Potential Anticancer Agents V: The Synthesis and Biochemical Studies of 5-Fluorinated Pyrimidine-6-carboxaldehydes and Derivatives

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Abstract □ A series of 5-fluoropyrimidine-6-carboxaldehydes with varying 2-substituents was prepared from which a number of 6-substituted 5-fluoropyrimidines were derived. Several compounds in this series were found to inhibit growth of the Ehrlich ascites carcinoma only slightly less effectively than 5-fluorouracil and the strongest inhibition of the series was 2-ethylthio-5-fluoropyrimidine-6-carboxaldehyde, I-8. The replacement of the formyl group by other substituents tended to decrease or to abolish the inhibitory activity. The various compounds in this series inhibit respiration and glycolysis of Ehrlich ascites carcinoma cells, but these inhibitory effects were obtained only at concentrations which were several fold higher than those required for inhibition of nucleic acid synthesis as reported previously.

Keyphrases □ 5-Fluorinated pyrimidine-6-carboxaldehydes, derivatives—synthesis □ Anticancer screening—5-fluorinated pyrimidine-6-carboxaldehydes □ Nucleic acid biosynthesis—inhibition □ Cell suspensions—respiration, glycolysis determinations

As a part of a continuing program (1–7) of research on potential anticancer agents, synthetic and biochemical mechanistic studies have been made on a series of fluorinated pyrimidine aldehydes and related derivatives. In the search for useful antimetabolites or for enhancing biological activities the substitution of a fluorine atom for hydrogen atom has been reported for many classes of medicinal agents (8–10). Notable examples among the anticancer agents are the 5-fluoro analogs of naturally occurring pyrimidines and pyrimidine nucleosides (11, 12).

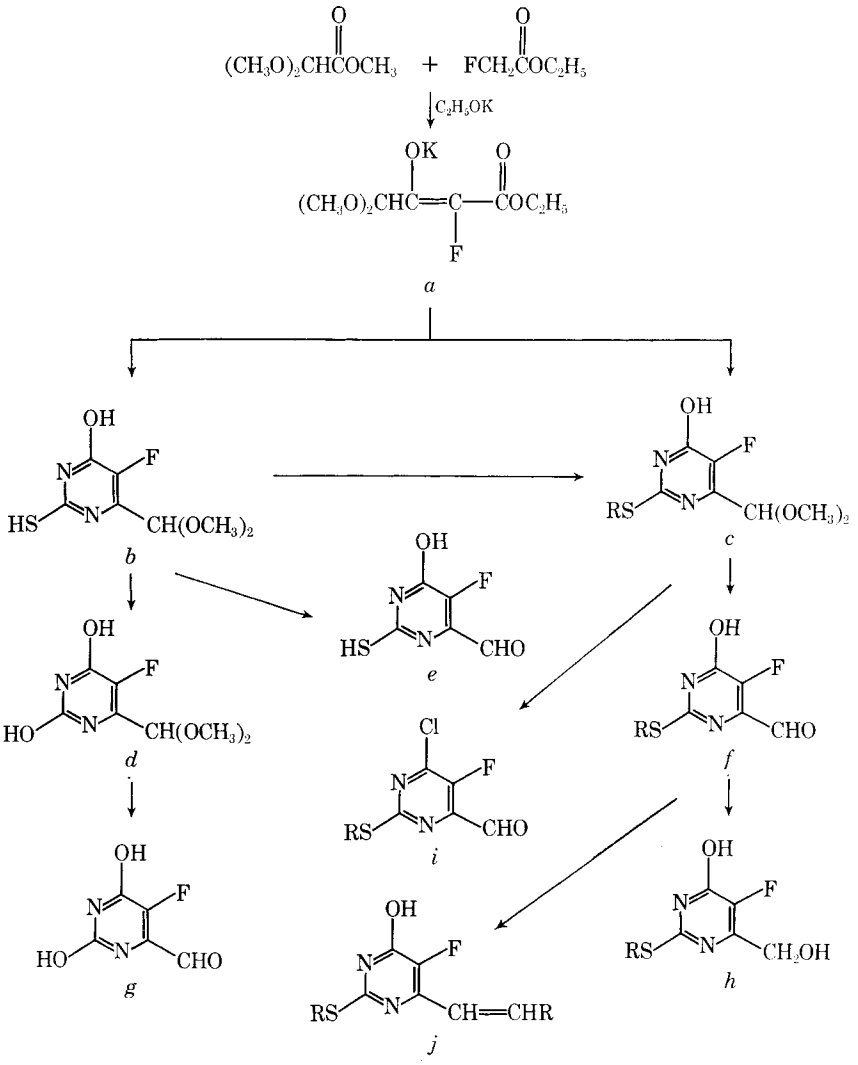
The introduction of a fluorine atom into the 5-position of 2,4-dihydroxy-pyrimidine-6-carboxaldehyde was thought to be of interest because the resulting 5-fluoro-6-formyluracil would be structurally analogous to orotic acid, a precursor of pyrimidine nucleotide biosynthesis. The chemical reactivity of the formyl group might also play a significant role by interacting with macromolecules. It could form a covalent bond with nucleophilic enzyme groups such as NH_2 and SH, hopefully in or near the active site of the enzyme. The sulfhydryl group, especially, has been implicated as participating in the catalytic mechanisms of a variety of enzymes and it is one of the most reactive groups with regard to the formation of covalent bonds. Thus, it was hoped that this pyrimidine derivative might act as an analog as well as sulfhydryl reagent (13). The substitution of the hydroxyl group at the 2-position of I-11 by an isosteric thiol group or by alkyl or aralkylthio groups was also considered of interest. Since hydrazones, Schiff bases, thiosemicarbazones, and azlactones of certain heterocyclic aldehydes (14–16) have been shown to inhibit the growth of neoplasms, it was thought to be worthwhile to prepare such derivatives of the pyrimidine aldehydes for biological studies. Various procedures were used to provide the desired compounds (see Scheme I), and the data are recorded in Table I.

