tempt to prepare the picrate in methanol solution resulted in the formation of the picrate of methyl 4-methoxynicotinate-1-oxide, m.p. 146–147°, identical with a sample prepared from methyl 4-methoxynicotinate-1-oxide.

2-Methoxy-3-cyanopyridine.—To a solution of 2.8 g. of sodium in 250 ml. of absolute methanol was added 10.0 g. of 2-chloronicotinonitrile.[1] The reaction mixture was stirred and heated under reflux on a steam-bath for 1.5 hours and with continued stirring the excess methanol was distilled under slightly reduced pressure. The residual light brown oil crystallized upon addition of 75–100 ml. of water. The white solid was filtered by suction, washed with cold water and dried *in vacuo* to give 7.6 g. (78.5%) of 2-methoxynicotinonitrile, m.p. 75–77°. By making the filtrates alkaline, extracting with chloroform, and evaporating the chloroform, a small amount of oil was obtained which yielded additional product (*ca.* 0.5 g.) on recrystallization from water. Extraction of the latter filtrates with ether yielded 0.34 g. of a second product, m.p. 117–122°, which was purified by vacuum sublimation at 100° to give 2-methoxynicotinamide, m.p. 128–130°, identified by a mixed melting point determination with an authentic sample. The analytical sample of 2-methoxynicotinonitrile was prepared by vacuum sublimation at 55–60°, m.p. 76.5–77.5°.

Anal. Calcd. for $C_7H_6N_2O$: C, 62.7; H, 4.5; N, 20.9. Found: C, 63.0; H, 4.7; N, 20.8.

2-Methoxynicotinamide.—To 40 ml. of absolute ethanol was added with stirring 2.0 g. of 2-methoxynicotinonitrile and 0.8 g. of potassium hydroxide. After solution occurred, 40 ml. of 30% hydrogen peroxide was added slowly. The reaction mixture was then heated at 65° (internal temperature) with stirring for 30 minutes, an additional 10 ml. of 30% hydrogen peroxide was added, and heating and stirring were continued for 30 minutes. The reaction mixture was reduced to a volume of 20–25 ml. and chilled. The resulting solid was filtered, washed and dried *in vacuo* to give 1.32 g. of 2-methoxynicotinamide, while concentration of the filtrates to 10 ml. gave an additional 0.27 g. for a total yield of 1.59 g. (70%), m.p. 130–131°. The analytical sample was prepared by vacuum sublimation at 100°.

Anal. Calcd. for $C_7H_8N_2O_2$: C, 55.3; H, 5.3; N, 18.4. Found: C, 55.5; H, 5.1; N, 18.7.

PRINCETON, NEW JERSEY

[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

Studies on Condensed Pyrimidine Systems. XIII. Some Amino-substituted Derivatives of Guanine and 6-Thioguanine

BY GERTRUDE B. ELION, WILLIAM H. LANGE AND GEORGE H. HITCHINGS

RECEIVED JULY 29, 1955

A variety of 2-substituted amino-6-hydroxypurines has been prepared by the reaction of 6-hydroxy-2-methylmercaptopurine with aliphatic, aromatic and heterocyclic amines. These purines have been converted to the corresponding 6-mercaptopurines by treatment with phosphorus pentasulfide in pyridine.

In pursuance of the investigation of analogs of the nucleic acid bases for possible antimetabolite activity,[1–4] the synthesis of a number of purines structurally related to guanine was undertaken. The finding that the replacement of the hydrogens of the amino groups of adenine and 2,6-diaminopurine weakened their microbiological activities[5,6] made it of interest to determine whether a similar effect would be observed with derivatives of guanine. The fact that 6-thioguanine[7] acts as an inhibitor of the growth of *Lactobacillus casei*,[6,8] embryonic tissue[9] and a number of neoplasms, *e.g.*, sarcoma 180[10] and leukemia L 1210[11,12] further stimulated the investigation of related compounds.

