yielded 1-aza-2-carboxy-4-thia-5-methylbicyclo[4.3.0]non-5-en-9-one (**17**) (3.2 g 75%) as fine needles, mp 140–160° dec. Five recrystallizations from AcOH gave needles, mp 145–165° dec; from Me_2CO, small cubes, mp 141–156° dec; and from $CHCl_3$, plates, mp 165–175° dec; ir ($CHCl_3$), 3500–2700, 1720, 1675, and 1650 cm^{-1}; pmr (DMSO-d_6), δ 1.75 (s, 3, $CH_3C{=}C$), 2.25–2.95 (A_2B_2, m, 4, CH_2CH_2CO), 2.95 (calcd) (d of d, 1, J_{AB} = 13 Hz, J_{AX} = 3.7 Hz, SCH_2CH), 3.24 (calcd) (d of d, 1, J_{BA} = 13 Hz, J_{BX} = 3.2 Hz, SCH_2CH), 5.04 ppm (broad t, 1, J = 3.2 Hz, SCH_2CH); COOH proton not detectable; pmr (pyridine), δ 1.19 (s, 3, CH_3), 2.06 (A_2B_2, broad s, 4, CH_2CH_2), 2.57 (calcd) (d of d, 1, J_{AB} = 13 Hz, J_{AX} = 3.5 Hz, SCH_2), 3.18 (calcd) (d of d, 1, J_{BA} = 13 Hz, J_{BX} = 3 Hz, SCH_2CH), 5.06 (t, 1, J = 3 Hz, SCH_2CH), and 15.88 ppm (s, 1, COOH); uv (MeOH), 256 mμ (ϵ 8000). *Anal.*[51] ($C_9H_{11}NO_3S$) C, H, N, S.

(b) **With α-Toluenethiol.**—A solution of **5** (0.56 g, 5 mmol) and α-toluenethiol (0.60 g, 5 mmol) in dioxane (3 ml) was kept at 20° for 24 hr during which time no reaction (by pmr) had occurred. Et_3N (1 mg) in dioxane (0.5 ml) was then added, and further pmr spectra were recorded at hourly intervals. A peak at δ 5.25, corresponding to the vinylic proton of a Δ^2-unsaturated γ-lactone, appeared and gradually increased in intensity during 5 hr; it then declined and was replaced by peaks, at δ 6.10 and 7.45 ppm, of the vinylic protons of the benzylthio analog of the Δ^2-unsaturated lactone **16**.

Reaction of 3,3-Dimethyl-4-hydroxyhex-4-enoic Acid Lactone (4b) with Benzylamine.—Benzylamine (0.53 g, 5 mmol) in petroleum ether (bp 40–60°, 2 ml) was added slowly to a stirred solution of the lactone **4b** (0.704 g, 5 mmol) in petroleum ether (bp 40–60°, 6 ml) and Et_2O (2 ml). After 18 hr the precipitated crystalline mass was collected and was thoroughly dried *in vacuo* to give 1-benzyl-4,4-dimethyl-5-ethyl-5-hydroxypyrrolidin-2-one (1.12 g, 91%): mp 102–103°; ir ($CHCl_3$), 3600, 3400, and 1688 cm^{-1}. Attempts to recrystallize this material from a variety of solvents led to its contamination with increasing amounts of the dehydrated material, 1-benzyl-4,4-dimethyl-5-ethylidenepyrrolidin-2-one. Elemental analysis was therefore performed on the material which had precipitated from the reaction solution. *Anal.* ($C_{15}H_{21}NO_2$) C, H, N.

Lactone **4b** did not react with cysteine in aqueous solution at pH 7 or with methyl cysteinate in EtOH.

Reaction of Vinyl Acetate with L-Cysteine.—Vinyl acetate (1.72 g, 20 mmol) was added to a stirred solution of L-cysteine (2.4 g, 20 mmol) in H_2O (15 ml) at pH 7.[50] Dilute NaOH (20 mmol) was added periodically to maintain pH 6–7,[50] and the solution was allowed to stand overnight at room temperature. Acidification with concentrated HCl and isolation in the usual manner gave N-acetyl-L-cysteine (1.0 g, 30%), mp 108°.

Reaction of Vinylene Carbonate with L-Cysteine.—Vinylene carbonate (1.72 g, 20 mmol) was added to a stirred solution of L-cysteine (2.4 g, 20 mmol) in H_2O (15 ml) at pH 7.[50] An exothermic reaction occurred and carbon dioxide was evolved. After 3 hr the solution was evaporated; on treatment with 1 *N* HCl and with ethanol, the residual gum gave L-cysteine hydrochloride monohydrate (3.1 g, 90%), mp 175–185°.

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(51) The analytical sample was obtained *via* AcOH recrystallization followed by thorough drying.

Potential Anticancer Agents. IV. 5-Substituted Pyrimidine-6-carboxaldehydes and Derivatives[1]

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Various 5-substituted pyrimidine-6-carboxaldehydes and derivatives were synthesized and tested for inhibition of growth of the Ehrlich ascites carcinoma and inhibition of incorporation of L-phenylalanine-1-^{14}C and glycine-1-^{14}C into proteins and orotic acid-5-^{3}H, thymidine-2-^{14}C, and formate-^{14}C into nucleic acids of tumor cells *in vitro*. The following compounds were found to be particularly active as inhibitors: 2-mercapto-4-hydroxy-5-(4-chlorobenzyl)pyrimidine-6-carboxaldehyde (VII-3), 2-mercapto-4-hydroxy-5-(4-bromobenzyl)pyrimidine-6-carboxaldehyde (VII-6), 2-ethylthio-4-hydroxy-5-(4-chlorobenzyl)pyrimidine-6-carboxaldehyde (VII-16), and 2-ethylthio-4-hydroxy-5-(4-bromobenzyl)pyrimidine-6-carboxaldehyde (VII-17). The best compounds of this series are equally as effective as 5-fluorouracil (FU) in inhibiting formate incorporation into DNA and growth of the tumor. They are more effective than FU in inhibiting incorporation of formate and orotic acid into RNA, thymidine into DNA, and phenylalanine and glycine into proteins. Although these compounds inhibit incorporation of formate into DNA and RNA, they have only negligible inhibitory activity against the folate reductases. Smaller *in vivo* and *in vitro* inhibitions are obtained with the 5-bromopyrimidine-6-carboxaldehydes. The diamino-5-phenylpyrimidinealdehyde exhibits the least inhibitory activity against the Ehrlich ascites carcinoma cells *in vivo*, but it shows greater inhibition of folate reductases although considerably less than antifols such as aminopterin.

