tive after 1 day and about equally effective after 2 days (Table II). The mechanism of this delayed toxicity is not yet understood but would indicate that the compound is metabolized differently from 3′-deoxyadenosine. It should be noted, however, that uridine-^{3}H incorporation was inhibited effectively during only 6 hr. of contact of the 6-methylamino compound with either KB cells or chick embryo fibroblasts.

The inhibitory mechanisms for the 6-dimethylamino- and 6-ethylaminopurine 3′-deoxyribosides probably resemble that of the 6-methylamino compound except for degree since they exhibited delayed cytotoxicity in KB cells, and also, the slopes of inhibition of uridine incorporation for all three compounds in each cell system were similar.

The results obtained with 2,6-diaminopurine 3′-deoxyriboside and 3′-deoxyguanosine presented a markedly different picture. The 2,6-diaminopurine derivative showed low toxicity in KB cells but was, if anything, more cytotoxic than 3′-deoxyadenosine in chick fibroblasts. Unfortunately, the supply of 3′-deoxyguanosine was insufficient to obtain similar comparisons in chick embryo fibroblasts. Both compounds effectively inhibited the incorporation of uridine-^{3}H into the acid-insoluble fraction of chick fibroblasts, but a meaningful comparison with the effect of 3′-deoxyadenosine could not be made since the slopes of the dose–response lines were different (Figure 2).

The effect of these two compounds on uridine-^{3}H incorporation was strikingly different in KB cells where actually stimulation rather than inhibition was observed (Table III). Until these two compounds are tested against a wider variety of cells in culture, one cannot speculate whether the observed qualitative differences between KB cells and chick fibroblasts with regard to uridine-^{3}H incorporation and cytotoxic effects reflect species differences, differences between a malignant and a normal cell, or differences between an established cell line and a primary explant of an embryonic cell.

The Synthesis and Properties of 6-Mercaptomethylpurine and Derivatives[1a]

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Reaction of 6-chloromethylpurine with either thioacetic acid or ammonium dithiocarbamate led to 6-acetylthiomethylpurine and 6-dithiocarbamylmethylpurine, respectively, which gave after ammonolysis, 6-mercaptomethylpurine (6-homomercaptopurine). This compound was reduced to 6-methylpurine with Raney nickel or by prolonged refluxing with thioacetic acid. Chlorination of 6-mercaptomethylpurine produced 6-trichloromethylpurine, which in turn, was reduced to 6-dichloromethylpurine with thioacetic acid. Reaction of equivalent amounts of 6-bromomethylpurine with potassium thiocyanate or with thiourea resulted in the synthesis of 6-purinylmethyl thiocyanate and 2-(6-purinylmethyl)pseudothiourea hydrobromide, respectively. The corresponding alkylthiomethylpurine derivatives were prepared from 6-chloromethylpurine and methyl, ethyl, and benzyl mercaptan, benzenethiol, and 6-mercaptopurine. Some physical and chemical properties of the new compounds are reported and results of animal screening tests are included. The tumor-inhibitory activity of 6-acetylthiomethylpurine on mouse Sarcoma 180 (ascites) was marked and its effect on mouse Glioma 26 was moderate; the other derivatives proved toxic in mice.

The inhibitory activity of 6-mercaptopurine and other thiopurines and their nucleosides on neoplastic growth[2] stimulated our interest in the synthesis of new mercaptopurine derivatives as potential chemotherapeutic agents. The outstanding toxic effects shown by 6-methylpurine[3] led also to a search for alterations in its structure that might result in derivatives of lower toxicity which still possessed carcinostatic properties. Previous studies on the fluorination,[4] oxidation,[5] chlorination, and bromination[6] of 6-methylpurine afforded derivatives with insignificant biological activity. In studies of the synthesis of thiated purines carried out recently in this laboratory, novel routes of the thiation of purine and 6-methylpurine[7] were developed. The 2-mercapto- and 8-mercapto-6-methylpurine and purine-6-thiocarboxaldehyde which were synthesized did not show any activity in tumor screening tests.

It has been established that the introduction of a methylene group into certain pharmacologically active compounds leads to an alteration or increase in their

(1) (a) This investigation was supported by funds from the National Institute, National Institutes of Health, Public Health Service (Grant CA 03190-08) and The Atomic Energy Commission (Contract No. AT[30-1], 910) and aided by Grant T-128D from the American Cancer Society and the First National City Bank Grant for Research from the American Cancer Society. Presented in part at the 149th Meeting of the American Chemical Society, Detroit, Mich., April 1965, Abstracts of Papers, p. 7N. (b) Recipient of a Public Health Service research career award (3-K6-CA-22,533-01S1) from the National Institutes of Health.

(2) (a) G. B. Elion and G. H. Hitchings, *J. Am. Chem. Soc.*, **69**, 2138 (1947); (b) G. H. Hitchings and C. P. Rhoads, *Ann. N. Y. Acad. Sci.*, **60**, 185 (1954); (c) G. B. Elion and G. H. Hitchings, *J. Am. Chem. Soc.*, **77**, 1676 (1955); (d) D. A. Clarke, G. B. Elion, G. H. Hitchings, and C. C. Stock, *Cancer Res.*, **18**, 445 (1958); (e) J. A. Johnson, Jr., and H. J. Thomas, *J. Am. Chem. Soc.*, **78**, 3863 (1956); (f) J. J. Fox, I. Wempen, A. Hampton, and I. L. Doerr, *ibid.*, **80**, 1669 (1958); (g) I. L. Doerr, I. Wempen, D. A. Clarke, and J. J. Fox, *J. Org. Chem.*, **26**, 3401 (1961).

