TABLE II

RELATIVE PROGESTATIONAL ACTIVITY (CLAUBERG ASSAY)

Progesterone	S.c.	Oral	Corresponding Δ4-3-ketone S.c.	Oral
Progesterone	1			
17α-Ethynyl-19-nortestosterone		1		
Ia	2	0.4		0.1[a]
Ib	2	0.4		
Ic	10	10	20[a]	10[a]
Ie	1	0.4		1[b]
IIa	1	0.05	1–2[c]	0.15[c]
IIc	1	0.4	1–2[c]	1–2[c]
IIIc	10	<10	4[a,d]	10[a,d]

[a] Determined in this laboratory. [b] A. Bowers, L. C. Ibanez, and H. J. Ringold, *J. Am. Chem. Soc.*, **81**, 5991 (1959). [c] J. Fried, E. F. Sabo, P. Grabowich, L. J. Lerner, W. B. Kessler, D. M. Brennan, and A. Borman, *Chem. Ind.* (London), 465 (1961). [d] R. Deghenghi and R. Gaudry, *J. Am. Chem. Soc.*, **83**, 4668 (1961).

ice-cooled solution of 39 mg. (1.04 mmoles) of sodium borohydride in 5 ml. of dry methanol. After stirring for 1.5 hr., 0.1 ml. of acetic acid was added and the solution was evaporated to dryness *in vacuo*. Extraction with chloroform gave a solid product which was identified as almost pure 3β-hydroxy-16α, 17α-isopropylidenedioxy-4-pregnen-20-one (IIa) by infrared spectral and thin layer chromatographic comparison with the compound obtained by reduction of the same starting material with lithium aluminum tri-*t*-butoxyhydride.

When 17-acetoxyprogesterone was reduced with sodium borohydride under the same conditions, the crude product consisted of the corresponding 3β-hydroxy compound (Ia) contaminated with a small amount of starting material.

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A Nitrogen Mustard Derivative of 6-Mercaptopurine[1]

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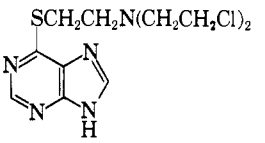
The carcinostatic effects of a variety of nitrogen mustards[2a] and of 6-mercaptopurine[2b] (6-MP) made it of interest to study a combination of the two in which the purine was the carrier for the alkylating group. Such a derivative, 6-[[2-[bis(2-chloroethyl)amino]-ethyl]thio]purine (I) was prepared by the alkylation

TABLE I

EFFECT OF THE NITROGEN MUSTARD DERIVATIVE OF 6-MERCAPTOPURINE (I) AND OF 6-MP ON MOUSE, RAT, AND HAMSTER TUMORS[a]

Tumor	I 2.5 mg./kg./day	HN2 0.5 mg./kg./day	6MP 30 mg./kg./day
Ehrlich carcinoma (ascitic)	+++	+++	++
Sarcoma 180 (ascitic)	+++	+++	+++
Mecca lymphosarcoma	+	±	−
Friend virus leukemia	+	+	++
Ehrlich carcinoma	±	±	+
Carcinoma 1025	±	+	++
Ridgway osteogenic sarcoma	±	−	++
Wagner osteogenic sarcoma	±	−	+
Glioma 26	±	−	++
Sarcoma 180	−	±	+++
Adenocarcinoma E 0771	−	±	++
Lewis lung carcinoma	−	−	−
Harding–Passey melanoma	−	−	−
Flexner–Jobling rat carcinoma	+++	+	++
Jensen rat sarcoma	++	+	+
Walker carcinosarcoma 256	+	+	±
Crabb hamster sarcoma	±	+	+

[a] −, no effect; ±, slight inhibition; +, moderate inhibition; ++, marked inhibition; +++, complete inhibition or regressions of tumors.



I

procedure of Johnston, *et al.*,[3] from tris(2-chloroethyl)-amine and 6-mercaptopurine in N,N-dimethylformamide containing triethylamine. It was isolated as a dihydrochloride and half of the halogen present was found to be ionic. Its ultraviolet absorption spectrum corresponded to that expected of a 6-alkylthiol derivative. The possibility of ring alkylation is eliminated since only hypoxanthine was formed by oxidative hydrolysis in dilute hydrogen peroxide at room temperature.

Compound I was tested at, or near, the maximum tolerated dose for carcinostatic activities against seventeen tumors. Similar tests with methyl bis(2-chloroethyl)amine (HN2) and with 6-MP are recorded in Table I.