2-Mercapto-4-hydroxypyrimidine-6-carboxaldehydes,[3] 2-phthalimidoaldehydes,[4] 5-fluoropyrimidine-6-carboxaldehydes,[5] and their derivatives have been synthesized and tested as inhibitors of growth and protein synthesis in Ehrlich ascites carcinoma in mice. The 5-fluoropyrimidine-6-carboxaldehydes have shown strong inhibitory activity against incorporation of amino acids and formate into proteins as well as thymidine, orotic acid, and formate into nucleic acids.

Several derivatives of different types of aldehydes have also been found to inhibit the growth of neoplasms, for example, pyridine-2-carboxaldehyde thiosemicarbazone,[6] octadecylthiosemicarbazones of aldehydes,[7] indole-3-carboxaldehyde *p*-bromophenylhydrazone,[8] and 3-ethoxy-2-ketobutanal bisthiosemicarbazone.[9]

Antagonism to the utilization of folates was recognized a number of years ago as a general property of

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2,4-diaminopyrimidine derivatives.[10-12] The effective folate reductase inhibitors all seem to have in common the C–N–C–N sequence of the pyrimidine ring, two functional groups attached at the 2 and 4 positions, a bulky group in the 5 position, and only small groups in the 6 position.[13]

The functional groups at the 2 and 4 positions are involved in hydrogen bonding to the enzyme surface and contribute strongly to the binding in the order OH < SH < NH_2. Strong hydrophobic bonding to dihydrofolic reductase by alkyl and aralkyl groups attached to the pyrimidine 5 position has been discovered. It was proposed that an aryl group in this position also complexed to dihydrofolic reductase by hydrophobic bonding.[14] It was therefore of interest to synthesize 5-substituted pyrimidine-6-carboxaldehydes and derivatives in order to determine whether an enhanced inhibitory activity could be obtained in comparison with the previous[3,5] pyrimidine-6-carboxaldehydes, as well as determine whether these derivatives possess antifolic activity. The work described in this paper deals with the synthesis and biochemical studies of 5-substituted pyrimidine-6-carboxaldehydes and their derivatives.

Chemistry.—The α-benzyl-β-keto esters were prepared by the reaction of γ,γ-dimethoxyaceto acetate[3] with a benzyl halide in the presence of sodium ethoxide in 70% yield. They can also be synthesized from the ethyl β-phenylpropionic esters and methyl dimethoxyacetate by a modification of the method in the literature.[15]

Condensation of the β-keto esters with thiourea gave the acetal in 30% yield and with guanidine hydrochloride the yield was 45%. Hydrolysis of the acetal was accomplished in dioxane with 10% H_2SO_4 and resulted in a 60% yield of the pyrimidine-6-carboxaldehyde. However, VI-4 and VI-8 (compounds **4** and 8 in Table VI) were hydrolyzed very slowly to VII-4 and VII-8, respectively. The 6-hr hydrolysis of VI-4 resulted in only 22% yield of VII-4 and most of the reaction mixture was the starting acetal. These aldehydes contained a molecule of EtOH when recrystallized from EtOH–H_2O. This molecule of EtOH was removed by heating at 100° for several hours. Compounds VII-7, VII-12, VII-13, and VII-14 were each found to have a molecule of H_2O. Alkylation of the thiol was accomplished by treating 2-mercapto-6-(dimethoxymethyl)-4-pyrimidols with an alkyl halide in the presence of NaOH. Compounds VI-15 and VI-22 were prepared from the corresponding 2-mercaptopyrimidines by the reaction of alkaline H_2O_2. Compounds VII-19, VII-20, and VII-21 were prepared from the corresponding acetals by bromination with Br_2 in HOAc in 60% yield. α-(Dimethoxyacetyl)-*p*-chlorophenylacetonitrile was synthesized by the procedure of Russell and Hitchings[12] from *p*-chlorophenylacetonitrile and methyl dimethoxyacetate in 55.5% yield. The α-acylphenylacetonitrile was converted to enol ether by diazomethane and then treated with guanidine to give VI-21 in 55% yield. The acetals of 2-aminopyrimidine were hydrolyzed by suitable modification of the procedure of Baker and Jordaan.[16] Compound VI-20 was prepared by catalytic hydrogenation from VI-13. Compound VI-19 was prepared from VI-12 by formylation with 37% HCHO. The azlactones were prepared in the usual manner.

Screening Test.—The compounds were tested *vs.* the Ehrlich ascites carcinoma in Swiss–Webster white mice by a slight modification of procedures described previously.[17,18] 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 7–9 days and diluted with 0.9% NaCl to a cell concentration of 10% by volume based upon an initial ascitocrit determination. The 0.1-ml inoculum contained an average of 7×10^6 carcinoma cells. 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, each control mouse received an intraperitoneal injection of 0.2 ml of DMSO–0.9% NaCl (3:1) and each experimental mouse received a solution of the tested compound in DMSO–0.9% NaCl (3:1). The intraperitoneal injections of control and experimental mice were continued twice daily for 6 days (total eleven injections). On day 7 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 standard deviations. The results of tests of representative compounds are recorded in Table I. The results showed that almost all compounds had an inhibitory activity and very low toxicity. Compound VII-3 was the most active compound, and it also showed no lethal toxicity at relatively high dosage. It is of great interest that this compound was as effective as 5-fluorouracil against the Ehrlich carcinoma in this test system. Compounds VI-3 and VIII-3 showed activity only at relatively high dosage. Compound VII-19 was moderately effective. In general, 5-benzylpyrimidine-6-carboxaldehydes showed strong inhibitory activity, and their derivatives, such as acetals and azlactones, were less active than the corresponding aldehydes.