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(4) A. Giner-Sorolla and A. Bendich, *J. Am. Chem. Soc.*, **80**, 5744 (1958).

(5) (a) A. Giner-Sorolla, I. Zimmerman, and A. Bendich, *ibid.*, **81**, 2515 (1959); (b) M. A. Stevens, A. Giner-Sorolla, H. W. Smith, and G. B. Brown, *J. Org. Chem.*, **27**, 567 (1962).

(6) S. Cohen, E. Thom, and A. Bendich, *ibid.*, **27**, 3545 (1962).

(7) A. Giner-Sorolla, E. Thom, and A. Bendich, *ibid.*, **29**, 3209 (1964).

biological activity.[8] However, several homologous purines, such as aminomethylpurine[9,10] (homoadenine), 6-hydroxymethylpurine (homohypoxanthine, obtained as its acetyl derivative),[5b] and 6-chloromethylpurine,[6] which were synthesized also failed to elicit any relevant biological activity.

A decrease in antitumor activity is observed in 6-alkyl- or arylthiopurines as compared with 6-mercaptopurine[11]; 6-methyl- and 6-benzylthiopurine showed an increase in antitumor activity.[12,13]

In the present work, 6-mercaptomethylpurine (the methylene homolog of 6-mercaptopurine) and its alkyl, alkylaryl, and aryl derivatives were found to be toxic in Sarcoma 180 tumor bearing mice at the usual levels of administration. Thus far, these compounds have exhibited no outstanding antitumor properties. A more detailed tumor screening study was carried out on 6-acetylthiomethylpurine, which exhibited marked inhibition on Sarcoma 180 (ascitic) and moderate effect on Glioma 26.

Synthetic Studies.—Attempts to introduce a sulfur atom into the methyl group of 6-methylpurine (I) or its 1-N-oxide (II) with either thioacetic acid or its anhydride were unsuccessful.[14] Accordingly, other routes for the thiation of this methyl group were studied. The thiation of 6-hydroxymethylpurine[5b] with P_2S_5 in pyridine or in quinoline could not be accomplished. Treatment of 6-methylpurine 1-N-oxide (II) with thiourea and acetic anhydride failed to give the desired thiated product.[15] It was found in the present investigation that an ethanolic solution of 6-chloromethylpurine[6] (III) was not affected by H_2S in the cold or at refluxing temperature, and that alkaline hydrosulfides were of no value for the synthesis of the methylene homolog of 6-mercaptopurine. Transformation of 6-chloromethylpurine (III) into 6-mercaptomethylpurine (6-homomercaptopurine) (V) could be attained, though in low yield (6%), by reaction with H_2S in a saturated ethanolic solution of elemental sulfur (see Scheme I).

The acetylating properties of thioacetic acid are well known.[16] Its thiating properties and those of its anhydride on several purines other than halogen derivatives have been described.[7] Substitution of bromine in acetylated bromo sugars by an acetylthio group with potassium thioacetate has been reported.[17] It was recently found[7] that 6-chloro-[18] and 6-iodopurine[19] can readily and quantitatively be transformed into 6-mercaptopurine[2a] by reaction with thioacetic acid. Similar replacements of halogen by sulfur in 6-halogenopurines can be effected by reaction with thioacetamide. A quantitative yield of 6-mercaptopurine from 6-chloropurine was obtained with thioacetamide; with the iodo analog the yield was 32%. Similar treatment of 6-N-hydroxylamino-[10] and 6-hydrazinopurine[20] did not afford 6-mercaptopurine.

Treatment of 6-chloromethylpurine (III) with thioacetic acid afforded 6-acetylthiomethylpurine (IV) in 66% yield. It is known that thioacetic acid decomposes at its boiling point liberating sulfur and H_2S.[21] However, the lack of appreciable thiation of III with sulfur and H_2S, as described before, indicates that the nucleophilic species is the thioacetic acid itself, since the reaction product is an acetylthio derivative.

Ammonolysis of 6-acetylthiomethylpurine in a nitrogen atmosphere provided 6-mercaptomethylpurine (V) in 62% yield. 6-Mercaptomethylpurine (V) was stable in ammoniacal solution under nitrogen; on exposure to air it decomposed with the formation of amorphous material. When buffered solutions (pH 7) of V or its acetyl derivative IV were treated with the calculated amount of iodine, according to the procedure for the oxidation of 6-mercaptopurine,[2g] the equivalent of iodine was completely absorbed, but no crystalline material could be isolated. 6-Acetylthiomethylpurine (IV) was obtained in 56% yield by acetylation of V with thioacetic acid.

When 6-chloromethylpurine (III) was refluxed in a methanolic solution of ammonium dithiocarbamate, the corresponding 6-dithiocarbamylmethylpurine (VIII) was obtained in 95% yield. Ammonolysis of VIII gave 6-mercaptomethylpurine (V) in 26% yield. Compound VIII was stable to mild acid treatment. In contrast to the 6-chloromethyl derivative, the bromo analog[6] (X) reacted with hydrogen sulfide at low temperature to provide a solution with the ultraviolet spectra and chromatographic characteristics of V. Prolonged treatment of 6-chloromethylpurine (III) with thioacetic acid resulted in its complete reduction to 6-methylpurine (I). A solution of 6-acetylthiomethylpurine (IV) in cold ethanol afforded the known[6] 6-trichloromethylpurine (VI) by treatment with chlorine.[22] Upon refluxing 6-trichloromethylpurine[6] (VI) with thioacetic acid for 2 hr., 6-dichloromethylpurine[6] (VII) was obtained in 76% yield. Treatment of 6-dichloromethylpurine (VII) with thioacetic acid failed to afford purine-6-thiocarboxaldehyde, as might be expected by analogy with the reaction described above and also the reaction with purine-6-carboxaldehyde

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(9) A. Giner-Sorolla and A. Bendich, *J. Am. Chem. Soc.*, **80**, 2932 (1958).

