2.00 g. (8.61 mmoles) of the β-keto ester (XXII), 0.49 g. (9.00 mmoles) of sodium methoxide, 0.76 g. (10.0 mmoles) of thiourea, and 10 ml. of absolute ethanol was heated under reflux with stirring for 7 hr. The mixture was evaporated *in vacuo* at 50–60°, the red residue was dissolved in 15 ml. of water and the solution heated at 70–80° for 10 min. After treatment with Norit the hot solution was filtered, the filtrate chilled and adjusted to *p*H 4 with 6*M* hydrochloric acid. A gummy material precipitated which crystallized on standing to yield 0.90 g. (43%) of crystalline solid, m.p. 144–147°; $\lambda_{max(\mu)}^{Nujol}$ 3.20–3.27 (NH), 5.93 (C=O), 6.12 (C=C), 6.42 (pyrimidine ring), 8.41 (C=S), 9.50 and 9.62 (C—O—C), 11.77 (olefinic CH). On paper chromatography in solvent B, the compound moved as a single spot with R_{Ad} 1.65.

Anal. Calcd. for $C_{10}H_{16}N_2O_3S$: C, 49.1; H, 6.59; S, 13.1. Found: C, 49.7; H, 6.89; S, 12.6.

6-Acetyl-2-thiouracil (XIX). A mixture of 18.0 g. (77.5 mmoles) of the β-keto ester (XXII), 9.90 g. (0.183 mole) of sodium methoxide, 11.8 g. (0.155 mole) of thiourea, and 90 ml. of absolute ethanol was stirred for 14 hr. at room temperature. The solution was heated under reflux for 6 hr. and evaporated *in vacuo*. Water (90 ml.) was added to the residue, the solution filtered and the filtrate adjusted to *p*H 3 with 6*M* hydrochloric acid. The acid solution was heated on the steam bath for 30 min., during which time crystallization began. After chilling the mixture, 8.50 g (51%) of product was obtained with m.p. 274–276° dec.; this product was identical with that prepared from XVIII.

Previously, by heating a mixture of 0.20 g. (0.82 mmole) of the diethylketal (XVIII), 5 drops of 6*M* hydrochloric acid, and 15 ml. of water at 70–80 for 30 min., a yellow, crystalline product, 0.14 g. (97%), m.p. 278–280° dec., had been obtained. This was recrystallized from 35 ml. of boiling water with the aid of Norit to yield 0.060 g. (58%) of solid, m.p. 278–280° dec.; $\lambda_{max(\mu)}^{Nujol}$ 3.18 and 3.22 (NH), 5.91 (thiouracil and ketone C=O), 6.15 (C=C), 6.40–6.50 (pyrimidine ring), 8.57 (C=S), 11.70 (olefinic CH); $\lambda_{max(m\mu)}^{pH\ 1}$ 272 (ε 20,200; $\lambda_{max(m\mu)}^{pH\ 7}$ 260 (ε 13,400); $\lambda_{max(m\mu)}^{pH\ 13}$ 267 (ε 14,700). On paper chromatography in solvents A and B, the product moved as a single spot with R_{Ad} 1.14 and 1.46, respectively.

Anal. Calcd. for $C_6H_6N_2O_2S$: C, 42.3; H, 3.54; S, 18.8. Found: C, 42.5; H, 3.64; S, 18.9.

6-Acetyluracil (XX). A. *From 6-(1,1-diethoxyethyl)-2-thiouracil* (XVIII). A stirred suspension of 0.22 g. (0.90 mmole) of the diethylketal (XVIII), 0.43 g. (4.5 mmoles) of chloroacetic acid, and 5 ml. of water was heated under reflux for 6 hr. The solution was chilled, causing the precipitation of 0.094 g. (68%) of product, m.p. 259–260° dec. This was recrystallized from 5 ml. of hot water, yielding 0.080 g. (58%) of solid, m.p. 265–266° dec. (lit.[20] m.p 255–260° dec.); $\lambda_{max(\mu)}^{Nujol}$ 3.00 and 3.19 (NH); 5.82 and 5.93 (uracil and ketone C=O), 6.10 (C=C), 11.50 (olefinic CH); $\lambda_{max(m\mu)}^{pH\ 1}$ 295 (ε 6,600); $\lambda_{max(m\mu)}^{pH\ 13}$ 295 (ε 5,400). On paper chromatography in solvents A and B, the compound moved as a single spot with R_{Ad} 0.97 and 0.92, respectively.

Anal. Calcd. for $C_6H_6N_2O_3$: C, 46.6; H, 3.92. Found: C, 46.5; H, 4.05.

In the same manner, the treatment of 7.0 g. of 6-acetyl-2-thiouracil (XIX) with a solution of 13.6 g. of chloroacetic acid in 130 ml. of water heated at reflux for 5 hr. gave 4.0 g. (63%) of 6-acetyluracil (XX), m.p. 264–265° dec., with infrared spectrum and paper chromatographic behavior identical to those of the analytical sample.

B. *From 2-amino-6-acetyl-4(3H)-pyrimidinone* (XXIII). To a stirred mixture of 2.00 g. (13.0 mmoles) of the aminopyrimidinone (XXIII), 10 ml. of 6*M* hydrochloric acid, 5 ml. of concd. sulfuric acid, and 15 ml. of water at room temperature was added dropwise a solution of 3.58 g. (52 mmoles) of sodium nitrite in 10 ml. of water over a period of 10 min. The mixture was stirred at room temperature 1.5 hr. and chilled to yield 1.40 g. (69%) of crystalline 6-acetyluracil (XX), m.p. 257–260° dec., which was identical with the analytical sample in infrared spectrum and paper chromatographic behavior.

Acknowledgment. The authors are indebted to Dr. Peter Lim for interpretation of the infrared spectra and to his staff for the paper chromatography. They also wish to thank Mr. O. P. Crews and his staff for the large-scale preparation of certain intermediates.

MENLO PARK, CALIF.

[CONTRIBUTION FROM THE DIVISIONS OF NUCLEOPROTEIN CHEMISTRY AND EXPERIMENTAL CHEMOTHERAPY, SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH; SLOAN-KETTERING DIVISION OF CORNELL UNIVERSITY MEDICAL COLLEGE]

Thiation of Nucleosides. III. Oxidation of 6-Mercaptopurines[1]

IRIS L. DOERR, I. WEMPEN, DONALD A. CLARKE, AND JACK J. FOX

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Syntheses of the disulfides of 6-mercaptopurine, 6-thioguanine, 6-thioinosine, and 6-thioguanosine from the corresponding mercaptans are described. Cleavage of the S—S bond in the disulfide of 6-mercaptopurine and 6-thioguanine by aqueous alkali yields purine-6-sulfinate and 2-aminopurine-6-sulfinate respectively. These sulfinates also result from oxidation of the mercaptopurines with alkaline iodine solution, while the corresponding sulfonates result from oxidation of the parent mercaptans or sulfinates with alkaline permanganate. The sulfonates are also prepared by the direct replacement of the chlorine atom in 6-chloropurine and 2-amino-6-chloropurine respectively by means of sodium sulfite. The sulfinates are useful in various syntheses since the 6-sulfinate can be replaced by a chloro, hydrogen, or hydroxyl with relative ease. The shifts in the ultraviolet spectra of the bis(6-purinyl) disulfides and their nucleosides and the influence of the sulfinates and sulfonates on the pK_a values of the imidazole dissociation, are discussed. A preliminary report of the effects of the disulfides, sulfinates, and sulfonates on transplantable mouse tumor, Sarcoma 180, is given.

