[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

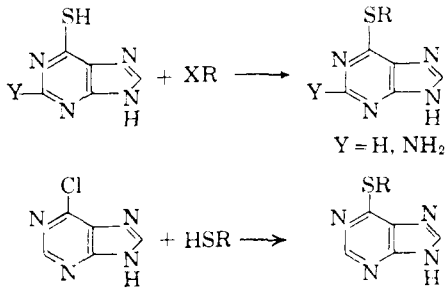
Condensed Pyrimidine Systems. XX. Purines Related to 6-Mercaptopurine and Thioguanine

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

RECEIVED SEPTEMBER 17, 1958

The syntheses of a number of new 8-substituted 6-mercaptopurines and a number of S-substituted derivatives of 6-mercaptopurine and thioguanine are reported. The 8-substituted derivatives were prepared from the 4,5-diaminopyrimidines by fusion with an appropriate urea or amide. The S-substituted derivatives were prepared either from the 6-mercaptopurine with an aryl or alkyl halide or by the reaction of 6-chloropurine with an appropriate mercaptan. 6-Purinylthiocyanate is rapidly and quantitatively hydrolyzed to 6-mercaptopurine in aqueous alkaline solution.

The synthesis of potential antagonists of the nucleic acid bases has been under way in these laboratories for a number of years.[1-5] With the finding that 6-mercaptopurine and 6-thioguanine are inhibitory to a number of transplantable animal tumors[6-12] as well as to human leukemia,[13,14] an extensive program was undertaken to study the relationship of chemical structure to antitumor activity among purines related to 6-mercaptopurine and 6-thioguanine.[15,16] The synthesis of some of



these compounds has been reported.[17-20] It is the purpose of this paper to describe the methods of preparation of a number of additional derivatives, whose effects on Sarcoma 180 have been reported.[16]

A group of purines was prepared in which the sulfur of 6-mercaptopurine and 6-thioguanine was replaced by alkylmercapto, benzylmercapto and arylmercapto groups. These compounds (Table I) were synthesized from the mercaptopurines by reaction with the appropriate halide in alkaline medium or by the reaction of 6-chloropurine with the desired mercaptan.

The ultraviolet absorption spectra of the alkylmercaptopurines differ strikingly from those of the parent "mercapto" purines.[17,21,22] Whereas at *p*H 1.0, 6-mercaptopurine and 6-thioguanine have bands at 325 and 347 mμ, respectively,[17,19] S-alkyl derivatives absorb at 298 and 318 mμ. At *p*H 11 the differences are less but still very pronounced. These observations suggest that the parent substances have the lactam (thioamide) rather than the mercaptan configuration. This is supported further by the close resemblance of the spectra of 1-methyl-6-purinethione and 6-mercaptopurine.[22] It is noteworthy also that the magnitude of the shift of absorption maximum with change in *p*H is diminished in the alkylmercapto derivatives. This might have been expected since in effect alkylation removes one potentially ionizable hydrogen atom.

The reaction of 6-chloropurine and potassium thiocyanate in methanol led to the formation of 6-thiocyanopurine. This compound was found to be extremely unstable in dilute aqueous alkali, forming 6-mercaptopurine extremely rapidly in 0.1 *N* sodium hydroxide (Fig. 1). The compound is, however, quite stable in acid and neutral solution, in which it possesses a spectrum typical of a 6-substituted mercaptopurine. Isomerization to the isothiocyanate, which was observed by Johnson and McCollum[23] when 4-thiocyanopyrimidines were heated with alcohol at reflux temperature, did not occur when the thiocyanopurine was heated under these conditions.

Of interest in the correlation of structure with biological activity were derivatives containing a variety of substituents at the 8-position of the purine, *e.g.*, methyl, hydroxyl, mercapto and methylmercapto. The 6-mercapto-8-methylpurine was prepared in two ways: by ring closure of the sodium salt of 5-acetamido-4-amino-6-hydroxypyrimidine to 6-hydroxy-8-methylpurine followed by treatment with phosphorus pentasulfide, and

