Anal. Calcd. for $C_5H_7N_3OS$: C, 38.20; H, 4.49; N, 26.73; S, 20.40. Found: C, 38.48, 38.57; H, 4.25, 4.42; N, 26.29, 26.42; S, 20.9.

This compound has recently been described by Koppel, *et al.*,[19] who record m.p. 294° dec., but the nitrogen analysis is unsatisfactory.

Cytosine (XIV). A. By Dethiation of 4-Amino-2-hydroxy-6-mercaptopyrimidine (XII).—To a nearly complete solution of 4-amino-2-hydroxy-6-mercaptopyrimidine (0.5 g., 0.0036 mole) in 35 ml. of 95% ethanol was added 5 ml. of Davison sponge nickel catalyst (apparent bulk density 0.85),[8] 20 ml. of 95% ethanol being used to transfer the nickel into the reaction flask. The suspension was refluxed with stirring for 1 hr.; the ultraviolet absorption spectrum indicated that the reaction had gone to completion within that time. The supernatant liquid, while still hot, was decanted and filtered by gravity. The nickel slurry in the flask was extracted three times with small portions of 95% ethanol and the extracts were filtered through the same funnel. After refrigeration overnight, the crystals that had formed were collected, washed with 2–3 ml. of 95% ethanol, and air-dried; colorless, thin, shiny prismatic needles; yield, 0.167 g. (43%). These crystals, which lost solvent of crystallization and became chalky on drying at 70° *in vacuo* over phosphorus pentoxide for 17 hr., were submitted directly for analysis, m.p. 323° dec.

Anal. Calcd. for $C_4H_5N_3O$: C, 43.24; H, 4.54; N, 37.82. Found: C, 43.16; H, 4.53; N, 37.90, 37.99.

An additional 100 mg. of XIV was obtained from the filtrate of the first crop of crystals on evaporation of the filtrate to dryness *in vacuo* and recrystallization of the dry residue (128 mg.) from 35 ml. of 95% ethanol; total yield, 267 mg. (68%).

B. By Dethiation of 4-Amino-2-hydroxy-6-methylthiopyrimidine (XIII).—4-Amino-2-hydroxy-6-methylthiopyrimidine (0.5 g., 0.0032 mole) was suspended in 100 ml. of 95% ethanol and warmed on a steam bath for approximately 0.5 hr. to dissolve most of the solid. The solution was cooled to room temperature and to it was added 5 ml. of Davison sponge nickel catalyst (apparent bulk density 0.85).[8] The suspension was refluxed with stirring for 1.5 hr. and then filtered by decantation from the nickel, as is procedure A, while still hot. The nickel was washed three times with small portions of 95% ethanol and the washings were added to the filtrate. The alcoholic solution was evaporated to dryness under reduced pressure and the colorless residue (200 mg.) was crystallized directly from 55 ml. of 95% ethanol. After overnight refrigeration the crystals were collected, washed with cold 95% ethanol, and air-dried; yield, 53 mg. (15%). Concentration of the mother liquor yielded an additional 74 mg. (21%). The total yield was 127 mg. (36%) of colorless, shiny prismatic plates, which, after recrystallization from 95% ethanol, melted at 320–322° dec.

Anal. Found: C, 43.29, 43.20; H, 4.58, 4.32; N, 37.58, 37.58.

These crystals were shown to be identical with those prepared by procedure A and to a sample of highly pure cytosine obtained from Nutritional Biochemicals Corp., having the same melting point and mixture melting point (321–323° dec.), ultraviolet and infrared absorption spectra, and ascending paper chromatographic behavior in two solvent systems: 5% acetic acid (R_f 0.79) and 2:1:1 1-propanol–ammonia (4.2% aqueous) (R_f 0.71).

Acknowledgment.—The assistance of Dr. Kurt Pollock, Mrs. M. Clifton Harrigan, Miss Charlene Horn, and Miss Dorothy H. Trites at various times during the course of this investigation is gratefully acknowledged. We wish to thank Mr. James H. Gunnerson for the infrared and ultraviolet absorption spectra. Larger quantities of the following pyrimidines were obtained through the courtesy of the Cancer Chemotherapy National Service Center, National Cancer Institute, National Institutes of Health, and were prepared according to the procedures outlined in the Experimental section: 4-amino-6-chloropyrimidine and 4-amino-6-mercaptopyrimidine (Francis Earle Co., Peekskill, N. Y.), and 4-amino-6-chloro-2-methylthiopyrimidine (Aldrich Chemical Co., Milwaukee, Wis.).