(10) A. Giner-Sorolla, Thesis, Cornell University, *Dissertation Abstr.*, **20**, 1148 (1959).

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(12) G. S. Tarnowsky and C. C. Stock, Proceedings of the 46th Meeting of the American Association for Cancer Research, San Francisco, Calif., 1955, p. 51.

(13) (a) C. G. Skinner, R. G. Ham, D. C. Fitzgerald, Jr., R. E. Eakin, and W. Shive, *J. Org. Chem.*, **21**, 1330 (1956); (b) T. P. Johnston, L. B. Holum, and J. A. Montgomery, *J. Am. Chem. Soc.*, **80**, 6265 (1958); (c) H. E. Skipper, J. A. Montgomery, J. R. Thompson, and F. M. Schabel, Jr., *Cancer Res.*, **19**, 425 (1959); (d) L. R. Lewis, C. W. Noell, A. G. Beaman, and R. K. Robins, *J. Med. Pharm. Chem.*, **5**, 607 (1962).

(14) 6-Methylpurine 1-N-oxide (II) gave 6-acetoxymethylpurine by treatment with acetic anhydride[5b]; with thioacetic acid or anhydride a mixture of 2- and 8-mercapto-6-methylpurines was obtained.[5] No reaction was found between II and thioacetamide.

(15) *Cf.* H. Kofod, *Acta Chem. Scand.*, **7**, 1302 (1953); "Organic Syntheses," Coll. Vol. IV, John Wiley and Sons, Inc., New York, N. Y., 1963, p. 491.

(16) E. E. Reid in "Organic Chemistry of Bivalent Sulfur," Vol. IV, Chemical Publishing Co., New York, N. Y., 1962, p. 14.

(17) (a) M. Gehrke and W. Kohler, *Ber.*, **64**, 2696 (1931); (b) W. A. Bonner, *J. Am. Chem. Soc.*, **73**, 2659 (1951); (c) D. Horton and M. L. Wolfrom, *J. Org. Chem.*, **27**, 1794 (1962).

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

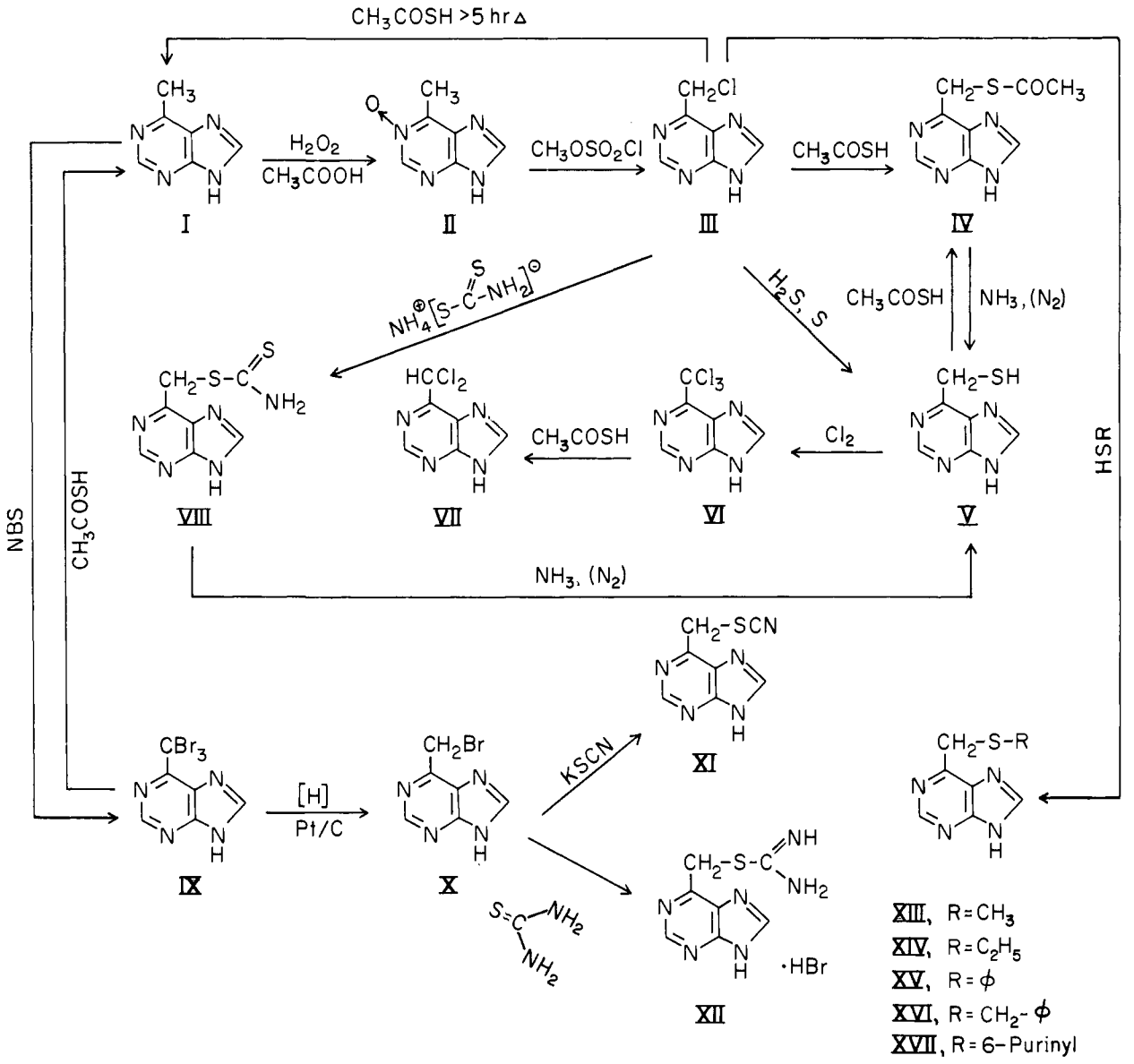
(19) G. B. Elion and G. H. Hitchings, *ibid.*, **78**, 3508 (1956).

