[CONTRIBUTION FROM THE WELLCOME RESEARCH LABORATORIES]

Studies on Condensed Pyrimidine Systems. XXI. The Isolation and Synthesis of 6-Mercapto-2,8-purinediol (6-Thiouric Acid)

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6-Mercapto-2,8-purinediol (6-thiouric acid), which has been isolated from the urine of patients receiving 6-purinethiol and prepared enzymatically by the action of xanthine oxidase on 6-purinethiol, has been synthesized chemically. Two synthetic methods have been employed: the thiation of uric acid with phosphorus pentasulfide and the conversion of 2,6-dichloro-8-purinol to 6-mercapto-2,8-purinediol *via* 2-chloro-6-mercapto-8-purinol and 8-hydroxy-6-mercaptopurine-2-sulfonic acid.

6-Mercapto-2,8-purinediol (6-thiouric acid) has been identified as a metabolite of 6-purinethiol in both the mouse[1] and human[2,3] and as a product of the action of xanthine oxidase on 6-purinethiol *in vitro*.[1] Recently it has been reported also as the product of the action of xanthine oxidase on 6-mercapto-2-purinol.[4] The characterization of 6-thiouric acid originally was based on analysis, spectra and on its formation by means of xanthine oxidase. In essence, the formation from 6-purinethiol by means of xanthine oxidase constitutes a proof of structure since only the 2- and 8-positions are available for the introduction of the two additional oxygen atoms. Furthermore, the ultraviolet absorption spectra (*v.i.* Fig. 1) are consistent with this conclusion since the relationship between the spectra of 6-purinethiol and 6-thiouric acid is closely analogous to that between hypoxanthine and uric acid.

The first synthetic material was prepared by means of the reaction of phosphorus pentasulfide and uric acid (I) in pyridine, a method previously used for the thiation of a number of hydroxypurines.[5-7] This reaction, besides failing to provide a completely unequivocal synthesis in this case, resulted in a mixture of 6-thiouric acid and unchanged starting material, together with small amounts of 6,8-dimercapto-2-purinol and decomposition products which could be separated only by column chromatography.

The formation of 6-thiouric acid by the treatment of uric acid with phosphorus pentasulfide in pyridine was expected since xanthine produces 6-mercapto-2-purinol[7] under these conditions. Two of the three possible dithiouric acids, 2,6-dimercapto-8-purinol[8] and 2,8-dimercapto-6-purinol[9] are known substances. Since the thiation by-product is different from these and its spectrum closely resembles that of 2-amino-6,8-purinedithiol[10] it appears to be 6,8-dimercapto-2-purinol.

Several alternative syntheses were investigated. The preparation of 2,8-dimethylthio-6-purinethiol from the 2,8-dimercapto-6-hydroxy[6] derivative was carried out *via* the 6-chloro derivative, since it was hoped that the alkylmercapto groups of the former might be preferentially hydrolyzable. However, on acid hydrolysis, the 6-mercapto group was hydrolyzed at least as fast as the alkylmercapto groups. This was not entirely unexpected since 2,8-bis-carboxymethylthio-6-purinol fails to yield uric acid on hydrolysis but results in a product which probably is 8-carboxymethylthio-2,6-purinediol.[9] A possibly more promising approach *via* the chlorination of 2-methylthio-6,8-purinediol[11] was not investigated.

Attempts were made to prepare 6-chloro-2,8-purinediol by deamination of 2-amino-6-chloro-8-purinol, but the deamination was accompanied by the simultaneous hydrolysis of the chloro group and the consequent formation of uric acid. Attention then was turned to the reaction of trichloropurine with thiourea or sodium hydrosulfide. Fischer[12] had reported that at elevated temperatures and with an excess of hydrosulfide, 2,6,8-purinetrithiol resulted.