Derivatives of Purinethiols. Purine Thiolcarbonates and Related Compounds[1]

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The ethyl carbonate and 1-pentyl carbonate derivatives of purine-6-thiol were prepared by the reaction of ethyl and 1-pentyl chloroformates, respectively, with purine-6-thiol. The structures were established by an alternate synthesis from 6-thiocyanatopurine. Analogous ethyl and 1-pentyl carbonate derivatives were obtained from purine-8-thiol, and methyl and ethyl carbonate derivatives from 9-methylpurine-6-thiol. The purine-6-thiol ethyl carbonate has tumor-inhibiting properties. Attempts to prepare thiolcarbamates by the action of isocyanates on purine-6-thiol or on its 9-methyl derivative were unsuccessful. Reaction of 6-methylthiopurine with alkyl chloroformates gave methyl and ethyl 6-(methylthiopurine)-7(or 9)carboxylates. Methyl 8-(methylthiopurine)-7(or 9)carboxylate was obtained similarly. Isocyanates reacted with 6-methylthiopurine to give the 7(or 9) phenyl- 1-naphthyl-, and 1-butylcarbamoyl derivatives.

Because of the inhibitory effect of purine-6-thiol[2] (6-mercaptopurine) on the growth of tumors[3] and on leukemia,[4] compounds that might decompose to give this purine are of possible interest. Such compounds include purine thiolcarbonates, $PSCO_2R$, and purine thiolcarbamates, PSCONHR (where P is the purine nucleus). Simple alkyl thiolcarbonates have shown[5] pharmacological effects similar to those of the thiols. Neither the thiolcarbonate nor thiolcarbamate derivatives of purines have been described, although there has been extensive work[6] on other derivatives of purine-6-thiol.

Purine thiolcarbonates (I–VI, Table I) were prepared

(1) (a) Supported by PHS Grant No. CY-3477 from the National Cancer Institute, Public Health Service; (b) From the Ph.D. thesis of Howard S. Bender, University of Delaware, 1962.

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

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

(4) G. H. Hitchings and C. P. Rhoads, *Ann. N. Y. Acad. Sci.*, **60**, 183 (1954).

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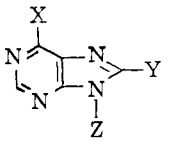
(6) (a) C. G. Skinner, W. Shive, R. G. Ham, D. C. Fitzgerald, and R. E. Eakin, *J. Am. Chem. Soc.*, **78**, 5097 (1956); (b) H. C. Koppel, D. E. O'Brien, and R. K. Robins, *J. Org. Chem.*, **24**, 259 (1959); (c) C. G. Skinner, J. R. Claybrook, D. L. Ross, and W. Shive, *ibid.*, **23**, 1223 (1958); (d) D. A. Clarke, G. B. Elion, G. H. Hitchings, and C. C. Stock, *Cancer Res.*, **18**, 445 (1958).