(20) J. A. Montgomery and L. B. Holum, *ibid.*, **79**, 2185 (1957).

(21) E. Ulrich, *Ann.*, **109**, 274 (1859).

(22) *Cf.* Wellcome Foundation Ltd., British Patent 767,216 (1957), *Chem. Abstr.*, **51**, 14796 (1957).

Scheme I



hydrazone.[7] The reducing properties of thioacetic acid were also manifested in the rapid transformation of the 6-bromomethyl- (X) or 6-tribromomethylpurine (IX) to 6-methylpurine (I) at reflux temperature. 6-Dibromomethylpurine (VII) was neither thiated to purine-6-thiocarboxaldehyde nor reduced to 6-methylpurine on prolonged refluxing with thioacetic acid. Desulfurization of IV or V with Raney nickel led to 6-methylpurine (I) in nearly quantitative yields.

An equivalent amount of potassium thiocyanate reacted with 6-chloro- (III) or 6-bromomethylpurine (X) to give 6-purinylmethyl thiocyanate (XI) in 84 and 71% yield, respectively. The synthesis of 2-(6-purinylmethyl)pseudothiourea hydrobromide (XII) was accomplished in 82% yield from 6-bromomethylpurine (X) and thiourea in methanolic solution under mild reaction conditions. The free base of XII was obtained from the hydrobromide by neutralization with sodium bicarbonate; it proved to be unstable in hot aqueous or methanolic solutions. Attempts to prepare XII from the chloro derivative (III) and thiourea failed, in contrast with the excellent yield in the synthesis of 6-mercaptopurine from 6-chloropurine.[18] Although alkaline treatment of XII gave a solution with the ultraviolet spectral characteristics of 6-mercaptomethylpurine (V), no crystalline product could be isolated.

Two procedures were investigated in the synthesis of S-substituted 6-mercaptomethylpurines. In the first method, the treatment of 6-chloromethylpurine (III) with methyl, ethyl, phenyl, and benzyl mercaptans, as well as 6-mercaptopurine, yielded the corresponding 6-alkylthiomethylpurine derivatives XIII (93%), XIV (24%), XV (64%), XVI (49%), and XVII (43%). In the second method, the treatment of 6-acetylthiomethylpurine (IV) with benzyl chloride or 6-chloropurine in sodium acetate solutions provided the S-substituted products XVI and XVII. As expected, the acetyl group of IV was easily removed in both cases by the alkali used in the reaction.

Physicochemical Properties.—The ultraviolet spectral data of the purine derivatives are listed in Table I.

Biological Activity.—The new 6-mercaptomethylpurine derivatives have been examined in the Division of

TABLE I

ULTRAVIOLET SPECTRAL PROPERTIES OF 6-SUBSTITUTED PURINES

Substituent	λ_{max}, mμ (ε × 10⁻³) 1 N HCl	λ_{max}, mμ (ε × 10⁻³) pH 7.65[a]	λ_{max}, mμ (ε × 10⁻³) 1 N NaOH	λ_{min}, mμ (ε × 10⁻³) 1 N HCl	λ_{min}, mμ (ε × 10⁻³) pH 7.65	λ_{min}, mμ (ε × 10⁻³) 1 N NaOH
CH_2SH (V)[b]	266 (7.31)	265 (8.13)	273 (9.19)	236 (3.74)	238 (4.81)	247 (4.46)
CH_2SCOCH_3 (IV)[b]	266 (7.35)	267 (9.58)	...	242 (4.12)	244 (5.41)	
		231 (5.89)				
CH_2SCH_3 (XIII)	267 (6.01)	268 (7.84)	278 (8.30)	235 (2.78)	238 (3.15)	239 (1.38)
	305 sh (0.57)					
$CH_2SC_2H_5$ (XIV)	266 (6.72)	267 (8.34)	277 (8.70)	233 (3.30)	237 (3.31)	239 (1.49)
	305 sh (1.22)					
$CH_2SC_6H_5$ (XV)	249 (7.43)	268 (9.41)	277 (6.52)	...	255 (8.26)	257 (3.05)
		250 (8.44)	251 (3.31)			
$CH_2SCH_2C_6H_5$ (XVI)	265 (4.04)	268 (6.19)	280 (6.87)	229 (5.10)	238 (3.35)	243 (1.41)
CH_2SCN (XI)[b]	265 (7.98)	272 (7.90)	275 (7.90)	241 (2.99)	234 (1.95)	252 (4.55)
$CH_2SC(=NH)NH_2 \cdot H_2O$ (free base) (XII)	267 (6.12)	268 (6.73)	...	242 (3.88)	235 (3.24)	...
$CH_2SC(=S)NH_2$ (VIII)[b]	267 (10.3)	271 (12.5)	...	240 (7.10)	230 (6.40)	...
	240 (6.66)	245 sh (7.58)	...	228 (6.44)	...	...