METHODS AND MATERIALS

The 2-mercapto-6-(dimethoxymethyl)-5-fluoro-4-pyrimidol (*b*) was prepared by the condensation of the potassium salt of ethyl α-fluoro-γ,γ-dimethoxyacetoacetate (*a*), prepared by an adaption of the method of Heidelberger *et al.* (17), with thiourea. 2-Alkylthio-6-(dimethoxymethyl)-5-fluoro-4-pyrimidol (*c*) was obtained by either condensation of *a* with *S*-alkyl substituted thiourea or treating *b* with alkylbromide in 1 *N* NaOH solution at room temperature. The 2,4-dihydroxy-5-fluoropyrimidine-6-carboxaldehyde (*g*) was obtained by treating *b* in sodium hydroxide solution with 30% H_2O_2 and subsequent treatment with acid. The acetals, *b* and *d*, were hydrolyzed with dilute HCl and *c* with 50% acetic acid to give the corresponding aldehydes. 2-Ethylthio-6-hydroxymethyl-5-fluoro-4-pyrimidol (*h*) was prepared by treating *f* with excess formaldehyde in the presence of potassium hydroxide. The treatment of *c* with excess $POCl_3$ gave 2-alkylthio-4-chloro-5-fluoropyrimidine-6-carboxaldehyde (*i*). Aldol condensation of *f* with acetone or acetaldehyde resulted in the introduction of an α,β-unsaturated ketone or aldehyde moiety (*j*), respectively. Schiff bases, hydrazones, and azlactones were prepared in the usual manner.

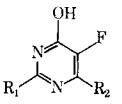
Screening Tests—The compounds were tested *versus* the Ehrlich ascites carcinoma in Swiss-Webster white mice by a slight modification of procedures described previously (18). Each mouse (initial weight approximately 30 g.) received an intraperitoneal injection of 0.1 ml. of pooled ascitic fluid, collected from donor mice which had borne the ascites carcinoma for 7–8 days and diluted with saline to a cell concentration of 10% by volume (average of 5.5×10^6 cells/0.1 ml.). For each assay the mice were divided into a control group of eight mice and several experimental groups of eight mice each. Twenty-four hours after the inoculation which was given on Day 1 of the study, each control mouse received an intraperitoneal injection of 0.2 ml. of 0.9% NaCl and each experimental mouse received 0.2 ml. of a solution or suspension of the test compound in 0.9% NaCl. The intraperitoneal injections for control and experimental mice were given twice daily (9 a.m. and 5 p.m.) on Days 2–6 and once on Day 7 of the study (total of 11 injections). On the seventh day all surviving mice in control and experimental groups were sacrificed. The volume of ascitic fluid was measured for each animal, and the percentage of cells by volume (ascitocrit) was determined for each sample of ascitic fluid by centrifugation in heparinized capillary tubes. The total packed-cell volume (TPCV) of tumor cells was calculated in each case together with average values and *SD*. The results of tests of representative compounds are recorded in Table II.

A marked decrease in the average total packed-cell volume (TPCV) of tumor cells of treated mice in comparison with corresponding controls is an index of the effectiveness of a compound against the growth of the Ehrlich ascites carcinoma. Of the compounds tested, the most active were those numbered I-12, I-8, I-10, I-26, I-27, I-15, and I-22 (Table II). Compound I-8 was the most effective member of this series, and it was only slightly less active than 5-fluorouracil against the Ehrlich ascites carcinoma. The dimethyl acetals had no activity at the dosages tested in contrast to the considerable activity exhibited by the free aldehydes at similar dosage. In general, the substituted aldehydes had less activity than the corresponding free aldehydes. The only exception to this was the cyclopropylamine Schiff base (Compound I-15) which had



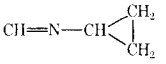
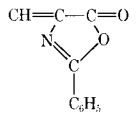
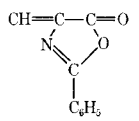
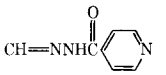
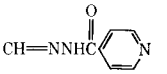
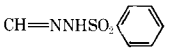
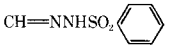
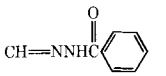
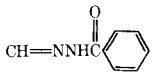
Scheme I