TABLE I

DERIVATIVES OF PURINETHIOLS



No.	X	Y	Z	M.p., °C.[a]	Yield, %	Recryst. solv.[b]	Formula	Calcd. C	Calcd. H	Calcd. N	Calcd. S	Found C	Found H	Found N	Found S
I	$SCO_2C_2H_5$	H	H	198–199	43	A	$C_8H_8N_4O_2S$	42.85	3.60	24.99	14.30	43.23	3.91	24.80	14.63
II	$SCO_2C_5H_{11}$	H	H	190–191	57	B	$C_{11}H_{14}N_4O_2S$	49.42	5.32	21.12	12.09	49.86	5.23	20.89	12.27
III	H	$SCO_2C_2H_5$	H	186–187	53	B	$C_8H_8N_4O_2S$	42.85	3.60	24.99	14.30	42.98	3.69	25.00	14.50
IV	H	$SCO_2C_5H_{11}$	H	167–170	39	C	$C_{11}H_{14}N_4O_2S$	49.42	5.32		12.09	49.87	5.63		12.32
V	SCO_2CH_3	H	CH_3	136–137	58	D	$C_8H_8N_4O_2S$	42.85	3.60	24.99	14.30	43.03	3.70	24.69	13.99
VI	$SCO_2C_2H_5$	H	CH_3	100–101	72	E	$C_9H_{10}N_4O_2S$	45.36	4.23	23.51	13.46	45.26	4.35	23.30	13.22
VII	SCH_3	H	CO_2CH_3	147	64	B	$C_8H_8N_4O_2S$	42.85	3.60	24.99	14.30	43.24	3.88	24.93	13.61
VIII	SCH_3	H	$CO_2C_2H_5$	142	70	B	$C_9H_{10}N_4O_2S$	45.36	4.23	23.51		45.26	4.31	23.21	
IX	H	SCH_3	CO_2CH_3	126–127	46	B	$C_8H_8N_4O_2S$	42.85	3.60	24.99	14.30	43.37	3.99	24.67	13.82
X	SCH_3	H	$CONHC_6H_5$	151–152	94	C	$C_{13}H_{11}N_5OS$	54.72	3.87	24.55	11.24	55.01	3.79	24.24	10.90
XI	SCH_3	H	$CONHC_{10}H_7$	182	25	F	$C_{17}H_{13}N_5OS$	60.88	3.91	20.88		61.22	3.87	20.88	
XII	SCH_3	H	$CONHC_4H_9$	95–96	44	C	$C_{11}H_{15}N_5OS$	49.79	5.70	26.40	12.09	49.95	5.69	26.15	11.86

[a] Melting points corrected, taken on a Fisher-Johns Block. [b] Solvents: A, methanol; B, ethanol; C, 50% water–ethanol; D, water; E, ether; F, chloroform–hexane.

TABLE II

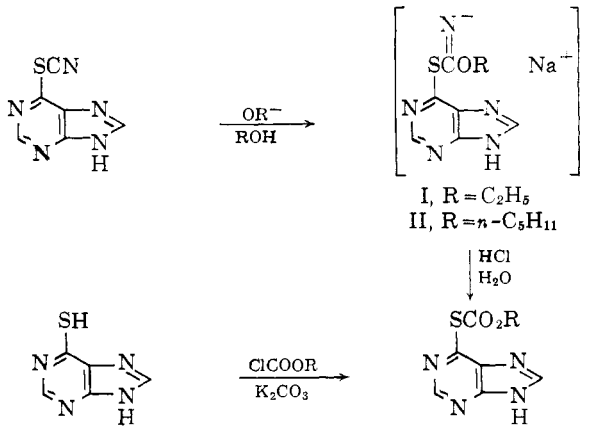
SPECTRAL DATA

Compound no.	Name	Ultraviolet absorption[a] pH 1 λ_{max}	pH 1 $\epsilon \times 10^{-4}$	pH 7 λ_{max}	pH 7 $\epsilon \times 10^{-4}$	Infrared absorpt.,[b] C=O, cm.$^{-1}$
I	Purine-6-thiol ethyl carbonate	321	1.74	322	1.89	1770
II	Purine-6-thiol pentyl carbonate	320				1754
III	Purine-8-thiol ethyl carbonate	318	1.70	318	0.96	1721
IV	Purine-8-thiol pentyl carbonate					1748
V	9-Methylpurine-6-thiol methyl carbonate	283	1.05	280	1.35	1724
VI	9-Methylpurine-6-thiol ethyl carbonate	280	1.08	282	1.50	1721
VII	Methyl 6-(methylthiopurine)-7(9) carboxylate	287	2.26	287	2.15	1748
VIII	Ethyl 6-(methylthiopurine)-7(9) carboxylate	288	2.02	288	1.82	1770
IX	Methyl 8-(methylthiopurine)-7(9) carboxylate	313	2.58	299	1.52	1754
X	6-Methylthio-N-phenyl purine-7(9)-carboxamide	275	1.09			1721
XI	6-Methylthio-N-1-naphthyl purine-7(9) carboxamide	280	0.55	280	1.13	1724
XII	6-Methylthio-N-butyl purine-7(9)-carboxamide	265	.74	260	0.62	1704