Inhibition of Protein Synthesis.—The effects of the pyrimidine derivatives upon protein synthesis were studied by determining the inhibition of incorporation of L-phenylalanine-1-^{14}C, glycine-1-^{14}C, and formate-^{14}C into the proteins of Ehrlich ascites carcinoma cells which were incubated aerobically for 1 hr with the labeled substrate and the pyrimidine *in vitro* in Krebs–Ringer phosphate buffer at 37 ± 1° by a procedure described previously in detail.[5] After the incubation, the total proteins were isolated and freed of lipids and nucleic acids. Each protein preparation was dissolved

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TABLE I

RESULTS OF SCREENING TESTS *vs.* THE EHRLICH ASCITES CARCINOMA[a]

Compd[b]	Dose, mg/kg/day	Mortality C	Mortality T	Av wt change T/C, g	Av TPCV T, C, ml	Av TPCV Std dev T, ± ml	Av TPCV T as % of C
VI-3	118.8	0/8	1/8	3.4/1.0	0.4/1.6	0.15	25
VII-3	30.0	0/8	0/8	3.4/8.1	0.3/2.4	0.10	12
	48.2	0/8	0/8	3.1/9.1	0.1/3.0	0.23	3
	90.0	0/8	0/8	0.2/7.7	0.1/2.5	0.70	4
VII-6	36.3	0/8	0/8	2.7/8.1	0.5/2.4	0.18	21
	52.6	0/8	0/8	1.1/9.1	0.4/3.0	0.49	15
VII-9	79.6	1/8	1/8	2.4/8.2	0.15/2.0	0.21	7
VII-11	26.4	0/8	0/8	7.0/8.1	1.4/2.4	0.36	58
	119.5	3/8	2/8	4.8/1.0	0.3/1.6	0.24	18
VII-12	91.6	0/8	0/8	1.1/7.7	0.4/2.5	0.67	16
VII-17	48.2	0/8	0/8	0.6/9.1	1.1/3.0	0.33	37
VII-19	30.5	0/8	0/8	0.1/8.1	1.4/2.4	0.24	58
	67.1	0/8	0/8	5.3/9.1	0.9/3.0	0.48	30
VIII-3	125.7	3/8	2/8	1.7/1.0	0.2/1.6	0.08	12
FU[c]	27.5	0/8	0/8	−0.3/5.3	0.15/1.5	0.10	10

[a] T = treated group, C = controls, TPCV = total packed-cell volume of tumor cells on final day of assay. The average standard deviation for TPCV of all control groups was ±0.50 ml. [b] In addition to the above results, VII-2 and VIII-4 showed 50% inhibition at 50–60-mg/kg/day dose; VI-15, VII-8, VII-15, and VII-16, 40–60% inhibition at 80–90 mg/kg/day; and VII-5 and VII-10, 90% inhibition at 90–120 mg/kg/day. [c] FU = 5-fluorouracil.

in 2.0 ml of 0.3 *N* NaOH, and 0.2 ml of the protein solution was transferred to a glass scintillation vial. To each vial 0.2 ml of Hyamine Hydroxide 10-X and 16 ml of POPOP–PPO Triton X-100[19] scintillation fluid were added for determination of radioactivity in a Packard liquid scintillation spectrometer, Model 3003.

The effects of the pyrimidine analogs upon incorporation of L-phenylalanine-1-^{14}C, glycine-1-^{14}C, and formate-^{14}C into proteins of the carcinoma cells are recorded in Tables II and IV. Compounds VII-3, VII-6, VII-16, VII-17, and VII-20 completely inhibited the incorporation of L-phenylalanine-1-^{14}C and glycine-1-^{14}C into proteins at concentrations of 0.83–0.96 m*M*. Compound VII-3 (Figure 1) inhibited incorporation of L-phenylalanine-1-^{14}C into proteins by 50 and 90% at a concentration of 0.2 m*M* and 0.57 m*M*, respectively. This compound lost the inhibitory activity at 0.09 m*M*.

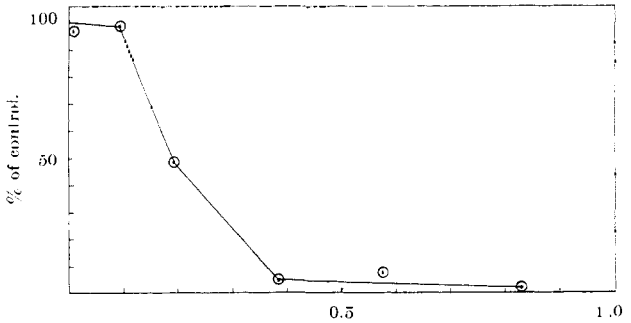

Figure 1.—Effects of VII-3 on incorporation of L-phenylalanine-1-^{14}C into proteins of Ehrlich ascites carcinoma cells *in vitro* (L-phenylalanine-1-^{14}C: 0.1 μmole/ml, 0.2 μCi).

The above analogs also completely inhibited the incorporation of formate-^{14}C into proteins at a concentration of 1.54 m*M*. Since VII-3, VII-6, VII-16, and VII-17 were more active than VII-1, VII-7, and VII-14, *p*-halogen substitution of the benzyl group on the pyrimidine 5 position seemed to be important for enhanced inhibitory activity.

