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A Comparative Study of the Anticancer Activity of Some *S*-Substituted Derivatives of 6-Mercaptopurine and their Ribonucleosides*

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I. Introduction

Because of the established anticancer activity of 6-mercaptopurine[1] [purine-6(1H)-thione], we prepared the ribonucleoside of this purine some time ago in these laboratories.[2] A careful comparative evaluation of the activity of this purine and its ribonucleoside against Carcinoma 755, a tumour unusually sensitive to purine antagonists, in BDF 1 mice showed the ribonucleoside to have a greater therapeutic index in this test system.[3] This initial result led us to synthesize and test a number of ribonucleosides of *S*-substituted derivatives of 6-mercaptopurine. The *S*-substituted 6-mercaptopurine derivatives themselves had been synthesized previously[4] and found to have activity against Carcinoma 755.[3]

We have now made a study of the activity of this series of purines and their ribonucleosides against not only Carcinoma 755 but also against Sarcoma 180 and Leukemia L 1210.††

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† Affiliated with the Sloan-Kettering Institute.

†† These are the three tumours employed in the primary screening programme of the Cancer Chemotherapy National Service Center, U.S. Public Health Service.

II. Discussion

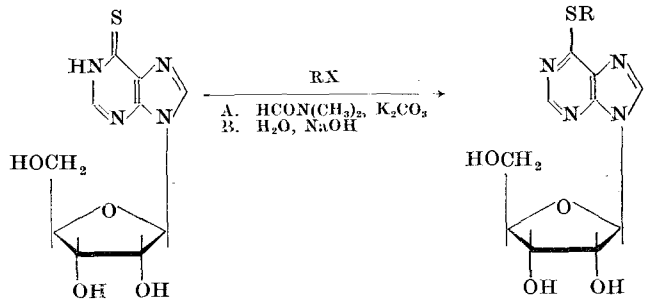
A. Synthesis

The facile conversion of inosine into 9-β-D-ribofuranosyl-9H-purine-6(1H)-thione via the thiation of 9-(2,3,5-tri-*O*-benzoyl-β-D-ribofuranosyl)hypoxanthine[5] has made the thione a readily available starting material for the preparation of *S*-substituted derivatives.* The direct methylation of 9-β-D-ribofuranosyl-9H-purine-6(1H)-thione with iodomethane in aqueous sodium hydroxide solution to give the 6-(methylthio) derivative has been described.[5] Previously, the preparation of the 6-(methylthio) derivative had been achieved by the condensation of the chloromercuri derivative of 6-(methylthio)purine with 2,3,5-tri-*O*-acetyl-D-ribosyl chloride, followed by removal of the acetyl groups.[6] The synthesis of 6-(benzylthio)-9-β-D-ribofuranosyl-9H-purine by the action of sodium α-toluenethiolate on 6-chloro-9-β-ribofuranosyl-9H-purine has been reported.[7]

The 6-(alkylthio)-9-β-D-ribofuranosyl-9H-purines whose preparations are summarized in Table I were all prepared by the direct alkylation of 9-β-D-ribofuranosyl-9H-purine-6(1H)-thione either in dimethylformamide with potassium carbonate as the acid acceptor (Method A) or in water with sodium hydroxide as the acid acceptor (Method B). Method A is essentially the method previously described for the preparation of a large number of 6-(alkylthio)purines in which dimethylformamide was employed advantageously as a solvent.[4] Dimethylformamide proved to be a good medium for most of the alkylations that produced the ribonucleosides of Table I, but its great solvating power complicated the isolation of pure products in some cases. In no instance in which dimethylformamide was used was the presence of unchanged 9-β-D-ribofuranosyl-9H-purine-6(1H)-thione detected in the isolated product either by ultraviolet absorption spectroscopy or by paper chromatography. When the substituent was a methyl, cyanomethyl, or acetonyl group, method B was the preferred procedure; when the substituent was a thenyl group, method A was preferred because of the competitive hydrolysis of 2-chloromethylthiophene in water. The products were obtained

* We wish to thank the Cancer Chemotherapy National Service Center for a generous supply of 9-β-D-ribofuranosyl-9H-purine-6(1H)-thione.

Table I. *S*-Substituted 9-β-D-ribofuranosyl-9H-purine-6-thiols



R	X	Method[a]	Recrystn. medium[b]	Drying *in vacuo* over P_2O_5 time, h	Drying *in vacuo* over P_2O_5 temp., °C	Water content in mole equivalents	Yield, %	Analysis, % Calcd. C	Calcd. H	Calcd. S	Found C	Found H	Found S
Methyl	I	A	A	40	80	1[c]	66[d]	41·76	5·10	10·13	42·11	5·00	10·22
	I	B	B	40	25	0[e]	78[f]	44·30	4·73	18·79	44·50	4·85	18·65
Ethyl	Br	A	C	7	80	⅔	54	44·42[g]	5·39	9·89	44·75	5·23	9·52
Cyanomethyl	Cl	A	A	40	80	0	81[d]	44·58	4·05	9·97	44·88	4·29	9·70
		B	D	4	80	1	62	42·22	4·43	9·40	42·50	4·36	8·89
Allyl	Cl	A	A	40	80	¼	87	47·50	5·06	9·75	47·55	5·01	9·62
Propyl	Br	A	A	24	80	1	80[d]	45·34	5·85	9·32	45·29	5·62	9·36
Acetonyl	Cl	A	B	9	80	¼	34	45·27	4·82	9·32	45·50	5·29	9·26
	Cl	B	D	4	80	0	75	45·88	4·72	—	45·95	5·12	—
Cyclopentyl	Br	A	A	40	80	¼	74[d]	50·47	5·79	8·98	50·39	5·94	8·96
2-Thenyl	Cl	A	E	8	60	½	71	46·26	4·40	16·47	46·38	4·65	16·56
Benzyl	Cl	B	A[h]	4	45	0	90	—	—	—	—	—	—
Cinnamyl	Cl	A	B	8	80	½[i]	83	55·73	5·17	7·83	55·66	5·53	7·53

[a] Indicated in the equation under the title of this table. [b] A, water; B, ethyl alcohol; C, methyl alcohol–ether; D, isopropyl alcohol; E, methyl alcohol. [c] m.p. indefinite (softening from 104° on Kofler Heizbank). [d] Crude yield. [e] m.p. 167° (Kofler Heizbank); lit.[5] m.p. 163–164°. [f] From 0·3 mole run. [g] % Nitrogen: Calcd., 17·27. Found, 16·90. [h] Gelled from aqueous reaction mixture; acetone solution of gel dried over magnesium sulphate and evaporated to dryness *in vacuo*; λ_{max} and $\epsilon \times 10^{-3}$ practically identical with lit.[7] values. [i] m.p. 214° with softening from 210° (Kofler Heizbank).

in varying degrees of hydration, the water content depending on the solvent used for recrystallization and the drying conditions; forced drying *in vacuo* at temperatures above 80° was avoided because several of the compounds were observed to be unstable when drying was attempted at 100°. Only one of the hydrated ribonucleosides [9-β-D-ribofuranosyl-6-(2-thenylthio)-9H-purine] had a definite melting point. Examples of typical procedures employed are given in the Experimental Section.