The synthesis of 2-substituted amino-6-hydroxypurines can be approached in a number of ways. Introduction of the substituted amino group into the 2-position of the pyrimidine ring can be accomplished in the first stage of the synthesis by reaction of an amine hydrochloride with dicyandiamide[13] or with cyanoacetic ester. The latter type of condensation leads, however, to a mixture of isomers.[13] It is also possible to replace the 2-methylmercapto group of 4-amino-6-hydroxy-2-methylmercaptopyrimidine by amines[13] and one could then proceed with the total synthesis of each individual purine *via* nitrosation, reduction, formylation and ring closure. However, the most direct method for the synthesis of a series of purines of this type is obviously the introduction of the substituted amino group in the last step, so that a common intermediate, *e.g.*, 6-hydroxy-2-methylmercaptopurine, can be used for all the desired compounds. This method was highly successful in the synthesis of 6-substituted aminopurines from 6-methylmercaptopurine[14] and 8-alkylamino-2-hydroxypurines from 2-hydroxy-8-methylmercaptopurine[15] but presents some difficulties when the methylmercapto group is in the 2-position. Andrews, *et al.*,[16] had found that the 2-methylmercapto group of 2-methylmercaptoadenine could not be replaced successfully by ammonia or amines under a variety of conditions and this experience was duplicated in these laboratories using alkylamines. The primary obstacle to this type of

(1) G. H. Hitchings, E. A. Falco and M. B. Sherwood, *Science*, **102**, 251 (1945).

(2) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell, M. B. Sherwood and H. VanderWerff, *J. Biol. Chem.*, **183**, 1 (1950).

(3) G. H. Hitchings, G. B. Elion, E. A. Falco, P. B. Russell and H. VanderWerff, *Ann. N. Y. Acad. Sci.*, **52**, 1318 (1950).

(4) G. H. Hitchings and G. B. Elion, *ibid.*, **60**, 195 (1954).

(5) G. B. Elion and G. H. Hitchings, *J. Biol. Chem.*, **185**, 651 (1950).

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

(7) G. B. Elion and G. H. Hitchings, THIS JOURNAL, **77**, 1676 (1955).

(8) G. B. Elion, S. Singer, G. H. Hitchings, M. E. Balis and G. B. Brown, *J. Biol. Chem.*, **202**, 647 (1953).

(9) S. Bieber, R. Bieber and G. H. Hitchings, *Ann. N. Y. Acad. Sci.*, **60**, 207 (1954).

(10) D. A. Clarke, F. S. Philips, S. S. Sternberg and C. C. Stock, *ibid.*, **60**, 235 (1954).

(11) L. W. Law, *Proc. Soc. Exptl. Biol. Med.*, **84**, 409 (1953).

(12) L. W. Law, V. Taormina and P. J. Boyle, *Ann. N. Y. Acad. Sci.*, **60**, 244 (1954).

(13) B. Roth, J. M. Smith, Jr., and M. E. Hultquist, THIS JOURNAL, **73**, 2864 (1951).

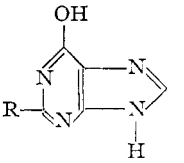
(14) G. B. Elion, E. Burgi and G. H. Hitchings, *ibid.*, **74**, 411 (1953).

(15) C. O. Johns, *J. Biol. Chem.*, **21**, 319 (1915).

(16) K. J. M. Andrews, N. Anand, A. R. Todd and A. Topham, *J. Chem. Soc.*, 2490 (1949).