Compound VII-18 showed low activity against protein synthesis (Table II), but it was the most active of the new compounds in inhibition of the folate reductases although far less active than the accepted antifols (Table V). Compound VII-20 was the most active inhibitor of protein synthesis among the 5-bromopyrimidine-6-carboxaldehydes. Each of the active compounds of Table II was found to have greater inhibitory

(19) In 1 l. of toluene 5 g of 2,5-diphenyloxazole (PPO) and 0.3 g of 1,4-bis(4-methyl-5-phenyloxazolyl)benzene (dimethyl-POPOP) were dissolved. Two liters of this POPOP–PPO solution and 1 l. of Triton X-100 were mixed.

TABLE II

EFFECTS OF PYRIMIDINE ANALOGS ON INCORPORATION OF L-PHENYLALANINE-1-^{14}C AND GLYCINE-1-^{14}C INTO PROTEINS OF EHRLICH ASCITES CARCINOMA CELLS

Compd	Concn of analog, m*M*	Av expt'l values (% of controls) Protein L-Phe-1-^{14}C[a]	Protein Gly-1-^{14}C[b]
None	0	100[c]	100[d]
VI-21	0.96	...	49
VII-1	0.48	15	...
	0.96	4	5
VII-3	0.57	8	...
	0.83	2	1
VII-6	0.83	4	2
VII-7	0.96	71	93
VII-12	0.96	13	8
VII-14	0.83	100	...
VII-16	0.96	1	4
VII-17	0.96	1	1
VII-18	0.96	61	87
VII-19	0.83	38	58
VII-20	0.83	10	5
VIII-4	0.83	82	...
	0.96	...	24
FOA[e]	0.56	82	80
	1.66	26	10
FU[f]	0.68	87	87
	2.0	81	91

[a] 0.1 μmole/ml (0.2 μCi). [b] 0.1 μmole/ml (0.2 μCi). [c] 1.122 × 10^3 cpm/mg. [d] 2.317 × 10^2 cpm/mg. [e] FOA = 5-fluoroorotic aldehyde; see ref 5. [f] FU = 5-fluorouracil.

activity against protein synthesis than 5-fluorouracil or the 5-fluoroorotic aldehydes previously reported.[5]

Inhibition of Nucleic Acid Synthesis.—The effects of the pyrimidine derivatives upon nucleic acid synthesis were studied by determining the inhibition of incorporation of orotic acid-5-^{3}H and formate-^{14}C into RNA and thymidine-2-^{14}C and formate-^{14}C into DNA of Ehrlich ascites carcinoma cells. These cells were incubated aerobically for 1 hr with the labeled substrate and the pyrimidine derivative *in vitro* in Krebs–Ringer phosphate buffer at 37 ± 1° by a procedure described previously in detail.[5]

a. Incorporation of Orotic Acid-5-^{3}H and Thymidine-2^{14}C into the Nucleic Acids.—Each incubation flask contained 5 ml of a 20% suspension of tumor cells in Krebs–Ringer phosphate buffer (pH 7.1–7.2), 1 ml of Krebs–Ringer phosphate buffer containing orotic acid-5-^{3}H (0.04 μmole/ml, 10 μCi), thymidine-2-^{14}C (0.1 μmole/ml, 1 μCi), glucose (5 mg/ml), and 0.2 ml of the pyrimidine in DMSO. After incubation, the acid-insoluble residue was obtained and washed as previously.[5] The acid-insoluble residue then was suspended in 5 ml of 0.5 *N* $HClO_4$ and heated for 30 min at 95°. At the end of heating the tubes were centrifuged, and the supernatant was neutralized with KOH. To each glass scintillation vial 0.2 ml of the neutralized solution, 0.2 ml of Hyamine Hydroxide 10-X, and 16 ml of the scintillation fluid were added for determining radioactivity. The radioactivities of ^{14}C and ^{3}H were determined according to Kabara, *et al.*,[20] and the exact channel ratios of ^{14}C and ^{3}H were determined as previously.[5] The concentration of RNA was determined by the orcinol reaction and corrected for the interference by DNA, and the amount of DNA was determined by the diphenylamine reaction.[21] The specific activities were calculated as cpm (^{3}H)/mg of RNA and cpm (^{14}C)/mg of DNA. The effects of the pyrimidine analogs upon incorporation of orotic acid-5-^{3}H into RNA and thymidine-2-^{14}C into DNA of the carcinoma cells are recorded in Table III. Compounds VII-3, VII-6, VII-17, and VII-20 showed only weak or no inhibitory activity at a concentration of 0.83 m*M*, but about twofold increase of the concentration (1.92 m*M*) of analogs showed complete inhibition of incorporation of thymidine-2-^{14}C into DNA-thymine. Similar results have also been obtained in previous work with 5-fluoropyrimidine-6-carboxaldehydes.[5] Compounds VII-3, VII-6, VII-17, and VII-20 showed 80–90% inhibition of incorporation of orotic acid-5-^{3}H into RNA at relatively high concentration (1.92 m*M*) of the analogs.

b. Incorporation of Formate-^{14}C into the Nucleic Acids.—Each incubation flask contained 5 ml of a 40% suspension of tumor cells in Krebs–Ringer phosphate buffer, 1 ml of glucose in KRP (3 mg/ml), 1 ml of formate-^{14}C in KRP (0.318 μmole/ml, 8 μCi), and 0.2 ml of the pyrimidine in DMSO. After incubation, nuc eic acids were isolated as sodium nucleates. The proteins were also isolated and used for inhibition stud es of protein synthesis. RNA was separated from DNA by hydrolyzing with 0.3 *N* KOH at 37° for 16–18 hr. After purification, the radioactivities were counted for each nucleic acid by a procedure described previously.[5]

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TABLE III

EFFECTS OF PYRIMIDINE ANALOGS ON INCORPORATION OF THYMIDINE-2-^{14}C AND OROTIC ACID-5-^{3}H INTO NUCLEIC ACIDS OF EHRLICH ASCITES CARCINOMA CELLS[a]

Compd	Concn of analog, m*M*	Av exptl values (% of control) DNA	RNA
None	0	100[b]	100[c]
VII-1	0.83	70	...
	1.92	20	80
VII-3	0.83	76	...
	1.92	0.5	13
VII-6	0.83	68	...
	1.92	0.7	16
VII-7	1.92	130	54
VII-17	0.83	88	...
	1.92	0.2	10
VII-19	0.83	100	...
	1.92	12	189
VII-20	0.83	92	...
	1.92	1	21
FOA[d]	0.83	87	...
	1.72	7	...
FU[e]	0.57	...	90
	1.60	330	...