In bacterial and mammalian systems, 6-mercaptopurine (6-MP) has been shown to inhibit *de novo* synthesis of nucleic acid, presumably through blocking the conversion of inosinic acid into other purine ribonucleotides.[2] Similarly, recent investigations into the mechanism of the action of 6-mercaptopurine, using microbiological systems[3] and enzyme preparations,[4] have indicated that in the form of its ribonucleotide, 6-mercaptopurine inhibits the normal enzymic conversions of inosinic acid. The complete metabolic mechanism in mammals of the 6-mercaptopurines (6-mercapto-

purine or 6-thioguanine) has not yet been elucidated. It appears, however, that biological oxidation might play a significant role, since studies[5,6] of the ultimate fate of 6-mercaptopurine-S-35 have resulted in the isolation of 6-thiouric acid (together with sulfate and other as yet uncharacterized degradation products) in the urine of treated mice and humans. 6-Thiouric acid has also been isolated in tissues from mice treated with 6-thioguanine.[7] In view of the established anti-tumor activity of 6-mercaptopurine,[8,9] thioguanine,[10] and thioguanosine[11], it would be of interest to test their disulfides, sulfinates, and sulfonates. The availability of analogs of 6-mercaptopurines in which the oxidation state of sulfur has been modified, may contribute to the overall knowledge of the metabolism of 6-mercaptopurine and thioguanine.

The oxidation products of 6-mercaptopurines thus far reported are the disulfide of 6-mercaptopurine[12] and a number of 6-methylsulfonylpurines.[13] No 6-mercaptopurine nucleoside with a higher oxidation state of sulfur has been prepared. This report deals with the synthesis of some of the oxidation products of 6-mercaptopurine and 6-thioguanine (TG), *i.e.*, the disulfides, the 6-sulfinates, and 6-sulfonates. The facile synthesis of the ribonucleosides of 6-mercaptopurine (6-mercapto-9-β-D-ribofuranosyl purine) and 6-thioguanine (2-amino-6-mercapto-9-β-D-ribofuranosylpurine) have been described in a previous paper in this series.[11] The disulfides of these nucleosides are also reported in this present paper.

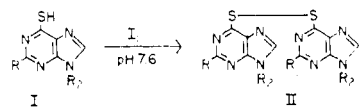


Figure 1

(a) $R_1 = R_2 = H$
(b) $R_1 = NH_2$, $R_2 = H$
(c) $R_1 = H$, R_2 = 9-β-ribofuranosyl-D
(d) $R_1 = NH_2$, R_2 = 9-β-D-ribofuranosyl

As shown in Fig. 1, the disulfides[14] of 6-mercaptopurine (IIa), and 6-thioguanine (IIb) were prepared by the rapid oxidation of the corresponding mercaptan by iodine in a phosphate buffer, *p*H 7.6, according to a procedure of Miller, Roblin, and Astwood.[15] In a similar manner, the disulfides[14] of 6-thioinosine (IIc) and 6-thioguanosine (IId) were easily prepared. The instability of the S—S bond of the disulfides to acid or base was determined by ultraviolet absorption studies in *N* hydrochloric acid[16] or *N* sodium hydroxide which demonstrated the formation of mercaptan equivalent to at least 50% of the disulfide used. The presence of mercaptan indicated, of course, the rupture of the disulfide linkage.

The mechanism most commonly proposed for the cleavage of a sulfur—sulfur bond in aqueous alkali is the nucleophilic displacement of a thioanion from the disulfide by an hydroxyl ion (Equation 1). This reaction would give rise to a mercaptan and a sulfenic acid. The sulfenic acid, however, has never been isolated from such a disulfide cleavage; its existence has been inferred from the formation of sulfinic and sulfonic acids in such reaction mixtures.[17,18] According to a number of investigators[17-20] sulfinic and sulfonic acids may arise from

(1) This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, Public Health Service (Grant No. CY-3190), the Ann Dickler League, and the American Cancer Society. A preliminary account appears in the Abstracts of the 137th American Chemical Society Meeting, Cleveland, Ohio, April 1960, p. 8N.

(2) G. B. Elion, S. Singer, and G. H. Hitchings, *J. Biol. Chem.*, **204**, 35 (1953).

(3) M. E. Balis, D. H. Levin, G. B. Brown, G. B. Elion, and G. H. Hitchings, *Arch. Biochem. Biophys.*, **71**, 358 (1957).

(4) A. Hampton, M. H. Maguire, J. M. Griffiths, Abstr. 4th Intl. Cong. Biochem., p. 40 (Vienna, 1958); A. Hampton, *Federation Proc.*, **19**, 310 (1960); J. S. Salser, D. J. Hutchison, and E. M. Balis, *J. Biol. Chem.*, **235**, 429 (1960).

(5) G. B. Elion, S. Bieber and G. H. Hitchings, *Ann. N. Y. Acad. Sci.*, **60**, 297 (1954).

(6) L. Hamilton and G. B. Elion, *Ann. N. Y. Acad. Sci.*, **60**, 304 (1954).

(7) E. C. Moore and G. A. LePage, *Cancer Research*, **18**, 1075 (1958).

(8) D. A. Clarke, F. S. Philips, S. S. Sternberg, and C. C. Stock, *Ann. N. Y. Acad. Sci.*, **60**, 235 (1954).

(9) D. A. Clarke, F. S. Philips, S. S. Sternberg, C. C. Stock, G. B. Elion, and G. H. Hitchings, *Cancer Research*, **13**, 593 (1953).

(10) F. S. Philips, S. S. Sternberg, L. D. Hamilton, and D. A. Clarke, *Cancer*, **9**, 1092 (1956).

(11) J. J. Fox, I. Wempen, A. Hampton, and I. L. Doerr, *J. Am. Chem. Soc.*, **80**, 1669 (1958).

(12) G. H. Hitchings and G. B. Elion, U. S. Patent **2,697,709** (Dec. 21, 1954). This compound has also been prepared by Dr. Max Hoffer of Hoffmann-LaRoche (unpublished data).

(13) C. W. Noell and R. K. Robins, *J. Am. Chem. Soc.*, **81**, 5997 (1959).

(14) The correct nomenclature for the disulfides of 6-mercaptopurine, 6-thioguanine, 6-thioinosine, and 6-thioguanosine is respectively: bis(6-purinyl) disulfide, bis(2-amino-6-purinyl) disulfide, bis(9-β-D-ribofuranosyl-6-purinyl) disulfide, and bis(2-amino-9-β-D-ribofuranosyl-6-purinyl) disulfide.

(15(a) W. H. Miller, R. O. Roblin, Jr., and E. B. Astwood, *J. Am. Chem. Soc.*, **67**, 2201 (1945). (b) As described in the previous paper of this series, an adaptation of this procedure has been applied to the preparation of disulfides from two 4-thiopyrimidine nucleosides, namely, 4-thiouridine and 4-thiothymidine. [J. J. Fox, D. Van Praag, I. Wempen, I. L. Doerr, L. Cheong, J. E. Knoll, M. L. Eidinoff, A. Bendich, and G. B. Brown, *J. Am. Chem. Soc.*, **81**, 178 (1959).]

(16) No attempt was made to study the effect of acid on the disulfides of the purine nucleosides because of the acid lability of the glycosyl bond.

(17) N. Kharasch, S. J. Potempa, and H. L. Wehrmeister, *Chem. Revs.*, **39**, 269 (1946).

(18) A. J. Parker and N. Kharasch, *Chem. Revs.*, **59**, 583 (1959).

(19) N. Kharasch, W. King, and T. C. Bruice, *J. Am. Chem. Soc.*, **77**, 931 (1955).

(20) E. L. Carr, G. E. P. Smith, Jr., and G. Alliger, *J. Org. Chem.*, **14**, 921 (1949).

