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 $C_{11}H_{15}Cl_2N_5S \cdot 2HCl$: 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.

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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).

for the Reimer–Tiemann reaction was used in each preparation.

For the 4,6-dihydroxy-2-thiopyrimidine-5-carboxaldehyde, the reaction mixture was cooled for a few hours in a refrigerator and filtered to give a mixture of potassium salts which included the salt of the aldehyde. This buff colored salt mixture was suspended in water to form a thick slurry and acidified with 6 *N* sulfuric acid until the color change to orange-red was complete. This suspension was heated to 60°, cooled, and filtered. The product was washed with approximately 1 *N* sulfuric acid and finally cold water until free of potassium. A final washing involving ether was used to facilitate drying the product. This unrecrystallized product was used in preparing derivatives.

TABLE II

HYDRAZONE DERIVATIVES OF 2-THIO-SUBSTITUTED PYRIMIDINE-5-CARBOXALDEHYDES

Aldehyde of[a]	Reagent used[b]	Procedure[c]	M.p., °C.	% N Calcd.	% N Found
A	2-Benzothiazolyl(H)	I	237 dec.	23.09	23.32
A	*p*-Bromo(PH)	II	261 dec.	17.29	17.43
A	*o*-Carboxy(PH)	II	242 dec.	19.30	19.28
A	*p*-Chloro(PH)	II	270 dec.	19.96	19.96
A	2,4-Dinitro(PH)	I	305 dec.	24.98	25.02
A	1,1-Diphenyl(H)	II	258 dec.	17.38	17.45
A	*p*-Fluoro(PH)	II	250 dec.	21.20	21.19
A	Nitroaminoguanidine[d]	I	>360	38.12	37.90
A	*p*-Nitro(PH)[e]	I	370 dec.	24.04	23.80
A	4-Phenylsemicarbazide	II	221 dec.	24.20	24.38
A	1-Naphthyl(H)	II	235 dec.	18.89	18.72
A	Benzoyl(H)	I	285 dec.	20.42	20.33
A	2,4-Dinitrophenyl-semicarbazide	I	260 dec.	25.91	25.75
A	*m*-Nitrobenzhydrazide	I	260 dec.	21.92	22.39
A	*p*-Nitro(PH)	II	297 dec.	24.04	24.15
A	*p*-Carboxy(PH)	I	297 dec.	19.30	19.70
B	*o*-Carboxy(PH)	II	322 dec.	18.41	18.61
A	2,4-Dinitro(PH)[f]	I	305 dec.	23.98	23.73
B	*p*-Nitro(PH)	I	322 dec.	22.94	23.13
C	2,4-Dinitro(PH)	I	319 dec.	22.21	21.90
C	*p*-Nitro(PH)	I	313 dec.	21.01	21.14

[a] A, 4-Hydroxy-2-thiopyrimidine; B, 4-hydroxy-6-methyl-2-thiopyrimidine; C, 4-hydroxy-6-propyl-2-thiopyrimidine. [b] H, Hydrazine; P, phenyl; D, hydrazide. [c] The following procedures were used in preparing derivatives: (1) all of the derivatives were prepared from solutions of the unisolated aldehyde. The Reimer–Tiemann reaction mixture was cooled and filtered to remove any precipitated salts. The filtrate was acidified with acetic acid and refiltered if necessary. To the hot, acidified filtrate was added an excess of the hydrazine in dilute acetic acid. The reaction mixture was boiled for 5–10 min. and then cooled. The product was collected on a filter, dried, and recrystallized. All derivatives were recrystallized from dimethylformamide and water unless otherwise specified. (2) The same procedure (1) except the hydrazine was first liberated from the hydrazine hydrochloride by treatment with dilute sodium hydroxide. [d] Not recrystallized. The sample was prepared from filtered solutions and washed with hot water. [e] *Anal.* Calcd. for $C_{11}H_9N_5O_3S$: C, 45.35; H, 3.11. Found: C, 45.46; H, 3.42. [f] *Anal.* Calcd. for $C_{12}H_{14}N_6O_5S$: C, 41.15; H, 2.85. Found: C, 41.26; H, 2.97.

Other pertinent experimental details for the aldehyde and derivative preparations are given as footnotes to Table I. The compounds were dried at 150° (1 mm.) for 8 hr. prior to analysis. In addition to the derivatives listed in the Tables a few other were prepared for which nonconfirmatory nitrogen analyses were obtained.

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Some Dichloroacetyl Derivatives and Their Antitumor Activity[1,2]

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Feitelson[3] and co-workers have shown that replacing the dichloroacetyl group with an acetyl group in chloramphenicol produces a sevenfold decrease in the potency of this antibiotic. It has also been shown that the compounds synthesized by Surrey,[4] with a dichloroacetyl group present, were strong amebicides. This evidence indicates the specificity of the dichloroacetyl group at specific receptor sites in the biological system.

Taking advantage of the increased potency potential of the dichloroacetyl group, Levi, *et al.*,[5] prepared N-dichloroacetyl-DL-serine and showed that it depressed the growth of Sarcoma 37 in mice, and in some cases the tumors sloughed off. Recent studies[6] report that this compound was effective in treating human tumors in combination with irradiation. Therefore, it was decided to prepare compounds which are related to physiologically active substances but which contain the dichloroacetyl group, and test them for carcinostatic activity on Sarcoma 180.

Inositol, a naturally occurring sugar in both plant and animal organisms, was tested by Laszlo and Leuchtenberger[7] and found to be effective in inhibiting the growth of Sarcoma 180 in mice. The hexadichloroacetate of inositol was prepared most effectively by the use of dichloroacetic anhydride.

Another compound which has shown anticancer possibilities is 9,10-phenanthraquinone. According to Powell,[8] when incorporated in the diet at the level of 1–2%, 9,10-phenanthraquinone inhibits the growth of several types of transplanted mouse tumors. In order to attach the dichloroacetyl group to the molecule, 2-amino-9,10-phenanthraquinone was first synthesized according to the procedure of Schmidt and Spoun[9] and the amine was then allowed to react with dichloroacetyl chloride.

Anthranilic acid has been demonstrated to be a precursor in the metabolic formation of tryptophan.[10] Therefore, the N-dichloroacetyl derivative of methyl anthranilate was prepared using dichloroacetyl chloride and methyl anthranilate.

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