[a] Thymidine-2-^{14}C: 0.1 μmole/ml (1 μCi), orotic acid-5-^{3}H: 0.04 μmole/ml (10 μCi). [b] 3.26 × 10^5 cpm/mg. [c] 1.43 × 10^4 cpm/mg. [d] FOA = 5-fluoroorotic aldehyde; see ref 5. [e] FU = 5-fluorouracil; data from ref 5.

The effects of the pyrimidine analogs upon incorporation of formate-^{14}C into the nucleic acids of carcinoma cells are recorded in Table IV.

TABLE IV

EFFECTS OF PYRIMIDINE ANALOGS ON INCORPORATION OF FORMATE-^{14}C INTO NUCLEIC ACIDS AND PROTEINS OF EHRLICH ASCITES CARCINOMA CELLS[a]

Compd	Concn of analog, m*M*	Av exptl values (% of control) RNA	DNA	Protein
None	0	100[b]	100[c]	100[d]
VII-3	0.77	4.4	5.3	2.2
	1.54	0.4	0.09	0.2
VII-6	0.77	6.7	12.1	4.5
	1.54	0.7	2	0.6
VII-16	0.77	7.8	4.4	3.5
	1.54	0.3	0.07	0.1
VII-17	0.77	7.4	4.5	3.4
	1.54	0.2	0.06	0.1
VII-18	0.77	90	77.6	71.5
	1.54	41	52	38
VII-19	0.77	53.5	30.6	38
	1.54	8	7	13
VII-20	0.77	30	22.3	8.7
	1.54	2	1	2
FOA[e]	1.7	14.8	4	38
FU[f]	0.64	126	7	...

[a] Formate-^{14}C: 0.318 μmole/ml (8 μCi). [b] 1.57 × 10^4 cpm/mg of RNA. [c] 2.26 × 10^4 cpm/mg of DNA. [d] 1.88 × 10^4 cpm/mg. [e] FOA = 5-fluoroorotic aldehyde; see ref 5. [f] FU = 5-fluorouracil; data from ref 5.

Compounds VII-3, VII-6, VII-16, and VII-17 showed almost complete inhibition of incorporation of formate into DNA and RNA at a concentration of 0.77 m*M*, and thus they are more than twice as active as 5-fluoroorotic aldehyde previously reported.[5] These compounds are practically identical in activity with FU in inhibiting formate incorporation into DNA, but they are more

active than FU in inhibiting formate incorporation into RNA. Compounds VII-18, VII-19, and VII-20 are considerably less active than compounds VII-3, VII-6, VII-16, and VII-17 in inhibiting formate incorporation into RNA, DNA, and proteins.

Inhibition of Folate Reductase.—The effects of the pyrimidine derivatives upon folate reductases of Ehrlich ascites carcinoma and *Escherichia coli* were studied by determining the inhibition of reduction of folic acid or dihydrofolic acid to tetrahydrofolic acid.

a. Ehrlich Ascites Carcinoma Cells.—The Ehrlich ascites carcinoma cells were washed with cold 0.9% NaCl and suspended in three volumes of cold 0.01 *M* Tris–HCl, pH 7.4. The cells were disrupted for 1 min by sonic vibration (Branson Sonifier, Branson Instruments, Inc., Stamford, Conn.). The high-speed supernatant fraction (S_3) was obtained by centrifuging the disrupted cells for 1 hr at 105,000*g*. The supernatant was stored at −15°.

Assay of Folic Reductase Activity.—The reaction mixtures of Roberts and Hall[22] were adopted. The complete system contained in a total of 0.5 ml, 20 μmoles of 3,3-dimethyl glutarate, pH 6.1, 2 μmoles of $MgCl_2$, 2 μmoles of citrate, 46 mμmoles of NADPH, 36 mμmoles of folic acid, 25 μl of DMSO or 25 μl of drug solution in DMSO, and 0.1 ml of S_3 fraction (1.8 mg of protein). After incubation at 37° for 10 min the mixture was deproteinized with 0.5 ml of 15% trichloroacetic acid,[23] and the supernatant fluid was assayed for the appearance of diazotizable *p*-aminobenzoylglutamate[24] from the quantitative hydrolysis of tetrahydrofolate.[25]

b. *E. coli.*—*E. coli* B, grown in phosphate-buffered medium, was purchased from Grain Processing Corp., Muscatine, Iowa. The cells were washed twice with 0.01 *M* potassium phosphate buffer, pH 7.0, containing 1 m*M* EDTA, and suspended in 3 vol of buffer. $(NH_4)_2SO_4$ fraction (55–95%) was obtained according to the procedure of Burchall and Hitchings[26] and dialyzed for 3 hr against 100 vol of 0.001 *M* potassium phosphate buffer pH 7.0 containing 1 m*M* EDTA. The dialyzed fraction was diluted ten times and stored at −15°.

