[CONTRIBUTION FROM DEPARTMENT OF CHEMISTRY, ARIZONA STATE UNIVERSITY]

Potential Purine Antagonists. XXIV. The Preparation and Reactions of Some 8-Diazopurines[1]

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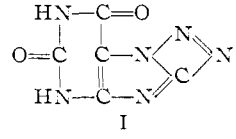
A number of 8-aminopurines have been diazotized to yield the corresponding 8-diazopurines isolated as rather stable solids. These compounds appear to possess a zwitterion type structure with the negative charge in the imidazole ring. A number of reactions of the 8-diazopurines have been studied. The introduction of a nitro group has been successfully achieved in several instances *via* the 8-diazo group and sodium nitrite. Several new purine derivatives have been prepared by coupling reactions of the 8-diazopurines.

The diazotization of an amino group in the "8"-position of the purine ring was first reported by Gomberg[2] who described the reaction of 8-aminocaffeine and nitrous acid as yielding a very unstable substance which he called "diazocaffeine." Gomberg[2] reported that this substance was obtained only in solution and was stable only at a low temperature.

Later studies by Fischer[3] describe a solid diazo derivative obtained from 8-aminotheophylline (IX) and a yellow crystalline substance similarly obtained from 8-aminoxanthine (XII) and sodium nitrite in acid solution which he termed "diazoxanthine." Although Fischer did not record any analytical data for these two substances, he described "diazoxanthine" as exploding when heated above 150° and assigned the compound formula I.



I

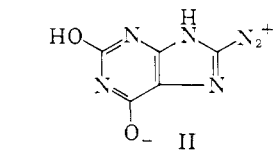
Fischer's formula for "diazoxanthine"

The present work was initiated to prepare and study a number of 8-diazopurines, especially to investigate the possibility of utilizing the diazonium group to introduce new functional groups into the purine ring.

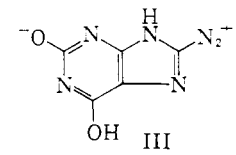
A modification of the procedure of Fischer[3] for the preparation of diazoxanthine gave a yellow solid which analyzed for $C_5H_2N_6O_2$ and showed the absence of the usual chloride anion of a normal diazonium salt. This substance exhibited absorption maxima at *p*H 11, λ_{max} 294 mμ, ϵ_{max} 11,400, and λ_{max} 340 mμ, ϵ_{max} 5,900; and at *p*H 1, λ_{max} 277 mμ, ϵ_{max} 4,100, and λ_{max} 368 mμ, ϵ_{max} 24,800.

Hot dilute hydrochloric acid and 8-diazoxanthine (I) gave uric acid as previously noted by Fischer.[3] A strong band in the infrared was noted at 2250–2400 cm.$^{-1}$ which is characteristic of a diazonium structure. The structure I assigned by Fischer[3] was eliminated by the absence of a —N=N— absorption band in the region 1550–1650 cm.$^{-1}$ in the infrared. It thus appeared that an internal zwitterion structure such as II or III might be assigned to 8-diazoxanthine.

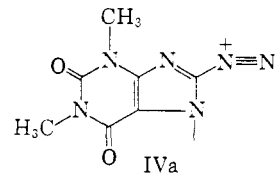
To obtain supporting evidence for either structure II or III, 8-amino-9-methylxanthine (XXXII) was prepared; however, all efforts to isolate a stable, solid 8-diazo derivative from 8-amino-9-methylxanthine (XXXII) failed. An extension of the earlier studies of Fischer[3] on 8-aminotheophylline (IX) revealed that IX was readily diazotized in 5% hydrochloric acid to yield a yellow solid, $C_7H_6N_6O_2$ (V), which showed no chloride ion and exhibited in the ultraviolet at *p*H 11, λ_{max} 382 mμ and at *p*H 1, λ_{max} 370 mμ. The presence of a diazonium group was indicated by a definite band at 2225 cm.$^{-1}$ in the infrared. Further examination of 8-diazotheophylline (V) in the infrared revealed the absence of –NH– absorption due to the imidazole hydrogen at position "7" or "9" in the region of 3200–3450 cm.$^{-1}$. These data strongly suggest structure V which could be considered a resonance hybrid of structures IVa, IVb, IVc and IVd.



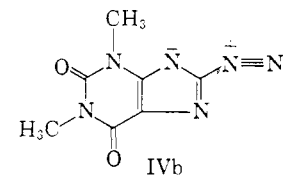
II



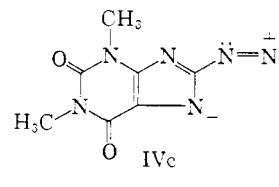
III



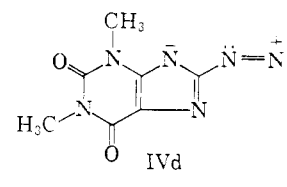
IVa



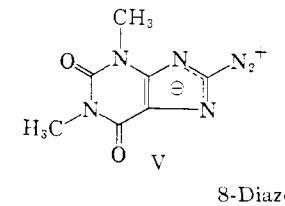
IVb



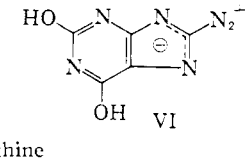
IVc



IVd



V



VI

8-Diazoxanthine

Structures of the type shown in formulas II and III are eliminated in the case of 8-diazotheophylline (V) due to the presence of the methyl groups at positions "1" and "3". On the basis of these studies it would appear that 8-diazoxanthine is best represented by structure VI.

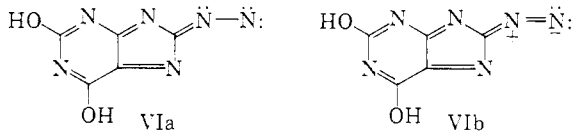
The stabilization of a diazo structure attached to a five-membered ring has been previously reported

(1) This research has been supported by grant NSF-G-5221 from the National Science Foundation.

(2) M. Gomberg, *Am. Chem. J.*, **23**, 51 (1901).