(1) G. H. Hitchings, E. A. Falco and M. B. Sherwood, *Science*, **102**, 251 (1945).
(2) G. H. Hitchings, G. B. Elion, *et al.*, *J. Biol. Chem.*, **183**, 1 (1950).
(3) G. H. Hitchings and G. B. Elion, *Ann. N. Y. Acad. Sci.*, **60**, 195 (1954).
(4) G. H. Hitchings, *Am. J. Clin. Nutrition*, **3**, 321 (1955).
(5) G. H. Hitchings and G. B. Elion, Proc. Intern. Congr. Biochem., 3rd Congr., Brussels, 1955.
(6) D. A. Clarke, F. S. Philips, S. S. Sternberg and C. C. Stock, *Ann. N. Y. Acad. Sci.*, **60**, 235 (1954).
(7) D. A. Clarke, F. S. Philips, S. S. Sternberg, C. C. Stock and G. H. Hitchings, *Proc. Am. Assoc. Cancer Research*, **1**, 9 (1954).
(8) D. A. Clarke, F. S. Philips, S. S. Sternberg, C. C. Stock, G. B. Elion and G. H. Hitchings, *Cancer Research*, **13**, 593 (1953).
(9) L. W. Law, V. Taormina and P. J. Boyle, *Ann. N. Y. Acad. Sci.*, **60**, 244 (1954).
(10) A. Goldin, J. M. Venditti, S. R. Humphreys, D. Dennis, N. Mantel and S. W. Greenhouse, *ibid.*, **60**, 251 (1954).
(11) H. E. Skipper, *ibid.*, **60**, 267 (1954).
(12) H. E. Skipper, J. R. Thomson, G. B. Elion and G. H. Hitchings, *Cancer Research*, **14**, 294 (1954).
(13) J. H. Burchenal, M. L. Murphy, R. R. Ellison, M. P. Sykes, T. C. Tan, L. A. Leone, D. A. Karnofsky, L. F. Craver, H. W. Dargeon and C. P. Rhoads, *Blood*, **8**, 965 (1953).
(14) B. E. Hall, M. D. Richards, F. M. Willett and T. V. Feichtmeier, *Ann. N. Y. Acad. Sci.*, **60**, 374 (1954).
(15) G. B. Elion, *Proc. Roy. Soc. Med.*, **50**, 7 (1957).
(16) D. A. Clarke, G. B. Elion, G. H. Hitchings and C. C. Stock, *Cancer Research*, **18**, 445 (1958).
(17) G. B. Elion, E. Burgi and G. H. Hitchings, THIS JOURNAL, **74**, 411 (1952).
(18) G. B. Elion and G. H. Hitchings, *ibid.*, **76**, 4027 (1954).
(19) G. B. Elion and G. H. Hitchings, *ibid.*, **77**, 1676 (1955).
(20) G. B. Elion, W. H. Lange and G. H. Hitchings, *ibid.*, **78**, 217 (1956).
(21) S. F. Mason, *J. Chem. Soc.*, 2071 (1954).
(22) G. B. Elion, "Chemistry and Biology of Purines," Ciba Foundation Symposium, Churchill, London, 1957, pp. 39–49.
(23) T. B. Johnson and E. V. McCollum, *Amer. Chem. J.*, **36**, 136 (1906).

directly by heating 4,5-diamino-6-mercaptopyrimidine with acetamide. It had been shown by Bredereck, *et al.*,[24] that 4,5-diaminouracil and acetamide give 8-methylxanthine. Fusion of the appropriate 4,5-diaminopyrimidines with urea or thiourea led to the corresponding 8-hydroxy or 8-mercaptopurines.[25-27] The 8-hydroxy derivative of thioguanine was prepared by treatment of 2-amino-6-chloro-8-hydroxypurine with sodium hydrosulfide.

The 6-hydroxyl group of two derivatives, 6-hydroxy-8-methyl- and 6-hydroxy-8-mercaptopurine, could be converted directly to a 6-mercapto group by treatment with phosphorus pentasulfide using tetralin as the solvent.[17] This method failed with 2-amino-6-hydroxy-8-mercaptopurine; however, when this was first methylated to the 8-methylmercapto derivative, the desired 2-amino-6,8-dimercaptopurine was obtained. (The replacement of an alkylmercapto by a mercapto group in this reaction had been observed earlier in the pyrimidine series.[28]) In later work, after the superiority of pyridine over tetralin as a solvent for the preparation of thioguanine had been discovered,[19] the reaction of 2-amino-6-hydroxy-8-mercaptopurine with phosphorus pentasulfide was restudied using pyridine as the solvent, and the aminodimercapto derivative was thus obtained directly. It seems probable that pyridine would prove superior in all the above reactions.

In Table II are given the ultraviolet absorption spectra of the various 8-substituted purines. As could be anticipated, the introduction of an 8-methyl group has little or no effect on the ultraviolet absorption spectrum of 6-mercaptopurine or hypoxanthine. The 8-hydroxyl group produces a bathochromic shift at *p*H 1 of about 7 mμ in the longer wave length band of 6-mercaptopurine, whereas the 8-mercapto group produces a bathochromic shift of considerably larger magnitude: 30–35 mμ for derivatives of hypoxanthine, guanine, 6-mercaptopurine and 6-thioguanine at *p*H 1, and *ca.* 25 mμ at *p*H 11. The bathochromic shift produced by the 8-methylmercapto group is somewhat less than that caused by the 8-mercapto group.

Experimental

Melting points were determined in a copper block apparatus preheated to within 10° of the melting temperature. In a number of instances a micro hot-stage apparatus also was used. No corrections were applied.

6-Alkylmercaptopurines (2 = H or NH_2). General Method.—To a solution of 6-mercaptopurine[17] or 2-amino-6-mercaptopurine[19] in 2 molecular equivalents of 0.3 *N* sodium hydroxide (either aqueous or 50% aqueous methanol) is added 1.1 molecular equivalents of an alkyl halide (bromide or iodide) and the mixture is heated in a sealed tube at 120° for 18 hours. The reaction mixture is then cooled, acidified to *p*H 5 with hydrochloric acid and the precipitate collected. The product is recrystallized from water or ethanol, as indicated in Table I. The preparation of 2-amino-6-ethylmercaptopurine is described in the example given below.