The ultraviolet absorption spectra of these ribonucleosides (Table II) are, in general, quite similar to those of the corresponding 6-(alkylthio)purines.[4] The greatest difference in the spectra

Table II. Ultraviolet absorption spectra of *S*-substituted 9-β-D-ribofuranosyl-9H-purine-6-thiols[a, b]

R of RS-group	0·1 N HCl		*p*H 7		0·1 N NaOH	
	λ_{max}, mμ	$\epsilon \times 10^{-3}$	λ_{max}, mμ	$\epsilon \times 10^{-3}$	λ_{max}, mμ	$\epsilon \times 10^{-3}$
Methyl	224	11·4	224	11·8	224	11·4
	293	17·4	289	18·9	289	18·9
Ethyl	224	10·4	224	10·6	224	10·5
	294	16·8	292	18·2	292	18·4
Cyanomethyl	277	16·3	278	16·1	278	16·0
Allyl	292	17·6	291	19·4	291	19·3
Propyl	225–226	11·0	225–226	10·8	225–226	10·7
	295	17·4	293	19·3	293	19·7
Acetonyl	300	13·8	282	17·2	255[c]	—
					281	—
Cyclopentyl	297	18·5	295	20·2	294–295	20·4
2-Thenyl	224	15·8	224	15·8	224	—
	292	18·0	293	18·6	292	19·1
Benzyl	293[d]	18·9	292	20·6	292	20·8
Cinnamyl	254–255	19·7	255	19·5	253–258[e]	19·6
	295	23·3	295	24·7	295	24·8

[a] Determined with either a Beckman Model DK-2 spectrophotometer (optical densities on a Beckman DU) or a Cary Model 14 spectrophotometer. [b] Shoulders not recorded but have patterns similar to those recorded for the corresponding 6-(alkylthio)purines.[4] [c] Undergoes partial hydrolysis to inosine at pH 13. [d] *cf.* ref. 7. [e] Plateau.

occurs in the case of 6-(cyanomethylthio)purine (289 mμ) at pH 13 *versus* 6-(cyanomethylthio)-9-β-D-ribofuranosyl-9H-purine (278 mμ). 6-(Acetonylthio)-9-β-D-ribofuranosyl-9H-purine underwent

partial hydrolysis to inosine during the course of determining its spectrum at pH 13. Paper chromatographic data and optical rotations of these compounds are recorded in Table III.

B. Screening Results

Carcinoma 755. The raw data obtained in screening these purines and their ribonucleosides against Carcinoma 755 in BDF 1 mice are presented in Table IV. With one exception, 6-(methylthio)-9-β-D-ribofuranosyl-9H-purine,* these materials exhibited a high degree of inhibitory action against this tumour. From these data the chemotherapeutic indices of the compounds were calculated. The results of these calculations are summarized in Table V in which the maximum tolerated dose (MTD); the minimum effective dose, which in this paper is defined as the lowest dose that will inhibit the growth of this tumour to 50 per cent of the untreated controls (ED_{50})†; the chemotherapeutic indices (MTD/ED_{50}); and the ratio of the chemotherapeutic index of the ribonucleoside to that of the purine from which it is derived (CI_R/CI_P) are given. The MTD's and the ED_{50}'s were calculated in μmoles/kg/day to give a reasonable comparison between these compounds that vary so widely in their molecular weights. On this basis, two of the ribonucleosides, *viz.* 6-(methylthio)-9-β-D-ribofuranosyl-9H-purine and 9-β-D-ribofuranosyl-6-(2-thenylthio)-9H-purine, were more toxic than the parent purines, and these two compounds were the only ones more toxic than 6-mercaptopurine itself. The only compound to exhibit a smaller ED_{50} than 6-mercaptopurine was its ribonucleoside, and this was the only ribonucleoside that was significantly more effective against Carcinoma 755 than the parent purine, as can be seen from the ratios of the chemotherapeutic indices. Five ribonucleosides were about as effective as the corresponding purines and four were considerably less effective, the ratios varying from 0·04 to 0·4.

* Sarcione and Stutzman have found that, in the rat, 6-(methylthio)purine is metabolized to 6-mercaptopurine, and 6-mercaptopurine to 6-(methylthio)purine, but neither conversion takes place to any great extent.[9] The significance of this observation with regard to the anticancer activity of 6-(methylthio)purine or its ribonucleoside is not certain, nor is it known whether other *S*-substituted derivatives of 6-mercaptopurine undergo the same type of cleavage *in vivo*.

† The Ed_{50} values were obtained by an extrapolation procedure.[8]

Table III. Chromatographic data[a] and optical rotations[b] of *S*-substituted 9-β-D-ribofuranosyl-9H-purine-6-thiols

R of RS Group	Solvent systems[c]								$[\alpha]_D^{27}$	*c*, %	Solvent[e]
	A		B		C		D				
	R_f	R_{Ad}[d]	R_f	R_{Ad}	R_f	R_{Ad}	R_f	R_{Ad}			
Methyl	0·64	1·43	0·76	1·20	0·76	1·53	0·66	1·85	—	—	—
Ethyl	0·73	1·69	0·83	1·30	0·81	1·75	0·69	1·95	—	—	—
Cyanomethyl	0·52	1·27	0·73	1·17	0·71	1·55	0·71	1·96	−79·4±1·4	0·97	A
Allyl	0·75	1·82	0·84	1·35	0·83	1·78	0·67	1·92	−55·7±1·4	0·98	B
Propyl	0·81	1·80	0·86	1·35	0·84	2·02	0·67	1·74	−49·3±1·5	0·96	B
Acetonyl	0·48	1·17	0·72	1·13	0·72	1·57	0·76	2·14	−60·1±2·3	0·61	C
Cyclopentyl	0·84	1·95	0·89	1·36	0·92	1·70	0·51	1·41	−62·1±1·5	0·94	C
2-Thenyl	0·79	1·91	0·87	1·43	0·85	1·88	0·37	1·04	−52·1±1·3	1·04	D
Benzyl	0·86	1·80	0·90	1·36	0·86	1·73	0·38	0·92	—	—	—
Cinnamyl	0·81	1·86	0·88	1·39	0·86	1·95	[f]	[f]	−71·3±5·7	0·24	A