[a] 0.1 *M* phosphate buffer. [b] A 3×10^{-3} *M* solution of ethylenediaminotetraacetic acid disodium salt (EDTA) was used to minimize metal-catalyzed oxidation. V was sufficiently stable (in EDTA solution) in 1 *N* NaOH at room temperature to permit spectroscopic examination. Under these solvent conditions, VIII was rapidly transformed into V; the transformation of IV and XII into V was more gradual; sh indicates shoulder.

Experimental Chemotherapy against Sarcoma 180 in mice *in vivo* and were found to be generally toxic at the low levels of administration (*ca.* 35 mg./kg.). Only 6-acetylthiomethylpurine (IV) showed an inhibition, and a more detailed screening study was made by Dr. K. Sugiura (Table II).

TABLE II

EFFECT[a] OF 6-ACETYLTHIOMETHYLPURINE (IV) ON MOUSE AND RAT TUMORS

Mouse tumor	Compd. IV, 32.5 mg./kg./day	6-Mercaptopurine, 30 mg./kg./day
Sarcoma 180 (solid)	±	+++
Sarcoma 180 (ascitic)	++	+++
Ehrlich carcinoma (solid)	−	+
Ehrlich carcinoma (ascitic)	±	++
Bashford carcinoma 63	±	++
Adenocarcinoma E0771	±	++
Carcinoma 1025	±	++
Lewis bladder carcinoma	−	−
Wagner osteogenic sarcoma	−	+
Ridgway osteogenic sarcoma	±	++
Mecca lymphosarcoma	−	−
Harding–Passey melanoma	±	−
Glioma 26	+	++
Taper hepatoma (solid)	±	
Friend virus leukemia	−	++
Rat tumor	**10 mg./kg./day**	**30 mg./kg./day**
Flexner–Jobling carcinoma	±	++
Walker carcinosarcoma 256	−	±
Jensen sarcoma	±	+

[a] −, no effect; ±, slight inhibition; +, moderate inhibition; ++, marked inhibition; +++, complete inhibition or destruction of tumors. For a more detailed study of the techniques employed, see ref. 23.

6-Acetylthiomethylpurine (IV) was tested at, or near, the maximum tolerated dose for antitumor activity against 17 tumors. Similar tests with 6-mercaptopurine are recorded in Table II for comparison. From the results on this spectrum of tumors, it is apparent that the antitumor activity of 6-acetylthiomethylpurine (IV) was definitely less pronounced than 6-mercaptopurine on both mouse and rat tumors. Compound IV exhibited marked inhibition only on Sarcoma 180 (ascitic) and a moderate effect on Glioma 26. The technique used in these assays has been described previously.[23]

Experimental

Ultraviolet absorption spectra were determined with a Cary Recording spectrophotometer Model 11. Paper chromatograms were run by the ascending method on Schleicher and Schuell No. 1 paper in the four following solvent systems: water saturated with 1-butanol (1:1, v./v.), 1-butanol saturated with water (same proportions) with or without 1% ammonia, and 1-butanol–formic acid–water (77:10:13, v./v.).[6] Melting points were taken in a Thomas–Hoover Unimelt melting point apparatus and are corrected. Microanalyses were carried out by Spang Microanalytical Laboratory, Ann Arbor, Mich.

6-Acetylthiomethylpurine (6-Acetylhomomercaptopurine) (IV).—A solution of 6-chloromethylpurine[24] (III, 3.0 g., 0.017 mmole) in thioacetic acid (4.0 ml.) (Eastman Kodak, redistilled) was refluxed for 30 min. The precipitate which formed was collected, thoroughly washed with ether, and dissolved in water (40 ml.). The resulting yellow solution was treated with charcoal and filtered, and the filtrate was neutralized to pH 6 with anhydrous sodium acetate. The abundant crystalline yellow precipitate which appeared was collected, washed with cold water, and dried to yield 2.46 g. (66%) of yellow product, m.p. 178–180°. After repeated recrystallizations from methanol, colorless prisms were obtained, m.p. 190–191°.

Anal. Calcd. for $C_8H_8N_4OS$: C, 46.37; H, 3.87; N, 27.03; S, 15.47. Found: C, 46.07; H, 3.78; N, 26.94; S, 15.60.

The synthesis of 6-acetylthiomethylpurine (IV) was also achieved when a solution of 6-chloromethylpurine (III) in 10% aqueous thioacetic acid was refluxed for 1 hr., though the yield was somewhat lower (59%).

6-Acetylthiomethylpurine (IV) gave a faint positive test, in alkaline solution, with phosphomolybdate[25a] and with sodium nitroprusside[25b] reagents; a neutral solution of IV gave a mercuric salt with $HgCl_2$. After heating IV with 1 *N* NaOH at 70° for 5 min., these tests became strongly positive (intense blue and deep purple colors, respectively), indicating liberation of a mercapto function from an acetylthio group.[26] Upon treatment of IV (115 mg.) with Raney nickel (0.45 g.) in boiling water suspension, complete dethiation to 6-methylpurine (I, 73 mg., 96%) was

(23) K. Sugiura in "Progress in Experimental Tumor Research," F. Homburger, Ed., S. Karger, Basel, 1961, pp. 332–376.

(24) 6-Chloromethylpurine (III) was obtained in pure form by the method described by Cohen and co-workers,[6] from 6-methylpurine 1-N-oxide (II)[5b] and methanesulfonyl chloride.

(25) (a) A. Bendich and G. C. Clements, *Biochim. Biophys. Acta*, **12**, 462 (1953); (b) H. Meyer in "Analyse und Konstitutionsermittlung organischer Verbindungen," Springer Verlag, Berlin, 1931, p. 631.