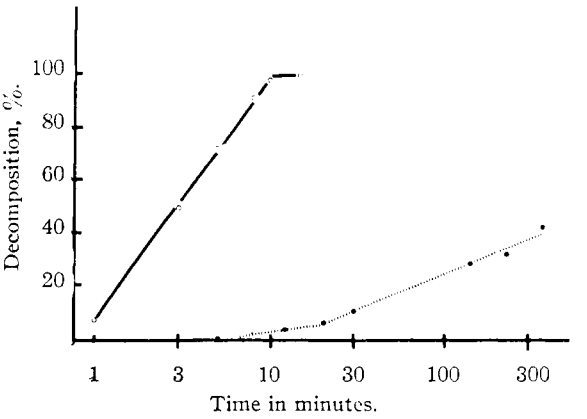


Fig. 1.—The rate of decomposition of 6-thiocyanopurine to 6-mercaptopurine at 25° as determined spectrophotometrically in 0.1 *N* sodium hydroxide, O—O—O, and in *p*H 11 buffer, ●- -●- -●- -●. For details see text.

Carboxymethylthiopurines were prepared by a somewhat atypical technique which also is illustrated. The 2-amino-6-methylmercaptopurine was prepared by the portionwise addition of dimethyl sulfate (in 10% excess of the calculated quantity) to the alkaline solution of thioguanine at room temperature, according to the procedure previously described for the methylation of 6-mercaptopurine.[17]

2-Amino-6-ethylmercaptopurine.—A mixture of 4 g. (0.024 mole) of thioguanine, 24 ml. of 2 *N* sodium hydroxide, 2.8 g. (0.026 mole) of ethyl bromide and 150 ml. of water was heated at 120° for 18 hours in a sealed vessel. The reaction mixture was acidified to *p*H 5 with hydrochloric acid, chilled and the precipitate was recovered by filtration. The crude reaction product was suspended in 150 ml. of water heated to boiling and filtered hot from some unreacted thioguanine. On chilling 2.55 g. of product precipitated, was collected, washed and dried in a vacuum desiccator. The compound was recrystallized for analysis by two additional recrystallizations from water.

6-Carboxymethylthiopurine.—To a solution of 5 g. of 6-mercaptopurine hydrate (0.0294 mole) in 60 ml. of 1 *N* sodium hydroxide was added 3 g. (0.0317 mole) of chloroacetic acid and the solution was heated to boiling for a few minutes and then acidified to *p*H 3–4 with 13 ml. of 2 *N* hydrochloric acid. The mixture was allowed to cool for 1 hour, chilled and filtered. The pale yellow crystalline precipitate (4.55 g.) was recrystallized from 1 liter of boiling water and then was collected, washed with water and dried at 100° (3.7 g.).

6-Benzylmercaptopurines (2 = H or NH_2). General Method.—To a stirred solution of 6-mercaptopurine or 2-amino-6-mercaptopurine in 2 molecular equivalents of 2 *N* sodium hydroxide diluted with 6 volumes of water or 50% aqueous methanol is added slowly, over a 2- or 3-hour period one molecular equivalent (5 to 10% excess in some cases) of the appropriate benzyl bromide or chloride. The reaction mixture is kept at room temperature and the stirring is continued for at least 5 hours or longer if solution is not complete. The product is precipitated by acidification to *p*H 5–6 with hydrochloric acid and recrystallized as indicated. The yields and properties of the compounds are given in Table I. Examples of the procedure follow.

2-Amino-6-(2′-chlorobenzylmercapto)-purine.—To a solution of thioguanine (4 g., 0.024 mole) in 24 ml. of 2 *N* sodium hydroxide and 150 ml. of 50% aqueous methanol was added, with stirring over a 2-hour period, 3.85 g. (0.024 mole) of *o*-chlorobenzyl chloride and stirring was continued for 3 hours. The acidity was adjusted to *p*H 5 by the addition of 2 *N* hydrochloric acid, and the mixture was chilled and the precipitate collected and washed with water. After recrystallization from 300 ml. of 50% aqueous methanol, the product (4.9 g.) precipitated as colorless needles, m.p. 200° dec. An additional 0.2 g. of product was obtained by dilution of the filtrate with 250 ml. of water.

6-(4′-Chlorobenzylmercapto)-purine.—To a solution of 8 g. (0.047 mole) of 6-mercaptopurine in 47 ml. of 2 *N* so-

(24) H. Bredereck, H. G. VonSchuh and A. Martini, *Ber.*, **83**, 201 (1950).

(25) S. Gabriel and J. Colman, *ibid.*, **34**, 1234 (1901).

(26) O. Isay, *ibid.*, **39**, 250 (1906).

(27) Since the completion of this work a paper appeared by M. Ishidate and H. Yuki, *Pharm. Bull.*, **5**, 240 (1957), which described the syntheses of 6-hydroxy-8-mercaptopurine and 6,8-dimercaptopurine by the fusion of the appropriate pyrimidine with thiourea.