The relative reactivities of the 2- and 6-chloro groups of 2,6-dichloro-8-purinol were investigated with respect to ammonia by Fischer[13] who found that in this substance and its 7- and 9-methyl derivatives,[14] as in 2,6,8-trichloropurine,[15] the 6-chloro group is much the most reactive. Only in two cases was a differential reaction toward hydrosulfide attempted. With 2,6-dichloro-7-methylpurine and sodium hydrosulfide at room temperature, 2-chloro-7-methyl-6-purinethiol was obtained whereas the same reagent at 100° gave the dimercapto derivative.[12] With 7-methyl-2,6,8-trichloropurine and potassium hydrosulfide, even at 0°, Fischer[12] reported the formation of a mixture of a dimercapto-chloropurine and a monomercapto-dichloropurine. However, in this case it was possible that the presence of the 7-methyl group was activating the 8-chloro group as it does in the reaction of 7-methyl-2,6,8-trichloropurine toward ammonia to give 8-amino-2,6-dichloro-7-methylpurine.[16] Therefore, it seemed possible that selective replacement of the chloro groups in 2,6,8-trichloropurine might be achieved. Some selectivity with sodium hydrosulfide at room tem-

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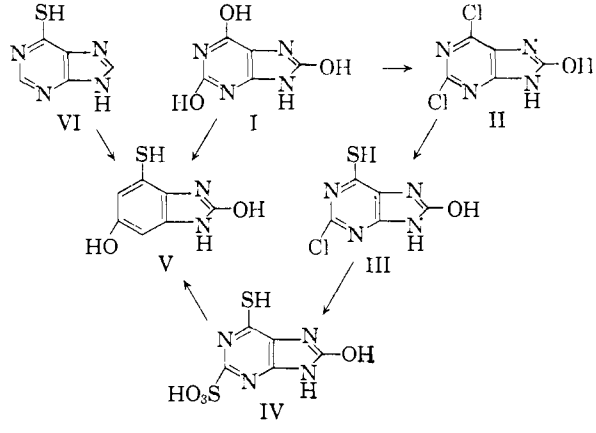
TABLE I
ULTRAVIOLET ABSORPTION SPECTRA

Purine substituents 2	6	8	pH 1 λ_{max}, mμ	pH 1 E_m	pH 1 λ_{min}, mμ	pH 1 E_m	pH 11 λ_{max}, mμ	pH 11 E_m	pH 11 λ_{min}, mμ	pH 11 E_m
			248	5,730						
Cl	Cl	OH	288	12,100	260	3800	295	14,350	255	3,700
			240	11,800	260	1540	247	16,300		
Cl	SH	OH	302	7,650	307	7400	320	23,900	278	1,920
			341	17,200						
			240	14,700			248	22,800		
SO_3Na	SH	OH	330	15,000	265	1900	313	20,500	275	4,900
			260	8,080			237	17,000		
OH	SH	OH	355	28,650	295	460	344	22,200	285	1,250
			263	9,400	242	4400	250	30,000		
SH	SH	OH	298	22,800	268	9200	353	16,400	315	5,000
			367	19,000	330	7400				
			243	11,200			243	12,800		
SH	OH	SH	310	20,400	265	7000	295	17,200	252	12,000
CH_3S	OH	CH_3S	283	19,600	248	7600	289	15,200	255	6,500
NH_2	Cl	OH	312	7,950	268	1670	310	10,300	272	2,780

perature was, in fact, observed and 2,8-dichloro-6-purinethiol was recovered in poor yield. However, this reaction always gave mixtures and offered no advantage over the original thiation procedure.

In the end, it was found that the reaction of 2,6-dichloro-8-purinol (II) with sodium hydrosulfide or ammonium hydrosulfide under controlled conditions led preponderantly to the 2-chloro-8-hydroxy-6-mercapto (III) isomer. The further conversion of this to 6-thiouric acid presented some difficulties since attempts to hydrolyze the halogen under either acid or alkaline conditions resulted in loss of sulfur. This difficulty was solved through the use of a two-stage procedure: conversion of the intermediate to 8-hydroxy-6-mercaptopurine-2-sulfonic acid (IV) and the acid hydrolysis of the latter to the 2,8-dihydroxyl derivative V.