[a] Paper chromatograms run by the descending technique on Whatman No. 1 paper; spots viewed in ultraviolet light. [b] Determined with a Standard Model D Keston polarimeter attachment to the Beckman DU spectrophotometer, calibrated with standard sucrose solutions [see K. G. Poulsen, *Anal. Chem.*, **32**, 410 (1960)]. [c] A, water-saturated butyl alcohol [J. G. Buchanan, C. A. Dekker and A. G. Long, *J. chem. Soc.*, 3162 (1950)]; B, 5 : 2 : 3 butyl alcohol–acetic acid–water [D. M. Brown, A. Todd, and S. Varadarajan, *J. chem. Soc.*, 2388 (1956)]; C, 70 : 5 : 25 isopropyl alcohol–concentrated ammonium hydroxide–water [R. Markham and J. D. Smith, *Nature, Lond.*, **168**, 406 (1951)]; D, phosphate buffer, pH 6·7 (0·1M K_2HPO_4–0·1M KH_2PO_4). [d] Adenine used as a standard and arbitrarily assigned a value of 1·00. Other spots assigned R_{Ad} values with reference to adenine. [e] A, 80% ethyl alcohol; B, water; C, ethyl alcohol; D, methyl alcohol. [f] Non-moving.

Table IV. Inhibition of Adenocarcinoma 755 by certain *S*-substituted derivatives of 6-mercaptopurine and their ribonucleosides

NSC no.[a]	Compound	Dosage, mg/kg/day	Mortality	Animal wt. change, g *T/C*	Animal wt. difference, g *C–T*	Av. tumour wts., mg *T/C*	% of control tumour wt.
20105	6-(Methylthio)purine	150	10/10				
		125	4/10	−0·8/+2·8	3·6		0
		100	0/10	−0·3/+3·7	4·0	0/1135	0
		50	0/10	+0·4/+3·7	3·3	28/1135	2
		20	0/10	+1·0/+3·0	2·0	132/1372	10
		10	0/10	+1·8/+3·7	1·9	350/1135	31
		5	1/10	+2·4/+3·1	0·7	527/1112	47
		2·5	0/10	+3·0/+3·7	0·7	697/1135	61
		1	1/10	+3·6/+3·7	0·1	746/1135	66
49555	6-(Methylthio)-9-β-D-ribofuranosyl-9H-purine	50	10/10				
		30	4/10	−2·5/+3·6	6·1		
		25	7/10				
		20	1/10	−0·4/+3·6	4·0	275/ 937	29
		15	0/10	+3·0/+5·3	2·3	1281/1907	67
		10	0/10	+1·9/+5·3	3·4	969/1907	51
		5	0/10	+2·7/+3·6	0·9	1502/1907	79
11588	6-(Ethylthio)purine	250	4/10	−4·0/+4·0	8·0		
		150	4/10	−3·0/+4·0	7·0		
		75	1/10	−4·0/+1·0	5·0	12/1432	1
		37	0/10	−1·0/+1·0	2·0	137/1432	10
		18	0/10	−1·0/+1·0	2·0	145/ 932	16
		9	0/10	−1·0/+1·0	2·0	407/1432	28
		4·5	0/ 9	+3·7/+4·5	0·8	278/ 714	39
		2·5	0/10	+3·2/+5·3	2·1	673/1680	40
		1	0/10	+2·0/+4·0	2·0	1672/2327	72

Table IV—*continued*

NSC no.[a]	Compound	Dosage, mg/kg/day	Mortality	Animal wt. change, g *T/C*	Animal wt. difference, g *C–T*	Av. tumour wts., mg *T/C*	% of control tumour wt.
39368	6-(Ethylthio)-9-β-D-ribofuranosyl-9H-purine	400	8/10				
		200	3/10	−2·9/+1·4	4·3	0/ 856	0
		100	0/10	−1·8/+1·4	3·2	5/ 856	1
		50	2/10	−1·8/+1·4	3·2	59/ 856	7
		25	0/10	−0·9/+1·4	2·3	167/ 856	20
		12	0/10	−0·5/+1·4	1·9	527/ 856	62
29421	6-(Benzylthio)purine	400	10/10				
		200	7/10				
		100	0/10	−1·4/+2·3	3·7	0/1200	0
		50	0/10	+0·1/+2·3	2·2	0/1200	0
		25	0/10	+0·2/+2·3	2·1	5/1200	0
		10	0/10	−0·5/+2·3	1·8	38/1200	3
		5	1/10	+2·5/+3·0	0·5	555/1372	40
		2·5	0/10	+1·6/+3·0	1·4	552/1372	40
		1·3	0/10	+2·7/+3·0	0·3	748/1372	55
26273	6-(Benzylthio)-9-β-D-ribofuranosyl-9H-purine	400	7/10				
		200	1/10	−1·7/+1·9	3·6	17/ 680	3
		100	1/10	−1·0/+4·0	5·0	73/1230	6
		50	1/10	+1·0/+3·0	2·0	86/1827	5
		25	0/10	+1·0/+3·0	2·0	512/1827	28
		12	0/10	+1·0/+4·0	3·0	1243/2448	51
		6	0/10	+6·0/+5·0	−1·0	2553/2703	93