(28) G. B. Elion and G. H. Hitchings, THIS JOURNAL, **69**, 2138 (1947)

TABLE I

S-SUBSTITUTED DERIVATIVES OF 6-MERCAPTOPURINE AND THIOGUANINE

Purine substituents 2	6	Yield, %	Solvent of recrystn.[a]	M.p., °C.	Empirical formula	Ultraviolet absorption pH 1 λ_{max}	pH 1 $Em \times 10^3$	pH 11 λ_{max}	pH 11 $Em \times 10^3$	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
	SC_2H_5	43	A	196–197	$C_7H_8N_4S$	298	15.6	292	15.9	46.7	46.6	4.4	4.2	31.1	31.3
	SC_2H_4OH	52	A	301 d.	$C_7H_8N_4OS \cdot H_2O$	293	15.2	228	11.9					26.1	26.3
								291	15.7						
	$SC_4H_9(n)$	47	C	150–151	$C_9H_{12}N_4S$	298	16.3	292	15.6	51.9	52.3	5.8	5.3	26.9	27.1
	SCH_2COCH_3	70	A	184–186	$C_8H_8N_4OS \cdot {}^1/_2H_2O$	227	13.0	227	10.9					25.8	25.9
						300	14.9	288	14.3						
	SCH_2COOH	60	A	230–240 d.	$C_7H_6N_4O_2S$	287	14.5	292	15.1	40.0	39.9	2.7	2.9	26.7	26.4[b]
	$SCH_2C_6H_4Cl(2')$	65	B	199–200	$C_{12}H_9N_4ClS$	293	16.5	292	15.6	52.1	52.0	3.3	3.5	20.3	20.4
	$SCH_2C_6H_4Cl(3')$	23	G	163	$C_{12}H_9N_4ClS$	293	16.3	293	16.2					20.3	20.2
	$SCH_2C_6H_4Cl(4')$	69	B	197–198	$C_{12}H_9N_4ClS$	295	15.5	293	14.9	52.1	52.4	3.3	3.3	20.3	20.4
	$SCH_2C_6H_4OH(3')$	57	G	233–236	$C_{12}H_{10}N_4OS$	294	17.7	294	19.4					21.7	21.2
	$SCH_2C_6H_4NO_2(3')$	88	G	198	$C_{12}H_9N_5O_2S$	292	19.5	291	17.8	50.2	50.8	3.1	3.3		
	$SCH_2C_6H_4CH_3(4')$	34	A	143–144	$C_{13}H_{12}N_4S$	295	15.6	293	15.1	60.9	61.1	4.7	4.6	21.9	22.3
NH_2	SCH_3	87	A	205–206	$C_6H_7N_5S \cdot H_2O$	242	7.5	312	10.9	36.1	36.3	4.5	4.6	35.1	34.7
						273	10.2								
						318	12.7								
NH_2	SC_6H_5	55	A	203–204	$C_7H_9N_5S$	275	11.9	313	11.8	43.1	43.2	4.6	4.4	35.9	35.3
						318	15.6								
NH_2	$SC_3H_7(n)$	14	A	189–190	$C_8H_{11}N_5S$	277	10.2	313	11.5	45.9	46.0	5.3	5.4	33.5	33.3[c]
						318	14.2								
NH_2	$SC_4H_9(n)$	31	A,B	200–202	$C_9H_{13}N_5S$	276	10.5	313	11.4	38.4	38.3	5.8	5.7	31.4	31.8
						318	14.6								
NH_2	SCH_2COOH	84	H	>300 d.	$C_7H_7N_5O_2S$	240	9.2	312	10.8	37.3	37.0	3.1	3.5	31.1	30.5
						268	10.3								
						319	12.8								
NH_2	SCH_2COCH_3	92	A	198–199	$C_8H_9ON_5S$	270	9.0	230	14.4					31.3	31.5
						318	10.1	311	9.0						
NH_2	$SCH_2C_6H_5$	86	E	205–207	$C_{12}H_{11}N_5S$	276	9.5	315	11.3	56.0	55.9	4.3	4.4	27.2	27.0
						320	14.6								
NH_2	$SCH_2C_6H_4Cl(2')$	73	C	198–200 d.	$C_{12}H_{10}N_5ClS$	274	10.6	316	10.9	49.4	49.8	3.4	3.4	24.0	24.2
						320	14.7								
NH_2	$SCH_2C_6H_4Cl(4')$	76	B	229–230	$C_{12}H_{10}N_5ClS$	275	10.2	315	11.3	49.4	49.2	3.4	3.6	24.0	23.6[d]
						318	14.9								
NH_2	$SCH_2C_6H_4CH_3(2')$	65	D	223–224	$C_{13}H_{13}N_5S$	275	10.6	315	11.6	57.6	57.8	4.8	4.9	25.8	25.8
						319	15.4								
NH_2	$SCH_2C_6H_4CH_3(3')$	61	A	178–180	$C_{13}H_{13}N_5S$	275	10.4	313	12.0	57.6	57.5	4.8	4.9	25.8	25.6
						320	16.1								
NH_2	$SCH_2C_6H_4CH_3(4')$	77	C	261–263	$C_{13}H_{13}N_5S$	275	11.1	313	12.3	57.6	57.8	4.8	4.8	25.8	25.5
						320	17.1								
NH_2	$SCH_2C_6H_3Cl_2(3',4')$	55	F	230 d.	$C_{12}H_9N_5Cl_2S$	274	9.8	316	9.3					21.5	21.5
						320	13.7								
	SC_6H_5	83	G	244–245	$C_{11}H_9N_4S$	295	14.9	293	15.0	57.9	57.6	3.5	3.7	24.6	24.4
	$SC_6H_4Cl(4')$	85	G	248–250	$C_{11}H_7N_4ClS$	250	6.8	294	14.7	50.3	50.1	2.7	2.8	21.3	21.6
						292	15.8								
	$SC_6H_4CH_3(2')$	64	D	165–166 d.	$C_{12}H_{10}N_4S$	295	16.7	293	16.9	59.5	59.8	4.1	4.2	23.2	23.2
	$SC_6H_4CH_3(3')$	53	D	214–215	$C_{12}H_{10}N_4S$	295	15.7	293	16.2	59.5	59.7	4.1	4.3	23.2	23.6
	$SC_6H_4CH_3(4')$	70	D	239–240 d.	$C_{12}H_{10}N_4S$	295	14.8	293	15.9	59.5	59.8	4.1	4.3	23.2	23.5
	SCN	90	A	235 d.	$C_6H_3N_5S$	277	12.1			40.9	40.9	1.7	1.6	39.5	39.7

