ehrlich ascites carcinoma systems, has shown that 5-fluoro-2′-deoxyuridine 5′-monophosphate (FUDRP, IX) is the specific, competitive inhibitor of the enzyme *thymidylate synthetase*, which is responsible for the metabolic conversion of 2′-deoxyuridylic acid to thymidylic acid. In order to inhibit *thymidylate synthetase*, 5-FU, as well as its nucleosides, 5-FUDR and 5-FUR must be metabolized *in vivo* to the nucleotide FUDRP (IX).

Various catabolic processes, however, decrease the efficiency with which the cell can incorporate and metabolize 5-FUDR and 5-FUR to FUDRP. In particular, cleavage of the glycosyl bonds of 5-FUDR and 5-FUR by *nucleoside phosphorylase* gives 5-FU, which undergoes further metabolic degradation.[13]

The therapeutic use of the (enzymically[14] or chemically[15]) preformed metabolite, FUDRP, for the inhibition of *thymidylate synthetase* appears to

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