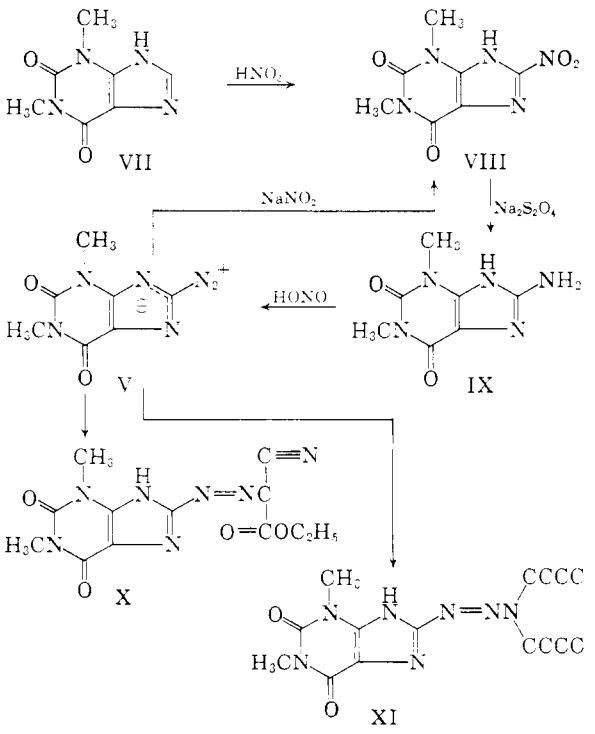
(3) H. Fischer, *Z. physiol. Chem.*, **60**, 69 (1909).

by Doering and DePuy[4] for diazocyclopentadiene. In the present case of the condensed imidazole nucleus it would appear that the five-membered ring can even more readily acquire a negative charge by the expulsion of a proton from the imidazole ring. This negative charge can then be equally shared by the two imidazole nitrogen atoms. Additional resonance contributions by conjugated structures of the type VIa and VIb could well account for the yellow color and the ultraviolet absorption maximum of the 8-diazopurines in the region of 340 to 370 mμ (see Table I).



VIa VIb

The large amount of 8-aminotheophylline (IX) required for the preparation of V prompted a new preparation of 8-aminotheophylline from 8-nitrotheophylline (VIII)[5] by reduction of VIII with sodium hydrosulfite. The nitration of theophylline has recently been reported by Morozowich and Bope[5] to give 8-nitrotheophylline (VIII). When 8-diazotheophylline (V) was treated with sodium

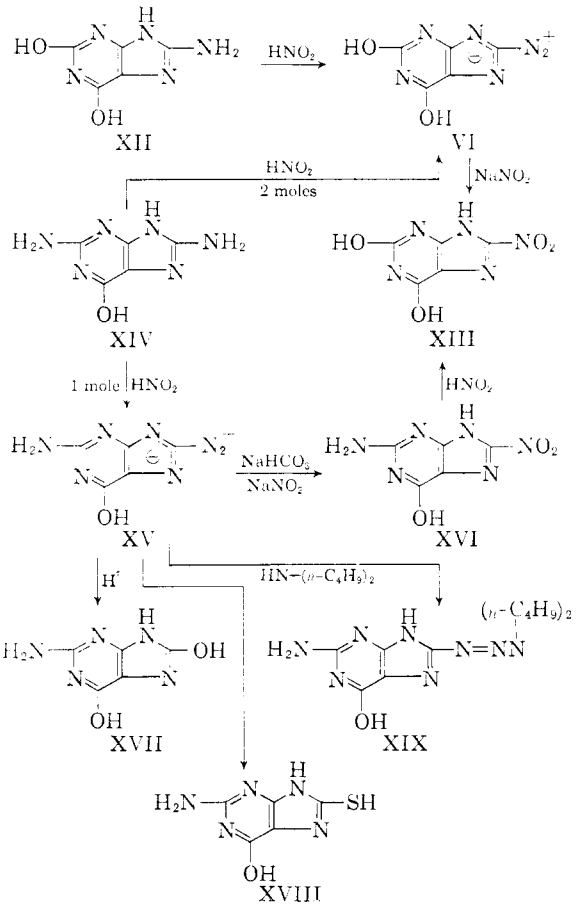


REACTION SCHEME I

nitrite in neutral aqueous solution, the diazo group was replaced to yield 8-nitrotheophylline (VIII), identical to the product reported by Morozowich and Bope[5] (see reaction scheme I). 8-Diazotheophylline (V) coupled with ethyl cyanoacetate to give compound X. When V was stirred with di-*n*-butylamine, coupling took place to yield compound XI.

The treatment of 8-diazoxanthine (VI) with sodium nitrite gave 8-nitroxanthine (XIII). This is the first reported introduction of a nitro group into an unmethylated purine.

The diazotization of 2,8-diamino-6-hydroxypurine (XIV) in the presence of fluoboric acid and an excess of sodium nitrite resulted in the isolation of 8-diazoxanthine (VI) even under carefully controlled conditions. Similar results were obtained when diazotization of XIV was studied in hydrochloric acid with two moles of sodium nitrite. However, when 2,8-diamino-6-hydroxy-



REACTION SCHEME II

purine (XIV) was dissolved in potassium hydroxide solution with one mole of sodium nitrite and the resulting solution was carefully added to concentrated hydrochloric acid, only one of the two possible amino groups reacted, and a stable diazopurine derivative was isolated. The product obtained was assigned the structure 2-amino-8-diazo-6-hydroxypurine (XV) since boiling dilute hydrochloric acid converted XV to 2-amino-6,8-dihydroxypurine (XVII).[6] Additional proof of diazotization of the 8-amino group in preference to the 2-amino group was supplied when 2-amino-8-diazo-6-hydroxypurine (XV) was converted to 2-

(4) W. von E. Doering and C. H. DePuy, THIS JOURNAL, **75**, 5955 (1953).

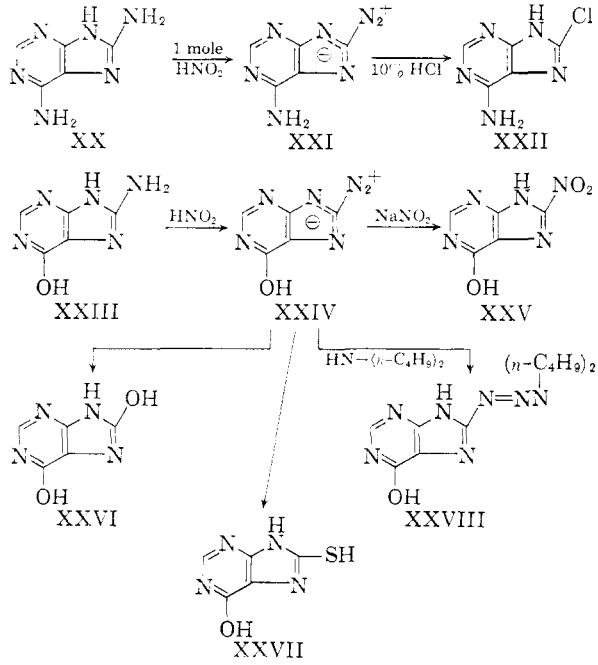
(5) W. W. Morozowich and F. W. Bope, *J. Am. Pharm. Assoc.*, **47**, 173 (1958).

