Synthesis and Antitumor Activity of 2-Amino-1-methylpurine-6-thione[1]

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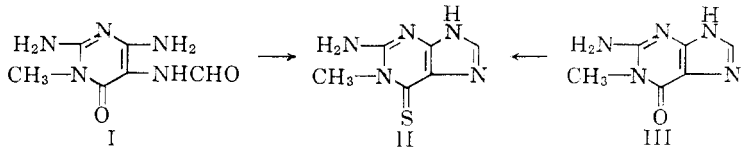
The synthesis of 2-amino-1-methylpurine-6-thione (II) has been achieved in a single step from 2,4-diamino-5-formamido-1-methyl-6-pyrimidone (I) and phosphorus pentasulfide. II is apparently as active as 6-thioguanine against Adenocarcinoma 755 and possesses a much superior therapeutic index. II is also significantly active against Leukemia L-1210 and Dunning Leukemia in experimental mice.

The antitumor activity of various 9-alkyl-2-amino-6-purinethiols[2] and certain 6-alkylthio-2-aminopurines[3] suggested that other alkyl derivatives of 2-amino-6-purinethiol might also exhibit tumor-inhibiting properties. The synthesis of 2-amino-1-methylpurine-6-thione (II) was accordingly investigated. The recently described synthesis[3] of the parent compound, 2-amino-6-purinethiol, by combined ring closure and thiation of the requisite pyrimidine intermediate, 2,4-diamino-5-formamido-6-hydroxypyrimidine, suggested that the desired compound might be obtained in a similar manner from 2,4-diamino-5-formamido-1-methyl-6-pyrimidone (I). This indeed proved to be the case. Compound II was prepared in reasonable yield by treating I with phosphorus pentasulfide in boiling pyridine. Verification of the reaction product was obtained by the preparation of II from 1-methylguanine.



I II III

(1) Supported by Contract SA-43-ph-1928 with the Cancer Chemotherapy National Service Center of the National Cancer Institute of the National Institutes of Health.

(2) C. W. Noell and R. K. Robins, *J. Med. Pharm. Chem.*, **5**, 558 (1962).

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

TABLE I

ACTION OF 2-AMINO-1-METHYLPURINE-6-THIONE (NSC 43405) AGAINST ADENOCARCINOMA 755

Dose, mg./kg.	Survivors	Wt. change (test/control)	Tumor wt. (test/control)	*T/C*
50	0/10	toxic		
25	1/10	−5.2/−0.2	0/749	0.00
12.5	6/10	−4.2/−0.2	0/749	0.00
7.5	7/10	−2.4/2.6	29/1326	0.02
7	9/10	−1.0/2.8	53/1094	0.04
6.25	7/10	−3.4/−0.2	0/1059	0.00
6	10/10	−0.5/2.8	58/1094	0.05
5	10/10	−0.5/2.8	50/1094	0.04
5.00	8/10	−0.4/2.6	58/1326	0.04
3.125	8/10	−2.5/−0.2	38/1059	0.03
2.50	10/10	0.2/2.6	61/1326	0.04
2.00	7/10	−3.8/2.6	50/1326	0.03
1.563	9/10	−2.9/−0.2	75/1059	0.07
1.250	9/10	0.9/2.6	247/1326	0.18
1.00	9/10	−3.2/2.6	40/1326	0.03
.781	10/10	−1.6/−0.2	106/1059	0.10
.39	8/10	−1.9/−0.9	356/1042	0.34
.310	9/10	2.5/2.6	434/1326	0.32
.195	10/10	−1.2/−0.9	390/1042	0.37
.150	10/10	2.9/2.6	1149/1326	0.86
.097	9/10	−1.0/−0.9	761/1042	0.73

Discussion of Antitumor Testing

Sartorelli and LePage[4] report that in Ehrlich ascites carcinoma, 6-thioguanine produces at least three different metabolic blocks in the area of purine biosynthesis. This would well account for the high toxicity exhibited by this compound. The possibility that certain derivatives of 6-thioguanine might be more selective and therefore less toxic has already been realized in the superior antitumor activity of such derivatives as 2-amino-9-*n*-propyl-6-purinethiol[2] against Adenocarcinoma 755. Inspection of Table I reveals that 2-amino-1-methylpurine-6-thione exhibits a therapeutic index[2] of approximately 64 as compared to 4 for 6-thioguanine against the same tumor.[5] This would appear to be a rather significant difference in considering an agent for clinical trial. The 1-methyl derivative (II) appears to be as active against Adenocarcinoma 755 per given dosage in the mouse as 6-thioguanine on the basis of available data.

LePage and Jones[6] have shown that 2-amino-1-methylpurine-6-

(4) A. C. Sartorelli and G. A. LePage, *Cancer Research*, **18**, 1329 (1958).