Table I—5-Fluoropyrimidine-6-carboxaldehyde and Derivatives



No.	R_1	R_2	M.p., °C.	Yield, %	Formula	C% Calcd. Found	H% Calcd. Found	F% Calcd. Found	N% Calcd. Found	S% Calcd. Found
1	SH	$CH(OCH_3)_2$	201–202	15	$C_7H_9FN_2O_3S$	38.18 38.14	4.09 4.12	8.63 8.87	12.72 12.56	14.54 14.47
2	CH_3S	$CH(OCH_3)_2$	135–137	70	$C_8H_{11}FN_2O_3S$	41.03 41.41	4.70 4.42	8.12 8.38	11.97 11.90	13.66 13.63
3	C_2H_5S	$CH(OCH_3)_2$	105–106	64	$C_9H_{13}FN_2O_3S$	43.55 44.02	5.24 5.56	11.29 11.22	12.90 12.85	7.66 7.84
4	nC_3H_7S	$CH(OCH_3)_2$	77–79	63	$C_{10}H_{15}FN_2O_3S$	45.80 46.25	5.73 5.57	7.25 7.59	10.69 10.94	12.21 12.12
5	$C_6H_5CH_2S$	$CH(OCH_3)_2$	132–134	6.5	$C_{14}H_{15}FN_2O_3S$	54.19 54.38	4.84 4.75	6.13 6.31	9.03 8.98	10.32 10.16
6	OH	$CH(OCH_3)_2$	215–216	75	$C_7H_9FN_2O_4$	41.18 41.61	4.41 4.66	9.31 9.22	13.71 13.61	— —
7	CH_3S	CHO	220–222	89	$C_6H_5FN_2O_2S$	38.30 38.71	2.66 2.26	10.10 10.23	14.89 15.20	17.21 17.53
8	C_2H_5S	CHO	180–182	87	$C_7H_7FN_2O_2S$	41.58 41.61	3.47 3.63	9.45 9.68	13.86 13.71	15.84 15.75
9	nC_3H_7S	CHO	129–131	70	$C_8H_9FN_2O_2S$	44.44 44.29	4.17 4.33	8.79 8.71	12.96 12.84	14.81 14.68
10	$C_6H_5CH_2S$	CHO	165–167	69	$C_{12}H_9FN_2O_2S$	54.55 53.99	4.41 4.46	7.20 7.18	10.61 10.24	12.12 12.00
11	OH	CHO	262–263d	75	$C_5H_3FN_2O_3$	37.97 38.06	1.89 1.84	12.03 12.34	17.72 17.66	— —

Table I (Continued)

No.	R_1	R_2	M.p., °C.	Yield, %	Formula	C% Calcd. Found	H% Calcd. Found	F% Calcd. Found	N% Calcd. Found	S% Calcd. Found
12	SH	CHO	>300d	65	$C_5H_3FN_2O_2S$	34.48 34.24	1.72 2.02	10.92 10.34	16.09 16.25	18.39 18.42
13	C_2H_5S	$CH{=}N{-}C_6H_5SO_2NH_2$	>350d	99	$C_{13}H_{13}FN_4O_3S_2$	43.82 43.45	3.65 3.96	5.34 5.43	15.73 15.12	17.98 17.49
14	C_2H_5S	$CH{=}N(CH_2)_2OH$	224–225d	73	$C_9H_{12}FN_3O_2S$	44.00 43.67	4.89 4.89	7.75 7.81	17.14 17.35	13.06 13.24
15	C_2H_5S		164–165	90	$C_{10}H_{12}FN_3OS$	49.79 49.77	4.98 5.08	7.88 8.05	17.43 17.31	13.28 13.29
16	OH		341–344d	65	$C_{14}H_8FN_3O_4$	55.80 55.66	2.65 2.60	6.31 6.40	13.95 14.09	— —
17	C_2H_5S		212–214d	45	$C_{16}H_{12}FN_3O_3S$	55.60 55.83	3.47 4.06	5.50 5.20	12.17 12.41	9.27 8.81
18	CH_3S	CH=NOH	261–262d	75	$C_6H_6FN_3O_3S$	35.47 35.67	2.96 3.02	9.34 9.63	20.69 20.52	15.76 15.63
19	C_2H_5S	CH=NOH	238–240	76	$C_7H_8FN_3O_3S$	38.71 38.69	3.69 4.22	8.76 8.48	19.35 19.18	14.75 14.25
20	C_2H_5S	$CH{=}NNHCSNH_2$	260–262	84	$C_5H_{10}FN_5OS_2$	34.91 34.98	3.64 3.92	6.91 6.83	25.45 25.23	23.27 23.04
21	C_2H_5S	$CH{=}CHCOCH_3$	157–160	75	$C_{10}H_{11}FN_2O_2S$	49.59 49.56	4.55 4.69	7.85 7.92	11.57 11.44	13.22 13.29
22	C_2H_5S	CH_2OH	136–138	46	$C_7H_9FN_2O_2S$	41.18 41.45	4.41 4.86	9.31 9.01	13.73 14.03	15.67 15.87
23	C_2H_5S	CH=CHCHO	136–138	41	$C_9H_9FN_2O_2S$	47.37 47.82	3.95 4.04	8.33 8.12	12.28 12.56	14.04 14.51
24	C_2H_5S		253–255d	78	$C_{13}H_{12}FN_5O_2S$	48.50 48.13	3.73 4.01	5.91 6.32	21.80 21.79	9.96 10.56
25	OH		350–352d	75	$C_{11}H_8FN_5O_3$	47.65 47.28	2.89 3.63	6.85 6.19	25.27 25.22	— —
26	C_2H_5S		172–174d	64	$C_{13}H_{13}FN_4O_3S_2$	43.80 43.99	3.65 3.76	5.33 4.65	15.73 15.79	17.97 17.71
27	SH		199–202	66	$C_{11}H_9FN_4O_4S$	42.31 42.78	2.88 3.20	6.09 7.26	17.95 17.26	10.26 9.81
28	C_2H_5S		250–251	73	$C_{14}H_{13}FN_4O_2S$	52.50 52.38	4.06 4.52	5.94 6.04	17.50 17.26	10.00 9.81
29	OH		325–326d	77	$C_{17}H_9FN_4O_3$	52.17 52.25	3.26 3.52	6.88 7.02	20.29 20.11	— —