[a] Solvents of recrystallization: A, water; B, 95% ethanol; C, 50% aqueous methanol; D, precipitated from ethanol by addition of water; E, 50% aqueous acetone; F, precipitated from acetone by addition of water; G, absolute ethanol; H, dissolved in alkali and precipitated with acetic acid. [b] *Anal.* Calcd.: S, 15.2. Found: S, 15.2. [c] *Anal.* Calcd.: S, 15.2. Found: S, 14.9. [d] *Anal.* Calcd.: S, 11.0. Found: S, 10.5.

dium hydroxide and 250 ml. of water was added, with stirring at room temperature, 8.4 g. (0.052 mole) of *p*-chlorobenzyl chloride over a 2-hour period. After 5 hours of stirring, hydrochloric acid was added until a *p*H value of about 6 was reached. The mixture was chilled, filtered and washed well with water. The precipitate (9.0 g., m.p. 193–195°), after recrystallization from 150 ml. of 95% ethanol and decolorization with Darco, melted at 197–198° dec.

6-Phenylmercaptopurines. General Method.—A solution of equimolecular amounts of 6-chloropurine[29] and the appropriate thiophenol in 2 equivalents of 2 *N* sodium hydroxide was allowed to stand at room temperature from 4 to 18 hours. In some cases, *e.g.*, *p*-chlorophenylmercaptopurine, the mixture was then heated to 60° for 1 hour. The acidity was adjusted to *p*H 5 and the product collected and purified as indicated in Table I.

6-Phenylmercaptopurine.—A solution of 2 g. (0.013 mole) of 6-chloropurine and 1.4 g. (0.013 mole) of thiophenol in 13 ml. of 2 *N* sodium hydroxide was allowed to stand at room temperature for 4 hours. The mixture was acidified to *p*H 5 by the addition of acetic acid and the crude precipitate collected and recrystallized from 250 ml. of absolute ethanol with the addition of Darco.

6-Purinylthiocyanate.—A mixture of 32 g. (0.206 mole) of 6-chloropurine and 21 g. (0.217 mole) of potassium thiocyanate in 600 ml. of methanol was heated under reflux conditions for 6 hours. The reaction mixture was chilled and filtered. The crude precipitate was collected and recrystallized from 13 liters of hot water. The product decomposes at about 235° when put into a preheated melting point apparatus. Its decomposition point depends on the rate of heating.

When the compound was dissolved in 1 *N* sodium hydroxide, it was essentially instantaneously converted to mercaptopurine. Time studies were carried out spectrophotometrically at *p*H 11 (in a Sørensen glycine–sodium hydroxide buffer) and in 0.1 *N* sodium hydroxide to determine the rate of hydrolysis of thiocyanopurine to 6-mercaptopurine. The amount of 6-mercaptopurine formed was determined by the increase in ultraviolet absorption at 310 mμ, at 25° and a concentration of 8.08 mg. per liter. The pertinent data are given in Table III. From these the transformation to 6-mercaptopurine in percent was calculated for each interval and the results were plotted in Fig. 1.

(29) A. Bendich, P. Russell and J. Fox, THIS JOURNAL, **76**, 6073 (1954).

TABLE II

ULTRAVIOLET ABSORPTION SPECTRA OF 8-SUBSTITUTED PURINES

Purine substituents 2	6	8	pH 1 λ_{max}, mμ	pH 1 E_{m} × 10^{3}	pH 11 λ_{max}, mμ	pH 11 E_{m} × 10^{3}
	OH	SH	233	7.9	233	26.7
			288	22.0	289	21.8
	SH	SH	270	17.7	241	12.6
			357	32.0	260	14.7
					333	31.4
	SH	OH	237	13.2	237	17.8
			290[a]	6.3	310	22.9
			333	19.5		
	SH	CH_3	326	18.6	311	19.4
	OH	CH_3	248	12.5	260	11.6
NH_2	OH	SH	269	16.4	237	16.8
			303	15.6	300	15.3
NH_2	OH	SCH_3	263	12.9	265[a]	9.9
			291	15.5	290	12.7
NH_2	SH	SH	272	25.4	251	16.8
			372	35.0	273	14.6
					347	25.0
NH_2	SH	OH	250	8.6	240	15.4
			350	22.0	325	17.0

[a] Inflection.