[a] Taken with a Perkin-Elmer Model 202 ultraviolet–visible spectrophotometer. The blank spaces indicate insufficient solubility in the aqueous solutions used for accurate measurement. [b] In potassium bromide pellets, using a Model B Baird infrared recording spectrophotometer.

from purine-6-thiol, purine-8-thiol, and 9-methylpurine-6-thiol by treatment with alkyl chloroformates. A satisfactory medium for preparing compounds I–IV was dimethylformamide with potassium carbonate as acid acceptor, as used for alkylations[7] on the thiol group.[7a] The method for compounds V and VI involved treating the sodium salt of the purinethiol with the chloroformate ester in benzene. Aqueous alkaline media were not effective.

The structures of compounds I and II were proved by synthesis from the known 6-thiocyanatopurine,[8] using Grant and Snyder's method[9] for converting a thiocyanate to a thiolcarbonate. The products isolated from the reaction of 6-thiocyanatopurine with sodium ethoxide or sodium 1-pentoxide, followed by hydrolysis, were identical with those obtained from the reaction of ethyl and 1-pentyl chloroformates with purine-6-thiol.



It is to be noted that the attack of the chloroformate is on the SH group in purine-6-thiol, whereas in 6-aminopurine (adenine) this occurs at the 7 or 9 position,[10] due probably to the greater electron density around the thiol or thiolate ion than around the 7- or 9-nitrogen or the 6-amino nitrogen of the purine ring. By analogy to the behavior of purine-6-thiol, it may be assumed that the reaction of chloroformates with purine-8-thiol and

(7) T. P. Johnston, L. P. Holum, and J. A. Montgomery, *J. Am. Chem. Soc.*, **80**, 6265 (1958).

(7a) NOTE ADDED IN PROOF.—Purine-6-thiol ethyl carbonate has been prepared in 70% yield by treating purine-6-thiol with ethyl chloroformate in aqueous sodium hydroxide. (Private communication from H. Tilles and D. G. Stoffey, Stauffer Chemical Company, Richmond Research Center.)

(8) G. B. Elion, I. Goodman, W. Lange, and G. H. Hitchings, *ibid.*, **81**, 1898 (1959).

(9) M. S. Grant and H. R. Snyder, *ibid.*, **82**, 2742 (1960).

(10) E. Dyer, J. M. Reitz, and R. E. Farris, Jr., *J. Med. Chem.*, **6**, 298 (1963).

with 9-methylpurine-6-thiol also took place at the thiol group.

All of the purine thiolcarbonates showed carbonyl absorption in the infrared (Table II) within the range 1721–1770 cm.$^{-1}$. In diethyl thiolcarbonate[11] this absorption is at 1700 cm.$^{-1}$. A weak, but narrow band at 662–666 cm.$^{-1}$ in all of the purine thiolcarbonates might be caused by the C–S stretching vibration.[12] The ultraviolet absorption of compounds V and VI is in the region typical[13] of other 6-substituted thiopurines (280–290 mμ), but compounds I, II, and III absorb at somewhat longer wavelengths.

The thiolcarbonates were rapidly hydrolyzed to 6-purinethiol at pH 11, less rapidly at pH 8, and were unaffected by dilute acids at room temperature for periods of several hours.

Efforts were made to prepare purine thiolcarbamates by the interaction of the thiol group with phenyl isocyanate under conditions known[14] to be favorable for the reaction of simple thiols with isocyanates. However, treatment of anhydrous purine-6-thiol with phenyl isocyanate in dimethylformamide in the presence of triethylamine gave only mixtures of unidentified products, and 9-methylpurine-6-thiol did not react when suspended in toluene and heated with the isocyanate in the presence of the powerful catalyst, 1,4-diazabicyclo-[2.2.2]octane.