TABLE I

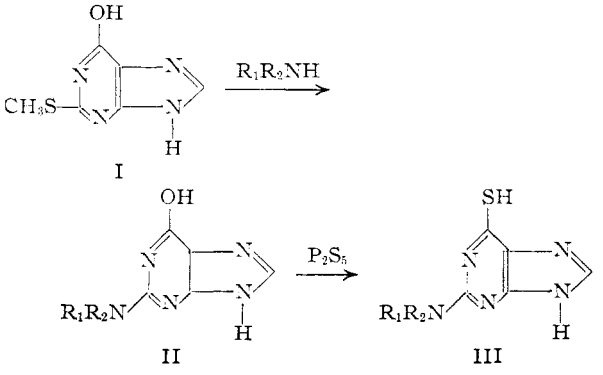
2-SUBSTITUTED AMINO-6-HYDROXYPURINES



R	Reaction conditions: Solvent	Concn. of amine, %	Yield, %	Empirical formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	H_2O, % Calcd.	H_2O, % Found
CH_3NH	H_2O	35	37	$C_6H_7N_5O$	43.6	43.0	4.2	4.6	42.4	41.9		
CH_3NH	CH_3OH	14	65									
C_2H_5NH	H_2O	33	30	$C_7H_9N_5O \cdot H_2O$	42.6	42.8	5.6	5.8	35.5	35.9	4.6[c]	4.7[d]
$(CH_3)_2N$	CH_3OH	12[a]	61	$C_7H_9N_5O \cdot HCl \cdot H_2O$	36.0	35.9	5.1	5.0	30.0	30.1	7.7	8.0[e]
$(CH_3)_2N$	CH_3OH	25	50									
C_6H_5NH	None		56	$C_{11}H_9N_5O \cdot HCl \cdot 2H_2O$	44.1	44.4	4.7	5.0	23.4	23.5	12.1	12.6[e]
p-ClC_6H_4NH	None		35	$C_{11}H_8ClN_5O \cdot HCl \cdot 2H_2O$	39.5	39.9	3.9	4.5	21.0	21.0	10.8	11.1[e]
$CH_2(CH_2)_4N$	None		33	$C_{10}H_{13}N_5O \cdot HCl \cdot H_2O$	43.9	44.0	5.9	6.1	25.6	24.8		
$CH_2(CH_2)_4N$	C_2H_5OH	50	10									
$CH_2(CH_2)_4N$	H_2O—HCl[b]		19									

[a] Two molecular equivalents of amine. [b] Four molecular equivalents of piperidine dissolved in 2 molecular equivalents of concentrated hydrochloric acid. [c] Calculated for loss of one-half mole of water; other half-mole is not lost at 140°. [d] Dried at 140°; regains the water on exposure to air. [e] Dried at 110°; regains the water on exposure to air.

replacement in the purine series appears to be the instability of the purine ring under strongly alkaline conditions at elevated temperatures. With aqueous alkylamines at 150° there is extensive decomposition of 6-amino-2-methylmercaptopurine, while at 130° there is very little replacement of the methylmercapto group. However, in the case of 6-hydroxy-2-methylmercaptopurine (I) it is possible to obtain 30–40% replacement of the methylmercapto group at 140°, with the simultaneous recovery of some starting material and only slight decomposition, when aqueous alkylamines are used, and to improve the yield to about 60% by using alcoholic amine solutions (Table I). With weak bases, such as aniline, one can raise the temperature to 160°, using no solvent, without extensive disruption of the purine ring. With piperidine, the most satisfactory yields are obtained either with no solvent or with a mixture of piperidine and piperidine hydrochloride (Table I). A series of amino-substituted guanines was therefore made by this direct method (II, R_1R_2N = CH_3NH, C_2H_5NH, $(CH_3)_2N$, C_6H_5NH, p-ClC_6H_4NH, piperidino). The conversion of these purines to the corresponding 2-substituted amino-6-mercaptopurines (III) (Table II) was accomplished by using phosphorus pentasulfide in dry pyridine, as previously described for the synthesis of 6-thioguanine,[7] a modification of the method used in the synthesis of 6-mercaptopurine.[14]



The effect on the ultraviolet absorption spectrum of replacing the 2-amino group of guanine by alkylamines or piperidine is, as might be expected, to shift the absorption maxima to slightly higher wave lengths without producing any marked changes in the curves (Table III). The 2-arylamino-6-hydroxypurines (II, R_2R_2N = C_6H_5NH, p-ClC_6H_4NH), on the other hand, have only one intense absorption band in the 270–275 mμ region at pH 1; at pH 11 their spectra resemble those of the other amino-substituted guanines except that the molecular extinction coefficients are higher. Similarly, the ultraviolet absorption spectra of the amino-substituted thioguanines (III) bear a close resemblance to that of 6-thioguanine.[7] Substitution of both hydrogens of the amino group, *e.g.*, $(CH_3)_2N$ or piperidino, results in a bathochromic shift at pH 1 as it does with the corresponding derivatives of guanine (Table III).

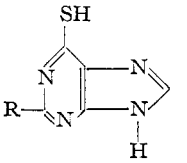
As was predictable from the effect of alkylation of the amino group of adenine,[5,6] 6-hydroxy-2-methylaminopurine acts as a purine source for *L. casei* but is only about 0.02 as potent as guanine, while 2-dimethylamino-6-hydroxypurine has only about 0.001 the activity of guanine. It is also of considerable interest that Weissmann, *et al.*,[17] have recently found a methylated guanine in urine which is identical with the synthetic 6-hydroxy-2-methylaminopurine. This is the first time that this compound has been demonstrated in natural material. Both 6-methylaminopurine and 6-dimethylaminopurine have been isolated recently from natural sources, the former from bacteria[18]

(17) B. Weissmann, P. A. Bromberg and A. B. Gutman, *Nature*, in press.