TABLE III

CHANGE IN ULTRAVIOLET ABSORPTION SPECTRUM OF 6-THIOCYANOPURINE WITH TIME

Time in min.	Optical density at 310 mμ 0.1 N NaOH	Optical density at 310 mμ pH 11	Time in min.	Optical density at 310 mμ 0.1 N NaOH	Optical density at 310 mμ pH 11
0	[a]	0.125	15	0.875	0.166
1	0.175	.135	20	.875	.175
3	.540	...	30	...	.202
5	.665	...	60	...	.257
8	.810	...	135	...	.345
10	.850	...	205	...	.405
12	...	.152	355	...	.478

[a] Changing rapidly.

5-Acetamido-4-amino-6-hydroxypyrimidine.—A mixture of 10 g. (0.057 mole) of 4,5-diamino-6-hydroxypyrimidine sulfate,[17] 4.67 g. (0.057 mole) of anhydrous sodium acetate and 5.85 g. (0.057 mole) of acetic anhydride in 200 ml. of glacial acetic acid was heated under reflux conditions for 6 hours. The reaction mixture was evaporated to dryness under reduced pressure and the residue was washed well with water and dried at 110°. The crude product (9.6 g.) was used for the preparation of 6-hydroxy-8-methylpurine without further purification. A sample was purified for analysis by two recrystallizations from 30 parts of water, with the use of Darco for decolorization. It was dried in a vacuum desiccator. The water of hydration was lost at 140° but was taken up again on exposure to air.

Anal. Calcd. for $C_6H_8N_4O_2 \cdot {}^1/_2H_2O$: C, 40.7; H, 5.1; H_2O, 5.1. Found: C, 40.4; H, 4.9; H_2O (140°), 4.5.

6-Hydroxy-8-methylpurine.—A solution of 9.25 g. (0.055 mole) of 5-acetamido-4-amino-6-hydroxypyrimidine in 27.5 ml. of 2 *N* sodium hydroxide was evaporated to dryness under reduced pressure. The dry sodium salt was heated at 260–280° for 3 hours, during which time water was evolved. The residue was dissolved in 150 ml. of water, filtered and acidified with acetic acid (*p*H 5). The precipitate was collected, washed with water and dried at 120° (6.5 g., 79%). A portion was recrystallized from 160 parts of hot water and decolorized with Darco. It was dried at 140° before analysis.

Anal. Calcd. for $C_6H_6N_4O$: C, 48.0; H, 4.0. Found: C, 47.5; H, 3.7.

6-Mercapto-8-methylpurine. A. From 6-Hydroxy-8-methylpurine.—A mixture of 4 g. of 6-hydroxy-8-methylpurine, 20 g. of freshly ground phosphorus pentasulfide and 200 ml. of tetralin was heated at 210° for 3 hours. The mixture was cooled, filtered and the insoluble residue was washed with benzene and petroleum ether. The solid was boiled with 300 ml. of water for 15 minutes and filtered hot. Ammonium hydroxide was added to the filtrate until *p*H 5 was reached and then the solution was evaporated to dryness under reduced pressure. The residue was leached with 100 ml. of cold water and then recrystallized by solution in 250 ml. of hot water, and concentration of the aqueous solution to 60 ml. The yellow precipitate (1.7 g., 39%) did not melt below 370°.

Anal. Calcd. for $C_6H_6N_4S$: C, 43.3: H, 3.6; N, 33.7. Found: C, 43.2; H, 4.0; N, 33.4.

B. From 4,5-Diamino-6-mercaptopyrimidine.—An intimate mixture of 5.1 g. (0.036 mole) of 4,5-diamino-6-mercaptopyrimidine[18] and 15 g. of acetamide was heated at 220° for 1.5 hours. The melt was cooled, 150 ml. of water was added and the orange precipitate (2 g.) of 6-mercapto-8-methylpurine was collected. An additional 1 g. of crude product was obtained after concentration of the filtrate to 50 ml. in an air stream.

8-Hydroxy-6-mercaptopurine.—A mixture of 4.2 g. of 4,5-diamino-6-mercaptopyrimidine[18] and 4 g. of urea was heated at 175° for 25 minutes. The mixture melted, evolved ammonia and resolidified. After cooling, the melt was dissolved in 50 ml. of 1 *N* sodium hydroxide, filtered and the filtrate acidified to *p*H 5 with acetic acid. The precipitate was collected, washed with water and dried at 110°. The product crystallizes with one-half molecule of water. On heating at 140° a portion of this water is lost but even this is regained rapidly on exposure to air.