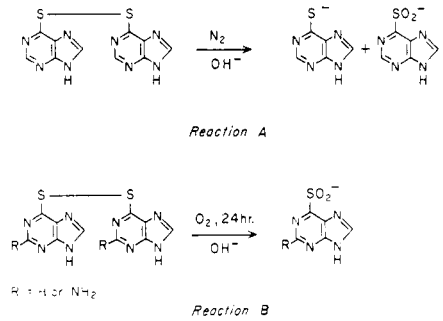


Figure 2

the disproportionation of the corresponding sulfenic and sulfinic acids. Carr and co-workers[20] observed that alkali cleavage of 2-benzothiazolyl disulfide gave a 75.5% recovery of mercaptan consistent with the following equations:

$$2\ RS{-}SR + 4\ OH^- \longrightarrow 2\ RSO^- + 2\ RS^- + 2\ H_2O \quad (1)$$

$$2\ RSO^- \longrightarrow RSO_2^- + RS^- \quad (2)$$

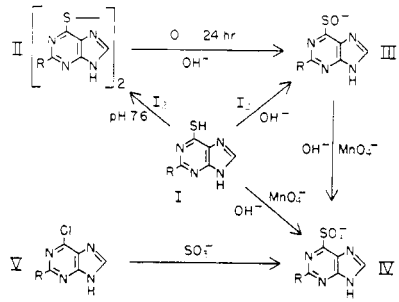
$$2\ RS{-}SR + 4\ OH^- \longrightarrow RSO_2^- + 3\ RS^- + 2H_2O \quad (3)$$

Accordingly, one would expect the mercaptan to be the major product of such a reaction, and indeed, the reaction of OH^- with the disulfide of 6-mercaptopurine (reaction A, Fig. 2)[21] was found to give an instantaneous conversion of the disulfide to the thiopurine (6-mercaptopurine) and purine-6-sulfinate. No other product could be detected. The yield of the 6-mercaptopurine isolated from the cleavage reaction was 80 ± 5%, while the amount of the sulfinic acid derivative formed was found to be 20 ± 5%; these values appear to be in fair agreement with those postulated by the above equations.

The reaction mixture resulting from the alkaline cleavage of the disulfides was found to be very susceptible to oxidation, and in fact, as shown in reaction B (Fig. 2), the addition of oxygen to such an alkaline solution over a period of twenty-four hours[22] resulted in the quantitative conversion to the corresponding sulfinic acid derivatives, *i.e.*, purine-6-sulfinate and 2-aminopurine-6-sulfinate, respectively. If the cleavage mechanism proposed by Carr is accepted, it would appear that the mercaptide anion was converted completely to the sulfinate under these reaction conditions. When the parent thiopurines, 6-mercaptopurine and 6-thioguanine, were subjected to the conditions of the disulfide oxidation (reaction B, Fig. 2), they were oxidized to the sulfinate to only a small extent (*ca.* 13%). Failure of the thiopurines themselves to be oxidized to the same extent as the disulfides was surprising since on the basis of the above equations,

(21) See Experimental for data on this reaction.

(22) If the reaction mixture were allowed to stand more than twenty-four hours, trace amounts of hypoxanthine and purine-6-sulfonate were detected by paper electrophoresis.



a series, R = H
b series, R = NH_2

Figure 3

oxidation of the mercaptide ion would be presumed to have occurred. These findings suggest the possible presence of hitherto undetected intermediate(s) in this disulfide cleavage reaction.

Although the mercaptans do not seem susceptible to oxidation in concentrated basic solutions, it has been previously reported[11] that 6-mercaptopurine, 6-thioguanine, and their respective nucleosides, on standing in *dilute* alkaline solution ($3–5 \times 10^{-5}$ *M*) for ninety-six hours, demonstrate a conversion of better than 60% (measured spectrophotometrically) of the original compounds to new substances. Repetition of the previous experiment, using 6-mercaptopurine, has now confirmed the suspicion that the new compound formed is indeed purine-6-sulfinate. (See Fig. 9 and Experimental).

As seen in Fig. 3 (and as previously discussed), 6-mercaptopurine and thioguanine (I) may be oxidized with iodine in a phosphate buffer (*p*H 7.6) to their respective disulfides (II) in 80% yield. Purine-6-sulfinate and 2-aminopurine-6-sulfinate (III) were obtained either from the treatment of the corresponding disulfide in alkaline solution with oxygen, or by the oxidation of the mercaptans (I) with two moles of iodine in excess sodium hydroxide. Both methods gave yields of over 80% of the sulfinic derivatives. Oxidation of the sulfinates (III) with alkaline permanganate yielded the corresponding purine-6-sulfonate and 2-aminopurine-6-sulfonate (IV). These sulfonates (IV) were also obtained directly from 6-mercaptopurine and thioguanine (I) by treatment with alkaline permanganate. It is of interest to note that under each of the oxidation conditions described, only sulfinate (III) or sulfonate (IV) derivatives are obtained rather than mixtures of these oxidation products. On the other hand, treatment of 6-mercaptopurine with hydrogen peroxide, under the alkaline conditions described above, gave a mixture of sulfinate, sulfonate, and trace amounts of hypoxanthine. The sulfonates (IV) could also be prepared by treatment of 6-chloropurine[23] or 2-amino-

(23) A. Bendich, P. J. Russell, Jr., and J. J. Fox, *J. Am. Chem. Soc.*, **76**, 6073 (1954).

6-chloropurine[24,25] (V) with sodium sulfite. This method is similar to that used by Elion[26] in the preparation of 8-hydroxy-6-mercaptopurine-2-sulfonate.

Chemical properties. Ultraviolet studies showed that the disulfide linkage in all the purinyl-6-disulfides (II) is unstable to base or acid as previously discussed. The lability of the disulfide linkage to base or acid is consistent with the properties exhibited by aromatic disulfides[17] and heterocyclic disulfides.[20]

Hydrolysis studies showed that the sulfinate and sulfonate groups are very labile to acid. For example, purine-6-sulfinate (IIIa) and 2-aminopurine-6-sulfinate (IIIb) in 0.1*N* hydrochloric acid are immediately converted to hypoxanthine and guanine, respectively, in 95–100% yields. Similarly the sulfonates (IV) are also hydrolyzed in aqueous acid to their respective 6-hydroxy compounds, although at a somewhat slower rate than the sulfinate derivatives.

Decomposition of the sulfinates (III), occurred in 98–100% formic acid, yielding purine and 2-aminopurine, respectively. By a similar method, Hoffer[27] reported the desulfination of 4,5,6-triaminopyrimidine-2-sulfinic acid.

Attempts to prepare the sulfinyl chloride or sulfonyl chloride of the above sulfinates or sulfonates with thionyl chloride have failed thus far. In the case of the sulfinates (III), treatment with thionyl chloride gave the respective 6-chloro derivatives, that is, 6-chloropurine and 2-amino-6-chloropurine. A similar treatment of the 6-sulfonates (IV) with thionyl chloride resulted only in recovery of the starting material. It was also noted that 6-chloropurines have a greater stability to dilute aqueous acid than either the sulfinates (III) or sulfonates (IV) (acid hydrolysis in all cases giving 6-hydroxypurines).