The ultraviolet absorption spectra of these compounds have been most useful in determining the course of these reactions, their completeness and the purity of the products. The pertinent absorption maxima and minima at *p*H 1 and 11 are presented in Table I. Moreover, the differences in the spectra of uric acid, 6-purinethiol and 6-thiouric acid at *p*H 1 (Fig. 1) have made it possible to follow the kinetics of the enzymatic conversion of 6-purinethiol to 6-thiouric acid and the efficiency of the separation of uric acid from thiouric acid in the chromatographic procedures. The optical density ratios which have been particularly important in this work are given in Table II.

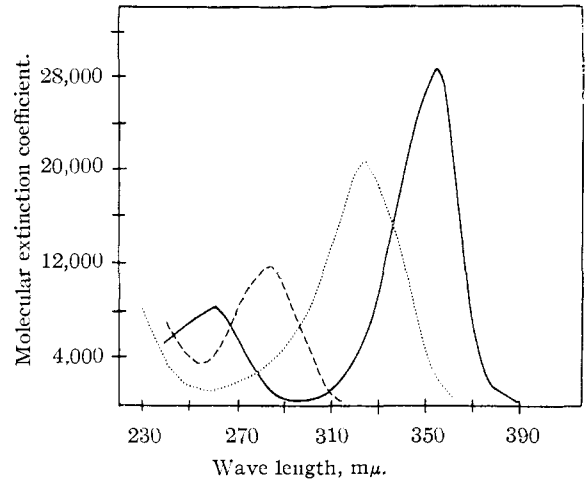


Fig. 1.—Ultraviolet absorption spectra of uric acid (– – – –), 6-mercaptopurine (·····) and 6-thiouric acid (———), at *p*H 1.

Acknowledgments.—We are indebted to Dr. Franz Bergel for a specimen of the xanthine oxidase concentrate. Microanalyses were performed by S. W. Blackman, C. Marr, P. R. V. Baker and V. Purdey to whom grateful acknowledgment is given. We are also indebted to William Lange for technical assistance in the synthetic work.

Experimental

6-Thiouric Acid. Isolation from Urine of Patients Receiving 6-purinethiol.—The urines of two leukemic patients receiving 5 mg./kg. of 6-purinethiol orally were collected for 3 days. The combined urines (6500 ml.) were filtered, acidified by the addition of 50 ml. of 90% formic acid followed by Dowex-50 (H+ form) resin sufficient to bring the *p*H value to 4 and filtered. (This freed an appreciable amount of carbon dioxide which would otherwise form on elution of the Dowex-1 column with acid.) The filtrate was passed through a Dowex-1 (formate) column 84 mm. in diameter and 140 mm. long. The column was washed with 1500 ml. of water and eluted with 10 liters of 0.1 *N* formic acid followed by 20 liters of 0.5 *N* formic acid. Fractions of 750 ml. each were collected. The last 10 liters of the 0.5 *N* formic acid

TABLE II

OPTICAL DENSITY RATIOS AT *p*H 1

Compound	$\frac{355\ m\mu}{325\ m\mu}$	$\frac{355\ m\mu}{285\ m\mu}$
6-Purinethiol	0.127	....
Uric acid	...	0
6-Thiouric acid	4.6	25-35[a]

[a] At *p*H 1 the E_m at 285 mμ for 6-thiouric acid is only 820 whereas the E_m at 355 mμ is 28,650. Slight variations in the optical density readings at 285 mμ (*e.g.*, 0.020 instead of 0.015), therefore, result in disproportionate changes in the ratio. Nevertheless, the ratio is useful in the estimation of the purity of samples of thiouric acid which may be contaminated with uric acid since the spectrum of uric acid has a maximum at 285 mμ.

eluate were richest in 6-thiouric acid, as determined spectrophotometrically. These 10 liters were combined and concentrated under reduced pressure to 250 ml. A precipitate formed which contained both orange and colorless crystals. The concentrate was chilled, filtered, washed with cold water and acetone. The acetone dissolved the colorless crystals leaving 97 mg. of orange thiouric acid. This was purified by solution in 50 ml. of dilute sodium hydroxide, filtration and acidification with hydrochloric acid. The 90 mg. of 6-thiouric acid which precipitated had an ultraviolet absorption spectrum identical with that described in Table I. At p*H* 1 the O.D. 355 mμ/O.D. 325 mμ was 4.6 and the O.D. 355 mμ/O.D. 285 mμ was 30 indicating that the product was free of both 6-purinethiol and uric acid.