11595	6-(Propylthio)purine	250	7/10				
		125	4/10	−1·5/+1·9	3·4		
		75	1/10	−2·0/+1·0	3·0	33/ 803	4
		40	0/10	0/+5·0	5·0	61/2604	2
		20	0/10	0/+5·0	5·0	95/2604	4
		10	0/10	+3·0/+5·0	2·0	735/2604	28
39044	6-(Propylthio)-9-β-D-ribofuranosyl-9H-purine	400	8/10				
		200	0/10	+0·9/+3·9	3·0	0/1321	0
		100	2/10	+0·9/+3·2	2·3	64/1236	5
		50	0/10	−0·4/+3·2	3·6	168/1236	14
		25	0/10	+0·7/+3·2	2·5	331/1236	27
		12	0/10	+2·1/+5·0	2·9	740/1173	63
		6	2/10	+3·4/+5·0	1·6	1078/1173	92
		3	2/10	+2·8/+5·0	2·2	875/1173	75
19862	6-(Allylthio)purine	250	8/11				
		125	13/20				
		63	1/10	0/+2·0	2·0	22/1553	1
		30	0/10	0/+2·0	2·0	83/1553	5
		15	0/10	0/+2·0	2·0	0/1553	0
		8	1/10	+1·0/+2·0	1·0	0/1553	0
		4	0/10	+2·0/+4·0	2·0	897/1382	65
39367	6-(Allylthio)-9-β-D-ribofuranosyl-9H-purine	400	4/10	−1·5/+2·5	4·0		
		200	1/10	−1·8/+4·5	6·3	35/1707	2
		100	0/10	−0·7/+5·5	6·2	16/1568	1
		50	0/10	+1·3/+3·1	1·8	0/ 943	0
		25	0/10	−0·3/+5·1	5·4	385/2029	19
		12	1/10	+2·9/+5·1	2·2	1550/2029	76
		6	0/10	+2·0/+5·1	3·1	1120/2029	55

Table IV—*continued*

NSC no.[a]	Compound	Dosage mg/kg/day	Mortality	Animal wt. change, g *T/C*	Animal wt. difference, g *C–T*	Av. tumour wts., mg *T/C*	% of control tumour wt.
19866	6-(Cyanomethylthio)purine	250	10/10				
		125	2/10	−2·0/+1·0	3·0	11/1717	1
		62	0/10	+2·0/+4·0	2·0	97/1382	7
		30	0/10	+1·0/+4·0	3·0	0/1582	0
		15	0/10	+1·0/+4·0	3·0	0/1882	0
		8	0/ 9	+1·0/+4·0	3·0	169/1382	12
		4	0/10	+1·0/+5·0	4·0	934/3883	24
		2	0/10	+2·0/+5·0	3·0	1818/3883	47
		1	0/10	+2·0/+3·0	1·0	977/1345	73
39848	6-(Cyanomethylthio)-9-β-D-ribofuranoysl-9H-purine	400	10/10				
		200	0/10	−0·1/+2·8	2·9	0/ 854	0
		100	0/10	+0·6/+1·7	1·1	5/ 684	1
		50	0/10	+1·0/+1·7	0·7	21/ 684	3
		25	0/12	+1·3/+2·0	0·7	109/ 703	16
		6	0/12	+3·1/+2·0	−1·1	855/ 703	122
20914	6-(Acetonylthio)purine	250	9/10				
		125	4/10	−1·0/ 0	1·0	0/ 557	0
		63	1/10	+2·0/+2·0	0	0/ 944	4
		30	0/10	+2·0/+2·0	0	48/ 944	5
		15	0/10	+1·0/+5·0	4·0	433/3883	11
		8	0/10	+2·0/+5·0	3·0	1077/3883	28
		4	0/10	+2·0/+3·0	1·0	1268/1345	94

39045	6-(Acetonylthio)-9-β-D-ribofuranosyl-9H-purine	450	0/10	−0·8/+2·7	3·5	41/1161	4
		200	0/10	−0·6/+3·2	3·8	59/ 884	7
		100	0/10	+1·0/+3·2	2·2	149/ 884	17
		50	0/10	+0·7/+3·2	2·5	256/ 884	29
		25	0/10	+0·7/+3·2	2·5	526/ 884	60
		12	0/10	+3·2/+3·2	0	784/ 884	89
22024	6-(Cyclopentylthio)purine	500	10/10				
		250	0/10	−1·0/+3·0	4·0	0/1612	0
		125	0/10	0/+5·0	5·0	0/1917	0
		63	0/10	−1·0/+4·0	5·0	9/1382	1
		30	0/10	+1·0/+4·0	3·0	0/1382	0
		15	0/10	+1·0/+4·0	3·0	84/1382	6
		8	0/10	+2·0/+4·0	2·0	783/1382	57
40630	6-(Cyclopentylthio)-9-β-D-ribofuranosyl-9H-purine	400	1/ 9	−2·5/+2·8	5·3	6/1098	1
		200	0/10	−1·5/+3·9	5·4	0/1463	0
		100	2/10	−0·2/+4·5	4·7	39/1576	2
		50	1/10	−0·4/+4·5	4·9	68/1576	4
		25	0/10	+0·8/+4·5	3·7	162/1576	10
		12	1/10	+0·2/+1·9	1·7	521/ 838	62
		6	1/10	+1·0/+1·9	0·9	604/ 838	72
		3	1/10	+2·1/+1·9	−0·2	865/ 838	103
20404	6-(Thenylthio)purine	250	9/10				
		125	0/10	−2·0/+2·0	4·0	30/1124	3
		63	0/10	−1·0/+2·0	3·0	0/1213	0
		31	0/10	0/+3·0	3·0	0/1997	0
		8	0/10	+1·0/+2·0	1·0	248/ 837	30
		4	0/10	+1·0/+5·0	4·0	2006/3883	52

Table IV—*continued*

NSC no.[a]	Compound	Dosage mg/kg/day	Mortality	Animal wt. change, g *T/C*	Animal wt. difference, g *C–T*	Av. tumour wts., mg *T/C*	% of control tumour wt.
41847	9-β-D-Ribofuranosyl-6-(2-thenylthio)-9H-purine	200	10/10				
		100	6/10				
		50	1/10	−1·3/+3·2	4·5	22/1018	2
		25	2/10	−0·5/+3·2	3·7	34/1018	3
		12	0/10	+0·1/+1·9	1·8	178/ 680	26
		6	1/10	+0·9/+1·9	1·0	262/ 680	39
		3	3/10	+1·4/+1·9	0·5	446/ 680	66
22416	6-(Cinnamylthio)purine	500	0/10	−3·0/+1·9	4·9	0/ 608	0
		250	1/10	−2·0/ 0	2·0	0/ 882	0
		125	0/10	0/+3·0	3·0	125/1133	11
		63	0/10	0/+3·0	3·0	153/1612	9
		30	1/10	+1·7/+1·9	0·2	371/ 680	55
		15	0/10	+1·0/+3·0	2·0	750/1612	47
39043	6-(Cinnamylthio)-9-β-D-ribofuranosyl-9-H-purine	400	0/10	+1·0/+2·5	1·5	264/ 731	36
		200	0/10	0/+4·1	4·1	408/1871	22
		100	0/10	+1·9/+5·5	3·6	875/ 731	56
		50	0/10	+2·1/+2·5	0·4	395/ 731	54
		25	0/10	+1·6/+2·5	0·9	434/ 731	59

[a] Consecutive Number of the Cancer Chemotherapy National Service Center, U.S.P.H.S., Bethesda, 14, Maryland.