Assay of Dihydrofolate Reductase Activity.—The reaction mixture (0.5 ml) contained 20 μmoles of potassium phosphate buffer, pH 7.0, 20 μmoles of 2-mercaptoethanol, 46 mμmoles of NADPH, 30 mμmoles of dihydrofolic acid, 25 μl of DMSO or 25 μl of drug solution of DMSO, and 0.1 ml of the diluted 55–95% $(NH_4)_2SO_4$ fraction (0.18 mg of protein). The mixture was incubated for 30 min at 37°, and the amount of the diazotizable amine released from tetrahydrofolate was measured as before. Dihydrofolic acid was prepared according to the method of Futterman,[27] modified by Blakley.[28]

The data of Table V clearly demonstrate that the pyrimidine-6-carboxaldehyde derivatives reported here are essentially inactive as folate reductase inhibitors except at concentrations 1000–100,000 times greater than inhibitory concentrations of the accepted antifols such as aminopterin, pyrimethamine, and trimethoprim. Also, it can be concluded from a comparison of the data in Tables IV and V that the inhibition of incorporation of formate into DNA and RNA by compounds VII-3, VII-6, VII-16, and VII-17 cannot be attributed to inhibition of the folate reductases. In this connection also it should be pointed out that VII-18 is a stronger inhibitor of folate reductases than VII-3 and VII-6, but the latter compounds are more effective than VII-18 in inhibiting incorporation of formate into DNA and RNA.

TABLE V

EFFECTS OF PYRIMIDINE ANALOGS ON FOLATE REDUCTASES OF EHRLICH ASCITES CARCINOMA CELLS AND *E. coli*

Compd	Concn of analog, m*M*	% of inhib[a]	
		Ehrlich	*E. coli*
VI-3	1	50	20
VI-4	0.1	40	20
	1	67	43
VI-17	1	20	25
VI-18	1	30	0
VI-21	0.1	45	35
	1	80	57
VII-3	0.1	14	
	1	46	0
VII-6	0.1	20	
	1	55	23
VII-10	1	60	30
VII-12	0.1	0	15
	1	70	45
VII-16	1	0	5
VII-17	1	0	0
VII-18	0.1	54	30
	1	80	70
VII-19	1	0	10
VIII-4	1	30	0
FOA[b]	1	0	0
Aminopterin	3.3×10^{-5}	50	100
Pyrimethamine	4×10^{-3}	50	0
	2×10^{-3}	100	50
Trimethoprim	7×10^{-6}	0	50
	1.1×10^{-2}	50	100

[a] VI-8, VII-1, VII-2, VII-5, and VII-7 also showed 40–50% inhibition at 1 m*M* against the reductase of tumor cells and 20–40% inhibition against the reductase of *E. coli* at the same concentration. [b] FOA = 5-fluoroorotic acid.

Experimental Section[29]

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 VI–VIII).

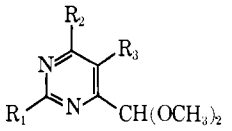
Ethyl α-Benzyl-γ,γ-dimethoxyacetoacetate.—To 11.5 g (0.5 g-atom) of Na in 250 ml of absolute EtOH, 95 g (0.5 mole) of ethyl γ,γ-dimethoxyacetoacetate[3] and 64 g (0.505 mole) of benzyl chloride were added, and the brown solution was refluxed gently with magnetic stirring for 9 hr during which time NaCl separated. The neutral reaction mixture was filtered and the NaCl was washed with absolute EtOH. The combined filtrate was dried ($MgSO_4$) and the EtOH was spin evaporated *in vacuo*. Subsequent fractionation gave a nearly colorless liquid which distilled at 122–124° (0.3 mm), yield 75 g (54%), $n^{20}D$ 1.4945. *Anal.* ($C_{15}H_{20}O_5$) C, H.

For the most part the α-substituted β-keto esters were prepared

(22) D. Roberts and T. C. Hall, *Cancer Res.*, **25**, 1894 (1965).
(23) W. C. Werkheiser, S. F. Zakrzewski, and C. A. Niehol, *J. Pharmacol. Exptl. Therap.*, **137**, 162 (1962).
(24) A. C. Bratton and E. K. Marshall, *J. Biol. Chem.*, **128**, 537 (1939).
(25) R. L. Blakley, *Biochem. J.*, **65**, 331 (1957).
(26) J. J. Burchall and G. H. Hitchings, *Mol. Pharmacol.*, **1**, 126 (1965).
(27) S. Futterman, *J. Biol. Chem.*, **228**, 1031 (1957).
(28) R. L. Blakley, *Nature*, **188**, 231 (1960).

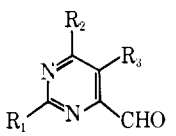
(29) Analyses were performed by M-H-W Laboratories, Garden City, Mich. 48135, and Spang Microanalytical Laboratory, Ann Arbor, Mich. 48106. Where analyses are indicated only by symbols of the elements analytical results obtained for those elements were within ±0.4% of the theoretical values. The melting points were determined with the Mel-Temp apparatus and have been corrected.