activity approximately equal to the free aldehyde (Compound I-8). Substitution of a hydroxymethyl group for the aldehyde group at position 6 (compare data for Compound I-22 with data for Compound I-8) resulted in a considerable decrease in inhibitory activity *versus* the Ehrlich carcinoma.

Inhibition of Energy-Yielding Processes—Previous studies (5) in this laboratory have shown that representative members of the series of compounds in Table II strongly inhibit the incorporation of various precursors into ribonucleic acid (RNA), deoxyribonucleic acid (DNA), and proteins. It seemed worthwhile to extend these studies to an examination of the effects of these compounds upon respiration and glycolysis, particularly since other carcinostatic compounds have been found to inhibit energy-yielding processes (20–22).

The experiments were performed *in vitro* with Ehrlich carcinoma cells which were incubated at 37° in Krebs-Ringer phosphate buffer for measurement of oxygen uptake and in Krebs-Ringer bicarbonate buffer for determination of glycolytic rate by measurement of the CO_2 released by lactic acid production. Details of these experiments are presented in the *Experimental* section of the paper, and the results are recorded in Table III.

The data of Table III show that the various 5-fluoropyrimidine-6-carboxaldehyde derivatives generally inhibit oxygen uptake more strongly than glycolysis, and the various members of this series of compounds exhibit considerable differences in degree of inhibition of respiration and glycolysis. Those compounds which are the most effective inhibitors of growth of the carcinoma cells, *viz.*—Compounds I-8, I-12, I-10, I-26, I-27, I-15, I-22, are also the most effective inhibitors of oxygen uptake by these cells with a few exceptions such as Compound I-22, which is a moderately good inhibitor of growth of the carcinoma but is less effective as an inhibitor of oxygen uptake than some of the other compounds. In general, the inhibition of oxygen uptake in the absence of added glucose (Q_{O_2}) was slightly greater than the inhibition of oxygen uptake in the presence of added glucose [$Q_{O_2}(G)$], but the differences were small in most cases and nonexistent for some.

Although the inhibition of oxygen uptake and glycolysis by these pyrimidine derivatives is of interest, it seems unlikely that these

Table II—Results of Screening Tests *versus* the Ehrlich Ascites Carcinoma[a]

Compd.	Dose mg./kg./day	Mortality C	Mortality T	Av. Wt. Change T/C, g.	Av. TPCV T/C ml.	Av. TPCV SD T ± ml.	Av. TPCV T as % of C
I-1	34.0	3/8	1/8	8.1/5.1	3.13/2.90	0.63	100
I-2	34.6	3/8	2/8	9.1/7.7	3.49/3.59	0.58	97
I-3	41.3	0/8	0/8	12.7/9.3	2.96/2.93	0.69	98
I-6	33.8	3/8	1/8	11.5/5.1	2.81/2.90	0.07	96
I-8	51.0	0/10	0/8	0.6/3.4	0.16/2.64	0.39	6
	29.0	0/10	0/8	2.4/3.6	0.61/2.30	0.41	23
I-10	49.0	0/10	0/8	4.2/3.4	1.40/2.64	0.64	53
I-11	10.3	0/8	0/8	8.7/6.1	2.06/2.72	0.86	76
I-12	28.6	0/8	0/8	7.6/6.1	1.81/2.72	0.30	69
I-15	28.0	0/10	2/8	1.1/3.4	0.50/2.64	0.34	29
I-16	27.1	3/8	0/8	3.5/5.1	2.48/2.90	0.77	85
I-18	35.0	0/8	0/8	3.6/3.6	2.01/2.30	0.66	77
I-22	49.1	3/8	0/8	3.8/12.2	1.58/3.47	1.14	45
I-24	61.8	2/8	4/8	10.2/6.7	2.29/2.79	0.47	82
I-25	52.9	2/8	0/8	8.3/6.7	2.40/2.79	1.40	86
I-26	29.0	3/8	1/8	7.3/12.2	2.20/3.47	1.04	63
I-27	50.0	0/10	0/8	4.0/3.4	1.22/2.64	0.56	46
FU[b]	27.5	0/8	0/8	0.3/5.3	0.15/1.5	0.10	10