Table V. Comparison of the chemotherapeutic indices of certain *S*-substituted derivatives of 6-mercaptopurine and their ribonucleosides against Adenocarcinoma 755

NSC no.	Compound	MTD[a] μmoles/kg/day	ED_{50}[b] μmoles/kg/day	CI MTD/ED_{50}	CI_R/CI_P
755	Purine-6(1H)-thione[c]	235	4·5	52	
4911	9-β-D-Ribofuranosyl-9H-purine-6(1H)-thione[c]	528	3·4	156	3·0
19862	6-(Allylthio)purine	328	22·4	15	
39367	6-(Allylthio)-9-β-D-ribofuranosyl-9H-purine	608	33·4	18	1·2
20914	6-(Acetonylthio)purine	303	24·0	13	
39045	6-(Acetonylthio)-9-β-D-ribofuranosyl-9H-purine	1324	91·2	15	1·2
22024	6-(Cyclopentylthio)purine	1135	38·6	29	
40630	6-(Cyclopentylthio)-9-β-D-ribofuranosyl-9H-purine	1121	36·5	31	1·1
11595	6-(Propylthio)purine	387	23·2	17	
39044	6-(Propylthio)-9-β-D-ribofuranosyl-9H-purine	582	37·8	15	0·9
11588	6-(Ethylthio)purine	417	17·8	23	
39368	6-(Ethylthio)-9-β-D-ribofuranosyl-9H-purine	617	40·1	15	0·2
29421	6-(Benzylthio)purine	413	10·3	40	
26273	6-(Benzylthio)-9-β-D-ribofuranosyl-9H-purine	535	34·8	15	0·4
20404	6-(2-Thenylthio)purine	503	19·0	27	
41847	9-β-D-Ribofuranosyl-6-(2-thenylthio)-9H-purine	129	12·3	11	0·4
22416	6-(Cinnamylthio)purine	1865	97·0	19	
39043	6-(Cinnamylthio)-9-β-D-ribofuranosyl-9H-purine	>980	19·1	>5	>0·3
19866	6-(Cyanomethylthio)purine	654	9·9	66	
39848	6-(Cyanomethylthio)-9-β-D-ribofuranosyl-9H-purine	620	40·2	15	0·2
20105	6-(Methylthio)purine	603	25·9	23	
49555	6-(Methylthio)-9-β-D-ribofuranosyl-9H-purine	70	70·0	1	0·04

[a] Defined as the maximum dose that causes not more than 20% mortality or a C—T weight difference of no more than 5 g. See data in Table IV.
[b] Defined as the minimum dose that inhibits tumour growth to 50% of untreated controls. See data in Table IV.
[c] See Reference 8.

Table VI. Inhibition of Sarcoma 180 by certain *S*-substituted derivatives of 6-mercaptopurine and their ribonucleosides

NSC no.	Compound	Dosage mg/kg/day	Mortality	Animal wt. change, g *T/C*	Animal wt. difference, g *C−T*	Av. tumour wts., mg *T/C*	% of control tumour wt.
755	Purine-6-(1H)-thiono	100	0/6	−2·2/+0·5	2·7	215/1493	14
		75	0/6	−0·9/+0·5	1·4	514/1493	34
		65	0/6	+0·2/+3·0	2·8	348/ 860	40
		60	0/6	+0·3/+3·0	2·7	267/ 860	31
		55	0/6	+0·4/+3·0	2·6	361/ 860	42
		50	0/6	+0·3/+3·0	2·7	328/ 860	38
		40	0/6	+5·8/+5·5	−0·3	181/ 553	32
		30	0/6	+1·0/+2·4	1·4	528/ 888	59
		20	0/6	+4·7/+5·5	0·8	384/ 553	69
		10	0/6	+6·4/+5·5	−0·9	401/ 553	73
		5	0/6	+5·6/+5·5	−0·1	609/ 553	110
4911	9-β-D-Ribofuranosyl-9H-purine-6-(1H)-thione	200	1/6	+1·0/+0·5	−0·5	367/1493	24
		150	0/6	−0·3/ 0	0·3	117/1033	11
		75	0/6	−1·5/ 0	1·5	159/1033	15
		37	1/6	−0·4/ 0	0·4	360/1033	35
		18	0/6	−1·6/ 0	1·6	623/1033	60
20105	6-(Methylthio)purine	200	1/6	−2·4/ 0	2·4	238/1033	23
		100	1/6	−0·9/ 0	0·9	598/1033	58
		50	2/6	−1·0/ 0	−1	423/1033	41
49555	6-(Methylthio)-9-β-D-ribofuranosyl-9H-purino	75	6/6				
		50	6/6				
		25	1/6	−1·4/ 0	1·4	455/1033	44
		12	0/6	−1·5/ 0	1·5	956/1033	93