The maximum tolerated dose of the compound was comparable to that of HN2 and was approximately one-tenth that of 6-MP. From the results (Table I) on the spectrum of tumors, it is apparent that the antitumor activity of I paralleled that of HN2 more closely than it did that of 6-MP. It was definitely less effective than 6-MP[4] on the mouse tumors.

Experimental

6-[[2-[Bis(2-chloroethyl)amino]ethyl]thio]purine (I) Dihydrochloride.—To a solution of 0.5 g. of 6-mercaptopurine in 15 ml. of dimethylformamide, 2.3 g. of tris(2-chloroethyl)amine hydro-

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(2) (a) E. Hirschberg, *Cancer Res.*, **23**, 548 (1963); (b) *ibid.*, **23**, 593 (1963).

(3) T. P. Johnston, L. B. Holm, and J. A. Montgomery, *J. Am. Chem. Soc.*, **80**, 6265 (1958).

(4) K. Sugiura, in "Progress in Experimental Tumor Research," Vol. II, F. Homburger, Ed., S. Karger, New York, N. Y., 1961, p. 332.

chloride[5] and 0.5 ml. of triethylamine were added. The mixture was heated at 80–90° for 12 hr., an additional 0.2 ml. of triethylamine was added, and the heating was continued for another 12 hr. The course of the reaction could be followed conveniently by the decrease of ultraviolet absorption at 320 mμ and the increase at 285 mμ. The reaction mixture was cooled, the precipitate of triethylamine hydrochloride was collected, and the solution was concentrated at 20 mm. and 80° to about half its initial volume, then poured into 250 ml. of acetone. The clear, pale-yellow solution was acidified with a few ml. of saturated ethanolic hydrogen chloride. A precipitate which formed promptly was discarded. The solution was left overnight at 0°. The product separated as white rosettes that were collected and washed with boiling acetone yielding 0.4 g. (32%): m.p. 220° dec.; λ_{max} 287 (ε 13,900) at pH 1, 286 (14,700) at pH 5, and 292 mμ (13,600) at pH 12.

Anal.[6] Calcd. for $C_{11}H_{13}Cl_2N_8S \cdot 2HCl$: C, 33.60; H, 4.36; Cl, 36.07; Cl^-, 18.03; N, 17.81; S, 8.15. Found: C, 33.40; H, 4.50; Cl, 35.94; Cl^-, 18.20; N, 17.59; S, 8.06.

About 10 mg. was dissolved in 0.2 ml. of water, about 0.1 ml. of 30% hydrogen peroxide was added, and the solution was left 2 days at room temperature. In a duplicate experiment a drop of ammonium hydroxide was also added. Paper chromatography of the reaction mixtures showed the one product to be hypoxanthine, which was identified by R_f values (0.50 in butanol–H_2O–acetic acid, 4:1:1, and 0.53 in 1% $(NH_4)_2SO_4$–2-propanol, 1:2), and from the spectra of eluates (λ_{max} 248 mμ in 0.1 *N* HCl, 250 in water, and 262 in 0.1 *N* NaOH).

Chemotherapy Assays.—With solid tumors,[7] subcutaneous implantations of tumor fragments were done by trocar. The progress of the tumors in the animals was recorded graphically by measuring the tumors at weekly intervals for 3 weeks after transplantation.

For ascites tumor growth,[8] intrapertioneal injection of 0.1 ml. of the ascitic fluid containing 1–2 million cancer cells was made into each mouse in the inguinal region. Treatment was started 24 hr. later as with the solid tumors, and was evaluated by measurement of the fluid volume after 10 days.

With the Friend virus leukemia,[9] intraperitoneal injections of 0.2 ml. of a 10% saline homogenate of leukemic spleens were given in the inguinal region of each mouse. The effect of the compounds upon leukemic mice was evaluated by comparison of the spleen weights in the treated and untreated infected mice after 3 weeks.

Intraperitoneal injection of 6-MP mustard at or near maximum tolerated doses was begun 24 hr. after inoculation with tumor material and was continued once daily for 7 days. The animals were maintained on a standard pellet diet (Purina Laboratory Chow) and water *ad libitum*. Saline solution of 6-MP mustard was prepared fresh daily; the usual injection volume was 0.5 ml. once a day.

(5) K. Ward, *J. Am. Chem. Soc.*, **57**, 914 (1935).

(6) Galbraith Laboratories, Inc., Knoxville, Tennessee. Ionic chlorine was determined by coulometric titration.

(7) K. Sugiura and C. C. Stock, *Cancer*, **5**, 382 (1952).

(8) K. Sugiura, *Ann. N. Y. Acad. Sci.*, **63**, 962 (1956).