Spectra properties. At *p*H 4.5 (neutral species) the disulfides of 6-mercaptopurine and its nucleoside, thioniosine (see section A and B of Fig. 4) both exhibit a hypsochromic shift (*ca.* 33 mμ) in the position of the ultraviolet absorption maximum from that of their parent thiopurines, 6-mercaptopurine[11,28] and thioinosine[11,29] (also *p*H 4.5, neutral species). An essentially similar relationship is observed when 6-methylmercaptopurine[28,30] and its ribonucleoside[31] are compared spectrally to 6-mercaptopurine and thioinosine. Similarly, a hypsochromic shift (*ca.* 20 mμ) can be observed in the comparisons of the absorption maxima (neutral species) of thioguanine disulfide *vs.* thioguanine[11,32] and that of thioguanosine disulfide (see section C and D of Fig. 4) *vs.* thioguanosine.[11] Again, it should be noted that in these cases, the hypsochromic shift is similar to that observed for 2-amino-6-methylmercaptopurine[33] as compared to thioguanine. In the case of the 6-alkyl-mercaptopurines, the hypsochromic shift has been attributed by other investigators[30,33,34] to the absence of the thione form in these compounds. The similarity of the hypsochromic shift exhibited by the 6-purinyl disulfides to that of the 6-alkylmercaptopurines is to be expected since the absence of the thione form is unequivocal in both these cases. These present observations would furnish further evidence, therefore, that 6-mercaptopurine, thioguanine, and their respective nucleosides, possess

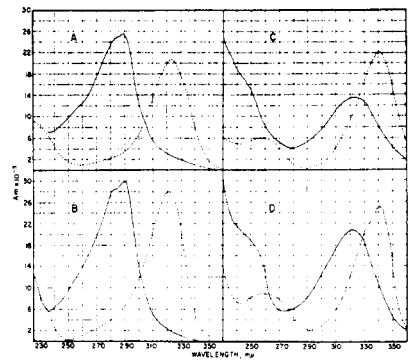


Fig. 4. Ultraviolet absorption spectra at *p*H 4.5 (neutral species) of purinyl disulfides and their respective mercaptans. A. (——) 6-mercaptopurine disulfide; (– – –) 6-mercaptopurine. B. (——) thioinisine disulfide; (– – –) thioinosine. C. (——) thioguanine disulfide. Due to the extreme insolubility of this compound extinction values could not be determined; the curve shown represents an arbitrary assignment. (– – –) thioguanine. D. (——) thioguanosine disulfide; (– – –) thioguanosine

(24) British Patent **767,216** (Jan. 30, 1957); *Chem. Abstr.*, **51**, 14796 (1957).

(25) G. D. Daves, Jr., C. W. Noell, R. K. Robins, H. C. Koppel, and A. G. Beaman, *J. Am. Chem. Soc.*, **82**, 2633 (1960).

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

(27) M. Hoffer, *Jubilee Volume Dedicated to Emil Barrell*, Basle, 1946, p. 428.

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

(29) J. A. Johnson, Jr., and H. J. Thomas, *J. Am. Chem. Soc.*, **78**, 3863 (1956).

(30) G. B. Elion in *The Chemistry and Biology of Purines*, A Ciba Foundation Symposium, J. and A. Churchill, Ltd., London, 1957, pp. 46–8.

(31) A. Hampton, J. J. Biesele, A. E. Moore and G. B. Brown, *J. Am. Chem. Soc.*, **78**, 5695 (1956). A sample of 6-methylmercapto-9-β-D-ribofuranosylpurine was supplied by Dr. A. Hampton of the Sloan-Kettering Institute. The ultraviolet spectra of this compound was similar to that of the disulfide of thioinosine (*p*H 4.5, max. 291 mμ, min. at 240 mμ).

(32) G. B. Elion, W. H. Lange, and G. H. Hitchings, *J. Am. Chem. Soc.*, **78**, 217 (1956).

(33) G. B. Elion, I. Goodman, W. H. Lange, and G. H. Hitchings, *J. Am. Chem. Soc.*, **81**, 1898 (1959).

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

(35) A. Albert and D. J. Brown, *J. Chem. Soc.*, 2060 (1954).

(36) H. F. W. Taylor, *J. Chem. Soc.*, 765 (1948).

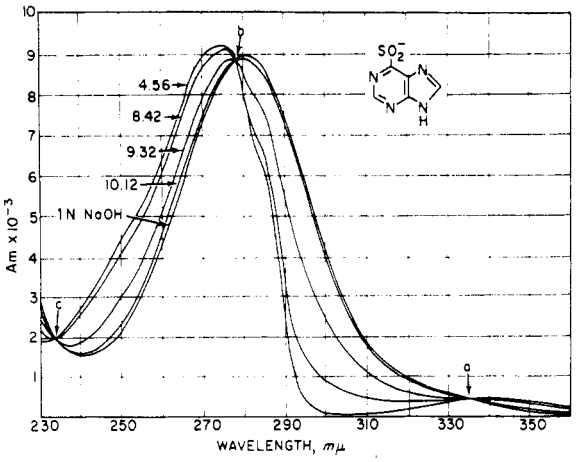


Figure 5

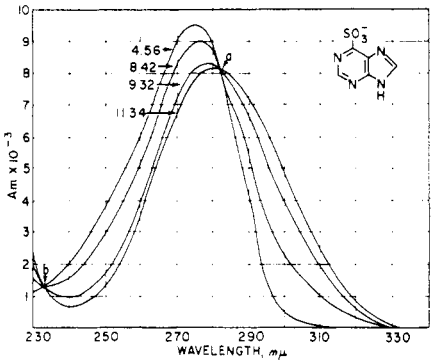


Figure 6

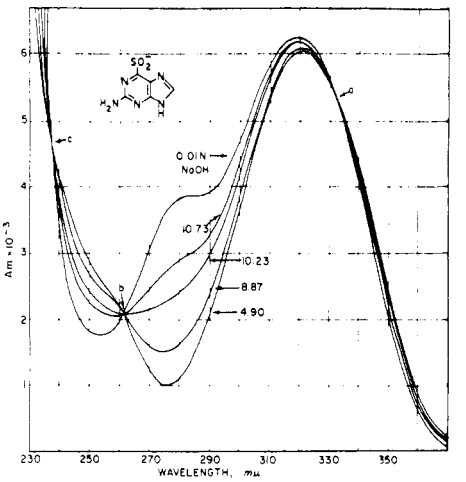


Figure 7

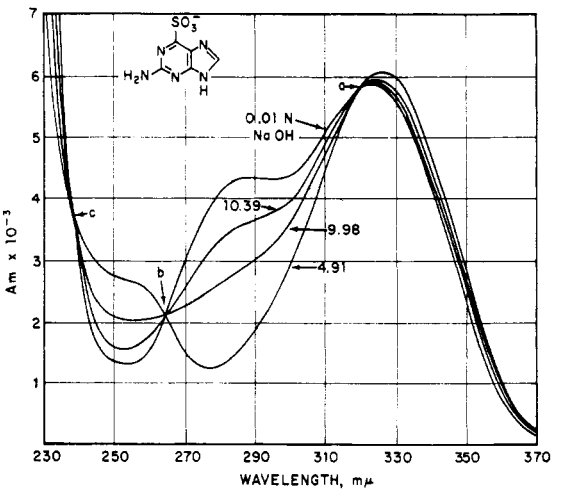


Figure 8

the thione ($-\overset{\overset{S}{|}}{C}-$) rather than the thiol ($=\overset{\overset{SH}{|}}{C}-$) structure. As can be seen in Fig. 5–6, the spectra of purine-6-sulfinate and purine-6-sulfonate exhibit similar equilibrium patterns for the acidic dissociation of the imidazole moiety. Likewise, 2-aminopurine-6-sulfinate possesses a spectral equilibrium pattern similar to that of 2-aminopurine-6-sulfonate, Fig. 7–8. The 6-sulfinate and 6-sulfonate groups exert a bathochromic effect on the position of the ultraviolet absorption maxima of purine and 2-aminopurine. This bathochromic displacement, relative to purine and 2-aminopurine has also been observed in the spectra of the 6-chloropurines, 6-mercaptopurines, 6-alkylmercaptopurines, and purinyl-6-disulfides.

Dissociation constants. All disulfides showed lability to both acid and base as previously noted, making pK_a determinations impossible.