11588	6-(Ethylthio)purine	200	3/6	−2·1/−1·2	0·9	305/1157	26
		150	2/6	−5·0/−3·0	2·0	396/ 761	52
		100	0/6	−2·4/−1·2	1·2	577/1157	50
		50	0/6	−2·3/−1·2	1·1	564/1157	49
39368	6-(Ethylthio)-9-β-D-ribofuranosyl-9H-purine	200	1/6	−4·4/−1·4	3·0	221/ 740	30
		100	0/6	−3·5/−1·4	2·1	632/ 846	75
		50	0/6	−2·1/−1·4	0·7	723/ 846	85
		25	0/6	−1·7/−1·4	0·3	739/ 846	87
11595	6-(Propylthio)purine	250	0/6	−1·9/ 0	1·9	293/1033	28
		125	0/6	−1·2/ 0	1·2	461/1033	45
		85	0/6	−1·0/−2·0	−1·0	547/ 931	59
39044	6-(Propylthio)-9-β-D-ribofuranosyl-9H-purine	250	0/6	−1·2/+0·6	0·6	558/1492	37
		125	0/6	−1·2/−1·6	−0·4	551/1328	41
		62	0/6	−2·1/−1·9	0·2	1084/1328	82
		31	0/6	−3·0/−1·6	1·4	1042/1328	72
19862	6-(Allylthio)purine	150	2/6	−2·2/ 0	2·2	406/1033	39
		100	0/6	−2·3/ 0	2·3	707/1033	68
		50	0/6	−0·7/ 0	0·7	741/1033	72
39367	6-(Allylthio)-9-β-D-ribofuranosyl-9H-purine	75	0/6	+0·1/+0·6	0·5	1448/1492	97
22024	6-(Cyclopentylthio)purine	500	5/6				
		250	1/6	−1·7/−1·2	0·5	301/1157	21
		125	0/6	−1·1/−1·2	0·1	704/1157	61
40630	6-(Cyclopentylthio)-9-β-D-ribofuranosyl-9H-purine	400	2/6	−2·7/−1·2	1·5	243/1157	21
		200	1/6	−1·9/−1·6	0·3	568/1328	43
		100	0/6	−1·3/−1·6	−0·3	744/1049	74
		50	1/6	−0·7/−1·6	0·9	641/1049	61
		25	1/6	−1·9/−1·6	0·3	719/1049	69

Table VI—*continued*

NSC no.	Compound	Dosage mg/kg/day	Mortality	Animal wt. change, g *T/C*	Animal wt. difference, g *C−T*	Av. tumour wts., mg *T/C*	% of control tumour wt.
29421	6-(Benzylthio)purine	200	0/6	−3·2/−1·2	2·0	813/1157	70
		100	1/6	−1·7/−1·2	0·5	692/1157	60
		50	0/6	−2·5/−1·2	1·3	1035/1157	89
26273	6-(Benzylthio)-9-β-D-ribofuranosyl-9H-purine	200	2/6	−2·7/−1·2	1·5	469/1157	41
		100	0/6	−2·6/−1·2	1·4	832/1157	72
		50	1/6	−1·6/−1·2	0·4	1280/1157	111
20914	6-(Acetonylthio)purine	150	1/6	−1·8/−1·2	0·6	738/1157	64
		100	0/6	−1·5/−1·2	0·3	729/1157	63
		50	1/6	−2·0/−1·2	0·8	922/1157	80
39045	6-(Acetonylthio)-9-β-D-ribofuranosyl-9H-purine	500	0/6	−1·6/−1·2	0·4	586/1435	39
		250	0/6	−0·8/−0·2	0·6	1151/1534	75
		125	1/6	−1·8/−0·2	1·6	1112/1534	72
		62	0/6	−0·8/−0·2	0·6	1193/1534	78
22416	6-(Cinnamylthio)purine	500	0/6	−3·4/−1·2	2·2	508/1157	44
		250	0/6	−1·0/−1·2	−0·2	554/1157	48
39043	6-(Cinnamylthio)-9-β-D-ribofuranosyl-9H-purine	500	0/6	−1·6/+0·1	1·7	528/1029	51
		150	0/6	−0·7/−0·8	−0·1	968/1293	75
19866	6-(Cyanomethylthio)purine	175	1/6	−0·7/ 0	0·7	584/1033	57
		87	0/6	−0·7/ 0	0·7	661/1033	64
		40	0/6	−1·5/ 0	1·5	888/1033	86
39848	6-(Cyanomethylthio)-9-β-D-ribofuranosyl-9H-purine	150	0/6	−2·4/−0·3	2·1	905/1456	62
41847	9-β-D-Ribofuranosyl-6-(2-thenylthio)-9H-purine	100	0/6	−3·5/−1·4	−2·1	169/ 700	23
		50	2/6	−2·5/−1·4	0·9	468/ 846	55
		25	1/6	−1·1/−1·4	−0·3	358/ 846	42
		12·5	0/6	−1·8/−1·5	0·3	837/ 846	99

The effect of ribonucleosidation of this series of purines on their activity against Carcinoma 755 is then, with the one exception noted, not beneficial.

Sarcoma 180 and Leukemia L1210. Because it does not necessarily follow that the results of screening these compounds against Sarcoma 180 and Leukemia L1210 would show the same pattern observed with Carcinoma 755, these two test systems were also used in our evaluation and some variation in results was observed.

6-Mercaptopurine is less effective against Sarcoma 180 than it is against Carcinoma 755, and its derivatives were also found to be less effective than they are against Carcinoma 755. In fact, the derivatives, with the exception of 6-mercaptopurine ribonucleoside, were all less effective than 6-mercaptopurine itself. 6-Mercaptopurine ribonucleoside, in these tests, inhibited Sarcoma 180 to at least the same extent as 6-mercaptopurine.

6-Mercaptopurine and its ribonucleoside seem equally effective against Leukemia L1210, causing a significant increase in life-span of the leukemic mice. None of the other compounds tested caused any greater increase in life-span when employed under standardized conditions and, indeed, only one other ribonucleoside showed 'plus' activity.

Table VI presents the Sarcoma 180 screening data and Table VII the Leukemia L1210 data. Three of the purines and the ribonucleosides of these same three purines caused a marked inhibition of Sarcoma 180, and three of the purines and two of the ribonucleosides produced a significant increase in life-span of the leukemic mice. A summary of these comparisons is given in Table VIII in which the compounds are listed in decreasing order of their chemotherapeutic indices against Carcinoma 755.

III. Conclusions

This study of closely related derivatives of 6-mercaptopurine shows that no increase in anticancer activity is obtained from the ribonucleosidation of these purines. Only in the case of the parent compound, 6-mercaptopurine, was an increase observed and in that case only against Carcinoma 755. A wide but not correlatable variation in host toxicity of the ribonucleosides was noticed.