(6) L. F. Cavalieri and A. Bendich, THIS JOURNAL, **72**, 2587 (1950).

amino-6-hydroxy-8-purinethiol (XVIII)[7] by boiling potassium hydrosulfide.

Treatment of 2-amino-8-diazo-6-hydroxypurine (XV) with sodium nitrite gave 2-amino-6-hydroxy-8-nitropurine (8-nitroguanine) (XVI). Nitrous acid converted 8-nitroguanine (XVI) to 8-nitroxanthine (XIII). 8-Diazoguanine (XV) coupled with di-*n*-butylamine to give XIX in good yield.

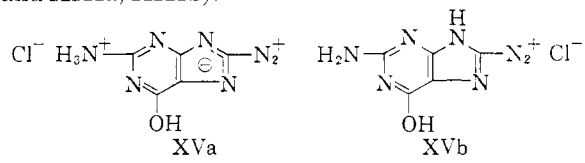
The diazotization of 8-amino-6-hydroxypurine (XXIII)[8] gave 8-diazo-6-hydroxypurine (XXIV) (8-diazohypoxanthine). This compound was rather unstable and appeared to be light-sensitive. When dry, it readily detonated with friction or upon heating. The freshly prepared damp product, however, if used immediately, was found suitable for further experimentation. Treatment of 8-diazo-6-hydroxypurine (XXIV) with sodium nitrite gave 6-hydroxy-8-nitropurine (XXV) which was isolated as the sodium salt. Reduction of XXV with sodium hydrosulfite in aqueous solution gave 8-amino-6-hydroxypurine (XXIII) which showed that the nitro group present in 6-hydroxy-8-nitropurine (XXV) could not be a nitrite group. Acid hydrolysis of XXIV gave 6,8-dihydroxypurine (XXVI).[8] Treatment of 8-diazo-6-hydroxypurine (XXIV) with boiling potassium hydrosulfide gave 6-hydroxy-8-purinethiol (XXVII)[8] which was characterized by its ultraviolet absorption spectra.[8] 8-Diazohypoxanthine (XXIV) coupled readily with di-*n*-butylamine to give XXVIII.



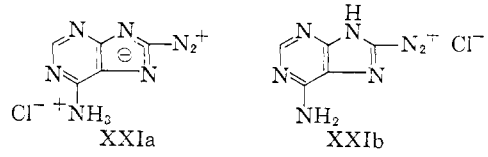
REACTION SCHEME III

Treatment of 6,8-diaminopurine (XX)[8] in 5% hydrochloric acid with an excess of sodium nitrite gave 6-amino-8-diazopurine hydrochloride (XXI). It is interesting to note the failure of the 6-amino group to diazotize under these conditions. The failure of adenine to undergo the Schiemann reaction has previously been noted.[9] The fact that diazotization had indeed taken place in position "8" was established by conversion of XXI to 6-amino-8-chloropurine (XXII)[8] with 10% hydrochloric acid.

It should be noted that 6-amino-8-diazopurine (8-diazoadenine) (XXI) and 2-amino-8-diazo-6-hydroxypurine (8-diazoguanine) (XV) were each isolated from an acid solution as the hydrochloride salts. Theoretically, the proton could be either in the imidazole ring or on the amino group in the pyrimidine portion of the ring (see XVa, XVb and XXIa, XXIb).



Since in each case XXI and XV were isolated from a hydrochloric acid solution, one would expect the amino group to be protonated; however, it should be pointed out that the structures XVb and XXIb have not been eliminated.



Various attempts to diazotize 6-amino-2,8-dihydroxypurine were unsuccessful as were attempts to effect diazotization of adenine under a variety of conditions.

The direct nitration of caffeine,[10] theobromine[11] and theophylline[5] have been reported to give the corresponding 8-nitro derivative. Presumably, the methyl groups in these compounds have resulted in sufficient electron density at position "8" so that electrophilic attack will take place. Biltz and Sauer[12] report the preparation of 2,6-dihydroxy-9-methyl-8-nitrosopurine (XXXI) from treatment of 2,6-dihydroxy-9-methyl-8-purinethiol with sodium nitrite and nitric acid. Since this work describes the only instance of preparation of a nitrosopurine reported in the literature, it seemed of interest to repeat this work. The preparation of Biltz and Sauer[12] was analyzed and found to consist of two substances; one was identified as 9-methylxanthine (XXIX)[13] and the other as 9-methyl-8-nitroxanthine (XXX). The preparation of XXX was independently achieved by nitration of 9-methylxanthine (XXIX) with boiling dilute nitric acid without the addition of sodium nitrite. Chromatographic analysis and identical ultraviolet and infrared spectra proved the compounds to be identical. Apparently the misleading analytical data of Biltz and Sauer was due to the presence of some 9-methylxanthine (XXIX) in their product. The reduction of 9-methyl-8-

(7) G. B. Elion, I. Goodman, W. Lange and G. H. Hitchings, THIS JOURNAL **81**, 1898 (1959).

(8) R. K. Robins, *ibid.*, **80**, 6671 (1958).

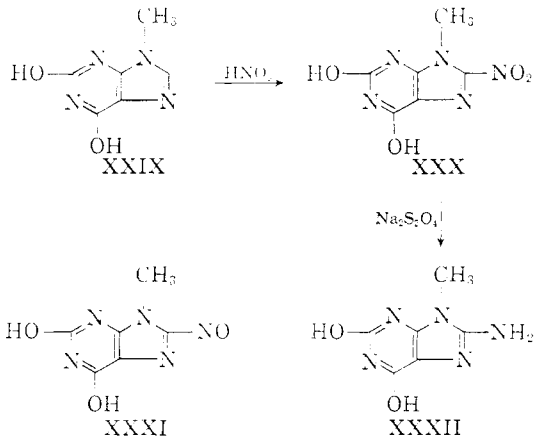
(9) A. Giner-Sorolla and A. Bendich, *ibid.*, **80**, 5744 (1958).

(10) H. Schultzen, *Z. physiol. Chem.*, 616 (1867).

(11) H. Brunner and H. Leins, *Ber.*, **30**, 2585 (1897).

(12) H. Biltz and J. Sauer, *ibid.*, **64**, 752 (1931).

(13) H. C. Koppel and R. K. Robins, THIS JOURNAL, **80**, 2751 (1958).



REACTION SCHEME IV

nitroxanthine (XXX) was accomplished with sodium hydrosulfite to give 8-amino-2,6-dihydroxy-9-methylpurine (XXXII) in good yield.