Although successful reaction of the thiol group of the purine with isocyanates has not been achieved, facile reaction at the 7- or 9-position took place with 6-methylthiopurine. Carbamoyl derivatives (X, XI, XII, Table I) were obtained from the action of phenyl, 1-naphthyl and 1-butyl isocyanates on 6-methylthiopurine in benzene solution at room temperature in the presence of triethylamine. These substances were crystalline, soluble in organic solvents, and showed strong carbonyl absorption in the infrared (Table II).

Reaction at the imidazole –NH group occurred also with chloroformates when the thiol group was alkylated. 6-Methylthiopurine and 8-methylthiopurine yielded the 7(or 9) carboxylate derivatives VII–IX (Table I) when treated with chloroformates. The compounds were very sensitive to basic hydrolysis, but were unaffected by dilute acid.

Attempts were made to determine whether the carboxylate group was at the 7- or 9-position by treatment of VII with sodium borohydride or aluminum hydride in the presence of aluminum chloride, which was expected to give a known 7-methyl or 9-methyl derivative of 6-methylthiopurine. However, the only product was 6-methylthiopurine. A similar result from hydrogenation of an N-carbalkoxy derivative of a heterocyclic compound was obtained by Dahlbom[15] using 10-carbethoxyphenothiazine.

On the basis of data on ultraviolet absorption, the location of the carboxylate group at the 9-position is somewhat more probable than at the 7-position because the 9-substituted thiopurines absorb at slightly lower wave lengths. For example, at pH 7, the λ_{max} of 6-purinethiol is 322 mμ, of 9-methyl-6-purinethiol 320 mμ, and of 7-methyl-6-purinethiol 327 mμ.[16] At the same pH VII and VIII absorb at 287 and 288 mμ, the parent 6-methylthiopurine absorbs at 290 mμ,[17] and 9-ethyl-6-methylthiopurine absorbs at 286 mμ.[18] However, these differences are too small to use for definite assignment of structure. Moreover, the analogy to the effect of alkyl substituents in the 7- and 9-positions might not be valid for carboxylate or carboxamide substituents, for which there are no standards.[18] The possibility of answering by synthetic approaches the question of 7- or 9-substitution on acylation of various purines is currently being studied.

Pharmacological Tests.—Testing by the Cancer Chemotherapy National Service Center[19] has shown that purine-6-thiol ethyl carbonate (I) is active against Sarcoma-180, adenocarcinoma-755, Leukemia-1210, and the KB cell culture. Details are given in Table III. The data for the Sa-180 and Ca-755 systems include statistical analysis (done by CCNSC) for a new "specificity test" designed by Skipper and co-workers.[20] Specificity at the 99.7% level is required for an active drug. Compound I is active at this level for Ca-755, and has an index of 3.5 for Sa-180 and 5.6 for Ca-755. 6-Purinethiol, active at the 99.7% confidence level, has a specificity index of 1.9 for Sa-180 and 10.0 for Ca-755.[20]

When the thiolcarbonate group was in the 8-position, however, as in III, no activity was observed against the same three systems as well as against solid Friend Virus Leukemia.

A limited number of tests on VIII, which is 6-methylthiopurine with a carboxylate group in the 9- or 7-position have shown activity, but the results are erratic. Toward Adenocarcinoma 755 the T/C was 7% at a dose of 50 mg./kg., 15% at 25 mg. kg. and 48% at 12.5 mg. kg.; while in another series of tests the T/C was 80% at 10 mg./kg.). Compound VIII was nontoxic and inactive toward the KB cell line. Hence, the carboxylate derivative probably has no advantage over the strongly active 6-methylthiopurine.[21]

Experimental

Purine Thiolcarbonates. A. From 6- and 8-Purinethiols and Alkyl Chloroformates (I–IV).—To a solution of 0.5 g. (0.0033 mole) of anhydrous 6-purinethiol[2] or 8-purinethiol[22] in 20 ml. of purified dimethylformamide[23] was added with stirring at room temperature 0.92 g. (0.0066 mole) of anhydrous potassium carbonate. The 6-purinethiol dissolved on stirring or on slight warming and the solution was cooled to room temperature. Then 0.0066 mole of ethyl or 1-pentyl chloroformate was added while stirring. The temperature rose about 5°. After stirring for another 2 hr. the mixture was poured into 50 g. of ice and the pH

(11) F. Feluoh, *Bull. Soc. Chim. France*, 890 (1957).