(5) H. E. Skipper, J. A. Montgomery, J. R. Thomson, and F. M. Schabel, Jr., *Cancer Research*, **19**, 425 (1959).

(6) G. A. LePage and M. Jones, *Cancer Research*, **21**, 642 (1961).

TABLE II

ACTION OF 2-AMINO-1-METHYLPURINE-6-THIONE AGAINST LEUKEMIA L-1210

Dose, mg./kg.	Survivors	Wt. change (test/control)	Survival (days) (test/control)	T/C
9	6/6	−1.8/0.6	12.2/9.3	1.31
6	6/6	−1.4/0.6	11.5/9.3	1.23
4	6/6	−1.1/0.6	14.0/9.3	1.50
2.7	5/6	−1.6/0.6	14.8/9.3	1.59

TABLE III

ACTION OF 2-AMINO-1-METHYLPURINE-6-THIONE AGAINST DUNNING LEUKEMIA

Dose (Mg./kg.)	Survivors	Wt. change (test/control)	Survival (days) (test/control)	T/C	Toxic deaths
50	0/6	+42.0	7.0/13.0	0.53	6/6
10	6/6	9.0/42.0	21.5/13.0	1.65	0/6

thione (II) is not converted to the nucleotide form *in vivo* in various ascites tumor cells. This strongly suggests that II and 6-thioguanine may not exert their antitumor effect by the same mechanism since the antitumor properties of 6-thioguanine can be correlated with its incorporation into the nucleic acid (DNA) as recently shown by LePage and Jones.[7]

Tables II and III show that 2-amino-1-methylpurine-6-thione is active against Leukemia L-1210 and the Dunning Leukemia. Further testing would indeed appear warranted to determine the antitumor spectrum and usefulness of this compound.

The testing procedures employed in obtaining the data in Tables I, II, and III have been adequately described previously.[7a]

Experimental

2-Amino-1-methylpurine-6-thione. Method 1.—Fifty grams of 2,4-diamino-5-formamido-1-methyl-6-pyrimidone[8] and 150 g. of phosphorus pentasulfide were added, with stirring, to 1800 ml. of pyridine. This mixture was stirred and refluxed for 30 hr. and then filtered. The precipitate was placed in 1000 ml. of water and allowed to stand 1 hr. The pyridine filtrate was reduced to a gummy residue *in vacuo* using a water bath as the source of heat. The resulting residue was added to the liter of aqueous mixture mentioned above and allowed to stand 3 hr. at room temperature. Finally, the mixture was placed on a steam bath for 3 hr. and then allowed to cool. The precipitate was filtered, washed with water, and dissolved in 1000 ml. of boiling 2% potassium hydroxide solution. The solution was treated with charcoal and filtered, and the hot filtrate was acidified with glacial acetic acid and allowed to cool to room temperature. The resulting precipitate was filtered and recrystallized from 2 l. of water. One more recrys-

(7) G. A. LePage and M. Jones, *Cancer Research.*, **21**, 1590 (1961).
(7a) J. Leiter, A. R. Bourke, S. A. Schepartz, and I. Wodinsky, *ibid.*, **20**, 734 (1960).
(8) F. G. Mann and J. W. G. Porter, *J. Chem. Soc.*, 751 (1945).

tallization from water gave 9 g. of pure product which was dried at 95° for analysis, m.p. 340–342°.

Anal. Calcd. for $C_6H_7N_5S$: C, 39.7; H, 3.9; N, 38.7. Found: C, 39.6; H, 3.9; N, 38.8; ultraviolet spectral data: pH 1, λ_{max} 256, 345 mμ, ϵ 9,400, 21,000; pH 11, λ_{max} 234, 336 mμ, ϵ 16,800, 21,000.

R_f values	System
0.29	5% aqueous Na_2HPO_4 saturated with isoamyl alcohol
.32	5% aqueous NH_4HCO_3
.62	MeOH-H_2O (7:3)

Method 2.—Two grams of 1-methylguanine[9] was placed in 70 ml. of pyridine, and then 6.0 g. of phosphorus pentasulfide was added with stirring. The mixture was refluxed for 6 hr. and reduced to a gummy residue *in vacuo* using a water bath as the source of heat. The residue was covered with 70 ml. of water, heated on a steam bath for 3 hr., and then allowed to cool. The precipitate was filtered and dissolved in 150 ml. of boiling 2% potassium hydroxide solution. The solution was treated with Norite and filtered and the filtrate acidified with glacial acetic acid. The mixture was allowed to cool to room temperature, and the precipitate was filtered, washed with water, and recrystallized twice from water to give 400 mg. of product. The product was identical with that prepared by method 1 as shown by ultraviolet and infrared spectral data and paper chromatography in three different solvent systems. A mixture melting point with the product of method 1 showed no depression.

(9) W. Traube and H. W. Dudley, *Ber.*, **46**, 3839 (1913).