[a] *T* = treated group, *C* = control group, TPCV = total packed cell volume of tumor cells on final day of assay. *SD* = standard deviation of TPCV of treated group. Compounds were dissolved or suspended in saline solution. The average *SD* of the control group was ±0.32 ml. [b] FU = 5-fluorouracil.

effects can account for the inhibition of growth of the carcinoma cells since the inhibition of oxygen uptake and glycolysis occurs only at relatively high concentration (1.95 moles) in contrast to the inhibition of orotic acid incorporation into RNA and DNA which is exhibited at concentrations of these compounds of 0.2–0.5 mmole (5). These pyrimidine derivatives also inhibit the incorporation of formate into DNA-thymine and RNA-purines (5) at concentrations which are one-third those required for inhibition of oxygen uptake by the carcinoma cells, and thus it is unlikely that the inhibition of respiration is responsible for the inhibition of nucleic acid synthesis by these compounds. On the other hand, it is quite possible that the inhibition of growth of the carcinoma cells by these compounds could be partly due to inhibition of respiration and glycolysis. However, it seems probable that the inhibition of RNA synthesis also is involved in the inhibition of protein synthesis.

EXPERIMENTAL[1]

The designation of a roman numeral followed by an arabic numeral indicates a specific compound number in the table indicated by the roman numeral (see Tables I–III).

Potassium Enolate of Ethyl α-Fluoro-γ,γ-dimethoxyacetoacetate—The method used was adapted from the procedure of Heidelberger *et al.* (17). The freshly prepared potassium ethoxide was suspended in 600 ml. of absolute ether and cooled in an ice bath. To this suspension, a mixture of 53 g. (0.5 mole) of ethyl fluoroacetate and 92.4 g. (0.7 mole) of methyl dimethoxyacetate was added dropwise, with stirring and cooling, over a period of 1.5 hr. After completing the addition, the mixture was stirred further for 3 hr. with cooling and then kept for 24 hr. at 20°. The precipitate was collected on a Büchner funnel, washed several times with absolute ether, and dried in a vacuum desiccator over P_2O_5. It was then used immediately for the next step without purification.

2-Mercepto-6-(dimethoxymethyl)-5-fluoro-4-pyrimidol (I-1)—A mixture of 123 g. (0.5 mole) of the freshly prepared potassium enolate of ethyl α-fluoro-γ,γ-dimethoxyacetoacetate, 21.6 g. (0.4 mole) of sodium methoxide and 30.4 g. (0.4 mole) of thiourea was stirred in 1,000 ml. of absolute methanol at room temperature for 24 hr. At the end of this time, the methanol was distilled off *in vacuo* (bath temperature: 50°). The residue was dissolved in 250 ml. of water and filtered, if incomplete dissolution was observed. The water solution was cooled in an ice bath and acidified by adding concentrated hydrochloric acid dropwise. The material which crystallized from the acid solution was filtered off, washed with ice water several times, and dried, yielding 22 g. of crude product. The crude product was dissolved in about 400 ml. of boiling methanol and treated with activated charcoal. The filtrate was concentrated to about 200 ml., cooled, and the analytical sample was recrystallized from 50% ethanol.

2-Methylthio-6-(dimethoxymethyl)-5-fluoro-4-pyrimidol (I-2)—Two and two-tenths grams (0.01 mole) of I-1 was dissolved in 14 ml. of 1 *N* NaOH solution. To this was added 4.2 g. (0.03 mole) of methyl iodide and the mixture was stirred at room temperature for 30 min. The reaction mixture was cooled in an ice bath and acidified with glacial acetic acid. Immediately, a crystalline material precipitated. The precipitate was collected, washed with cold water, and recrystallized from 50% ethanol.

2-Ethylthio-6-(dimethoxymethyl)-5-fluoro-4-pyrimidol (I-3)—(*a*) Two and two-tenths grams (0.01 mole) of I-1 in 14 ml. of 1 *N* NaOH was treated with 3.2 g. (0.03 mole) of ethylbromide at room temperature for 1 hr. The reaction mixture was cooled, then acidified with glacial acetic acid. The separated crystals were recrystallized from 50% ethanol.

(*b*) A mixture of 123 g. (0.5 mole) of freshly prepared potassium enolate of ethyl α-fluoro-γ,γ-dimethoxyacetoacetate, 74 g. (0.4 mole) of *S*-ethylthiouronium bromide and 21.6 g. (0.4 mole) of sodium methoxide in 1,000 ml. of absolute methanol was stirred at room temperature for 1 day. The methanol was distilled off *in vacuo*. The residue was dissolved in 250 ml. of water and acidified with concentrated HCl, while the mixture was cooling. The material which precipitated from the acid solution was filtered off and washed with cold water. The crude product was dissolved in absolute ether and the ether insoluble impurities were removed. The evaporation of the ether gave 13.7 g. (14%) of product, m.p. 87–104°. Three recrystallizations from 50% ethanol gave a pure product, m.p. 105–106°.