(12) L. S. Bellamy, "The Infrared Spectra of Complex Molecules", John Wiley and Sons, New York, N. Y., 1951, p. 291.

(13) C. G. Skinner, R. G. Ham, D. C. Fitzgerald, Jr., R. E. Eakin, and W. Shive, *J. Org. Chem.*, **21**, 1330 (1956).

(14) E. Dyer, J. F. Glenn, and E. G. Lendrat, *ibid.*, **26**, 2919 (1961).

(15) R. Dahlbom, *Acta Chem. Scand.*, **6**, 310 (1952).

(16) G. B. Elion in Ciba Foundation Symposium, "Chemistry and Biology of Purines," G. E. W. Wolstenholme and C. M. O'Connor, Ed., Little Brown and Co., Boston, Mass., 1957, p. 46.

(17) J. A. Montgomery and C. Temple, *J. Am. Chem. Soc.*, **79**, 5238 (1957).

(18) A 6-chloro-9(or 7)-acetylpurine was found to have practically the same absorption maximum as 6-chloropurine by J. A. Montgomery, *ibid.*, **78**, 1928 (1956).

(19) The procedures for Sa-180, Ca-755, and L-1210 have been described by J. Leiter, A. R. Bourke, S. A. Schepartz, and I. Wodinsky, *Cancer Research, Cancer Chemotherapy Screening Data*, V, **20**, 734 (1960).

(20) H. E. Skipper, W. S. Wilcox, F. M. Schabel, Jr., W. R. Laster, Jr., and L. Mattil, *Cancer Chemotherapy Rept.*, **29**, 1 (1963).

(21) J. A. Montgomery, *Cancer Res.*, **19**, 447 (1959).

(22) R. W. Balsiger, A. L. Fikes, T. P. Johnston, and J. A. Montgomery, *J. Org. Chem.*, **26**, 3386 (1961).

(23) G. R. Lealer and J. F. Gormley, *J. Am. Chem. Soc.*, **73**, 5731 (1951).

TABLE III

SCREENING DATA[a,b] ON PURINE-6-THIOL ETHYL CARBONATE

Dose, mg./kg.	Survivors ( ) of ( )		Animal wt. diff.	Sarcoma-180[c,d,e] Tumor wt. Test	Control	T/C, %	Specificity test[f]	Confidence,[f] %	Index[f]
112	5	6	−1.7	195	875	22			
100	6	6	−1.3	303	843	35			
66.0	6	6	−1.2	517	843	61			
56.0	6	6	−1.1	268	875	30			
44.0	6	6	−0.5	473	843	56			
28.0	6	6	1.0	405	875	46			
14.0	6	6	−1.0	364	875	41			
7.00	6	6	−0.9	800	777	102	Below 8,		
0.80	6	6	−0.8	1063	777	136	above 0	95.0	3.5
				Adenocarcinoma-755[d,g,h]					
112	7	10	−4.1	50	675	7			
56.0	10	10	−2.3	56	675	8			
28.0	10	10	−1.3	59	675	8			
28.0	10	10	−3.3	50	875	5			
14.0	10	10	−1.3	56	675	8			
14.0	10	10	−1.8	56	875	6			
3.50	10	10	−1.2	43	948	4			
1.75	10	10	−2.0	206	1663	12			
0.85	10	10	−0.9	847	1663	50	Below 14,		
0.21	10	10	−0.6	1449	1663	87	above 0	99.7	5.6
				Lymphoid Leukemia L-1210[d,g] Survival (days)					
160	6	6	−2.7	9.3	8.6	108			
80.0	6	6	−1.8	15.0	10.2	147			
40.0	6	6	−2.0	15.0	10.2	147			
20.0	6	6	−1.1	15.0	10.2	147			
16.0	6	6	−1.4	11.2	8.3	134			
8.0	6	6	−1.7	10.2	8.3	122			

KB Cell Culture[i] Slope	ED_{50}, μg./ml.
	<1.0
	<0.25
− .47	.93
−1.2	.17

[a] Portions, not the whole of the data. [b] One dose per day for all of the tumor systems. [c] Host, Swiss. [d] Vehicle, carboxymethylcellulose. [e] Seven IP. injections, sacrifice on day 8. [f] Ref. 20. [g] Host, BDF1. [h] Eleven IP. injections, sacrifice on day 12. [i] Vehicle, dimethylformamide.

was at once adjusted to 5 with glacial acetic acid. The solid product was recrystallized to constant melting point from the solvent specified in Table I. These substances were slightly soluble in water and moderately soluble in alcohols.