Table VII. Effect of certain *S*-substituted derivatives of 6-mercaptopurine and their ribonucleosides on the life-span of mice with Leukemia L1210

NSC no.	Compound	Dosage mg/kg/day	Animal wt. change, g *T/C*	Survivors on day 5	Mean survival time, *T/C* in days	% Increase in life-span
755	Purine-6(1H)thione	50	−1·1/+1·5	6/ 6	10·7/ 9·9	8
		40	+1 /+2	10/10	14·9/10·1	48
		20	+1 /+2	10/10	15·8/10·1	59
		10	0 /+1·5	6/ 6	14·5/ 9·9	46
		5	+1·3/+1·5	6/ 6	11·8/ 9·9	19
4911	9-β-D-Ribofuranosyl-9H-purine-6(1H)-thione	150	+0·1/+1·5	6/ 6	14·5/ 9·9	46
		125	+0·4/+0·8	6/ 6	14·0/ 9·1	53
		100	+0·5/+0·8	6/ 6	13·0/ 9·1	42
		75	+1·2/+1·5	6/ 6	13·2/ 9·9	33
		50	+1·0/+1·5	6/ 6	13·2/ 9·9	33
		34	+1 /+2	10/10	11·9/10·1	18
20105	6-(Methylthio)purine	300		0/ 6		
		200	−0·8/+0·5	5/ 6	12·2/10·3	18
		100	−0·8/+0·5	6/ 6	10·3/10·3	0
49555	6-(Methylthio)-9-β-D-ribofuranosyl-9H-purine	50	−1·8/+1·0	6/ 6	8·5/ 9·3	—
		30	−1·5/+1·5	6/ 6	9·6/ 9·9	—
		20	−0·2/+1·5	5/ 6	12·6/ 9·9	27
		10	+1·0/+1·5	5/ 6	9·8/ 9·9	—
11588	6-(Ethylthio)purine	500		0/ 6		
		250	−1·0/+0·5	6/ 6	10·2/ 9·3	10
		175	−1·5/+0·5	6/ 6	10·6/ 9·3	14
		100	+1 /+1	6/ 6	10·7/ 9·9	8
		50	+0·9/−1·1	6/ 6	11·6/10·3	14
39368	6-(Ethylthio)-9-β-D-ribofuranosyl-9H-purine	200	−1·2/+0·5	6/ 6	12·3/10·2	21

11595	6-(Propylthio)purine	500		0/ 6		
		375		1/ 6		
		250	0 /+1	5/ 6	10·2/ 9·9	3
		125	−1·3/−0·4	5/ 6	10·0/ 9·3	8
		85	−1 /+1	6/ 6	9·5/ 8·2	16
19862	6-(Allylthio)purine	200	−0·4/+0·5	4/ 6	10·3/10·3	0
		150	−1·1/+0·5	5/ 6	11·4/10·3	11
		100	−0·7/+0·5	6/ 6	10·3/10·3	0
		75	−0·5/+0·5	6/ 6	9·0/10·3	—
22024	6-(Cyclopentylthio)purine	500	−0·7/+0·5	6/ 6	7·5/10·3	—
		375	−1·2/+0·5	6/ 6	8·2/10·3	—
		250	+1 /+1	6/ 6	13·2/ 9·9	33
20914	6-(Acetonylthio)purine	150	−0·7/−0·3	6/ 6	13·7/ 9·0	52
		100	−1·0/−0·3	6/ 6	11·8/ 9·0	31
		50	−0·2/−0·3	6/ 6	9·8/ 9·0	9
39045	6-(Acetonylthio)-9-β-D-ribofuranosyl-9H-purine	400	−1·2/−0·3	6/ 6	13·3/ 9·0	48
29421	6-(Benzylthio)purine	300	−1·7/−0·7	6/ 6	12·3/ 9·6	28
		200	−2·6/−0·7	5/ 6	12·2/ 9·6	27
		100	−0·9/−0·7	6/ 6	11·5/ 9·6	20
		50	−0·3/−0·4	6/ 6	10·3/ 9·3	11
		25	−0·3/−0·4	6/ 6	10·2/ 9·3	10
26273	6-(Benzylthio)-9-β-D-ribofuranosyl-9H-purine	200	−0·3/+0·5	5/ 6	12·0/10·2	18
		100	0 /+0·5	6/ 6	12·3/10·2	21
		50	+1·2/+0·5	6/ 6	9·3/10·2	—
22416	6-(Cinnamylthio)purine	500	−1·9/−0·7	6/ 6	14·0/ 9·6	46
		375	−1·3/−0·7	6/ 6	15·5/ 9·6	59
		250	−1·1/−0·7	6/ 6	12·3/ 9·6	28
39043	6-(Cinnamylthio)-9-β-D-ribofuranosyl-9H-purine	500	−0·8/−0·3	6/ 6	10·2/ 9·0	13
		250	−0·4/−0·4	6/ 6	10·0/ 9·0	11

Table VIII. Summary of the anticancer activity of some *S*-substituted derivatives of 6-mercaptopurine and their ribonucleosides

NSC no.	Compound	Ca755 CI	S180[a] Rating	L1210[b] Rating
4911	9-β-D-Ribofuranosyl-9H-purine-6(1H)-thione	156	+	+
19866	6-(Cyanomethylthio)purine	66	−	...
755	Purine-6(1H)-thione	52	±	+
29421	6-(Benzylthio)purine	40	−	±
40630	6-(Cyclopentylthio)-9-β-D-ribofuranosyl-9H-purine	31	+	...
22024	6-(Cyclopenthylthio)purine	29	+	...
41847	6-(2-Thenylthio)purine	27	+	...
20105	6-(Methylthio)purine	23	+	−
11588	6-(Ethylthio)purine	23	±	−
22416	6-(Cinnamylthio)purine	19	±	+
39367	6-(Allylthio)-9-β-D-ribofuranosyl-9H-purine	18	...	...
11595	6-(Propylthio)purine	17	±	...
19862	6-(Allylthio)purine	15	±	...
39044	6-(Propylthio)-9-β-D-ribofuranosyl-9H-purine	15	±	...
39045	6-(Acetonylthio)-9-β-D-ribofuranosyl-9H-purine	15	±	±
39368	6-(Ethylthio)-9-β-D-ribofuranosyl-9H-purine	15	±	±
26273	6-(Benzylthio)-9-β-D-ribofuranosyl-9H-purine	15	±	±
39848	6-(Cyanomethylthio)-9-β-D-ribofuranosyl-9H-purine	15	−	...
20914	6-(Acetonylthio)purine	13	−	+
41847	9-β-D-Ribofuranosyl-6-(2-thenylthio)-9H-purine	11	+	...
39043	6-(Cinnamylthio)-9-β-D-ribofuranosyl-9H-purine	>5	−	−
49555	6-(Methylthio)-9-β-D-ribofuranosyl-9H-purine	1	±	±

[a] Degree of activity: − = >50% untreated control tumour weight; ± = 50–26% of untreated control tumour weight; + = 25–11% of untreated control tumour weight.

[b] Degree of activity: − = >20% increase in life-span of untreated control; ± = 20–50% increase in life-span of untreated control; + = 51–150% increase in life-span of untreated control.