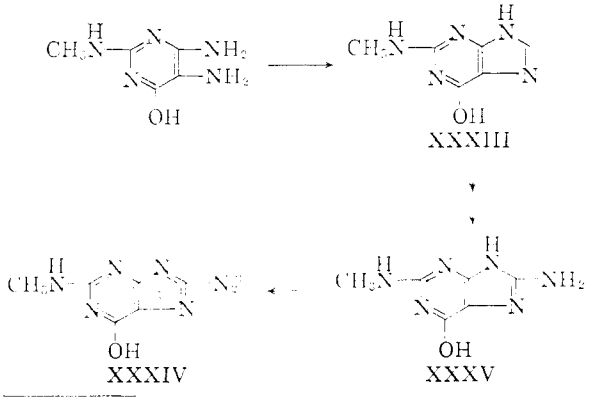
When 2,6-dihydroxy-9-methyl-8-purinethiol was treated with boiling hydrochloric acid and sodium nitrite instead of nitric acid and sodium nitrite as employed by Biltz and Sauer,[12] 9-methylxanthine (XXIX) was obtained, but no nitrosation took place.

It is interesting that although 9-methylxanthine (XXIX) can be nitrated by boiling dilute nitric acid, similar conditions failed to nitrate xanthine. This work suggests that other 9-substituted purines with electron-donating substituents in positions "2" and "6" might also undergo electrophilic substitution.

6-Hydroxy-2-methylaminopurine (XXXIII) was prepared by formamide ring closure of 4,5-diamino-6-hydroxy-2-methylaminopyrimidine.[14] 6-Hydroxy-2-methylaminopurine (XXXIII) was coupled with *p*-chlorobenzenediazonium chloride, and the product was reduced with sodium hydrosulfite to yield 8-amino-6-hydroxy-2-methylaminopurine (XXXV).

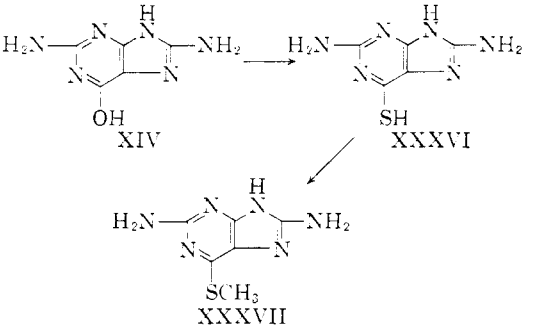
When XXXV was diazotized in a manner similar to that employed for 2,8-diamino-6-hydroxypurine (XIV) with only one mole of sodium nitrite, the solid 8-diazo-6-hydroxy-2-methylaminopurine (XXXIV) was obtained.

2,8-Diamino-6-hydroxypurine (XIV) was treated with phosphorus pentasulfide in pyridine to yield



(14) B. Roth, J. M. Smith, Jr., and M. E. Hultquist, *ibid.*, **73**, 2864 (1951).

2,8-diamino-6-purinethiol (XXXVI). This compound failed to yield an isolable diazo derivative. Methylation of 2,8-diamino-6-purinethiol with methyl iodide gave 2,8-diamino-6-methylthiopurine (XXXVII).

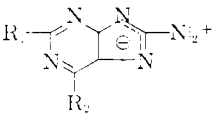


Treatment of 8-amino-6-hydroxy-2-methylaminopurine (XXXV) with phosphorus pentasulfide in pyridine similarly gave 8-amino-2-methylamino-6-purinethiol.

A study of the ultraviolet absorption spectra of the diazo purine derivatives (see Table I) revealed that a diazo group in position "8" shows, in general,

TABLE I

ULTRAVIOLET ABSORPTION SPECTRA OF SOME 8-DIAZOPURINES

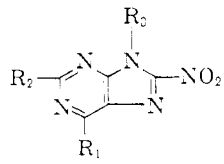


R_1	R_2	λ_{max}, mμ, *p*H 1	ε	λ_{max}, mμ, *p*H 11	ε
H	NH_2	234	9,300	288	12,000
		358	11,600		
CH_3NH	OH	254	7,700	247	5,400
		367	12,400	291	5,900
				340	4,800
NH_2	OH	233	24,800	250	16,200
		290	6,900	272	15,500
		365	46,000	344	12,900
OH	OH	277	4,100	294	11,400
		368	24,800	340	5,900
H	OH	234	7,500	243	6,800
		282	4,150	290	8,200
		364	12,000	342	5,800
8-Diazotheophylline		241	10,100	234	14,900
		287	4,500	320	15,300
		377	20,000		

absorption in the region of 340–370 mμ. The nitro group at position "8" similarly exhibits absorption in the same area (see Table II). It is quite notable that 8-nitroxanthine (XIII) exhibits a maximum at 430 mμ at *p*H 11. This compound in basic solution gives a deep, red-orange color. This absorption in the ultraviolet is at a longer wave length than any other previously recorded purine derivative. The spectra of other purines described in this work are listed in Table III.

TABLE II

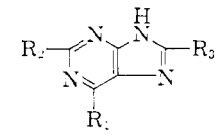
ULTRAVIOLET ABSORPTION SPECTRA OF SOME 8-NITROPURINES



R_1	R_2	R_3	λ_{max}, mμ, pH 1	ε	λ_{max}, mμ, pH 11	ε
OH	OH	H	360	10,600	262	8,300
					430	12,800
OH	H	H	236	21,100	240	15,800
			334	14,000	382	12,900
OH	OH	CH_3	355	12,900	254	9,100
					422	10,700
8-Nitrotheophylline[5]			245	8,800	240	8,500
			370	9,100	390	11,100
8-Nitrocaffeine[10]			244	9,100	244	9,800
			370	8,100	370	7,900

TABLE III

ULTRAVIOLET ABSORPTION SPECTRA OF CERTAIN 2,6,8-TRISUBSTITUTED PURINES



R_1	R_2	R_3	λ_{max}, mμ, pH 1	ε	λ_{max}, mμ, pH 11	ε
OH	NH_2	—N=NN(n-C_4H_9)$_2$	255	11,900	265	10,200
			353	14,300	352	11,600
OH	H	—N=NN(n-C_4H_9)$_2$	244	10,200	253	11,600
			338	24,400	338	19,500
OH	OH	—N=NN(n-C_4H_9)$_2$	268	12,900	254	7,800
			347	17,800	352	21,500
SH	NH_2	NH_2	252	10,500	260	9,600
			355	25,300	327	15,700
SCH_3	NH_2	NH_2	234	17,200	234	19,400
			333	11,800	325	12,400
OH	CH_3NH	NH_2	244	16,700	282	8,900
			294	8,400		
SH	CH_3NH	NH_2	225	23,200	235	19,600
			366	13,800	338	12,200

Experimental[15]

8-Amino-2,6-dihydroxypurine (XII).—*p*-Chloroaniline (well-ground, 92 g.) was diazotized in 400 ml. of concentrated hydrochloric acid at 0–5° by slow addition of 52 g. of sodium nitrite dissolved in 100 ml. of water. The solution was stirred 10 min. after the addition which required 30 min.