When 50 mg. of compound I was allowed to stand for 0.5 hr. in a buffer of pH 8 at room temperature, the solution acidified to pH 5, and extracted with ethyl acetate four times, 17 mg. of the thiolcarbamate was recovered unchanged. However, at pH 11 complete hydrolysis to 6-purinethiol took place in 0.5 hr.

B. From 6-Thiocyanatopurine and Alkoxides (I and II).—To 50 ml. of ethanol or 1-pentanol was added 1.04 g. (0.0455 g.-atom) of sodium. After the sodium had reacted, 1.00 g. (0.0057 mole) of 6-thiocyanatopurine[8] was added and the solution refluxed for 12 hr. The precipitate obtained on cooling was filtered, added to 50 ml. of 3 *M* hydrochloric acid, and the mixture refluxed for 48 hr. The cooled solution was treated with sodium bicarbonate to bring the pH to 5 and extracted with four 50-ml. portions of ethyl acetate. The thiolcarbonates were obtained in 19% yield from evaporation of the dried extracts at room temperature. Mixture melting points of the recrystallized products prepared by methods (A) and (B) showed no depression and the infrared spectra of the compounds were identical. No other product was obtained.

C. From the Sodium Derivative of 9-Methylpurine-6-thiol and Alkyl Chloroformates (V, VI).—A mixture of 50 ml. of dry benzene, 0.50 g. (0.0030 mole) of 9-methylpurine-6-thiol[24] (the purity of which was checked by ultraviolet absorption), and 0.079 g. (0.0033 mole) of sodium hydride was stirred until reaction was complete, treated with 0.0060 mole of methyl or ethyl chloroformate, and the mixture stirred overnight at room temperature. Evaporation of the filtered benzene solution at room temperature yielded the product.

Carboxylate Derivatives of 6- and 8-Methylthiopurine (VII-IX).—A mixture of 50 ml. of water, 0.2 g. (0.005 mole) of sodium hydroxide, and 0.5 g. (0.0033 mole) of 6- or 8-methylthiopurine[2,25] was stirred until all of the solid dissolved. Then 0.012 mole of methyl or ethyl chloroformate was added and the solution stirred at room temperature for 3 hr. The precipitate that formed in the now acid solution was filtered and recrystallized.

A solution of 50 mg. of VII in 0.1 *M* sodium hydroxide was completely hydrolyzed to 6-methylthiopurine in 15 min. at room temperature, but was unchanged in 0.1 *M* sulfuric acid.

Carbamoyl Derivatives of 6-Methylthiopurine (X-XII).—A mixture of 50 ml. of dry benzene, 0.5 g. (0.003 mole) of 6-methylthiopurine,[2] 0.006 mole of phenyl, 1-naphthyl, or 1-butyl isocyanate, and 11 mg. of triethylamine was stirred for 12 hr. with protection from moisture. The products were obtained by evaporation of the solvent at room temperature. At temperatures above their melting points, these compounds decomposed to the isocyanate and 6-methylthiopurine. The carbamoyl derivative X was hydrolyzed to 6-methylthiopurine when a 100 mg. sample was allowed to stand with 10 ml. of 0.01 *M* sodium hydroxide overnight at room temperature.

Hydrogenation of Methyl 6-(methylthiopurine)-7(or 9)carboxylate.—A 100 mg. sample of VII dissolved in tetrahydrofuran

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was treated with lithium aluminum hydride in the presence of aluminum chloride to maintain acidity. During the decomposition of the hydride complex the pH of the solution was never allowed to rise above 5. The product, isolated by continuous extraction with ethyl acetate, was 87 mg. of 6-methylthiopurine. A control experiment with all reagents except the lithium aluminum hydride showed that VII was not hydrolyzed under the acid conditions maintained. Therefore, since hydrolysis is not the cause of the formation of 6-methylthiopurine, hydrogenation must have occurred.