2-n-Propylthio-6-(dimethoxymethyl)-5-fluoro-4-pyrimidol (I-4)—(*a*) Two and two-tenths grams (0.01 mole) of I-1 was dissolved in 14 ml. of 1 *N* NaOH solution and treated with 4.3 g. (0.003 mole) of *n*-propyl bromide at room temperature for 90 min. The reaction mixture was cooled in an ice bath and acidified with glacial acetic acid. The precipitate was filtered off, washed with cold water, and dried. Recrystallization from 50% ethanol gave a pure compound, 1.65 g. (63%), m.p. 77–79°.

(*b*) A mixture of 123 g. (0.5 mole) of freshly prepared potassium enolate of ethyl α-fluoro-γ,γ-dimethoxyacetoacetate, 21.6 g. (0.4 mole) of sodium methoxide and 70.6 g. (0.4 mole) of *S*-*n*-propylthiourea hydrobromide in 1,000 ml. of absolute methanol was stirred at room temperature for 24 hr. The reaction mixture was evaporated to dryness *in vacuo* at 50° in a water bath. The residue was dissolved in 250 ml. of water, cooled, and acidified with concentrated HCl. The material which crystallized from the acid solution was filtered off, and then washed with cold water. Recrystallization from 50% alcohol gave 7.4 g. (7.1% of product, m.p. 77–79°).

2-Benzylthio-6-(dimethoxymethyl)-5-fluoro-4-pyrimidol (I-5)—This compound was prepared from the potassium enolate of ethyl

[1] Elemental analyses were performed by Alfred Bernhardt, Microanalytical Laboratory, Mulheim, Hohenweg, West Germany, and Spang Microanalytical Laboratory, Ann Arbor, Mich. The melting points were determined with the Mel-Temp apparatus and have been corrected.

Table III—Effect of Various 5-Fluoropyrimidine-6-Carboxaldehyde Derivatives on Respiration and Glycolysis of Ehrlich Ascites Carcinoma Cells

Compd.	Concn. (final) (m*M*)	Respiration				Glycolysis	
		Q_{O_2}	% of Control	Q_{O_2} (G)	% of Control	$Q^{N_2}_{CO_2}$	% of Control
I-3	1.30	10.3	100.9	—	—	—	—
I-7	1.95	3.3	32.4	1.9	36.5	11.9	61.7
I-8	1.95	2.1	20.6	1.6	30.7	17.7	59.7
I-9	1.95	4.0	39.3	3.3	63.5	15.7	80.1
I-10	1.95	2.4	23.5	3.4	65.4	15.1	77.0
I-11	1.95	3.1	30.4	2.7	51.9	11.9	60.7
I-12	1.95	7.4	72.5	3.1	59.6	10.9	55.4
I-13	1.95	10.1	99.0	5.1	98.1	—	—
I-14	1.30	10.0	98.0	—	—	—	—
I-15	1.95	2.3	22.5	3.4	65.4	14.9	76.0
I-16	1.95	5.9	57.8	3.8	73.1	17.1	87.2
I-17	1.30	8.2	80.4	—	—	—	—
I-18	1.30	9.7	95.1	—	—	—	—
I-19	1.30	10.3	100.9	—	—	—	—
I-20	1.30	9.4	92.2	—	—	—	—
I-21	1.95	3.6	35.3	1.9	36.5	16.8	85.7
I-22	1.95	8.7	85.3	4.5	86.5	19.5	99.5
I-23	1.95	7.2	70.6	3.6	69.2	16.1	82.1
I-26	1.95	2.5	24.5	3.5	67.3	14.8	75.5
I-28	1.95	5.7	55.9	3.0	57.7	16.9	86.2

For calculation of Q values, 2 ml. of 10% cell suspension was considered as equivalent to 32 mg. dry weight.

α-fluoro-γ,γ-dimethoxyacetoacetate and *S*-benzylthiourea hydrochloride in the same manner described for the preparation of I-3. The product was purified from water.

2,4-Dihydroxy-6-(dimethoxymethyl)-5-fluoropyrimidine (I-6)—One and one-tenths grams (0.005 mole) of I-1 was dissolved in 10 ml. of 1.5 *N* NaOH solution and cooled in an ice bath. To this was added 3.0 ml. of 30% hydrogen peroxide dropwise and the mixture was boiled gently for 1.5 hr. in a water bath. It was then concentrated *in vacuo* to a volume of 5 ml., cooled, and acidified with concentrated sulfuric acid. It was allowed to stand at room temperature for 1 hr. and then cooled in an ice bath. The crystallization took place spontaneously. It was then recrystallized from hot water.

2-Methylthio-5-fluoropyrimidine-6-carboxaldehyde (I-7)—One gram of I-2 was dissolved in 10 ml. of 50% glacial acetic acid. The solution was heated on a steam bath for 40 min. and evaporated to dryness. The residue was washed with cold water and recrystallized from 50% alcohol.

The acetals of 2-ethylthio-(I-8), 2-*n*-propylthio-(I-9), and 2-benzylthio-(I-10) derivatives were hydrolyzed in an analogous manner.

2,4-Dihydroxy-5-fluoropyrimidine-6-carboxaldehyde (I-11)—One gram (0.005 mole) of I-6 was dissolved in 12 ml. of 6 *N* HCl and boiled for 3 min. The solution was cooled in an ice bath. The material which crystallized from the solution was collected by filtration and washed with cold water. It was recrystallized from 50% alcohol.