Because of the acid lability of the sulfinate and sulfonate derivatives, only the pK_a values due to the acidic dissociation of the imidazole moiety of the purine ring could be measured. These pK_a values (spectrophotometrically determined, Fig. 5–8) are listed in Table I together with the corresponding pK_a values (potentiometrically and/or spectrophotometrically determined) for some unsubstituted purines, 6-hydroxypurines, and 6-mercaptopurines. It is interesting to note the effect of different substituents in the 6-position on the imidazole dissociation of purine and 2-aminopurine. With the exception of 2-amino-6-chloropurine, all of the 6-substituted purines in Table I already exist in a monoanionic form before the imidazole dissociation occurs, and therefore, removal of a proton from the imidazole moiety involves dissociation from an anion. A comparison of the pK_a values of these purinyl monoanions (see Table I) reveals the decreasing basicity of these heterocycles due to the decreasing mesomeric effect $(+M)$[37] of the negatively charged group $O^- > S^- \gg SO_2^- > SO_3^-$. A further examination of the data in Table I demonstrates that the presence of O^-, S^-, SO_2^- exerts an acid-weakening effect on the acidic imidazole dissociation as indicated by the higher pK_a values of these 6-substituted purines. It is to be noted that the SO_2^- group shows only a small acid-weakening

(37) C. K. Ingold, *Structure and Mechanism in Organic Chemistry*, Cornell University Press, Ithaca, N. Y., 1953, pp. 73–87.

TABLE I

COMPARISON OF THE EFFECT OF DIFFERENT 6-SUBSTITUENTS ON THE ACIDIC DISSOCIATION CONSTANTS OF PURINE AND 2-AMINOPURINE

	pK_a Imidazole Dissociation
Purine	8.92[b,23]
Hypoxanthine	12.10(±0.03)[a,35]
6-Mercaptopurine	11.17(±0.06)[a,11]
Purine-6-sulfinate	9.30[b]
Purine-6-sulfonate	8.56[b]
2-Aminopurine	9.93(±0.03)[a,c,35]
Guanine	12.3[a,36]
2-Amino-6-mercaptopurine	11.6(±0.1)[a,11]
2-Aminopurine-6-sulfinate	10.25[b]
2-Aminopurine-6-sulfonate	9.95[b]
2-Amino-6-chloropurine	9.15[b,d]

[a] Potentiometrically determined. [b] Spectrophotometrically determined values (±0.05). [c] The basic dissociation is 3.80 (±0.01). [d] A basic dissociation (pK_a = 1.79 ± 0.05) has been determined spectrally for this compound.

effect as compared to the O^- and S^- substituent. In contrast, the SO_3^- group in purine has an acid-strengthening[38] effect as indicated by a pK_a value lower than that of purine (8.92) due probably to the greater inductive effect (−I) of the sulfonate group as *vs.* the sulfinate function. On the other hand, 2-aminopurine-6-sulfonate has a pK_a value (9.95) almost identical with that of 2-aminopurine (9.93). This apparent lack of an acid-strengthening effect on the 2-aminopurine moiety, due to the presence of the SO_3^- group, was not anticipated.

In the case of 2-amino-6-chloropurine, the spectrophotometrically measured pK_a values of 1.79 (basic dissociation) and 9.15 (acidic dissociation, see Table I) are lower than the corresponding pK_a values (9.93 and 3.80 respectively) for 2-aminopurine. This lowering of the pK_a values reflects the usual base-weakening and acid-strengthening properties of a chloro-group.

Screening studies. In a preliminary study of tumor-inhibitory capacity using the transplantable mouse tumor, Sarcoma 180, a comparison of 6-mercaptopurine derivatives, that is, 6-mercaptopurine disulfide, thioinosine, and thioinosine disulfide *vs.* 6-mercaptopurine, was made on a molar basis. The disulfide of 6-mercaptopurine was found to be too toxic to evaluate. The disulfide of thioinosine was found to have a greater activity than 6-mercaptopurine. This was provocative since the 9-β-D-ribofuranosyl derivative (thioinosine) was without activity. On the other hand, a similar comparison of the antitumor activity of thioguanine derivatives *vs.* thioguanine on a molar basis revealed the disulfide of thioguanine to be a more potent inhibitor of S-180 and also to be less toxic than thioguanine. Whereas, thioguanosine proved to be equally as active as thioguanine, the disulfide of thioguanosine proved to be a more potent inhibitor than either thioguanosine or thioguanine on a molar basis. It is conceivable that the disulfides may serve as a "reservoir" dose of the active antimetabolite. In preliminary studies, purine-6-sulfinate or -sulfonate and 2-aminopurine-6-sulfinate or -sulfonate were not inhibitors of S-180.

(38) It has been noted that the presence of a SO_3^- substituent in benzoic acid and phenol causes an increase of acid strength. Both these cases involve dissociation from a monoanion. See H. C. Brown, D. H. McDaniel, and O. Haflinger in *Determination of Organic Structure by Physical Methods*, E. A. Braude and F. C. Nachod, eds., Academic Press, Inc., New York, 1955, pp. 585–6.

EXPERIMENTAL[39]

Disulfide of 6-mercaptopurine (IIa). 6-Mercaptopurine (Ia) (5.0 g., 0.033 mole) was dissolved in 3 l. of warm phosphate buffer,[40] *p*H 7.6, the solution filtered gravimetrically, and cooled to 33°.[41] To the stirred solution was added, over a period of 5 min., 30 ml. (0.016 mole) of aqueous *N* iodine solution (containing sodium iodide in the proportion of 2 parts of sodium iodide, to 1 part iodine by weight). The precipitate of short yellow needles was filtered *immediately*, washed thoroughly with water to remove traces of buffer, and triturated with ether-alcohol until powdery. After drying *in vacuo* at 100°, 4.0 g. (80%) of the disulfide was obtained, m.p. 245° (eff.). Recrystallization from 85% ethanol yielded cream-colored needles. Ultraviolet absorption data: at *p*H 3–5, maximum at 289 mμ, $A_{M(max)}$ 25,000; minimum at 237 mμ, $A_{M(min)}$ 6,000. (See Fig. 4.)

In *N* hydrochloric acid, 6-mercaptopurine disulfide (concn., 3×10^{-5} *M*) decomposed over a period of 24 hr. to give an amount of 6-mercaptopurine equivalent to 79% of the disulfide used, as measured by the ultraviolet absorption maximum at 325 mμ. In *N* sodium hydroxide, IIa decomposed immediately, affording a solution whose ultraviolet spectra revealed one absorption maximum at 310 mμ (due to 6-mercaptopurine) and a second unexpected maximum at 342 mμ, having an O.D. *ca.* one-half that at 310 mμ. The structure of the substance responsible for the latter maximum is unknown and is not observed spectrally in the more concentrated basic solutions (0.4−0.5 *M*) used for the oxidation of the disulfide. This observation may be an indication that the mechanism of disulfide cleavage in dilute solutions differs from that which occurs in concentrated solutions.

Anal. Calcd. for $C_{10}H_6N_8S_2 \cdot H_2O$: C, 37.51; H, 2.50; N, 35.00; S, 20.00; O, 5.03. Found: C, 37.40; H, 2.97; N, 35.20; S, 20.35; O, 5.07.

Reaction of disulfide (IIa) *with base under nitrogen.* When the disulfide of 6-mercaptopurine was dissolved in dilute sodium hydroxide under an atmosphere of nitrogen, it was converted essentially instantaneously to a mixture consisting of 80 ± 5% of 6-mercaptopurine and 20 ± 5% purine-6-sulfinate as previously discussed.

A suspension of 0.5 g. (1.56×10^{-3} mole) of IIa in 25 ml. of water (previously boiled and cooled by a stream of nitrogen) was treated, under a continuous atmosphere of nitrogen, with a 6.8 molar excess of *N* sodium hydroxide. The solution

(39) Melting points were determined by the capillary method and are uncorrected. Analyses were performed by the Schwarzkopf Microanalytical Laboratory and by the Spang Microanalytical Laboratory. Paper chromatograms were run by the ascending method using Schleicher and Schuell No. 597 paper in two systems: butanol-water (86:14) and water saturated with butanol.