Anal. Calcd. for $C_5H_4N_4OS \cdot {}^1/_2H_2O$: C, 33.9; H, 2.8; N, 31.6; S, 18.1; H_2O, 5.1. Found: C, 33.6; H, 2.4; N, 31.8; S, 17.6; H_2O (140°), 3.3.

6-Hydroxy-8-mercaptopurine.—A mixture of 11 g. of 4,5-diamino-6-hydroxypyrimidine sulfate,[17] 4.9 g. of anhydrous sodium acetate and 9 g. of thiourea was heated at 200° for one hour. The melt resolidified while still hot. After cooling, the melt was leached with 100 ml. of cold water to remove excess thiourea and inorganic salts. The insoluble residue was dissolved in 200 ml. of 0.2 *N* sodium hydroxide and filtered into 600 ml. of hot dilute excess acetic acid. The acidified solution was heated to boiling, filtered to remove a small amount of brown precipitate and then chilled. The product deposited as orange flaky crystals which lost their crystalline structure and became tan in color when dried at 140° (5.95 g., 56%). A portion was recrystallized from 140 parts of hot water and the precipitate dried at 60° *in vacuo* before analysis. This material showed a tendency to be slightly hygroscopic.

Anal. Calcd. for $C_5H_4N_4OS \cdot {}^3/_4H_2O$: C, 33.0; H, 3.0; N, 30.9; H_2O, 7.5. Found: C, 33.4; H, 3.1; N, 31.4; H_2O (140°), 7.7.

6,8-Dimercaptopurine. A. From 6-Hydroxy-8-mercaptopurine.—A mixture of 6.8 g. (0.040 mole) of 6-hydroxy-8-mercaptopurine, 8 g. of "liver of sulfur," 35 g. of freshly pulverized phosphorus pentasulfide and 200 ml. of tetralin was heated at 200° for 6.5 hours. The reaction mixture was cooled, filtered and the solid material was washed with benzene and petroleum ether. The crude product was boiled for 15 minutes with 300 ml. of water and then made alkaline with ammonium hydroxide. The ammoniacal solution was filtered, acidified to *p*H 5 with acetic acid, cooled and the precipitate of 6,8-dimercaptopurine (4.95 g., 55.5%) was collected. The product was identical with the one prepared by method B.

B. From 4,5-Diamino-6-mercaptopyrimidine.—A mixture of 5 g. of 4,5-diamino-6-mercaptopyrimidine and 5 g. of thiourea was heated at 210° for 15 minutes. A clear melt formed and after 15 minutes the evolution of gas ceased and the melt resolidified. The solid was treated with 25 ml. of 2 *N* sodium hydroxide and 150 ml. of water and a small insoluble residue was removed by filtration. The solution was acidified with acetic acid whereupon the 6,8-dimercaptopurine hydrate (3.6 g., 51%) precipitated as a bright yellow precipitate. A sample was purified for analysis by recrystallization from 250 parts of boiling water, and drying at 50° *in vacuo*. The monohydrate loses its water of crystallization at 140° but the water is regained on exposure to air.

Anal. Calcd. for $C_5H_4N_4S_2 \cdot H_2O$: C, 29.7; H, 2.97; N, 27.6; S, 31.6; H_2O, 8.9. Found: C, 30.0; H, 3.13; N, 27.3; S, 31.5; H_2O (140°), 9.2.

2-Amino-6-hydroxy-8-mercaptopurine.—A powdered mixture of 25 g. (0.125 mole) of 6-hydroxy-2,4,5-triaminopyrimidine sulfate and 30 g. of thiourea was heated at 190–200° for 30 minutes. The melt was cooled and dissolved in 600 ml. of water and 60 ml. of 17 *N* sodium hydroxide. After filtration, the product was precipitated by acidification of the solution to *p*H 5 with acetic acid, filtered, washed and dried in a vacuum desiccator (16 g., 67%).

Anal. Calcd. for $C_5H_5N_5OS \cdot {}^1/_2H_2O$: C, 31.3; H, 3.1; N, 36.5; H_2O, 4.7. Found: C, 31.9; H, 3.1; N, 36.7; H_2O (140°), 4.4.

2-Amino-6-hydroxy-8-methylmercaptopurine.—To a solution of 9.4 g. (0.0515 mole) of 2-amino-6-hydroxy-8-mercaptopurine in 220 ml. of 0.5 *N* sodium hydroxide was added slowly, with shaking, 8 g. (0.0563 mole) of methyl iodide. After one hour at room temperature, the mixture was acidified to *p*H 5 with acetic acid and the precipitate collected. The product was purified by solution in dilute sodium hydroxide and reprecipitation by acetic acid twice more (7.2 g., 65%). It was dried in a vacuum desiccator. The water of hydration is lost at 140° but regained rapidly on exposure to air.

Anal. Calcd. for $C_6H_7N_5OS \cdot H_2O$: C, 33.5; H, 4.2; H_2O, 8.4. Found: C, 33.6; H, 4.2; H_2O (140°), 7.4.