6-Thiouric Acid. From Uric Acid.—A mixture of 10 g. of finely powdered uric acid, 30 g. of powdered phosphorus pentasulfide and 500 ml. of dry pyridine was heated under reflux conditions for 11 hours. The excess pyridine was removed under reduced pressure and the residue was boiled with 500 ml. of water, cooled and filtered. This precipitate (4 g.) consisted almost entirely of unchanged uric acid and a little sulfur. The filtrate was acidified to *p*H 3 with concentrated hydrochloric acid, chilled overnight and filtered. The precipitate (5.2 g.) contained some uric acid, but about 60% of it was 6-thiouric acid according to its ultraviolet absorption spectrum. Purification was achieved by column chromatography on a Dowex-1 (formate) column.

In the course of studies of this reaction, a number of different reaction times and methods of purification were tried. The optimum reflux time was found to be 7 to 12 hours. At 2.5 hours, 70% of the uric acid was recovered, at 5 hours 48% was unchanged. When the reaction time was increased to 17 hours, little uric acid was recovered but a considerable amount of the 6-thiouric acid was transformed to di- or trimercapto derivatives; this was evident from the relatively high absorption in the 380 mμ region. Whereas the ultraviolet absorption of 6-thiouric acid at *p*H 1 has a ratio of O.D. 355 mμ/O.D. 380 mμ = 16.1, the product of the 17-hour reaction had a ratio of 1.5.

After recovery of unreacted uric acid from the reaction mixture (after water treatment) the second precipitate (obtained at *p*H 4) usually had a purity of 40 to 60%. It was contaminated with uric acid, pyridine, some dimercaptopurine derivatives and miscellaneous colored impurities. Attempts to purify the 6-thiouric acid by recrystallization in a variety of ways failed to achieve any noticeable improvement in the product. Purification could, however, be accomplished by ion-exchange chromatography. Both batch and column methods were tried and the latter was found to be superior. It was also found less time-consuming to employ a relatively small column and to load it with sufficient material so that much of the uric acid appeared in the effluent and only a small amount remained on the column. Elution with 0.5 *N* formic acid effected a good separation of the uric acid from 6-thiouric acid and eluted the thiouric acid in sufficient concentration so that it precipitated from the eluates. With 2 *N* formic acid it was possible to elute a further small amount of material from the Dowex with λ_{max} = 272, 373 mμ at *p*H 1. O.D. 373 mμ/O.D. 273 mμ = 1.5. The close resemblance of this spectrum to that of 2-amino-6,8-purinedithiol (λ_{max} 272, 372 mμ at *p*H 1, O.D. 372 mμ/O.D. 272 mμ = 1.4)[10] suggests that it is 6,8-dimercapto-2-purinol. Since the resin remained highly colored even after elution with 4 *N* formic acid, it is possible that some purinetrithiol was also formed. Examples of purification of the crude thiation product by the batch method and by the column method are given below.

Purification of 6-Thiouric Acid by Chromatography with Dowex-1. Batch Method.—A solution of 1.35 g. of 6-thiouric acid (48% pure) in 450 ml. of hot 0.1 *N* formic acid was shaken with 35 ml. of settled Dowex-1 (200–400 mesh) resin in the formate form. The mixture was filtered while still warm. The filtrate contained about 200 mg. of uric acid and 100 mg. of thiouric acid. The Dowex, now highly colored, was resuspended three times in 1-liter portions of 0.5 *N* formic acid and refiltered after each suspension. The first two eluates contained 450 mg. of 6-thiouric acid (75% pure) and the third 100 mg. of cruder material. Final purification was accomplished on a small Dowex-1 (formate) column.