(26) Because of these tests, compound IV is assigned the 6-acetylthiomethylpurine structure rather than 9- (or 7-) N-acetyl-6-thiomethylpurine previously proposed at the 149th National Meeting of the American Chemical Society, Detroit, Mich., April 1965, Abstracts, p. 7N.

achieved. Prolonged refluxing of IV (over 5 hr.) in excess thioacetic acid resulted in its complete reduction to 6-methylpurine.[27] A solution of IV in cold ethanol gave the known 6-trichloromethylpurine[6] (VI) by reaction with a stream of chlorine.

Attempts to prepare the corresponding disulfide from 6-acetylthiomethylpurine (IV) or from 6-mercaptomethylpurine (V) with the following oxidizing agents: iodine in buffered solution, peracetic acid, H_2O_2, dimethyl sulfoxide, and oxygen, were all unsuccessful. Treatment of 6-chloromethylpurine (III) with potassium disulfide also failed to yield the desired bis(6-purinylmethylene) disulfide.

6-Mercaptomethylpurine (V) (6-Homomercaptopurine). A.—Analytically pure 6-acetylthiomethylpurine (IV, 620 mg., 3 mmoles) was dissolved in concentrated NH_3 (5 ml.) and the solution was kept at room temperature for 1 hr. in a nitrogen atmosphere. The solution was concentrated under reduced pressure to about 1 ml., water (5 ml.) was added, and this operation was repeated three times. The resulting crystalline product was collected, washed with cold water (10 ml.), and dried *in vacuo* over P_2O_5, to yield 308 mg. (62%), m.p. 146–147° (effervescence). A sample of this material was recrystallized from ethyl acetate to give colorless prisms, m.p. 146–147° (effervescence).

Anal. Calcd. for $C_6H_6N_4S$: C, 43.35; H, 3.64; N, 33.71; S, 19.29. Found: C, 43.51; H, 3.62; N, 33.84; S, 19.21.

6-Mercaptomethylpurine (V) gave a strong positive sodium nitroprusside (intense purple) and phosphomolybdate (immediate deep blue color) tests. It was also reduced with Raney nickel to 6-methylpurine (I). A cold ethanolic solution of V afforded 6-trichloromethylpurine (VI) by reaction with Cl_2. Ammoniacal solutions of V were stable in a nitrogen atmosphere; on exposure to air they decomposed with the formation of amorphous material.

Other syntheses and the properties of two isomeric thiopurines of V, namely 8-mercapto-6-methylpurine (m.p. >350°) and 2-mercapto-6-methylpurine (m.p. >325°), have been previously described.[7]

B.—To a suspension of 6-dithiocarbamylmethylpurine (VIII, 0.213 g., 1 mmole) in water (2 ml.), 30% aqueous NH_3 (0.25 ml.) was added and the solution was heated at 55° for 15 min. The solution, which gave a positive test for thiol with sodium nitroprusside and also a positive phosphomolybdate test, was evaporated to dryness *in vacuo*. The residue was taken up in a little cold water and collected to yield 46 mg. (26%) of colorless needles, m.p. 145°.

A sample of this product was recrystallized from methanol to yield colorless needles, m.p. 147–149°.

Anal. Calcd. for $C_6H_6N_4S$: C, 43.35; H, 3.64; N, 33.71; S, 19.29. Found: C, 43.47; H, 3.54; N, 33.70; S, 19.30.

The ultraviolet absorption spectra at several pH ranges and R_f values in three different solvent systems of this product were identical with those of V. A mixture melting point of this substance with the product obtained by method A showed no depression. The product obtained by method B gave also positive nitroprusside and phosphomolybdate tests.

C.—6-Chloromethylpurine (III, 168 mg., 1 mmole) was dissolved in a solution of sulfur (32 mg., 1 mmole) in ethanol (10 ml.). Hydrogen sulfide was then bubbled through and the temperature gradually was raised to the boiling point. The solution was refluxed for 1 hr. with continuous stirring and bubbling of H_2S. The reaction mixture was evaporated *in vacuo* under reduced pressure and the residue was washed and recrystallized from ethyl acetate to yield 20 mg. (6%) of a product which was identical with those obtained by methods A and B.

Treatment of 6-bromomethylpurine (X) in ethanol with H_2S or KSH gave a solution with the same ultraviolet spectra as V but no crystalline material could be isolated. Attempts to rearrange 6-methylpurine 1-N-oxide (II) to 6-mercaptomethylpurine (V) with a solution of thioacetamide in dioxane failed. The starting material was recovered unaltered.

Acetylation of 6-Mercaptomethylpurine (V).—A solution of 6-mercaptomethylpurine (V, 16.8 mg.) in thioacetic acid (0.10 ml.) was heated at 70° for 30 min. The reaction mixture was concentrated to dryness under reduced pressure, and taken up in ether to yield 12.2 mg. (56%) of yellow needles, m.p. 178–180°. This product was found to be identical with 6-acetylthiomethylpurine (IV) by its mixture melting point, R_f values in three solvent systems, and ultraviolet spectra at various pH values.

Reduction of 6-Trichloromethylpurine (VI) to 6-Dichloromethylpurine (VII).—6-Trichloromethylpurine[6] (VI, 1.0 g., 4.2 mmoles) was dissolved in thioacetic acid (2.5 ml.) and refluxed for 2 hr. The resulting suspension was cooled, collected, washed with ether, and dissolved in water (15 ml.). Sodium acetate was added to pH 6.5, and the resulting precipitate was collected. The crystalline product, 0.65 g. (76%), proved to be identical with the known 6-dichloromethylpurine[6] (VII) in terms of ultraviolet spectra at different pH ranges, R_f values in several solvent systems, and mixture melting point. Compound VII remained unchanged after refluxing with thioacetic acid for 3 hr.