2-Mercapto-4-hydroxy-5-fluoropyrimidine-6-carboxaldehyde (I-12)—Two and two-tenths grams (0.01 mole) of I-1 was dissolved in 35 ml. of 6 *N* HCl with warming. The resulting yellow solution was heated to boiling for 5–7 min., and filtered. The filtrate was cooled in an ice bath. The yellow precipitate was filtered, washed with ice water thoroughly, and recrystallized from alcohol. The product did not melt and carbonized above 300°.

p-Aminosulfonyl-N-(2-ethylthio-4-hydroxy-5-fluoro-6-pyrimidylmethylidene) aniline (I-13)—A solution of 0.5 g. (0.0025 mole) of I-8 and 0.43 g. (0.0025 mole) of *p*-aminobenzenesulfonamide in 7 ml. of absolute ethanol was refluxed for 30 min. in a water bath. During this period, orange-colored crystals precipitated out. The crystals were collected by filtration and washed with a large amount of warm alcohol to remove any unreacted starting materials. The product did not melt and decomposed above 350°.

N-(2-Ethylthio-4-hydroxy-5-fluoro-6-pyrimidylmethylidene) ethanolamine (I-14)—To a solution of 0.4 g. (0.002 mole) of I-8 in 3 ml. of absolute alcohol was added 0.13 g. (0.002 mole) of ethanolamine. The mixture was refluxed for 30 min. in a water bath and placed in a refrigerator overnight. The product was collected on a filter and recrystallized from ethanol.

N-(2-Ethylthio-4-hydroxy-5-fluoro-6-pyrimidylmethylidene) cyclopropylamine (I-15)—To a solution of 0.6 g. (0.003 mole) of I-8 in 4 ml. of absolute ethanol was added 0.17 g. (0.003 mole) of cyclopropylamine. On shaking the mixture at room temperature for a few minutes, yellow crystals precipitated out. The crystals were collected, washed with cold alcohol, and recrystallized from a minimum amount of alcohol.

2-Phenyl-4-(2,4-dihydroxy-5-fluoro-6-pyrimidylmethylidene)-2-oxazoline-5-one (I-16)—A mixture of 0.45 g. (0.0025 mole) of hippuric acid and 0.1 g. of potassium bicarbonate in 2.5 ml. of acetic anhydride was stirred until a solution was obtained, and cooled in a cold water bath to maintain a temperature around 20°. To this solution was added 0.4 g. (0.0025 mole) of I-6 and the mixture was stirred for 2 hr. The reaction mixture was poured into 10 ml. of distilled water. The precipitate was filtered, washed with distilled water, and after being triturated with ethanol several times, the product was obtained. The 2-phenyl-4-(2-ethylthio-4-hydroxy-5-fluoro-6-pyrimidylmethylidene)-2-oxazoline-5-one (I-17) was prepared in an analogous manner.

2-Methylthio-4-hydroxy-5-fluoro-6-pyrimidinecarboxaldoxime (I-18)—The hydrolysis of the acetal and the oxime formation were accomplished in a one-step reaction. A solution of 0.47 g. (0.002 mole) of I-2 and 0.28 g. (0.004 mole) of hydroxylamine hydrochloride in 10 ml. of 50% ethanol was refluxed for 10 min. and then cooled in an ice bath. The crystalline solid was filtered, washed with cold water, and recrystallized from ethanol.

2-Ethylthio-4-hydroxy-5-fluoro-6-pyrimidinecarboxaldoxime (I-19)—This compound was prepared in an analogous manner from I-3.

Thiosemicarbazone of 2-Ethylthio-5-fluoropyrimidine-6-carboxaldehyde (I-20)—To a solution of 0.5 g. (0.0025 mole) of I-8 in 5 ml. of ethanol was added 0.23 g. (0.0025 mole) of thiosemicarbazide in 10 ml. of 80% ethanol and the solution was refluxed for 20 min. The yellow crystalline product was collected by filtration, washed with cold water and alcohol, and recrystallized from ethanol.

4-(2-Ethylthio-4-hydroxy-5-fluoro-6-pyrimidyl)-3-butene-2-one (I-21)—To a mixture of 0.5 g. (0.0025 mole) of I-8 and 0.58 g. (0.01 mole) of acetone was added dropwise 3 ml. of 6% aqueous NaOH solution while the solution was stirred and cooled. The mixture was stirred for 2.5 hr. at room temperature. Dilute hydrochloric acid was added until the mixture was acid to litmus. Immediately a yellow precipitate formed. It was filtered off and washed with cold water to yield 0.45 g. (75%), m.p. 154–157°. The crude material was dissolved in a minimum amount of warm ethanol. To this, water was added dropwise until the solution became cloudy and when cooled in an ice bath, a pure product precipitated.

2-Ethylthio-6-hydroxymethyl-5-fluoro-4-pyrimidol (I-22)—The method used was adapted from the procedure for the preparation of *p*-tolyl carbinol (23). Into a 100-ml. three-necked flask fitted with a dropping funnel, magnetic stirrer, and reflux condenser was introduced a solution of 2.5 g. of potassium hydroxide in 5 ml. of meth-

anol. The flask was surrounded by cold water while a solution of 3.03 g. (0.015 mole) of I-8 and 1.5 ml. (37%) of formalin in 5 ml. of methanol was added at such a rate that the internal temperature remained at 60–70°. The internal temperature was then maintained at 60–70° for 3 hr. At the end of this time methanol was distilled out. The residue was dissolved in a minimum amount of water and acidified with glacial acetic acid. The precipitate was collected by filtration and recrystallized from alcohol.