One-half of the diazo mixture was slowly added to a mechanically stirred solution of xanthine (100 g.) in 2 l. of 8.5% potassium hydroxide at 0–10°. A solution of 170 g. of potassium hydroxide in 300 ml. of water, previously prepared and cooled, was then carefully added. The remaining diazo solution was then added dropwise as before. The viscose, dark mixture was stirred for 2.5 hr. and filtered.

The precipitate was dissolved in 3000 ml. of 5% potassium hydroxide, and 500 g. of sodium hydrosulfite was carefully added at 80–90°. The solution was heated to boiling, treated with charcoal and Celite to adsorb the *p*-chloraniline, and filtered. Acidification of the hot filtrate gave a white product which was filtered and washed with water. The crude product obtained was reprecipitated from boiling 5% potassium hydroxide with acetic acid to yield 98 g. The ultraviolet absorption spectra were identical to those previously reported.[6]

Anal. Calcd. for $C_5H_5N_5O_2 \cdot {}^1/_2H_2O$: N, 39.8. Found: N, 39.5.

2,6-Dihydroxy-9-methyl-8-nitropurine (XXX). Method 1.—Two grams of 2,6-dihydroxy-9-methyl-8-purinethiol[12] was added to a solution of 25 ml. of concentrated nitric acid and 25 ml. of water in a 125-ml. erlenmeyer flask. The solution was boiled gently for 20 min. and then made ammoniacal with concentrated aqueous ammonium hydroxide. A precipitate separated as needles in the hot solution and was filtered immediately. The product (1 g.) was dissolved in hot dilute potassium hydroxide, treated with charcoal, and filtered. Finally, the hot solution was acidified with concentrated hydrochloric acid and filtered while hot. The residue was discarded. The filtrate was allowed to stand at room temperature for 12 hr. The precipitate that separated as yellow needles was filtered, washed with water, and dried at 80–90° for 12 hr. The compound showed λ_{max} 355 mμ, ϵ_{max} 12,900 at *p*H 1; at *p*H 11, λ_{max} 254 mμ, ϵ_{max} 9,100 and λ_{max} 422 mμ, ϵ_{max} 10,700. R_f 0.52 in 1-butanol (5)–acetic acid (2)–water(3).

Anal. Calcd. for $C_6H_5O_4N_5$: C, 34.1; H, 2.4; N, 33.2. Found: C, 33.8; H, 2.7; N, 33.6.

Method 2.—9-Methylxanthine (XXIX) (0.75 g.) was boiled gently for 20 min. in a solution of concentrated nitric acid (15 ml.) and water (15 ml.). The hot solution was made ammoniacal with concentrated aqueous ammonium hydroxide and treated as in method 1. The R_f (solvent for method 1), ultraviolet and infrared data on this compound were shown to be identical to those of the product isolated by method 1; R_f 0.52 in 1-butanol (5)–acetic acid (2)–water (3).

8-Amino-2,6-dihydroxy-9-methylpurine (XXXII).—One gram of 2,6-dihydroxy-9-methyl-8-nitropurine (XXX), method 1, was dissolved in 20 ml. of 10% potassium hydroxide. The solution was heated to 60–70° and treated with 2 g. of sodium hydrosulfite. A white product (0.8 g.) was collected after cooling at 5° for 12 hr. The precipitate was dissolved in hot dilute potassium hydroxide, treated with charcoal, and filtered. The hot solution was acidified with concentrated hydrochloric acid and filtered. A crystalline product was deposited from the solution while standing at room temperature for 12 hr. The hydrochloride was filtered, washed with water, and dried at 80–85° for 24 hr.

Anal. Calcd. for $C_6H_7N_5O_2 \cdot HCl$: C, 33.2; H, 3.7; N, 32.2. Found: C, 33.2; H, 3.9; N, 31.9.

8-Diazoadenine (XXI).—One gram of 6,8-diaminopurine hydrochloride (XX)[8] was mechanically stirred in 10 ml. of 5% hydrochloric acid, and 1 g. of solid sodium nitrite was added in small portions. Stirring was continued for 1 hr., and the solution was allowed to stand at 5–10° for 10 hr. A white product (1 g.) was collected and washed first with water then methanol. The product was allowed to dry and kept in a vacuum desiccator over calcium chloride. It gradually darkened when exposed to light.

Anal. Calcd. for $C_5H_3N_7 \cdot HCl$: C, 30.5; H, 2.1; N, 49.8. Found: C, 30.7; H, 2.1; N, 49.6.

8-Diazo-6-hydroxy-2-methylaminopurine (XXXIV).—Two grams of 8-amino-6-hydroxy-2-methylaminopurine (XXXV) was dissolved in 25 ml. of 5% potassium hydroxide containing 1 g. of sodium nitrite. This solution was added dropwise to a vigorously stirred solution of concentrated hydrochloric acid (30 ml.) at 10°. Stirring was continued for 1 hr. at which time 1.5 g. of a crystalline solid was collected.

Anal. Calcd. for $C_6H_5ON_7 \cdot HCl$: C, 31.6; H, 2.6; N, 43.0. Found: C, 31.0; H, 2.4; N, 42.9.

8-Diazotheophylline (V).—Fifteen grams of 8-aminotheophylline (IX) was dissolved in 300 ml. of 5% hydrochloric acid and cooled to 5–10°. To the vigorously stirred solution was added 7.5 g. of solid sodium nitrite in small portions. A heavy precipitate appeared after 10 min. Stirring was continued for 1.5 hr., and the mixture was filtered and washed first with water then methanol to yield 11.5 g. (85%) of yellow solid. The product was air-dried and analyzed directly.

Anal. Calcd. for $C_7H_6O_2N_8$: C, 40.8; H, 2.9; N, 40.8. Found: C, 41.1; H, 2.8; N, 40.9.

(15) All melting points were taken on a Fisher–Johns melting point apparatus and are uncorrected.