(18) D. B. Dunn and J. D. Smith, *Nature*, **175**, 336 (1955).

TABLE II

2-SUBSTITUTED AMINO-6-MERCAPTOPURINES

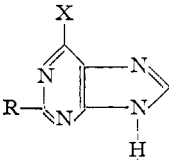


R	Yield, %	Empirical formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found	H_2O, % Calcd.	H_2O, % Found
CH_3NH	64	$C_6H_7N_5S \cdot {}^1/_4H_2O$	38.8	38.7	4.0	4.0	37.7	36.5[a]	17.3	17.4	2.4	2.7[d]
C_2H_5NH	45	$C_7H_9N_5S \cdot {}^1/_2H_2O$	41.2	41.3	4.9	5.4	34.3	33.6			4.4	3.7[d]
$(CH_3)_2N$	52	$C_7H_9N_5S \cdot H_2O$	39.5	39.3	5.2	4.7			15.0	15.7	4.2[b]	3.7[d]
C_6H_5NH	34	$C_{11}H_9N_5S \cdot 1.5H_2O$	48.9	48.9	4.4	4.4	25.9	26.0	11.9	12.1	6.7[c]	6.2[d]
$CH_2(CH_2)_4N$	51	$C_{10}H_{13}N_5S \cdot {}^1/_2H_2O$	49.2	49.3	5.7	5.2	28.7	28.1[a]	13.1	13.5	3.7	3.5[d]

[a] Nitrogen by Dumas method. [b] Calcd. for loss of one-half mole of water; other half-mole is not lost at 140°. [c] Calcd, for loss of one mole of water; other half-mole is not lost at 140°. [d] Dried at 140°; regains the water on exposure to air.

TABLE III

ULTRAVIOLET ABSORPTION SPECTRA



R	X	pH 1 λmax mμ	pH 1 Em	pH 1 λmin mμ	pH 1 Em	pH 11 λmax mμ	pH 11 Em	pH 11 λmin mμ	pH 11 Em
SH	OH	285	21,600	248	3200	278	16,800	248	6400
CH_3S	OH	265	16,400	238	6800	270	14,600	252	9400
		248	10,800			245	6,400		
NH_2	OH	271[a]	7,100			273	7,000	258	5700
CH_3NH	OH	250	12,300			245	8,000		
		280	6,900	272	6600	279	7,250	263	6100
C_2H_5NH	OH	253	14,800			245	9,500		
		280[a]	8,100			275	9,300	262	8100
$(CH_3)_2N$	OH	258	14,500			245[a]	9,200		
		290[a]	6,300	235	6900	277	8,000	265	7100
C_6H_5NH	OH					238	15,300		
		270	20,400	240	8700	274	20,400	250	11,000
p-ClC_6H_4NH	OH	274	20,200	245	8400	240	14,600	252	10,000
						280	21,100		
$CH_2(CH_2)_4N$	OH	260	19,800	235	7000	252	12,700	240	11,900
		290[a]	6,150			280[a]	8,100		
CH_3NH	SH	261	11,500	240	5500	245	11,100	240	10,000
		350	18,900	300	2800	275	10,200	265	9,200
						325	13,900	295	5,900
C_2H_5NH	SH	263	11,500	240	6500	250	11,200	245	11,000
		350	17,100	300	2800	276	10,100	265	9,100
						325	12,600	295	6,100
$(CH_3)_2N$	SH	268	12,700	245	5700	253	11,800	245	10,900
		358	17,200	309	2100	283	11,800	270	10,000
						322	11,200	302	6,200
C_6H_5NH	SH					245	12,700	238	12,200
		278	17,600	250	11,200	283	28,800	252	11,900
		352	17,900	315	6500	328	11,700	310	9,800
$CH_2(CH_2)_4N$	SH	272	14,500	248	7200	257	13,100	245	12,200
		359	14,400	313	2900	282	11,100	273	10,600
						328	9,400	305	6,600

[a] Inflection.

and the latter from fungi.[19] The 2-substituted amino-6-mercaptopurines behave like purine antagonists in *L. casei*. These results and results of the Sarcoma 180 trials will be published elsewhere.

(19) C. W. Waller, P. W. Fryth, B. L. Hutchings and J. H. Williams, THIS JOURNAL, **75**, 2025 (1953).