1,4-Bismethanesulfonates of the Stereoisomeric Butanetetraols and Related Compounds

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The synthesis of the stereoisomeric butane-1,2,3,4-tetraol 1,4-bismethanesulfonates and some of their cyclic acetals and ketals is described. The preparation of L-threitol 1,4-bismethanesulfonate from L(+)-tartaric acid as starting material makes this compound easily available for clinical evaluation.

The synthesis and cytoactivity of D-mannitol 1,6-bismethanesulfonate has led to a more detailed investigation of methanesulfonated sugars and polyhydric alcohols which are considered water-soluble analogs of 1,4-dimethanesulfonyloxybutane (busulfan).[1-3]

As already briefly reported we[4] synthesized the 1,4-bismethanesulfonates of the stereoisomeric 1,2,3,4-butanetetraols (I).

$$CH_3SO_2OCH_2CHOHCHOHCH_2OSO_2CH_3 \qquad I$$

L, (D), DL, *meso*)

We suggested that these compounds, although identical with busulfan with respect to their chain length, might show an alkylating mechanism different from that of busulfan owing to the hydroxyl groups in the α-position to the sulfonyloxy groups. Furthermore, our interest in these compounds was based on the known correlation in the busulfan series ($CH_3SO_2O(CH_2)_{2-10}OSO_2CH_3$) between chain length and both water–ether solubility ratio and the biological activity.[5] In addition, it could be expected that the stereoisomers would be different in their alkylation reactions *in vivo*.

In continuation of our search we prepared certain cyclic acetals and ketals (1,3-dioxolanes) of I with the purpose of obtaining substances for investigation as anticancer agents. Such dioxolanes may be regarded either as camouflaged 1,4-bismethanesulfonates of butanetetraol with initially decreased water solubility, or alternatively, when no ring hydrolysis is assumed, as bismethanesulfonates with a fixed distance between the alkylating groups. The latter assumption means that only the compounds with an *erythro*-, but not with a *threo*-configuration, have the ability to react under cycloalkylation, and this is proved to be the main reaction of busulfan *in vivo*.[6]

Of course, both the solubility of these compounds and the stability of the ring will depend on the nature of the substituents originating from the aldehyde or ketone forming the basis for the dioxolane. Moreover, the ring formation should influence the alkylating properties of these bismethanesulfonates.

Chemistry.—The synthetic routes for the preparation of the stereoisomeric bismethanesulfonates I are summarized for the L-isomers. Since no attack on the asymmetric carbon atoms is involved, the configuration of the compound is given by that of the starting material. The ring opening of the stereoisomeric 1,2:3,4-diepoxybutanes (VII) with methanesulfonic acid resulted in yields of *ca.* 30% of the corresponding I when the reaction was carried out in a diethyl ether–*t*-butyl alcohol mixture. Using pure diethyl ether as the solvent only small quantities of *meso*-I were obtainable, which is in agreement with recently reported results.[3]

The reaction of the stereoisomeric 1,4-dibromo-2,3-butanediols (VI) with silver methanesulfonate in boiling acetonitrile gave the corresponding I in 30–40% yield. To develop the preparation of L-I on a larger scale and consequently to make a clinical evaluation of the compound possible, the synthesis was based on L(+)-tartaric acid as an easily available starting material. Diethyl L-tartrate (II), obtained from natural tartaric acid by azeotropic esterification, was converted into diethyl 2,3-*O*-isopropylidene-L-tartrate (III) by an acid-catalyzed reaction with acetone in low boiling petroleum ether under simultaneous azeotropic removal of the reaction water. This procedure proved superior to the previously described ketalization with copper sulfate as a water-removing agent.[7] Compound III was then reduced with Li-AlH_4 to 2,3-*O*-isopropylidene-L-threitol (IV).[8] Ketal hydrolysis was avoided by an alkaline isolation

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