8-Diazohypoxanthine (XXIV).—Five grams of 8-amino-6-hydroxypurine sulfate (XXIII)[8] was dissolved in 20 ml. of 5% hydrochloric acid, and the solution was cooled to 0–5°. Sodium nitrite (2.5 g., solid) was added in small portions, and the solution was stirred for 2 hr. Methanol (150 ml.) was carefully added, and the mixture was allowed to stand at 0° for 12 hr. The yellow crystalline solid was filtered and washed with methanol. The product was allowed to air-dry and analyzed directly. This compound explodes violently when heated above 100° and is detonated by pressure or friction.

Anal. Calcd. for $C_5H_2N_6O \cdot {}^1/_4H_2O$: C, 36.0; H, 1.5; N, 50.4. Found: C, 35.8; H, 1.8; N, 50.4.

8-Diazoxanthine (VI). Method 1.—To a solution of 10 g. of 8-amino-2,6-dihydroxypurine (XII), in 100 ml. of 5% potassium hydroxide, was added 5 g. of sodium nitrite. This solution was then added dropwise to a vigorously stirred solution of concentrated hydrochloric acid (100 ml.) at 5–10°. After the addition, which required 30 min., stirring was continued for an additional 30 min. The precipitate was collected and washed first with cold water (until negative for Cl^-) and then cold methanol. The yellow product (12 g., 87%) was allowed to air-dry and analyzed directly.

Anal. Calcd. for $C_5H_2O_2N_6$: C, 33.7; H, 1.1; N, 47.2. Found: C, 33.4; H, 1.8; N, 47.2.

Method 2.—To a suspension of 7 g. of 2,8-diamino-6-hydroxypurine (XIV), in 35 ml. of fluoboric acid (48–50%) at 0–5°, was added 6.5 g. of sodium nitrite in small portions. Vigorous stirring was continued for 30 min. after all the solid had dissolved. The solution was allowed to stand for 12 hr. at 5°. The yellow crystalline solid which resulted was filtered and washed first with water then methanol. Infrared and ultraviolet spectra comparison with 8-diazoxanthine (VI), prepared by method 1, proved to be identical.

8-Aminotheophylline (IX).—Fifty grams of 8-nitrotheophylline (VIII)[5] was suspended in 500 ml. of water and heated to 80–90°. To the mixture was added 130 g. of sodium hydrosulfite in small portions. The solution was allowed to stand at 5–10° for 10–12 hr. and then filtered and washed with water. The white solid was dissolved in hot dilute potassium hydroxide, treated with charcoal, and filtered. The solution was adjusted to *p*H 1 with concentrated hydrochloric acid and filtered while hot. The solution was allowed to cool at room temperature to yield 44 g. of the crystalline hydrochloride.

Anal. Calcd. for $C_7H_9N_5O_2 \cdot HCl \cdot H_2O$: C, 33.7; H, 4.8; N, 28.1. Found: C, 33.9; H, 4.1; N, 27.8.

8-Diazoguanine (XV).—Seven grams of 2,8-diamino-6-hydroxypurine (XIV) was dissolved in 5% potassium hydroxide (50 ml.), containing 3.0 g. of sodium nitrite. The solution was added dropwise to a mechanically-stirred solution of concentrated hydrochloric acid (100 ml.) at 10–15° over a period of 10 min. It was allowed to stir for an additional 45 min. at 10°. The precipitate was collected and washed first with water then methanol. The yellow solid was dried over calcium chloride in a vacuum desiccator.

Anal. Calcd. for $C_5H_3N_7O \cdot HCl \cdot H_2O$: N, 42.4. Found: N, 42.4.

8-Nitroxanthine (XIII). Method 1.—Nine grams of 8-diazoxanthine (VI), suspended in 50 ml. of methanol, was added to 200 ml. of water containing 9 g. of sodium nitrite. The mixture was stirred for 2 hr. at 30° then heated to boiling and filtered. The filtrate was set aside. The residue was dissolved in hot dilute potassium hydroxide, treated with charcoal, and filtered. The hot solution was acidified with hydrochloric acid and filtered immediately. The filtrates were cooled, and the precipitates were collected. The combined precipitates were dissolved in 400 ml. of 5% hot aqueous potassium hydroxide. The solution was treated with charcoal and filtered. The hot filtrate was acidified with hydrochloric acid, and the solution was filtered immediately. The residue was discarded. A yellow precipitate (3.5 g.) settled from the filtrate on cooling at 5° for 12 hr. A small sample was recrystallized from a methanol–water solution; R_f 0.23 in butanol (5)–acetic acid (2)–water (3).

Anal. Calcd. for $C_5H_3N_5O_4$: C, 30.5; H, 1.5; N, 35.5. Found: C, 30.8; H, 1.7; N, 35.4.

Method 2.—8-Nitroguanine (XVI) (500 mg.) was dissolved in 5% hydrochloric acid (25 ml.) and heated to 80–90°. To the solution was added 500 mg. of sodium nitrite in small portions. Heating was continued for 10 min. after the addition was complete. The ultraviolet spectra of the solution were determined after proper dilution and were identical to 8-nitroxanthine. Also, a paper chromatograph of the solution revealed a visible yellow spot identical to 8-nitroxanthine.

8-Nitroguanine (XVI).—8-Diazoguanine (XV) (7 g.) was suspended in 100 ml. of water and stirred for 5 min. at room temperature. To the suspension was added sodium bicarbonate (4 g. in 25 ml. of water) followed by a solution of sodium nitrite (7 g. in 25 ml. of water). Stirring was continued for 12 hr. at room temperature. Finally, the mixture was heated to boiling and filtered immediately. The dark-brown residue was washed with 50 ml. of hot water. The residue was discarded, and the filtrate was allowed to cool at room temperature for 2 hr. The red precipitate that resulted was filtered, washed with cold water (25 ml.), and dissolved in hot 5% sodium hydroxide (100 ml.). The solution was treated with charcoal, filtered, and acidified with glacial acetic acid. The hot solution was then filtered immediately to remove a tan residue, which was discarded. A yellow-orange precipitate (3 g.) separated from the solution at room temperature. The product was further purified by precipitation from hot dilute potassium hydroxide with acetic acid.

Anal. Calcd. for $C_5H_4N_6O_3$: C, 30.6; H, 2.0; N, 42.8. Found: C, 30.2; H, 2.3; N, 42.5.

8-Nitrohypoxanthine (XXV).—Three grams of 8-diazohypoxanthine (XXIV) was added to a solution of 3 g. of sodium nitrite in 50 ml. of water. The mixture was stirred for 2 hr. and allowed to stand at room temperature for 12 hr. The solution was then heated to boiling and filtered. The filtrate, on cooling, yielded 2 g. of yellow solid which proved to be the sodium salt of 8-nitrohypoxanthine. This product was purified by recrystallization from water.