Experimental

6-Hydroxy-2-mercaptopurine.—This compound was synthesized by Traube's method[20] with several modifications. These modifications were mainly in the preparation of the intermediate 4,5-diamino-6-hydroxy-2-mercaptopyrimidine, as described previously,[14] and in the method of ring closure

(20) W. Traube, *Ann.*, **331**, 77 (1904).

of the 5-formamido derivative, as described below. The crude 4,5-diamino-6-hydroxy-2-mercaptopyrimidine (100 g.), as obtained after the reduction step, was formulated by heating with 500 ml. of 90% formic acid, under reflux conditions, for 2 hours. The reaction mixture was cooled and the crude 4-amino-5-formamido-6-hydroxy-2-mercaptopyrimidine filtered off and pressed as dry as possible on a sintered glass funnel. The filter cake was then suspended in 200 ml. of formamide and heated in an open flask in a Wood metal-bath, at 175–185° for 2 hours. The mixture was cooled and the precipitate of 6-hydroxy-2-mercaptopurine (100.5 g.) collected, washed with water and dried at 120°. The crude product was purified by solution 2 liters of 1 *N* sodium hydroxide, filtration and precipitation by acidification to *p*H 5 with glacial acetic acid. The precipitate (90 g.) was washed with water and dried at 120°. The ultraviolet absorption spectrum of the product was identical with that of a sample of 6-hydroxy-2-mercaptopurine prepared by Traube's method of ring closure[20] from the dry potassium salt of the formamido derivative (Table III).

Anal. Calcd. for $C_5H_4N_4OS$: N, 33.3. Found: N, 33.5.

6-Hydroxy-2-methylmercaptopurine.—To a solution of 42 g. (0.25 mole) of 6-hydroxy-2-mercaptopurine in 250 ml. of 2 *N* sodium hydroxide and 100 ml. of water was added slowly, with stirring, 31.5 g. (28.3 ml., 0.25 mole) of dimethyl sulfate. The temperature was kept between 25 and 40° by use of a cold water-bath when necessary. Stirring was continued for one hour after all the dimethyl sulfate had been added and the reaction mixture was allowed to stand at room temperature overnight. The mixture was adjusted to *p*H 5 with glacial acetic acid, chilled and the precipitate of 6-hydroxy-2-methylmercaptopurine collected, washed with cold water and dried at 110° (44 g., 95.5%). This material was 98% pure, as judged by its ultraviolet absorption spectrum and was used for the subsequent reactions without further purification. A 10-g. sample was purified for analysis by recrystallization from 600 ml. of hot water, using Darco for decolorization. The ultraviolet absorption spectrum is given in Table III. The compound does not melt below 300°. It loses its water of hydration at 110° but regains it on exposure to the atmosphere.

Anal. Calcd. for $C_8H_6N_4OS \cdot 1.5H_2O$: C, 34.4; H, 4.3; N, 26.8; H_2O, 12.9. Found: C, 34.9; H, 4.7; N, 27.5; H_2O, 12.4.

Reaction of 6-Hydroxy-2-methylmercaptopurine with Amines. General Method.—The reaction was carried out by heating 6-hydroxy-2-methylmercaptopurine with 3 or 4 molecular equivalents of the amine in a sealed tube at 140° for 24 hours, unless otherwise indicated. With the aromatic amines, *e.g.*, aniline and *p*-chloroaniline, the temperature was maintained at 160° for 48 hours. The solvents employed in the reaction varied: water, methanol, ethanol or no solvent at all; the concentration of the amine in the solvent is given in Table I. With alkylamines the reaction proceeded in better yield in methanol than in water; nevertheless, aqueous solutions were used when these were the only commercially available forms of the amine. The isolation of the reaction product depended on the amine used. Yields and analyses are given in Table I and the ultraviolet absorption spectra of the products are in Table III.

A. Methylamine or Ethylamine.—The reaction mixture was evaporated to dryness under reduced pressure. The solid residue was taken up in *ca.* 5 volumes of water at room temperature, and the 2-substituted amino-6-hydroxypurine filtered off. Neutralization of the filtrate with acetic acid to *p*H 5 resulted in the recovery of some starting material. The 2-methylamino- and 2-ethylamino-6-hydroxypurines were purified by solution in 40 volumes of hot 0.3 *N* hydrochloric acid and neutralization to *p*H 6 with ammonium hydroxide. The 2-methylamino derivative was dried at 140° for analysis; the 2-ethylamino derivative was dried at room temperature and contained one molecule of water of hydration.