6-Tribromo- (IX) and monobromomethylpurine (X) were rapidly reduced to 6-methylpurine (I) upon treatment with thioacetic acid. 6-Dibromomethylpurine was recovered unaltered after prolonged refluxing with thioacetic acid.

6-Dithiocarbamylmethylpurine (VIII).—A solution of 6-chloromethylpurine (III, 1.10 g., 6.5 mmoles) in methanol (10 ml.) and ammonium dithiocarbamate (1.32 g., 12 mmoles) was heated at 45° for 15 min. during which time a precipitate appeared. The mixture was kept at room temperature for 1 hr. and filtered to give 1.40 g. (95%) of slightly pink crystals, m.p. 174–175° dec. Recrystallization from methanol afforded colorless plates, m.p. 181° dec.

Anal. Calcd. for $C_7H_7N_5S_2$: C, 37.31; H, 3.13; N, 31.09; S, 28.46. Found: C, 37.32; H, 3.33; N, 31.21; S, 28.30.

6-Dithiocarbamylmethylpurine (VIII) could be recovered unaltered after treatment with 1 *N* HCl at 70° for 30 min.

6-Purinylmethyl Thiocyanate (XI).—Addition of potassium thiocyanate (260 mg., 3.7 mmoles) caused a suspension of 6-bromomethylpurine[6] (X, 580 mg., 3.7 mmoles) in methanol (5 ml.) to become a clear solution. After a few minutes at 60°, a precipitate appeared. The reaction mixture was heated at 60° for 1 hr. and cooled, and the resulting precipitate was collected, washed with a little cold water, and dried to yield a crude crystalline product (355 mg., 71%), m.p. 190°. A sample was repeatedly recrystallized from methanol to afford colorless prisms, m.p. 208–210°.

Anal. Calcd. for $C_7H_5N_5S$: C, 43.97; H, 2.64; N, 36.62; S, 16.77. Found: C, 43.92; H, 2.62; N, 36.61; S, 16.80.

Similar treatment of 6-chloromethylpurine (III) afforded XI in 84% yield.

2-(6-Purinylmethyl)pseudothiourea Hydrobromide (XII).—6-Bromomethylpurine[6] (X, 996 mg., 4.6 mmoles) and thiourea (356 mg., 5.1 mmoles) were dissolved in ethanol (12 ml.). After a few minutes of heating at 65°, a copious crystalline precipitate appeared. The heating was continued for 1 hr. and the precipitate was collected to yield 1.12 g. (83%), m.p. 182° dec. A sample was recrystallized from methanol–ethyl acetate; colorless needles, m.p. 192° dec., were obtained.

Anal. Calcd. for $C_7H_9BrN_6S$: C, 29.08; H, 3.14; Br, 26.64; N, 29.06; S, 11.09. Found: C, 29.17; H, 3.14; Br, 27.78; N, 29.09; S, 11.17.

Attempts to obtain XII from 6-chloromethylpurine (III) and thiourea in methanolic solution were unsuccessful. Compound XII (or its free base), when treated with nitrous acid or with alkali at room temperature, gave solutions with the ultraviolet spectral and chromatographic characteristics of 6-mercaptomethylpurine (V), though no crystalline product could be isolated from the reaction mixture.

2-(6-Purinylmethyl)pseudothiourea Hydrate (Free Base).—A solution of the hydrobromide XII (1.0 g., 4.8 mmoles) in water (20 ml.) was treated with charcoal and filtered. To the filtrate, sodium bicarbonate was added until no more precipitation occurred. The precipitate was collected, washed with cold water, and dried to yield 0.47 g. (59%) of a crystalline product, m.p. 158–160° dec.

A sample was purified by repeated treatment with $NaHCO_3$ and dilute HCl, giving colorless needles, m.p. 168° dec.

Anal. Calcd. for $C_7H_8N_6S \cdot H_2O$: C, 37.16; H, 4.45; N, 37.14; S, 14.17. Found: C, 37.16; H, 4.53; N, 37.20; S, 14.05.

The free base decomposed on boiling with water or heating with methanol.

6-Methylthiomethylpurine (XIII).—To a suspension of 6-chloromethylpurine (III, 2.8 g., 0.016 mole) in 25 ml. of ethanol, 5 g. of hydrated sodium methyl mercaptide (4.5 moles of water) (prepared from methyl mercaptan and NaOH) was added and the mixture, heated at 70° for 2 hr., was allowed to stand at room

(27) This new reduction of 6-halogenomethylpurines with thioacetic acid is now under study.

temperature overnight. The resulting suspension was filtered and an amorphous precipitate which formed was discarded. The filtrate was evaporated to dryness *in vacuo*, and the residue was suspended in cold water (5 ml.) and filtered to give 2.1 g. (93%) of a crystalline product, m.p. 165–170°. Upon repeated recrystallization from ethyl acetate colorless crystals shaped like arrow heads were obtained, m.p. 175–176°.

Anal. Calcd. for $C_7H_8N_4S$: C, 46.64; H, 4.47; N, 31.09; S, 17.79. Found: C, 46.45; H, 4.98; N, 31.14; S, 17.74.

The same material was obtained by reaction of an ethanolic solution of III with methyl mercaptan in a sealed tube at 100°, in lower yield.