(40) Phosphate buffer, *p*H 7.5–7.6 was prepared by dilution of a mixture of 0.2*M* dibasic sodium phosphate (560 ml.) and 0.2*M* monobasic sodium phosphate (320 ml.) to 4 l. with water.

(41) The temperature of the buffered solution at the time of addition of the aqueous *N* iodine solution was found to be critical for isolation of pure disulfides.

which formed within a few minutes was acidified with *N* hydrochloric acid to *p*H 7. The precipitated 6-mercaptopurine, which was isolated, represented a 77% yield and was spectrophotometrically and chromatographically identical with an authentic sample of 6-mercaptopurine. The filtrate was analysed, to determine the amount of residual 6-mercaptopurine and the yield of purine-6-sulfinate formed in the reaction, in the following way: the filtrate was diluted to 100 ml. with water and aliquots were removed from this stock solution for ultraviolet spectral analysis. The ultraviolet spectra at *p*H 4.5 revealed two maxima, one at 276 mμ corresponding to a yield of 19% purine-6-sulfinate, the other maximum at 322 mμ corresponding to 5% residual 6-mercaptopurine. The total amount of 6-mercaptopurine recovered from the disulfide cleavage was 82%. Confirmation of the yield of purine-6-sulfinate was accomplished by subjecting an aliquot of the above stock solution to ionophoretic migration in bicarbonate–carbonate buffer, *p*H 9.4. After 80 min. at 750 volts and 17–19 milliamps., the paper was dried and viewed under ultraviolet light. Two spots corresponding to those of pure 6-mercaptopurine and purine-6-sulfinate were observed. The sulfinate-containing spot was eluted with portions of water totaling 7 ml. (a blank from the ionophoretic paper was prepared in a similar way) and the yield of purine-6-sulfinate was found to be 17% (spectrophotometrically determined at 276 mμ).

Disulfide of 6-thioguanine (IIb). Two grams (0.0012 mole) of 2-amino-6-mercaptopurine (Ib) was dissolved with heating in a solution consisting of 2 l. of phosphate buffer (*p*H 7.6) and 1.5 l. of water. The solution was filtered gravimetrically and cooled to 38°. To the stirred solution was added, dropwise, 11.4 ml. (0.0006 mole) of an aqueous *N* iodine solution. There occurred an immediate precipitation of a white solid which gradually became yellow upon the addition of the final amount of the iodine solution. Yield of disulfide was 1.85 g. (93%), m.p. > 300°. The solubility of the compound proved to be so poor that no recrystallization could be carried out, nor any quantitative spectrophotometric measurements made. The qualitative ultraviolet absorption spectra at *p*H 4.5 exhibits a maximum at 322 mμ and a minimum at 280 mμ. (See Fig. 4.)

In *N* sodium hydroxide,[16] the disulfide decomposed to give a solution having a maximum at 322 mμ and a shoulder at 265 mμ. On treatment with *N* hydrochloric acid, IIb decomposed to give a solution having maxima at 347 mμ and 257 mμ. Thus, in both basic and acidic solutions, thioguanine disulfide appears to yield thioguanine as the major product.

Anal. Calcd. for $C_{10}H_8N_{10}S_2$: C, 36.17; H, 2.41; N, 42.14; S, 19.29. Found: C, 35.67; H, 2.82; N, 41.66; S, 19.24.

Disulfide of 6-thioinosine (IIc). Seven grams (0.025 mole) of 6-thioinosine (Ic) was dissolved in 1.4 l. of warm phosphate buffer, *p*H 7.6, the solution was filtered gravimetrically and cooled to 24°. To the stirred solution was added, dropwise, 23.4 ml. (0.012 mole) of *N* iodine. The addition required 5 min. during which time a colorless gel precipitated. After rapid filtration, the solid was washed with water to remove excess buffer, and the spongy white product was triturated with an ether-alcohol mixture until powdery. The yield of crude disulfide, dried *in vacuo* at 60° for 4 hr., was 7.0 g., m.p. 145° (eff.). The product could be recrystallized from 95% alcohol and yielded white needles which, after drying *in vacuo* at 100°, weighed 6.0 g., (85%), m.p. 205° (eff.). Ultraviolet absorption data: at *p*H 3–5, maximum at 290 mμ, $A_{M(max)}$ 30,000; minimum at 237 mμ, $A_{M(min)}$ 5770. (See Fig. 4.)

In *N* sodium hydroxide, the disulfide of thioinosine decomposed immediately, affording a solution whose ultraviolet absorption spectra revealed two maxima, one at 312 mμ (due to thioinosine) and one at 355 mμ (due to an unknown component; see discussion of similar phenomenon observed for the disulfide of 6-mercaptopurine).

Anal. Calcd. for $C_{20}H_{22}N_8O_8S_2$: C, 42.37; H, 3.88; N, 19.78; S, 11.32. Found: C, 42.18; H, 4.06; N, 20.02; S, 11.18.

Disulfide of 6-thioguanosine (IId). Thioguanosine (Id) (4.0 g., 0.0134 mole) was dissolved with heating in 3 l. of phosphate buffer, *p*H 7.6. The resulting solution was filtered gravimetrically, cooled to 24–27°, and with stirring, 12 ml. (0.0067 mole) of a *N* iodine solution was added dropwise. The addition required about 5 min., during which time a colorless, gel-like precipitate formed. After immediate filtration, the precipitate was washed with water until all traces of the buffer solution were removed. The white solid was then triturated with alcohol, finally washed with ether, and dried *in vacuo* at 80° for 3 hr. During drying, the color of the solid changed to a pale yellow. The yield of crude disulfide was 3.18 g. (79%), m.p. 205° (eff.) with some shrinking at 187°. The product could be recrystallized from 120 ml. of pyridine by the addition of 600 ml. of dry ether. The yield of recrystallized hydrated product was 3.0 g. (75%), m.p. 205–210° (eff.). Ultraviolet absorption data: at *p*H 4.6, maximum at 322 mμ, $A_{M(max)}$ 22,100; minimum at 275 mμ, $A_{M(min)}$ 5920. (See Fig. 4.)

In *N* sodium hydroxide,[16] the disulfide of thioguanosine decomposed immediately to give a solution which possessed ultraviolet absorption maxima at 252 mμ and 319.5 mμ and a shoulder at 270 mμ. The overall spectral pattern was similar to that of thioguanosine. The recovery of thioguanosine (measured spectrophotometrically) accounted for *ca.* 80% of the disulfide used.

Anal. Calcd. for $C_{20}H_{24}N_{10}O_8S_2 \cdot H_2O$: C, 37.96; H, 4.43; N, 22.15; S, 10.14. Found: C, 37.88; H, 4.63; N, 22.16; S, 9.87.