2-Amino-6,8-dimercaptopurine. A. From 2-Amino-6-hydroxy-8-mercaptopurine.—A mixture of 10 g. of 2-amino-6-hydroxy-8-mercaptopurine, 30 g. of powdered phosphorus pentasulfide and 500 ml. of pyridine was heated under reflux conditions for 16 hours. After removal of the pyridine under reduced pressure, the residue was boiled with 400 ml. of water for 15 minutes and filtered while hot. The insoluble residue (8.6 g., 77%) consisted of 2-amino-6,8-dimercaptopurine of 97% purity. On cooling of the aqueous filtrate an addition 2.85 g. was obtained, of 35% purity as judged by its ultraviolet absorption spectrum. A portion of the main batch was purified by solution in 10 parts of dimethylformamide, filtration and dilution with 20 volumes of water. After centrifugation the precipitate was washed with water and alcohol and dried in a vacuum desiccator. The product forms a monohydrate which loses only three-fourths of its water of hydration at 140° and regains this on exposure to air.

Anal. Calcd. for $C_5H_5N_5S_2 \cdot H_2O$: N, 32.2; S, 29.7; H_2O, 8.3. Found: N, 32.2; S, 29.8; H_2O (140°), 6.2.

B. **From 2-Amino-6-hydroxy-8-methylmercaptopurine.**—A mixture of 29.5 g. (0.15 mole) of 2-amino-6-hydroxy-8-methylmercaptopurine and 90 g. of phosphorus pentasulfide in 250 ml. of tetralin was heated at 200°, with stirring, for 3.5 hours. The mixture was cooled, filtered and washed with benzene. The solid residue was boiled with 800 ml. of water for 15 minutes and the hot mixture was filtered. The water-insoluble residue was treated with 300 ml. of water and 250 ml. of concentrated ammonium hydroxide and filtered to remove a small insoluble residue. The filtrate was brought to *p*H 8.5 by the addition of concentrated hydrochloric acid and filtered once more to remove a small dark precipitate. Upon acidification of the filtrate to *p*H 5–6, a yellow precipitate formed. This precipitate was collected and purified by re-solution in 500 ml. of water containing 25 ml. of ammonium hydroxide, filtration and acidification to *p*H 5 (9.3 g., 31%). The compound was identical with that prepared by method A above.

2-Amino-6-chloro-8-hydroxypurine was prepared from 2-amino-6,8-dihydroxypurine by the method of Fischer,[30] with a slight modification. After the chlorination was complete, the phosphorus oxychloride was removed under reduced pressure and the residue was treated with ice-water. After collection of the initial orange-colored precipitate, the filtrate was kept cold and treated with ammonium hydroxide until a *p*H of 4 was reached. This resulted in the precipitation of a large amount of additional 2-amino-6-chloro-8-hydroxypurine. This product was collected, washed, dried and used for the next step without further purification.

2-Amino-8-hydroxy-6-mercaptopurine.—A mixture of 25 g. of crude 2-amino-6-chloro-8-hydroxypurine[30] and 300 ml. of aqueous 2 *N* sodium hydrosulfide was heated in a sealed vessel at 100° for 16 hours. After cooling, the mixture, which already contained a considerable amount of precipitate, was acidified to *p*H 5 with acetic acid and filtered. The precipitate was dissolved in 40 ml. of 2 *N* sodium hydroxide and 200 ml. of water and the alkaline solution was filtered to remove a small amount of sulfur. Upon acidification to *p*H 5, the 2-amino-8-hydroxy-6-mercaptopurine which had precipitated was collected, washed and dried at room temperature. This material (10.8 g.) was 97% pure as judged by its ultraviolet absorption spectrum, but was brown in color. In order to obtain a pure sample it was found necessary to employ an ion-exchange column, followed by fractional crystallization. A 5-g. portion, dissolved in 22 ml. of 2 *N* sodium hydroxide plus 300 ml. of water, was passed through a Dowex-1 (formate) column (11 mm. diameter × 50 mm. high). To the effluent was added 10 ml. of 2 *N* hydrochloric acid, whereupon an amorphous brown precipitate formed and was removed by filtration and discarded. The yellow filtrate was acidified by the addition of 13 ml. of 2 *N* hydrochloric acid and the yellow precipitate (2.8 g.) was collected, washed with water and acetone and dried in a vacuum desiccator. The water of hydration was lost at 140° and regained on exposure to air. An additional 0.4 g. of the product was recovered from the Dowex-1 column by elution with 0.3 *N* sodium formate solution and acidification with hydrochloric acid.

Anal. Calcd. for $C_5H_5N_5OS \cdot {}^1/_2H_2O$: C, 31.2; H, 3.1; N, 36.5; H_2O, 4.7. Found: C, 31.3; H, 3.3; N, 35.9; H_2O (140°), 4.7.

Ultraviolet absorption spectra were determined with a Beckman DU spectrophotometer at a concentration of 10 mg./l. at *p*H 1.0 (0.1 *N* hydrochloric acid) and *p*H 11 (Sørensen glycinate buffer).

Acknowledgments.—We are indebted to Susan Mueller and Ludwig Salce for technical assistance in the syntheses and to P. R. V. Baker, Samuel Blackman and Veronica Purdey for Microanalyses.

TUCKAHOE, N. Y.

(30) E. Fischer, *Ber.*, **31**, 2619 (1898).