TABLE VI
6-(DIMETHOXYMETHYL)-5-SUBSTITUTED PYRIMIDINES



No.	R_1	R_2	R_3	Yield, %	Mp, °C	Formula	Analyses
1	SH	OH	$CH_2C_6H_5$	30	161–162	$C_{14}H_{16}N_2O_3S$	C, H, N, S
2	SH	OH	$CH_2C_6H_4F$-4	23.5	160–161	$C_{14}H_{15}FN_2O_3S$	C, H, N
3	SH	OH	$CH_2C_6H_4Cl$-4	29	195–197	$C_{14}H_{15}ClN_2O_3S$	C, H, N, S, Cl
4	SH	OH	$CH_2C_6H_4Cl$-2	13.5	156–158	$C_{14}H_{15}ClN_2O_3S$	C, H
5	SH	OH	$CH_2C_6H_4Cl$-3	25.2	141–143	$C_{14}H_{15}ClN_2O_3S$	C, H
6	SH	OH	$CH_2C_6H_4Br$-4	32	199–201	$C_{14}H_{15}BrN_2O_3S$	C, H, N, S, Br
7	SH	OH	$CH_2C_6H_4NO_2$-4	30	227–228	$C_{14}H_{15}N_3O_5S$	C, H, N, S
8	SH	OH	$CH_2C_6H_3Cl_2$-2,6	11.1	234–236	$C_{14}H_{14}Cl_2N_2O_3S$	C, H
9	SH	OH	$CH_2C_6H_3(CH_3)_2$-3,4	25.5	137–139	$C_{16}H_{20}N_2O_3S$	C, H
10	SH	OH	$CH_2(\alpha)C_{10}H_7$	24.1	223–225	$C_{18}H_{18}N_2O_3S$	C, H
11	NH_2	OH	$CH_2C_6H_5$	50	233-235 dec	$C_{14}H_{17}N_3O_3$	C, H, N
12	NH_2	OH	$CH_2C_6H_4Cl$-4	45.5	239–241	$C_{14}H_{16}ClN_3O_3$	C, H, N
13	NH_2	OH	$CH_2C_6H_4NO_2$-4	45	249–250 dec	$C_{14}H_{16}N_4O_5$	C, H, N
14	OH	OH	$CH_2C_6H_4NO_2$-4	70	247–248	$C_{14}H_{15}N_3O_6$	C, H, N
15	OH	OH	$CH_2C_6H_4Cl$-4	100	233–235	$C_{14}H_{15}ClN_2O_4$	C, H
16	C_2H_5S	OH	$CH_2C_6H_5$	94	97–98	$C_{16}H_{20}N_2O_3S$	C, H
17	C_2H_5S	OH	$CH_2C_6H_4Cl$-4	100	123–124	$C_{16}H_{19}ClN_2O_3S$	C, H
18	C_2H_5S	OH	$CH_2C_6H_4Br$-4	100	121–122	$C_{16}H_{19}BrN_2O_3S$	C, H
19	$(HOCH_2)_2N$	OH	$CH_2C_6H_4Cl$-4	28	70–75	$C_{18}H_{20}ClN_3O_5$	C, H
20	CH_3CONH	OH	$CH_2C_6H_4NH_2$-4	81	164–165	$C_{16}H_{20}N_4O_4$	C, H
21	NH_2	NH_2	C_6H_5Cl-4	55	261–263	$C_{13}H_{13}ClN_4O_2$	C, H
22	OH	OH	H	65.5	182–183	$C_7H_{10}N_2O_4$	C, H
23	C_2H_5S	OH	H	65	121–122	$C_9H_{14}N_2O_3S$	C, H
24	CH_3S	OH	H	94	154–155	$C_8H_{12}N_2O_3S$	C, H

TABLE VII
5-SUBSTITUTED PYRIMIDINE-6-CARBOXALDEHYDES



No.	R_1	R_2	R_3	Yield, %	Mp, °C	Formula	Analyses
1	SH	OH	$CH_2C_6H_5$	95	180–181	$C_{12}H_{10}N_2O_2S$	C, H, N, S
2	SH	OH	$CH_2C_6H_4F$-4	62.5	198–199	$C_{12}H_9FN_2O_2S$	C, H
3	SH	OH	$CH_2C_6H_4Cl$-4	62	210–211	$C_{12}H_9ClN_2O_2S$	C, H, N, S, Cl
4	SH	OH	$CH_2C_6H_4Cl$-2	55.8	202–203	$C_{12}H_9ClN_2O_2S$	C, H
5	SH	OH	$CH_2C_6H_4Cl$-3	42.5	205–210	$C_{12}H_9ClN_2O_2S$	C, H
6	SH	OH	$CH_2C_6H_4Br$-4	61.8	217–219	$C_{12}H_9BrN_2O_2S$	C, H, N, S, Br
7	SH	OH	$CH_2C_6H_4NO_2$-4	60	218–220	$C_{12}H_9N_3O_4S \cdot H_2O$	C, H, N, S
8	SH	OH	$CH_2C_6H_3Cl_2$-2,6	57.5	215–217	$C_{12}H_8Cl_2N_2O_2S$	C, H
9	SH	OH	$CH_2C_6H_3(CH_3)_2$-3,4	72	140–145	$C_{14}H_{14}N_2O_2S$	C, H
10	SH	OH	$CH_2(\alpha)C_{10}H_7$	76	199–205	$C_{16}H_{12}N_2O_2S$	C, H
11	NH_2	OH	$CH_2C_6H_5$	41.2	>300 dec	$C_{12}H_{11}N_3O_2$	C, H
12	NH_2	OH	$CH_2C_6H_4Cl$-4	67	>300 dec	$C_{12}H_{10}ClN_3O_2 \cdot H_2O$	C, H
13	NH_2	OH	$CH_2C_6H_4NO_2$-4	92.5	>300 dec	$C_{12}H_{10}N_4O_4 \cdot H_2O$	C, H, N
14	NH_2	OH	$CH_2C_6H_4NH_2$-4	86	>300 dec	$C_{12}H_{12}N_4O_2 \cdot H_2O$	C, H
15	C_2H_5S	OH	$CH_2C_6H_5$	47	151–154	$C_{14}H_{14}N_2O_2S$	C, H
16	C_2H_5S	OH	$CH_2C_6H_4Cl$-4	57.5	187–188	$C_{14}H_{13}ClN_2O_2S$	C, H
17	C_2H_5S	OH	$CH_2C_6H_4Br$-4	46.5	193–194	$C_{14}H_{13}BrN_2O_2S$	C, H
18[a]	NH_2	NH_2	C_6H_5Cl-4	89.5	>300 dec	$C_{11}H_9ClN_4O$	C, H
19	OH	OH	Br	63.5	268–270 dec	$C_5H_3BrN_2O_3$	C, H, N, Br
20	C_2H_5S	OH	Br	60	183–185	$C_7H_7BrN_2OS$	C, H
21	CH_3S	OH	Br	61	231–232 dec	$C_6H_5BrN_2OS$	C, H
22	OH	OH	$CH_2C_6H_4Cl$-4	80	236–237	$C_{12}H_9ClN_2O_3$	C, H

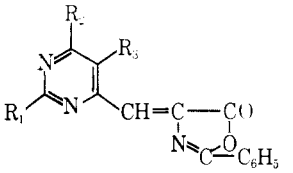
[a] This compound was reported by B. R. Baker and J. H. Jordaan, *J. Heterocyclic Chem.*, **4**, 31 (1967).

in an analogous manner. Because of extensive decomposition during fractionation, the crude esters were used for the next step.