Chromatography with Dowex-1. Column Method.—A solution of 2.25 g. of crude 6-thiouric acid (43% pure) in 7 ml. of 2.5 *N* sodium hydroxide was filtered and diluted with 125 ml. of water. This was put through a Dowex-1 (formate) column 20 mm. in diameter and 68 mm. long. The column was washed with 150 ml. of water and elution with 0.5 *N* formic acid was then begun. The effluent had a high end absorption and was dark in color, the water washings showed the presence of uric acid (λ_{max} 285 mμ). The 0.5 *N* formic acid eluates were collected in approximately 30-ml. portions and absorption spectra were determined. Particular attention was paid to the ratio of optical density at 355 mμ/285 mμ since the presence of uric acid increases the absorption at 285 mμ, thereby lowering the ratio. The first 5 fractions (150 ml.) of the formic acid eluate contained 73 mg. of 6-thiouric acid, but the O.D. 355 mμ/O.D. 285 mμ ratio ranged from 0.18 to 3.5. This ratio gradually rose to 8.7 in fraction 13 of the acid eluate. Precipitation began in fractions 9–12 soon after they came off the column. Fractions 6–17 (360 ml.) were combined and chilled at 4°, whereupon 300 mg. of 6-thiouric acid (95% pure) precipitated. An additional 100 mg. (65% pure) was recovered by partial neutralization (*p*H 4) with ammonium hydroxide of the combined filtrate and eluate fractions 18–26, and concentration to 30 ml. under reduced pressure. Recrystallization of the 95% pure sample from 450 parts of hot water, followed by drying in a vacuum desiccator, gave a yellow crystalline solid which was a monohydrate. On drying at 140°, only one-half of the water of crystallization was lost. This water was regained slowly on exposure to air for one week. The dried sample, when analyzed immediately after drying, gave an analysis for a hemihydrate.

Anal. Calcd. for $C_5H_4N_4O_2S.H_2O$: C, 29.7; H, 2.97; N, 27.7; S, 15.7; H_2O, 8.9. Found: C, 30.1; H, 3.2; N, 28.1; S, 15.9; H_2O (140°), 4.5. Calcd. for $C_5H_4N_4O_2S\cdot\frac{1}{2}H_2O$: C, 31.1; H, 2.59. Found: C, 31.4; H, 2.25.

The ultraviolet absorption spectrum for this sample is given in Table I. At *p*H 1 the O.D. 355 mμ/O.D. 325 mμ was 4.6 and O.D. 355 mμ/O.D. 285 mμ was 25. The compound gave a single spot on chromatography with the following R_f values: R_f 0.25 in solvent A, R_f 0.03 in solvent B, R_f 0.57 in solvent C. Uric acid has an R_f 0.40 in solvent A and R_f 0.45 in solvent C.

6-Thiouric Acid. From 6-Purinethiol. Enzymatic Method.—A solution of 60 mg. (0.2 millimole) of 6-purinethiol in 4 ml. of 0.1 *N* sodium hydroxide was diluted with 40 ml. of glass-distilled water and 4 ml. of *M*/15 potassium dihydrogen phosphate solution. To this was added 0.2 ml. of purified xanthine oxidase concentrate[17] and a few drops of toluene and the mixture was incubated at 37° for one week. After 1 day the O.D. 355 mμ/O.D. 325 mμ was 0.29 at *p*H 1 and an additional 0.1 ml. of enzyme was added. After one week the O.D. 355 mμ/O.D. 325 mμ ratio was 2.90. The mixture was filtered, acidified to *p*H 3 with hydrochloric acid and allowed to stand at 4° overnight. The yellow precipitate of 6-thiouric acid was collected by centrifugation, washed with cold water and dried in a vacuum desiccator (25 mg.). This sample had a spectrum identical with that of the analytical sample described above; at *p*H 1 the O.D. 355 mμ/O.D. 325 mμ was 4.5. An additional 15 mg., obtained by concentration of the filtrate under reduced pressure to 10 ml., was only 60% pure and contained some 6-mercaptopurine as evidenced by a O.D. 355 mμ/O.D. 325 mμ ratio of 1.5.