B. Dimethylamine.—After cooling, the reaction mixture was diluted with 3 volumes of methanol, chilled and the crude product filtered off. The 2-dimethylamino-6-hydroxypurine was recrystallized from 100 parts of boiling water. A sample was purified for analysis as the hydrochloride by recrystallization from 10 parts of 2 *N* hydrochloric acid.

C. Aniline or *p*-Chloroaniline.—The excess amine was removed by diluting the reaction mixture with 20 volumes of a 1:1 mixture of absolute ethanol and ether. The solid residue was collected, washed with ether and the products purified as their hydrochlorides by recrystallization from 1 *N* hydrochloric acid.

D. Piperidine.—The separation of 6-hydroxy-2-piperidinopurine from unchanged 6-hydroxy-2-methylmercaptopurine takes advantage of the tendency of the 2-piperidino derivative to form supersaturated solutions. The reaction mixture was diluted with *ca.* 4 parts of water, filtered and acidified to *p*H 5 with hydrochloric acid and the precipitate containing both product and starting material collected. The filtrate, containing 6-hydroxy-2-piperidinopurine, was evaporated to dryness under reduced pressure and the residue treated with 50 parts of a 1:1 mixture of 6 *N* hydrochloric acid and acetone and chilled to precipitate the hydrochloride of the product. The original precipitate was then extracted several times with 10 parts of boiling water, the extracts chilled and filtered and the filtrates treated in the same way as the original filtrate. The samples of crude 6-hydroxy-2-piperidinopurine hydrochloride were pooled and recrystallized by solution in 50 parts of hot 6 *N* hydrochloric acid, treated with Darco, diluted with an equal volume of acetone and chilled.

The 2-Substituted Amino-6-mercaptopurines. General Method.—Whenever the 2-substituted amino-6-hydroxypurine was isolated as its hydrochloride, this was first converted to the free base by neutralization of a hot aqueous suspension of the hydrochloride with the calculated amount of sodium hydroxide. The free bases were collected after chilling, washed with water and dried at 140° before treatment with phosphorus pentasulfide. A mixture of the 2-substituted amino-6-hydroxypurine, 5 parts of freshly pulverized phosphorus pentasulfide and 50 parts of dry pyridine was heated under reflux conditions for 3 hours. The pyridine was removed under reduced pressure and the residue heated with 40 volumes of water until the foaming subsided (*ca.* 15 to 20 minutes) to decompose the excess phosphorus pentasulfide. In the case of the 2-dimethylamino derivatives, the product could then be isolated simply by chilling and collecting the precipitate. In all the other cases, the aqueous reaction mixtures were chilled and diluted cautiously with an equal volume of concentrated ammonium hydroxide. After chilling, the copious precipitate of ammonium phosphate was filtered off and the filtrate evaporated to a small volume (*ca.* 25 ml. for a 5-g. run) under reduced pressure. The temperature was kept below 30° in the early stages of the evaporation and later permitted to reach 50°. The solution was adjusted to *p*H 5, chilled and the product collected by filtration.

The methylamino, ethylamino and piperidino derivatives were purified by solution in dilute ammonium hydroxide and acidification to *p*H 5 with acetic acid. The dimethylamino derivative was dissolved in 60 parts of hot 2 *N* hydrochloric acid and the filtered solution brought to *p*H 5 with ammonium hydroxide. Difficulty was encountered in ridding the 2-anilino-6-mercaptopurine of phosphorus by the usual methods; it was purified by solution in 12 parts of cold dimethylformamide, in which the phosphorus-containing material was insoluble, and precipitated by dilution with 8 volumes of water. All samples were washed with water and dried in a vacuum desiccator or at 100° before analysis. Samples which were dried at 140° showed a tendency to regain all or part of their water of crystallization when exposed to air at room temperature. The anilino derivative lost only one of its 1.5 molecules of water of hydration at 140° and regained this loss on exposure to the atmosphere. Yields and analyses are given in Table II, spectra in Table III.

Ultraviolet Absorption Spectra.—The spectra were measured with a model DU Beckman spectrophotometer, at a concentration of 10 mg. per liter. For solutions of *p*H 1, 0.1 *N* hydrochloric acid was used and for *p*H 11, a Sørensen glycine–sodium hydroxide buffer.

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