Experimental

6-(Propylthio)-9-β-D-ribofuranosyl-9H-purine monohydrate (Method A). To a stirred mixture of 9-β-D-ribofuranosyl-9H-purine-6(1H)-thione (1·00 g, 3·52 mmoles) and potassium carbonate (511 mg, 3·70 mmoles) in dimethylformamide (7 ml) was added 1-bromopropane (455 mg, 0·34 ml, 3·70 mmoles). When the exothermic reaction had ceased, the mixture was heated at 40–50° for 1 h. The mixture was then dissolved in 20 ml of water, and the solution, after being neutralized with 6 N hydrochloric acid, was evaporated to dryness under reduced pressure. The residue was extracted with dimethylformamide (3 × 5 ml), and the extract evaporated to dryness under reduced pressure at 60°, care being taken to remove all of the solvent. The residue was dissolved in 50 ml of hot water, and the solution was treated with Norit, filtered and cooled. The white gel that formed was collected and dried at 80° for 24 h *in vacuo* over phosphorus pentoxide; yield 667 mg (55 per cent) of analytically and chromatographically pure ribonucleoside monohydrate, m.p. indefinite with softening from 74° (Kofler Heizbank). An additional 343 mg (about 80 per cent pure by ultraviolet spectral analysis) was obtained as a second crop when the filtrate was evaporated under nitrogen to one-half volume; total yield 80 per cent.

9-β-D-Ribofuranosyl-6-(2-thenylthio)-9H-purine hemihydrate (Method A). 2-Chloromethylthiophene (245 mg, 1·85 mmoles) was added dropwise to a stirred mixture of 9-β-D-ribofuranosyl-9H-purine-6(1H)-thione (500 mg, 1·76 mmoles), anhydrous potassium carbonate (256 mg, 1·85 mmoles), and dimethylformamide (5 ml). After the exothermic reaction had ceased, the mixture was stirred and heated at 40–50° for 1 h. The mixture was then poured into 20 ml of water, and the pH of the resulting solution was adjusted from 9 to 7 with 6N hydrochloric acid. The crude ribonucleoside precipitated as a gel when the solution was cooled. The mixture was evaporated to dryness under reduced pressure at 60°, the residue triturated in 5 ml of cold water, and the remaining white solid collected and dried for 40 h *in vacuo* over phosphorus pentoxide at room temperature; yield 673 mg (shown by ultraviolet absorption spectra to be about 87 per cent pure). Two recrystallizations of a 550-mg sample of the crude product from methyl

alcohol (Norit) gave 350 mg (corresponding to a 62 per cent yield) of analytically and chromatographically pure 9-β-D-ribofuranosyl-6-(2-thenylthio)-9H-purine hemihydrate (dried *in vacuo* over phosphorus pentoxide at room temperature for 16 h and then at 60° for 8 h), m.p. indefinite, with softening from 97° (Kofler Heizbank).

From a larger run (10·6 mmoles of the thione) the yield of pure product was 71 per cent.

6-(Ethylthio)-9-β-D-ribofuranosyl-9H-purine two-thirds hydrate (Method A). To a stirred mixture of 9-β-D-ribofuranosyl-9H-purine-6(1H)-thione (8·20 g, 28·9 mmoles) and anhydrous potassium carbonate (4·20 g, 30·4 mmoles) in dimethylformamide (55 ml) was added dropwise bromoethane (3·32 g, 2·34 ml, 30·4 mmoles). The mixture was then heated at 45–50° for 1 h with stirring and poured into 165 ml of water. The pH of the resulting solution was adjusted from 9 to 7 with 6 N hydrochloric acid, and the solution evaporated to dryness under reduced pressure. The residue was extracted with warm (60°) dimethylformamide (4 × 24 ml), and the extract evaporated to dryness at about 60° under reduced pressure. The residue was then extracted with 50 ml of hot ethyl alcohol, a small amount of insoluble material being removed by filtration. Because crystallization of the ribonucleoside did not occur during prolonged refrigeration of the filtrate, the solvent was removed by evaporation *in vacuo*, and the residue was similarly treated with isopropyl alcohol. A small amount of hot isopropyl alcohol-insoluble material was removed, and the filtrate when cooled deposited a few crystals, which were collected and found to be impure ribonucleoside by paper chromatography. The filtrate was then evaporated to dryness *in vacuo*, and the residue dissolved in 20 ml of warm methyl alcohol. The methanolic solution was drawn by suction through a capillary attached to a sintered glass filter into 1·5 l. of ethyl ether. The resulting white precipitate was collected and dried *in vacuo* over phosphorus pentoxide for 7 h at 80°; yield 4·86 g (54 per cent, as the two-thirds hydrate).

6-(Cyanomethylthio)-9-β-D-ribofuranosyl-9H-purine monohydrate (Method B). To a stirred solution of 9-β-D-ribofuranosyl-9H-purine-6(1H)-thione (6·00 g, 21·1 mmoles) in approximately 0·4 N aqueous sodium hydroxide solution (54 ml) was added chloroaceto-

nitrile (1·75 g, 21·1 mmoles). When the exothermic reaction had ceased, more chloroacetonitrile (0·1 g) was added. The mixture was then stirred at room temperature for 30 min. The resulting solution, adjusted to pH 7 with 6 N hydrochloric acid, was chilled for about 5 h. The reddish-brown gel that formed was collected and dried *in vacuo* over phosphorus pentoxide first at room temperature for 1 h, then at 80° for 2 h. Recrystallization of the crude light-tan solid from isopropyl alcohol gave 4·45 g (62 per cent) of 6-(cyanomethylthio)-9-β-D-ribofuranosyl-9H-purine monohydrate (dried *in vacuo* over phosphorus pentoxide for 1 h at room temperature and then 4 h at 80°) as an off-white powder.

Screening procedures. The animal assay procedures were performed according to the specifications established by the Cancer Chemotherapy National Service Center. Since these specifications have been set forth in detail,[10] no description of the procedures will be included here.

Summary. The ribonucleosides of ten *S*-substituted derivatives of 6-mercaptopurine have been prepared by the alkylation of 6-mercaptopurine ribonucleoside.

The activity of these ribonucleosides against Carcinoma 755, Sarcoma 180, and Leukemia L1210 was compared with the activity of the parent purines against these same neoplasms.

None of the ribonucleosides of these derivatives of 6-mercaptopurine showed any increase in anticancer activity over the parent.

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