2-Chloro-6-mercapto-8-purinol (III).—To a suspension of 5 g. of 2,6-dichloro-8-hydroxypurine[18] in 250 ml. of water at

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

40° was added 50 ml. of a commercial 8% (dark) aqueous solution of ammonium hydrosulfide. All of the solid dissolved. The solution was kept at 35–40° in a stoppered flask and the course of the reaction was followed by intermittent removal of small samples and measurement of the optical densities at 290, 340 and 370 mμ at *p*H 1. The calculated optical density ratios for 2-chloro-6-mercapto-8-purinol are O.D. 340 mμ/O.D. 290 mμ = 2.68, O.D. 340 mμ/O.D. 370 mμ = 6.7. However, these limiting values are not attained in practice. Some 2,6-dimercapto-8-purinol with an O.D. 340 mμ/O.D. 370 mμ ratio of 0.48 is formed in the later stages and the ratio passes through a maximum of about 5.3. The reaction was interrupted at this point (2 hours) to facilitate the purification of the monomercapto product. The mixture was subjected to reduced pressure at room temperature for 1 hour to remove excess ammonia and hydrogen sulfide, and then acidified to *p*H 5 with acetic acid. After removal of some sulfur, the filtrate was evaporated to dryness under reduced pressure and the inorganic salts were removed by leaching with 25 ml. of cold water. The residue (4 g.) consisted of 2-chloro-6-mercapto-8-purinol of 85% purity (65% yield). A sample was recrystallized from 250 parts of 95% ethanol for analysis, and dried at 60° *in vacuo*. It showed single spots on chromatography in solvents A, B and C: R_f 0.25 (A), R_f 0.08 (B), R_f 0.36 (C).

Anal. Calcd. for $C_5H_3N_4ClOS$: C, 29.6; H, 1.48; N, 27.6; S, 15.8. Found: C, 30.0; H, 1.57; N, 27.3; S, 15.5.

The reaction was also carried out by allowing a suspension of 1.5 g. of 2,6-dichloro-8-purinol and 20 ml. of 2 *N* sodium hydrosulfide to stand in a stoppered flask for 3 days at room temperature. The mixture was then acidified to *p*H 5 with acetic acid and the crude precipitate of 2-chloro-6-mercapto-8-purinol (0.9 g.) collected. This fraction had a purity of 90% as determined spectrophotometrically; at *p*H 1 its O.D. 340 mμ/O.D. 290 mμ = 2.4 and O.D. 340 mμ/O.D. 370 mμ = 5.1. A second fraction (0.45 g., purity 80%) precipitated from the mother liquors after further standing for several days. The product was purified by recrystallization from 95% ethanol and was identical with the sample described above.

Sodium 8-Hydroxy-6-mercaptopurine-2-sulfonate (IV).—A mixture of 435 mg. (0.00215 mole) of 2-chloro-6-mercapto-8-purinol, 290 mg. (0.00230 mole) of sodium sulfite and 10 ml. of water was heated on the steam-bath for 4 hours. The reaction mixture formed a clear solution after a few minutes of heating. The mixture was cooled, acidified with 1 ml. of 2 *N* hydrochloric acid and filtered to remove a small amount of gelatinous precipitate. The filtrate was diluted with an equal volume of ethanol and chilled. The pale yellow precipitate was collected, washed with 2 ml. of cold 50% ethanol and dried at 100° (350 mg., 60% yield). On chromatography it gave the following R_f values: R_f 0.44 (A), R_f 0 (B), R_f 0.80 (C).

Anal. Calcd. for $C_5H_3N_4O_4S_2Na$: C, 22.1; H, 1.10; N, 20.6. Found: C, 22.4; H, 1.35; N, 20.5.

6-Thiouric Acid. From Sodium 8-Hydroxy-6-mercaptopurine-2-sulfonate.—A solution of 250 mg. (0.92 millimole) of sodium 8-hydroxy-6-mercaptopurine-2-sulfonate in 25 ml. of 2 *N* hydrochloric acid was heated under reflux conditions for 15 minutes. At this time the ultraviolet absorption spectrum indicated that the hydrolysis of the sulfonic acid group was complete. The mixture was chilled and the yellow precipitate was collected, washed with water and dried in a vacuum desiccator (170 mg.). The ultraviolet absorption spectrum indicated that the product was 6-thiouric acid of 90% purity (82.5% yield). After recrystallization from water the spectrum of the product was identical with the one described in Table I, with an O.D. 355 mμ/O.D. 285 mμ 34 at *p*H 1.