Anal. Calcd. for $C_5H_2O_3N_5Na \cdot 1{}^1/_2H_2O$: C, 26.1; H, 2.2; N, 30.4. Found: C, 25.9; H, 2.7; N, 30.3.

8-(1,1′-Bis-di-*n*-butyl)-triazinoxanthine.—To 1 g. of 8-diazoxanthine (VI), suspended in 100 ml. of absolute ethanol, was added 1 g. of di-*n*-butylamine. The mixture was stirred for 2 hr. at room temperature. A yellow solid (1.2 g.) was collected and washed with ethanol. A sample for analysis was recrystallized from methanol.

Anal. Calcd. for $C_{13}H_{21}O_2N_7$: C, 50.9; H, 6.8; N, 31.9. Found: C, 51.3; H, 6.9; N, 31.9.

2,6-Dihydroxy-8-purinethiol.—Diazoxanthine (VI) (1.5 g.) was suspended in 50 ml. of water and treated with 1.5 g. of thiourea (in 25 ml. of water) at room temperature. The mixture was stirred for 45 min. Solid sodium hydroxide (2 g.) was added, and the solution was heated at 90–95° for 15 min. The boiling solution was adjusted to *p*H 1 with concentrated hydrochloric acid and allowed to cool. A precipitate (1 g.) settled out and was identified by comparison of its ultraviolet spectra with the spectra of an authentic sample of 2,6-dihydroxy-8-purinethiol.[16]

Preparation of 6-Amino-8-chloropurine (XXII) from 8-Diazoadenine (XXI).—8-Diazoadenine (XXI) (250 mg.) was boiled gently in 10 ml. of 10% hydrochloric acid for 15 min. The solution was cooled, and the ultraviolet spectra of the solution was determined directly. The ultraviolet spectra were shown to be identical to those of 6-amino-8-chloropurine.[8]

Preparation of 6,8-Dihydroxypurine (XXVI) from 8-Diazohypoxanthine (XXIV).—One gram of 8-diazohypoxanthine (XXIV) was heated on the steam-bath in 20 ml. of fluoboric acid (48–50%) for 20 min. A precipitate settled out after cooling at room temperature. The precipitate was filtered, washed with water, and dried at 80–90°. The ultraviolet spectra were identical to 6,8-dihydroxypurine.[3]

Preparation of 6-Hydroxy-8-purinethiol (XXVII) from 8-Diazohypoxanthine (XXIV).—Two grams of 8-diazohypoxanthine (XXIV) was added to a boiling solution of 2 *N* sodium hydrosulfide. The solution was boiled for 20 min. and acidified with concentrated hydrochloric acid. The precipitate that formed on cooling at room temperature was filtered and washed with water. The product was reprecipitated from hot potassium hydroxide with acetic acid and dried at 80–90°. The ultraviolet spectra were shown to be identical to 6-hydroxy-8-purinethiol.[8]

(16) T. L. Loo, M. E. Michael, A. J. Garceau and J. C. Reid, THIS JOURNAL, **81**, 3039 (1959).

2-Amino-6,8-dihydroxypurine (XVII).—One-half gram of 8-diazoguanine (XV) was suspended in 100 ml. of 5% hydrochloric acid and refluxed for 3 hr. The solution was cooled to room temperature and neutralized with 5% potassium hydroxide. The proper dilution (1:500) was made, and the ultraviolet spectrum of the solution was determined at *p*H 1 and *p*H 11. The resulting spectra were shown to be identical to known spectra of 2-amino-6,8-dihydroxypurine (XVII).[6]

Coupling of 8-Diazotheophylline (V) with Ethyl Cyanoacetate.—To 2 g. of 8-diazotheophylline (V), suspended in 50 ml. of ethanol, was added 2 g. of ethyl cyanoacetate in 50 ml. of pyridine. The mixture was stirred for 3 hr. at room temperature. The mixture was filtered to yield 3 g. of crude yellow solid. A small sample was recrystallized from acetone and air-dried, m.p. 209–211° (block preheated to 200°). At *p*H 1 the compound exhibited ultraviolet absorption maxima of λ_{max} 263 mμ, ϵ_{max} 14,100, and λ_{max} 384 mμ, ϵ_{max} 13,500; at *p*H 11, λ_{max} 251 mμ, ϵ_{max} 12,100, and λ_{max} 420 mμ, ϵ_{max} 24,600.

Anal. Calcd. for $C_{12}H_{13}O_4N_7 \cdot H_2O$: C, 42.8; H, 4.6; N, 29.1. Found: C, 42.6; H, 4.7; N, 28.9.

Coupling of 8-Diazotheophylline (V) with Di-*n*-butylamine.—One gram of 8-diazotheophylline (V) was suspended in 50 ml. of acetone and treated with 1 g. of di-*n*-butylamine. The solution was stirred at room temperature for 30 min. and allowed to stand at 5° for 12 hr. The yellow product (1 g.) was filtered and washed with cold acetone. A small sample was recrystallized from acetone to give a melting point of 189–190°. At *p*H 1 the compound exhibited ultraviolet absorption maxima of λ_{max} 257 mμ, ϵ_{max} 11,500 and λ_{max} 352 mμ, ϵ_{max} 21,500; at *p*H 11, λ_{max} 237 mμ, ϵ_{max} 17,000, and λ_{max} 347 mμ, ϵ_{max} 21,500.

Anal. Calcd. for $C_{15}H_{25}N_7O_2$: C, 53.8; H, 7.5; N, 29.3. Found: C, 54.0; H, 7.6; N, 29.4.

Preparation of Uric Acid from 8-Diazoxanthine (VI).—One gram of 8-diazoxanthine (VI) was dissolved in 20 ml. of fluoboric acid. The solution was heated on the steam-bath for 1 hr. The precipitate that separated on cooling was filtered, washed with water, and dried at 80–90°. The ultraviolet spectra were identical to known spectra of uric acid.

2,8-Diamino-6-purinethiol.—Twenty grams of 2,8-diamino-6-hydroxypurine (XIV) was ground with 90 g. of phosphorus pentasulfide and refluxed in 1 l. of dry pyridine for 10 hr. The excess pyridine was removed under reduced pressure using a steam-bath as the source of heat. Water (500 ml.) was added to the residue, and the mixture was heated for 30 min. on the steam-bath. The mixture was filtered and washed with water. The precipitate was dissolved in hot 5% potassium hydroxide, treated with charcoal, and filtered. The hot solution was acidified with acetic acid, cooled to room temperature, and filtered. Sixteen grams of tan solid were collected.

Anal. Calcd. for $C_5H_6N_6S$: C, 33.0; H, 3.3; N, 46.1. Found: C, 33.1; H, 3.7; N, 45.6.