The synthesis of purine-6-sulfinate (IIIa) *or 2-aminopurine-6-sulfinate* (IIIb). A. *Via the action of oxygen on purinyl disulfides* (II). 1. *Purine 6-sulfinate* (IIIa). Four grams (0.013 mole) of IIa was suspended in 200 ml. of water and aerated with oxygen for 10 min. A 7-fold molar excess of a 2*N* sodium hydroxide solution was added and a slow stream of oxygen was passed through the bright yellow solution at room temperature for 24 hr. During this time, the color faded to a pale yellow. Progress of the reaction was followed spectrophotometrically by the appearance of a new peak at 275 mμ and the concurrent disappearance of the mercaptan peak at 320 mμ. The reaction mixture was evaporated *in vacuo* to half-volume, or until precipitation occurred, and the *p*H was adjusted to 8 with glacial acetic acid. The sulfinate was removed by filtration and washed sequentially with 85% alcohol, absolute alcohol, and ether. A yield of 90% of purine-6-sodium sulfinate (IIIa)[42] was obtained as long white needles; the product has no definite melting point, decomposition begins *ca.* 175°. Recrystallization was accomplished by reprecipitation from a *N* sodium hydroxide solution using glacial acetic acid. Paper chromatography showed the presence of only one substance. The purity of IIIa was confirmed by subjecting it to ionophoretic migration in bicarbonate–carbonate buffer, *p*H 9.4. After 80 min. at 750 volts and 17–19 milliamp., only one spot was observed under ultraviolet light, anodic migration +14.7 cm. Ultraviolet absorption data: at *p*H 4–6 (monoanionic species) maxima at 276 mμ and 340 mμ, $A_{M(max)}$ 9320 and 440, respectively; minima at 231 mμ and 305 mμ, $A_{M(min)}$ 1870 and 50, respectively.

Anal. Calcd. for $C_5H_3N_4SO_3Na \cdot H_2O$: C, 25.79; H, 2.23; N, 24.99; S, 14.27. Found: C, 27.22; H, 2.29; N, 25.13; S, 14.44.

2. *2-Aminopurine-6-sulfinate* (IIIb). A procedure similar to that described above for the synthesis of IIIa was carried out on IIb and afforded an 80% yield of IIIb, m.p. *ca.* 210° dec. Paper chromatography revealed no contaminant. In order to confirm its purity, IIIb was subjected to ionophoretic migration. After 150 min. (750 volts, 17–19 milliamp.), only one spot was observed under ultraviolet light,

(42) Storage of the sulfinate derivatives has been found inadvisable due to the tendency of these substances to decompose to the sulfonate and to corresponding 6-hydroxypurine.

anodic migration, +18.3 cm. Ultraviolet absorption data: at *p*H 5–7 (monoanionic species), maximum at 322 mμ $A_{M(max)}$ 6030; minimum at 276 mμ, $A_{M(min)}$ 990.

Anal. Calcd. for $C_5H_4N_5SO_2Na \cdot H_2O$: C, 26.09; H, 2.20; N, 30.43; S, 13.93. Found: C, 26.19; H, 2.32; N, 29.77; S, 13.50.

B. *Via the action of iodine on 6-mercaptopurine* (I). One gram of Ia or Ib was dissolved in a 10-fold molar excess of *N* sodium hydroxide. To this was added an amount of aqueous *N* iodine solution (1.9 mmoles/mmole of compound), slightly less than the theoretical quantity required. The product was isolated in a manner similar to that outlined in method A. A yield of 80% of IIIa or 85% of IIIb was obtained. The products, IIIa or IIIb, were spectrally and ionophoretically identical with those derived by method A.

The synthesis of purine-6-sulfonate (IVa) *or 2-aminopurine-6-sulfonate* (IVb). A. *Via the action of alkaline potassium permanganate on 6-mercaptopurines* (I). 1. *Purine-6-sulfonate* (IVa). One gram (0.0065 mole) of Ia was dissolved in a 1.6 molar excess of 0.5*N* potassium hydroxide solution to which an aqueous solution of 0.013*M* potassium permanganate was added, dropwise. The reaction mixture was allowed to stand 0.5 hr. Any excess potassium permanganate was then destroyed by addition of a solution of potassium bisulfite. The precipitated manganese dioxide was removed by filtration through a celite pad and washed thoroughly with water. The *p*H of the colorless filtrate was adjusted to 9 with glacial acetic acid and the solution evaporated to *ca.* 15 ml. The *p*H was brought to 7.9 with 2*N* acetic acid. The sulfonate (IVa) precipitated as white cube-like crystals and, after chilling for 1 hr., was filtered and washed sequentially with 85% alcohol, alcohol, and finally ether. A yield of 85% was obtained, m.p. >300°. Recrystallization was accomplished by reprecipitation from a *N* potassium hydroxide solution with dilute acetic acid. The R_f values of the sulfonate (IVa) and sulfinate (IIIa) were identical in all paper chromatographic systems tried and, therefore, the purity of the sulfonate (IVa) was evaluated by ionophoretic migration in bicarbonate–carbonate buffer, *p*H 9.4. After 80 min. at 750 volts and 17–19 milliamp., only one spot was observed under ultraviolet light, anodic migration, +18.0 cm. A pure sample of purine-6-sulfinate (IIIa) run concurrently with IVa gave an anodic migration of +14.7 cm. Ultraviolet absorption data: at *p*H 4–6 (monoanionic species), maximum at 275 mμ, $A_{M(max)}$ 9540; minimum at 228 mμ, $A_{M(min)}$ 1080.

Anal. Calcd. for $C_5H_3N_5SO_3K$: C, 25.20; H, 1.27; N, 23.52; S, 13.46; O, 20.16; K, 16.41. Found: C, 25.30; H, 1.63; N, 23.77; S, 13.33; O, 20.40; K, 16.59.

2. *2-Aminopurine-6-sulfonate* (IVb). A procedure similar to that used in the preceding synthesis gave an 80% yield of IVb, m.p. >300°. Only one bright blue fluorescent spot was observed by paper chromatography. Since the R_f values were identical with those of 2-aminopurine-6-sulfinate (IIIb) in all systems tried, the purity of the sulfonate was evaluated by ionophoretic migration in bicarbonate–carbonate buffer, *p*H 9.4. After 150 min. at 750 volts and 17–19 milliamp., only one spot was observed under ultraviolet light, anodic migration, +22.6 cm. A pure sample of 2-aminopurine-6-sulfinate (IIIb) run concurrently with IVb gave an anodic migration of +18.1 cm. Ultraviolet absorption data: at *p*H 5–7 (monoanionic species), maximum at 327 mμ, $A_{M(max)}$ 6100; minimum at 277 mμ, $A_{M(min)}$ 1240.

Anal. Calcd. for $C_5H_4N_5SO_3K$: C, 23.71; H, 1.59; N, 27.66; S, 12.60; K, 15.44. Found: C, 23.95; H, 1.83; N, 27.85; S, 12.49; K, 15.68.

B. *Via the action of alkaline potassium permanganate on purine sulfinates* (II). To a solution of IIIa or IIIb in a 2.6 molar excess of 0.5*N* potassium hydroxide was added, dropwise, an aqueous solution of potassium permanganate (2 mmoles/3 mmoles of sulfinate). The product was isolated in a manner similar to that outlined in the preceding synthesis affording a yield of 81% of IVa or 78% of IVb. These products were identical in all respects to those prepared directly from the thiopurines.

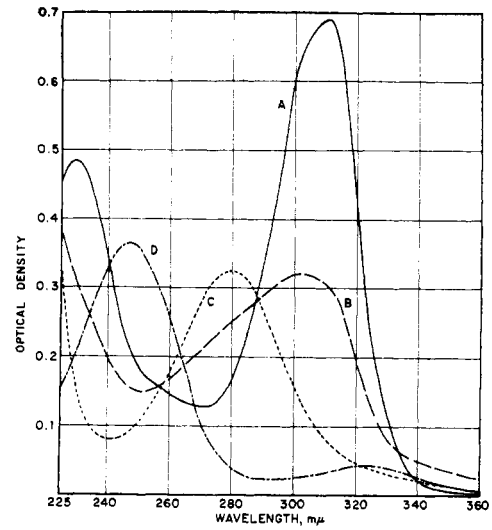


Fig. 9. Variation of 6-mercaptopurine ultraviolet absorption spectra with time at *p*H 11.9. A. *p*H 11.9 at 0 hr. B. *p*H 11.9 after standing 19 hr. C. *p*H 11.9 after standing 40 hr. D. *p*H 1.2 after standing 40 hr. at *p*H 11.9.