3-(2-Ethylthio-4-hydroxy-5-fluoro-6-pyrimidyl)-2-propenal (I-23)—A solution of 0.8 g. (0.004 mole) of I-8 and 1.2 g. (0.028 mole) of acetaldehyde in 10 ml. of 95% ethanol was cooled in an ice bath. To this was added dropwise 6 ml. of 1 *N* NaOH and the mixture was stirred for 1.5 hr. at 16–20°. It was then acidified with glacial acetic acid. Red crystals precipitated on standing and these were recrystallized from a minimum amount of ethanol.

2-Ethylthio-4-chloro-5-fluoro-6-dimethoxymethylpyrimidine—Two and one-half grams (0.01 mole) of I-2 was suspended in 10 ml. of phosphorous oxychloride, stirred, and treated dropwise with 1.49 g. (0.01 mole) of diethylaniline at 50–55°. The mixture was stirred at 50–55° for 1.5 hr. and then cooled. The reaction mixture was poured into ice in a beaker and extracted with ether. The ether layer was washed with water and dried over sodium sulfate, filtered to remove salts, and the ether was evaporated The residue was distilled under reduced pressure and the fraction at 102–103°/0.2 mm. Hg was collected. The yield was 0.96 g. (37%).

Anal.—Calcd. for $C_9H_{12}ClFN_2O_2S$: C, 40.53; H, 4.50; Cl, 13.30; F, 7.13; N, 10.51; S, 12.01. Found: C, 40.01; H, 3.92; Cl, 13.97; F, 7.34; N, 10.94; S, 12.60.

Hydrazones of the Pyrimidine Aldehydes (I-24-29)—The hydrazones of the pyrimidine aldehydes were prepared by the interaction of the pyrimidine aldehydes with the appropriate hydrazides.

To a solution of 0.0025 mole of the aldehyde in 5 ml. of 50% acetic acid was added 0.0025 mole of the hydrazide dissolved in 5 ml. of 50% acetic acid. The mixture was kept at 80° for 10 min. The crystalline precipitate was collected by filtration and recrystallized from ethanol.

EXPERIMENTS ON ENERGY-GENERATING SYSTEM

Preparation of Cell Suspension—The ascitic fluid, harvested from mice (Swiss-Webster) on the eighth day after inoculation of Ehrlich ascites cells, was centrifuged for 10 min. at 2,000 r.p.m.[2] The supernatant was discarded. The residue was washed three times by suspending in Krebs-Ringer solution and centrifuging for 5–7 min. at 700 r.p.m. to remove blood elements and soluble ascitic constituents. The cells were centrifuged for 15 min. at 2,300 r.p.m. to obtain the packed cells. The cells thus obtained were diluted to a 10% cell suspension with Krebs-Ringer phosphate buffer (pH 7.2–7.3) for the measurement of respiration or with Krebs-Ringer bicarbonate buffer for the measurement of glycolysis.

Measurement of Respiration—The rate of oxygen uptake by the cells was measured manometrically in a conventional Warburg apparatus. The main compartment of the flasks contained 2 ml. of 10% cell suspension in the Krebs-Ringer phosphate solution. Three-tenths milliliter of each of the solutions or suspensions of the compounds in phosphate buffer (pH 7.4) or in 0.1 *M* glucose was placed in the side-arm of the flasks. The center well contained 0.2 ml. of 20% potassium hydroxide and a strip of filter paper for the absorption of carbon dioxide. The flasks were attached to the manometers, gassed for 5 min. with oxygen, and then placed in the constant-temperature bath (37°). The compounds were tipped into the main compartment of the flasks from the side-arm after 10 min. of equilibration. The readings were taken for 60 min. at 10-min. intervals after the introduction of the compounds under study.

Q_{O_2} = μl. O_2 taken up/mg. dry weight of cell/hr. Q_{O_2} (G) = μl. O_2 taken up/mg. dry weight of cell/hr. with added glucose.

Measurement of Glycolysis—The rate of glycolysis was measured manometrically by measuring CO_2 production resulting from the action of lactic acid on bicarbonate in the media. The main compartment of the flasks contained 2 ml. of 10% cell suspension in Krebs-Ringer bicarbonate. Three-tenths milliliter of the solution or suspension of the compounds in 0.1 *M* glucose solution was placed in the side-arm of the flasks. The flasks were attached to the manometers and gassed for 10 min. with 95% nitrogen and 5% carbon dioxide. The compounds were tipped into the main compartment from the side-arm after 10 min. of equilibration in the constant-temperature bath (37°). The readings were taken for 60 min. at 10-min. intervals.

$Q_{CO_2}^{N_2}$ = μl. CO_2 given off in an atmosphere of nitrogen/mg. dry weight of cells/hr.

[2] Refrigerated in the International Refrigerated Centrifuge model B-20.

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