6-Ethylthiomethylpurine (XIV).—6-Chloromethylpurine (III, 3.4 g., 0.020 mole) was dissolved in 70% aqueous ethanol (30 ml.), ethyl mercaptan (6 ml.) was added, and the solution was heated to 50°. After 1 hr., sodium acetate (2 g.) was added, and the mixture was heated at the same temperature for an additional 5 hr. The resulting solution was evaporated to dryness *in vacuo*, and the residue was recrystallized from ethyl acetate to give a colorless crystalline product (0.95 g., 24%), m.p. 144°. Upon three recrystallizations from ethyl acetate, short needles were obtained, m.p. 154°.

Anal. Calcd. for $C_7H_{10}N_4S$: C, 49.46; H, 5.19; N, 28.84; S, 16.51. Found: C, 49.39; H, 5.20; N, 28.80; S, 16.45.

6-Phenylthiomethylpurine (XV).—Benzenethiol (1.25 ml., 12 mmoles) was added to a stirred solution of 6-chloromethylpurine (III) (1.68 g., 10 mmoles) and sodium acetate (2 g.) in 70% aqueous ethanol (15 ml.). The mixture was heated at 80° for 16 hr. and a small precipitate of amorphous material was filtered off and discarded. The filtrate was concentrated to dryness *in vacuo* and taken up in water (10 ml.). The resulting crystalline material was washed with a little cold water and then thoroughly with ether, to yield 1.55 g. (64%) of a yellow product, m.p. 148°. Repeated recrystallizations from ethyl acetate gave thin, light yellow needles, m.p. 150°.

Anal. Calcd. for $C_{12}H_{10}N_4S$: C, 59.48; H, 4.16; N, 23.12; S, 13.24. Found: C, 59.54; H, 4.09; N, 23.28; S, 13.18.

6-Benzylthiomethylpurine (XVI).—6-Chloromethylpurine (III, 2.50 g., 0.0148 mole) was dissolved in a solution of anhydrous sodium acetate (2.50 g.) in 70% aqueous ethanol (25 ml.). Benzyl mercaptan (5.5 ml., 0.042 mole) was added and the mixture refluxed for 6 hr. The resulting solution was evaporated to dryness *in vacuo*, washed with cold water, and recrystallized from water to yield 1.87 g. (49%) of colorless crystals, m.p. 94°. Further recrystallization from water afforded rectangular plates, m.p. 96°.

Anal. Calcd. for $C_{13}H_{12}N_4S$: C, 60.91; H, 4.72; N, 21.86; S, 12.51. Found: C, 61.12; H, 4.71; N, 22.10; S, 12.83.

The same product was obtained by treatment of 6-acetylthiomethylpurine (IV) with benzyl chloride in similar conditions to those described above.

6-S-Purinyl(6'-thiomethylpurine) (XVII).—A solution of 6-chloromethylpurine (III, 0.340 g., 2 mmoles) in 5 ml. of ethanol was mixed with 6-mercaptopurine (0.320 g., 2 mmoles), and water (30 ml.) was added. After a few minutes of boiling, the pH dropped to 4. Anhydrous sodium acetate (0.5 g.) was added, and the solution refluxed for 1 hr. The precipitate which appeared on cooling was collected, washed with cold water, and dried to yield 0.27 g. (43%) of brown-yellow needles, m.p. 290–292°. Upon recrystallization from water, thin yellow needles, m.p. >300°, were obtained.

Anal. Calcd. for $C_{11}H_8N_8S$: C, 46.47; H, 2.84; N, 39.42; S, 11.28. Found: C, 46.44; H, 3.03; N, 39.01; S, 11.58.

XVII could also be obtained in similar yield from 6-chloropurine and 6-acetylthiomethylpurine (IV) using the conditions described above.

Reaction of 6-Substituted Purines with Thioacetamide.—To a solution of 6-chloropurine[17] (0.65 g., 4 mmoles) in ethanol (25 ml.) thioacetamide (0.90 g., 12 mmoles) was added and the mixture refluxed for 3 hr. On cooling, a precipitate of 6-mercaptopurine (0.60 g., quantitative yield) was obtained. A yield of 32% was obtained when 6-iodopurine was used.

Similar treatment of 6-N-hydroxylaminopurine[19] and 6-hydrazinopurine[20] did not lead to 6-mercaptopurine, and the starting materials were recovered unaltered.

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Structural Studies of an Active Principle from *Croton tiglium* L.

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The isolation, semisynthesis, and structure elucidation of a pure crystalline highly active tumor-enhancing principle from the seed of *Croton tiglium* L. is described. The alkaline hydrolysis of the pure crystalline cocarcinogen called C-3 (I) yielded myristic and acetic acids and a polyhydroxy compound, $C_{20}H_{28}O_6$. Crystalline derivatives of the active compound were prepared. The chemistry of the active material, the parent alcohol, and their crystalline derivatives is discussed.

In a recent communication[2] we reported the isolation and partial structure for an active cocarcinogen from *Croton tiglium* L. Additional n.m.r. studies with decoupling suggested a revision of several discrepancies in the proposed structure.

We now present the results of further work on the active principle from *Croton tiglium* L. In earlier communications[3,4] Hecker, *et al.*, reported the isolation of three cocarcinogens A_1, B_1, and B_2 having the same parent alcohol, but B_1 and B_2 differing in the acids forming the two ester functions. However, these workers failed in their attempts to obtain a crystalline compound, indicating that cocarcinogens, A_1, B_1, and B_2 are not pure chemical entities but amorphous chromatographic fractions.

In 1941 Berenblum[5] showed that croton oil is a potent tumor-enhancing agent, *i.e.*, it stimulates the appearance and rapid growth of tumors on mouse skin after application of a minute dose of a carcinogenic

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