C. *Via the action of sodium sulfite on 6-chloropurines* (V). One-half gram of Va was added to 15 ml. of an aqueous solution containing a molar equivalent of sodium sulfite. The reaction mixture was heated to 80° for 0.5 hr. On cooling crystalline purine-6-sodium sulfonate precipitated, yield 85%. A similar procedure was used for the preparation of 2-aminopurine-6-sulfonate (IVb) from 2-amino-6-chloropurine (Vb) except that it was necessary to heat the reaction mixture at 80–85° for 2 hr. A small amount of unchanged starting material was filtered and the filtrate concentrated to half-volume. On cooling, crystalline 2-aminopurine-6-sodium sulfonate precipitated, yield 50%. The sulfonates (IV) were spectrally and electrophoretically identical with those prepared by other methods described above.

Replacement reactions of the 6-sulfinate group. A. *Replacement by hydroxyl.* Purine-6-sulfinate (IIIa) or 2-aminopurine-6-sulfinate (IIIb) are converted immediately in 0.1*N* hydrochloric acid solutions to hypoxanthine and guanine respectively in quantitative yields.

B. *Replacement by hydrogen.* IIIa or IIIb were heated on a steam bath with formic acid (98–100%) until no more sulfur dioxide was evolved. The excess formic acid was evaporated *in vacuo* affording a good yield of purine of 2-aminopurine. The identity of the products was confirmed by spectrophotometric and paper chromatographic methods.

C. *Replacement by chlorine.* One-half gram of IIIa or IIIb (dried at 80° *in vacuo*) was treated dropwise with 5 ml. of thionyl chloride keeping the temperature below 50°. The reaction mixture was allowed to stand overnight at room temperature. The yellow product was filtered and washed with ether. Recrystallization was accomplished by dissolving the compound in dilute aqueous ammonia and filtering. Evaporation of the excess ammonia afforded a good yield of 6-chloropurine (Va) of 2-amino-6-chloropurine (Vb)[43] which were identical spectrally, analytically and chromatographically with authentic samples of these substances.

The instability of 6-mercaptopurine in 0.01 N sodium hydroxide. The gradual oxidation of the thiol group of 6-

(43) Ultraviolet absorption data: in *N* hydrochloric acid (monocationic species), maximum at 317 mμ, $A_{M(max)}$ 7640; at *p*H 11 (monoanionic species), maxima at 308 mμ, and 274 mμ $A_{M(max)}$ 6670 and 4080. The spectral data at *p*H 11 were similar to that reported by C. W. Noell *et al.* (see Ref. 13).

mercaptopurine in *dilute* ($3–5 \times 10^{-5}M$) solutions in 0.01N sodium hydroxide was followed spectrophotometrically. A stock solution was prepared by dissolving 13.4 mg. of 6-mercaptopurine in 25 ml. of water containing 2 drops of 10N sodium hydroxide. A 50-*lambda* aliquot of this stock solution was added to 5 ml. of 0.01N sodium hydroxide (pH 11.9). The solution was covered to prevent evaporation and the ultraviolet absorption spectra was determined at intervals as indicated in Fig. 9. Curve A (0 time) shows the ultraviolet spectra of 6-mercaptopurine in 0.01N sodium hydroxide. Curve B (19 hr.) shows the spectra resulting from the partial oxidation of 6-mercaptopurine, while curve C(40 hr.) demonstrates that almost complete conversion to purine-6-sulfinate has occurred. Using the final absorption at 280 mμ (curve C), the amount of sulfinate present was calculated to be equivalent to 98% of the original mercaptan. Acidification of the 0.01N sodium hydroxide solution at the end of 40 hr. afforded hypoxanthine (curve D, maximum at 247 mμ) equivalent to 90% of the original 6-mercaptopurine. A small amount of unoxidized 6-mercaptopurine still appeared to be present as indicated by the small maximum at 325 mμ. Over the same period of time, conversion of 6-mercaptopurine to its sulfinate derivative also was found to occur at pH 9.4 (bicarbonate-carbonate buffer) to about the same extent as in the case of the more basic pH. In contrast however, 6-mercaptopurine treated with N sodium hydroxide (same conditions of concentration and time) demonstrated a reduction of only 30% in the original quantity of mercaptan.

Electrophoretic experiments. All studies were made using an E. C. electrophoresis apparatus.[44] Whatman 3MM paper was employed. Carbonate-bicarbonate buffer, pH 9.4 was prepared by dilution of a mixture of 200 ml. of a 0.2M sodium carbonate solution and 800 ml. of a 0.2M sodium bicarbonate solution to 4 liters with water.

Spectrophotometric studies. Ultraviolet absorption data were determined with a Cary recording spectrophotometer, model 11, using buffers and techniques previously described.[45,46] Solutions of the disulfides (II) for spectrophotometric determinations were prepared in alcohol-water mixtures (85:15) in which II were found to have greater stability than in water alone. Aliquots of these stock solutions were then measured at various pH values.

Acknowledgment. The authors are indebted to Drs. G. B. Brown and C. C. Stock for helpful discussions and continued interest.

NEW YORK 21, N. Y.

(44) Manufactured by E. C. Apparatus Co., Swarthmore, Pa.

(45) J. J. Fox and D. Shugar, *Bull. soc. chim. Belges*, **61**, 44 (1952).

(46) D. Shugar and J. J. Fox, *Biochem. Biophys. Acta*, 9, 199 (1952).

[CONTRIBUTION FROM THE INSTITUTE FOR CANCER RESEARCH]

Mono- and Difunctional Analogs of Some Quinoline and Acridine Nitrogen Mustards[1]

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The pronounced antitumor activity displayed by two ethyl-2-chloroethylaminoalkylamino derivatives of acridine led to the synthesis of fifteen quinoline and acridine analogs of this type for studies of their structure-activity relationships compared with those of the corresponding bis(2-chloroethyl) forms. Sixteen other alkylating agents which incorporated a variety of modifications both in the side chain and in the quinoline or acridine nucleus were also prepared.

The initial phase[2] of this program was concerned primarily with the synthesis of bis(2-chloroethyl)-aminoalkyl derivatives of 4-aminoquinoline and 9-aminoacridine which usually contained methyl, chloro, or methoxy groups as additional nuclear substituents. In tests,[3] in which the prolongation of survival time of mice bearing ascites tumors served as a criterion of antitumor activity, it was found that the hydrochlorides of the propyl-, methylbutyl-, and hexylamino derivatives were highly active at molar dosages that approximated those required for nitrogen mustard itself. This is in striking contrast to the alkyl-type nitrogen mustards of benzimidazole and certain aryl-type nitrogen mustards which were effective only at molar levels that were at least ten times greater than those needed for nitrogen mustard.[3]

The current series of bis(2-chloroethyl) derivatives of quinoline and acridine includes several nuclear variants and a variety of compounds modified in the side chain. Among the latter are a bis(2-bromoethyl) analog, two hydrazines, an N-oxide, and some aryl-type nitrogen mustards and ethyleneimines. Several mono 2-chloroethylamino derivatives were also prepared. Four derivatives of acridine (I), in which R was CH_3CH_2— and n was 2 or 3, and in which R was CH_3— or CH_3CH_2-CH_2— and n was 3, displayed outstanding activity against ascites tumors at molar levels that were only slightly greater than those required for the corresponding "bis" structures. Only moderate activity was apparent when R was $(CH_3)_2CH$—:

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(2) R. M. Peck, R. K. Preston, and H. J. Creech, *J. Am. Chem. Soc.*, **81**, 3984 (1959).

(3) H. J. Creech, E. Breuninger, R. F. Hankwitz, Jr., G. Polsky, and M. L. Wilson, *Cancer Research*, **20** (No. 7, part 2) 471 (1960).