(9) K. Sugiura, *Gann*, **50**, 251 (1959).

Hydroxy-2-thiopyrimidine-5-carboxaldehyde Derivatives in Cancer Chemotherapy

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In continuing a study of pyrimidine aldehyde derivatives[1] we have prepared a series of substituted hydrazone and anil derivatives of 4,6-dihydroxy-2-thiopyrimidine-5-carboxaldehyde (Table I) and some substituted hydrazone derivatives of 4-hydroxy-2-thio-, 4-hydroxy-6-methyl-2-thio-, and 4-hydroxy-6-propyl-2-thiopyrimidine-5-carboxaldehydes (Table II). Screening data[4] for these compounds have shown no significant or reproducible antitumor effects in Sarcoma 180 tests.

(1) R. H. Wiley and Y. Yamamoto, *J. Org. Chem.*, **25**, 1906 (1960).

TABLE I

DERIVATIVES OF 4,6-DIHYDROXY-2-THIOPYRIMIDINE-5-CARBOXALDEHYDE

Reagent used	Procedure[a]	M.p., °C.	% N Calcd.	% N Found
Substituted Hydrazones				
Aminoguanidine	I	278 dec.	36.82	36.83
Dimethylhydrazine	III	261 dec.	26.15	26.31
2,4-Dinitrophenylhydrazine	IV	295 dec.	23.86	23.81
Isonicotinoylhydrazide	IV	>360	24.04	24.03
Anils				
p-Aminophenol	IV	366 dec.	15.96	15.85
m-Anisidine	IV	311 dec.	15.15	15.40
p-Anisidine	IV	325 dec.	15.15	14.83
3,4-Dichloraniline	IV	350 dec.	13.29	13.54
p-Diethylaminoaniline	V	278 dec.	17.59	17.53
2,5-Difluoroaniline	IV	351 dec.	14.83	14.77
p-Fluoroaniline	IV	330 dec.	15.83	15.82
Pyridoxamine	V	310 dec.	17.38	17.49
Sulfadiazine	IV	>360	20.78	20.99
Sulfaguanidine	IV	325 dec.	22.81	22.75
Sulfamethazine	IV	320 dec.	19.43	19.20
Sulfamerazine	IV	331 dec.	20.08	20.33
Sulfapyridine	IV	339 dec.	17.36	17.24
Sulfathiazole	IV	>360	17.10	16.93

[a] The following procedures were used in preparing derivatives listed in Table I and II. (1) To 1.5 g. of the crude aldehyde dissolved in a minimum amount of hot water was added a solution of 1.5 g. each of sodium acetate and aminoguanidine sulfate in 50 ml. of water. The resulting mixture was heated on a steam cone for 30 min., cooled, and filtered to yield 0.25 g. of the product. (2) The hydrazine hydrochloride was suspended in dilute sodium hydroxide to liberate the organic base. The resulting mixture was acidified with enough acetic acid to assure a slight excess and filtered into a prepared solution of the crude aldehyde dissolved in a minimum amount of boiling water. The resulting mixture was boiled for 5–10 min., cooled, and the product collected on a filter, dried, and recrystallized. (3) The same as procedure 2 except the free hydrazine in acetic acid was used. (4) The hydrazine or amine in acetic acid was added to a hot solution of the crude aldehyde in a minimum amount of dimethylformamide. The resulting mixture was boiled for a few min., cooled, and water added to assure complete precipitation of the product which was collected on a filter and recrystallized. (5) The same as procedure 4 except the amine was first liberated from the amine hydrochloride by treating the hydrochloride with dilute sodium hydroxide. The products were recrystallized from dimethylformamide or dimethyl sulfoxide and water. The sulfathiazole anil was not recrystallized.

Experimental

2-Thiobarbituric acid, 2-thiouracil, 6-methyl-2-thiouracil, and 6-propyl-2-thiouracil were commercial pyrimidines used as received. The 5-carboxaldehydes were prepared by the Reimer Tiemann reaction but no attempts were made to isolate and purify the aldehydes. The previously described procedure[2]

(2) The authors are indebted to Dr. C. C. Stock, Dr. R. K. Barclay, Dr. Christine Reilly, Dr. Elvira Falco, and Dr. Sophronia Myron, Sloan-Kettering Institute for Cancer Research, for conducting these tests. The rating scales and procedures for the Sarcoma 180 test are given in *Cancer Res.*, Suppl. No. 1, 91 (1953); *ibid.*, Suppl. No. 2, 179 (1955); *ibid.*, **18**, 49 (1958).