Deamination of 2-Amino-6-chloro-8-purinol.—To 50 ml. of an acidic aqueous solution[19] containing 1 g. of 2-amino-6-chloro-8-purinol[18] was added over the course of 4 hours, 1.7 g. of sodium nitrite. The mixture was allowed to stand at room temperature for 24 hours. At the end of this time, the ultraviolet absorption spectrum and paper chromatography revealed that the product was uric acid.

Reaction of 2,6,8-Trichloropurine with Sodium Hydrosulfide.—A solution of 3.7 g. (0.016 mole) of 2,6,8-trichloropurine and 40 ml. of 2 *N* sodium hydrosulfide was allowed to stand at room temperature for 3 days in a stoppered flask. The precipitate (apparently a sodium salt) was collected and dissolved in 25 ml. of water; upon acidification with acetic acid to *p*H 5, a pale yellow precipitate formed (0.8 g.), which showed ultraviolet absorption maxima at 240 and 335 mμ (O.D. at 10 mg./per liter 0.45 and 0.79) at *p*H 1 and maxima at 240 and 320 mμ (O.D. at 10 mg. per liter 0.61, 0.87) at *p*H 11. Despite the appearance of homogeneity given by the sharpness of the absorption bands, this product gave three spots on chromatography in solvent A. The reaction mixture filtrate showed ultraviolet absorption maxima at 245, 260, 295 and 345 mμ at *p*H 1 and gave four spots on chromatography in solvent A.

Reaction of 2,6,8-Trichloropurine with Thiourea.—A mixture of 13.8 g. (0.061 mole) of 2,6,8-trichloropurine, 4.7 g. (0.062 mole) of thiourea and 300 ml. of absolute ethanol was heated under reflux conditions for 16 hours. The yellow precipitate (5.1 g.) which formed on cooling showed a complex absorption spectrum indicative of a mixture of products. The alcoholic filtrate was evaporated to dryness and the residue was leached with 40 ml. of water and dried at 100° (7.9 g.). This second fraction likewise showed a multiplicity of ultraviolet absorption bands at *p*H 1 (245, 290, 330, 355 mμ). Attempts to separate this mixture by a variety of solvent extractions and by the use of a Solka-Floc column were time-consuming and gave unsatisfactory results.

2,8-Dimethylthio-6-purinol.—To a solution of 20 g. (0.1 mole) of 2,8-dimercapto-6-purinol[9] in 300 ml. of 1 *N* sodium hydroxide was added slowly, with stirring, 18.8 ml. (0.2 mole) of dimethyl sulfate. The mixture was allowed to stand at room temperature overnight, acidified to *p*H 5 with acetic acid and chilled. The precipitate of crude 2,8-dimethylthio-6-purinol was collected, washed with water and dried at 100° (20.9 g., 91%). The bulk of this material was used for the next step without purification. A small sample was recrystallized from 125 parts of boiling water for analysis.

Anal. Calcd. for $C_7H_8N_2OS_2$: N, 24.6. Found: N, 24.3.

Ultraviolet Absorption Spectra.—The spectra were determined on a model DU Beckman spectrophotometer with solutions containing either 5 mg. or 10 mg. per liter. Measurements were made in 0.1 *N* hydrochloric acid and in a Sørensen glycine–sodium hydroxide buffer of *p*H 11.

Paper Chromatography.—Chromatograms were run in ascending fashion on S + S #597 paper in the following solvent systems: solvent A, water containing 5% ammonium sulfate and 5% isopropyl alcohol; solvent B, 1-butanol saturated with water, ammonia atmosphere; solvent C, 5% disodium phosphate–isoamyl alcohol.

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(19) This was a filtrate obtained in the preparation of 2-amino-6-chloro-8-purinol from 2-amino-6,8-purinediol. When the chlorination mixture was poured on ice, only a portion of the product precipitated, while the rest remained in solution. The concentration of the purine in the solution was determined spectrophotometrically using the molecular extinction values for a purified sample (Table I). The solution was 2 *N* with respect to acid.