8-Amino-6-hydroxy-2-methylaminopurine (XXXV).—*p*-Chloroaniline (well-ground, 13 g.) was diazotized in 50 ml. of concentrated hydrochloric acid at 0–5° by the slow addition of 8 g. of sodium nitrite in 10 ml. of water. The solution was stirred 10 min. after the addition was complete.

One-half of the diazo mixture was slowly added to a mechanically-stirred solution of 2-methylamino-6-hydroxypurine (XXXIII) (12 g.) in 150 ml. of 3.3 *N* potassium hydroxide at 0–10°. Potassium hydroxide (28 g.) in 50 ml. of water, previously prepared and cooled, was then added. The remaining diazo solution was then added dropwise as before. The mixture was stirred for 2.5 hr. then filtered.

The precipitate was dissolved in 200 ml. of 5% potassium hydroxide and treated in small portions with sodium hydrosulfite (70 g.) at 80–90°. Charcoal was then added, and the solution was heated to boiling and filtered. The hot solution was acidified with acetic acid, cooled to room temperature, and filtered. The precipitate was dissolved in hot 5% potassium hydroxide, charcoaled, filtered, and acidified with hydrochloric acid. The precipitate that resulted on cooling was reprecipitated from hot potassium hydroxide with acetic acid. The hydrochloride was formed by dissolving the crude precipitate (7.5 g.) in 5% hydrochloric acid and cooling at 5° for 12 hr. The product was filtered, washed with water, and dried at 80–90°.

Anal. Calcd. for $C_6H_8N_6O \cdot HCl \cdot 2H_2O$: C, 28.5; H, 5.2; N, 33.3. Found: C, 29.1; H, 5.2; N, 33.5.

Coupling of 8-Diazohypoxanthine (XXIV) with Di-*n*-butylamine.—8-Diazohypoxanthine (XXIV) (1.2 g.) was stirred in 75 ml. of acetone and treated with 1 g. of di-*n*-butylamine. The mixture was stirred for 1 hr. at 40–50° and then evaporated to dryness on the steam-bath. The residue was dissolved in 50 ml. of 5% hydrochloric acid, charcoaled, and filtered. The hot solution was then made ammoniacal with concentrated aqueous ammonium hydroxide. The precipitate (XXVIII) that separated in the hot solution was filtered, washed with water, and dried at 40–50° for 24 hr.

Anal. Calcd. for $C_{13}H_{21}N_7O$: C, 53.7; H, 7.2; N, 33.7. Found: C, 54.1; H, 7.3; N, 34.2.

Coupling of 8-Diazoguanine (XV) with Di-*n*-butylamine.—8-Diazoguanine (XV) (1 g.) was stirred in 50 ml. of acetone with 2 g. of di-*n*-butylamine for 1.5 hr. at room temperature. The mixture was filtered, and the precipitate was dissolved in 50 ml. of hot 5% hydrochloric acid. The solution was charcoaled, filtered, and allowed to cool at 5° for 12 hr. The precipitate was filtered and washed first with water then acetone. The yellow solid was dried at 40–50° for 12 hr.

Anal. Calcd. for $C_{13}H_{22}N_8O \cdot HCl$: N, 32.8. Found: N, 32.7.

8-Amino-2-methylamino-6-purinethiol.—8-Amino-6-hydroxy-2-methylaminopurine (XXXV) (1.5 g.) was finely ground with 7 g. of phosphorus pentasulfide and added to 100 ml. of dry pyridine. The mixture was refluxed for 10 hr. and allowed to stand at room temperature for 15 hr. The solvent was decanted from the solid that separated. The solid was then treated with 100 ml. of water and heated on the steam-bath for 30 min. The mixture was filtered, washed with water, and dried. The crude product (1.3 g.) was dissolved in hot dilute potassium hydroxide (50 ml.), charcoaled, filtered, and acidified with acetic acid. The precipitate was filtered, washed with water, then added to 25 ml. of hot water and dissolved by adding concentrated aqueous ammonium hydroxide. The hot solution was filtered and heated to expel the excess ammonia. The precipitate that settled while standing for 12 hr. at 5° was filtered, washed with water, and dried at 80–90° for 12 hr.

Anal. Calcd. for $C_6H_8N_6S \cdot {}^1/_4H_2O$: C, 35.9; H, 4.3; N, 41.9. Found: C, 35.4; H, 4.2; N, 41.6.

6-Hydroxy-2-methylaminopurine (XXXIII).[17]—4,5-Diamino-6-hydroxy-2-methylaminopyrimidine[14] (12 g.) was added to 120 ml. of boiling formamide. The solid dissolved immediately. Heating was continued for 20 min. during which time a precipitate separated in the boiling solution. After cooling the mixture to room temperature, 50 ml. of water was added. The mixture was allowed to stand at 5° for 15 hr. and was then filtered, washed with water, and dried to yield 11.5 g. A white product was obtained by reprecipitation from hot potassium hydroxide with glacial acetic acid. The ultraviolet spectrum was identical to those previously reported.[17]

Anal. Calcd. for $C_6H_7N_5O$: N, 42.4. Found: N, 42.8.

2,8-Diamino-6-methylthiopurine (XXXVII).—2,8-Diamino-6-purinethiol (XXXVI) (6 g.) was dissolved in 150 ml. of 2 *N* potassium hydroxide and treated with 5.2 g. of methyl iodide in one portion. The mixture was stirred for 1.5 hr. at 10°. Finally, the mixture was heated to boiling and acidified with acetic acid. A small amount of solid separated on standing at room temperature for 10 hr. The mixture was filtered, and the residue was discarded. The filtrate was then adjusted to *p*H 8 with concentrated aqueous ammonium hydroxide. The white precipitate that separated at room temperature was filtered and washed with water. The precipitate was then suspended in hot water and dissolved by adding concentrated ammonium hydroxide. The solution was treated with charcoal and filtered. The excess ammonia was expelled by heating the solution. A white precipitate (3.5 g.) settled at room temperature and was filtered, washed with water, and dried at 40–50° for 15 hr.

Anal. Calcd. for $C_6H_8N_6S \cdot {}^1/_2H_2O$: C, 35.1; H, 4.4. Found: C, 35.1; H, 4.3.

A small sample was recrystallized from water and dried at 90°, m.p. 306–308°.

Anal. Calcd. for $C_6H_8N_6S$: C, 36.7; H, 4.1; N, 42.7. Found: C, 36.8; H, 4.0; N, 42.8.

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(17) G. B. Elion, W. Lange and G. H. Hitchings, THIS JOURNAL, **78**, 217 (1956).