2-Mercapto-6-(dimethoxymethyl)-5-benzyl-4-pyrimidol (VI-1).—To 5.34 g (0.232 g-atom) of Na in 150 ml of absolute EtOH, 65 g (0.232 mole) of ethyl α-benzyl-γ,γ-dimethoxyacetoacetate and 17.6 g (0.232 mole) of thiourea were added, and the mixture was refluxed with stirring for 9 hr. The solution was evaporated to dryness and the gummy residue was dissolved in H_2O (600 ml). The aqueous solution was extracted with two 200-ml portions of ether and acidified with 10% aqueous HCl. The

TABLE VIII

4-(6-PYRIMIDYLMETHYLIDENE)-2-PHENYL-5-OXAZOLONES



No.	R_1	R_2	R_3	Yield, %	Mp, °C dec	Formula	Analyses
1	SH	OH	$CH_2C_6H_5$	23.4	310–311	$C_{21}H_{15}N_3O_3S$	C, H
2	SH	OH	$CH_2C_6H_4Cl$-4	30.7	311–312	$C_{21}H_{14}ClN_3O_3S$	C, H, N
3	SH	OH	$CH_2C_6H_4Br$-4	12.3	315–316	$C_{21}H_{14}BrN_3O_3S$	C, H, N, S
4	OH	OH	Br	66.3	352–353	$C_{14}H_8BrN_3O_4$	C, H

product precipitated gradually in an ice bath, was collected on a filter, washed (Et_2O), and then recrystallized (EtOH); yield, 20 g (30%) of white crystals, mp 161–162°. Table VI lists the compounds prepared by a similar procedure.

2-Mercapto-4-hydroxy-5-benzylpyrimidine-6-carboxaldehyde (VII-1).—A mixture of 5 g (0.017 mole) of VI-1, 30 ml of 10% H_2SO_4, and 50 ml of dioxane was refluxed for 30 min. The cooled yellow solution was diluted with EtOH–H_2O (1:3) to turbidity. After being cooled at 0° for several hours, the precipitate was filtered, washed (cold H_2O), and dried. The product was recrystallized from EtOH–H_2O (2:1) and dried at 100°; yield, 4 g (95%) of yellow crystals, mp 180–181°. Table VII lists the compounds prepared by an analogous procedure. However, VII-4 and VII-8 needed 6–8 hr for hydrolysis, and VII-15, VII-16, and VII-17 were hydrolyzed for a 5-min period because longer reaction time liberated mercaptan.

2-Amino-6-(dimethoxymethyl)-5-(4-chlorobenzyl)-4-pyrimidol (VI-12).—A mixture of 67 g (about 0.213 mole) of crude ethyl α-(4-chlorobenzyl)-γ,γ-dimethoxyacetoacetate and a solution of guanidine [from 20.4 g (0.213 mole) of guanidine hydrochloride and 4.9 g (0.213 g-atom) of Na in 250 ml of EtOH] was refluxed gently with stirring for 6 hr. The solution was evaporated to dryness and 500 ml of H_2O added. The mixture was acidified with HOAc. After cooling at 0° for several hours, the gummy precipitate was filtered and washed with cold EtOH and ether. The product was recrystallized from EtOH; yield, 30 g (45.5%) of white crystals, mp 239–241° dec. Table VI lists the compounds prepared by a similar procedure.

2-Amino-4-hydroxy-5-(4-chlorobenzyl)pyrimidine-6-carboxaldehyde (VII-12) was prepared by suitable modification of the known procedure[16] from VI-12. A mixture of 5.27 g (17 mmoles) of VI-12, 50 ml of dioxane, and 30 ml of 2 *N* HCl was refluxed for 1 hr. The solution was spin evaporated *in vacuo* leaving the HCl salt of VII-12. The residue was triturated with 25 ml of cold H_2O, then dissolved in warm 2-methoxyethanol. The solution was decolorized with charcoal, then poured into 100 ml of saturated aqueous $NaHCO_3$. The pale yellow powder was collected on a filter and washed (H_2O). Recrystallization with EtOH–H_2O (1:1) and drying at 100° gave 3 g (67%) of yellow crystals, mp >300° dec. Table VII lists the compounds prepared by a similar procedure.

2-Ethylthio-6-(dimethoxymethyl)-5-benzyl-4-pyrimidol (VI-16).—To a cooled solution of 2.93 g (10 mmoles) of VI-1 in NaOH [0.43 g (10.7 mmoles) of NaOH in 15 ml of H_2O] and 30 ml of EtOH, 0.8 ml (1.16 g, 10.7 mmoles) of EtBr was added and heated gently at 60–70° for 1 hr. After cooling at 0° for 2 hr, the precipitate was filtered and washed with cold H_2O. The filtrate was evaporated to small volume to yield additional product. The product was recrystallized from EtOH; yield, 3 g (94%) of white crystals, mp 97–98°. Table VI lists the compounds prepared by a similar procedure.

2,4-Dihydroxy-6-(dimethoxymethyl)-5-(4-chlorobenzyl)pyrimidine (VI-15).—To a solution of 3.27 g (10 mmoles) of VI-3 in 6.6 ml of 4 *N* NaOH, 4 ml of 30% H_2O_2 (35 mmoles) in 4 ml of H_2O was added cautiously with stirring at room temperature. When all the H_2O_2 was added, the reaction mixture was heated on a steam bath for 3 min to remove the excess H_2O_2. The cooled solution was acidified cautiously with concentrated HCl and cooled at 0° for 2 hr, resulting in white crystals. The product was filtered and washed (cold H_2O). It was recrystallized from EtOH–H_2O (1:1); yield, 2.64 g (100%) of product, mp 233–235°.

2,4-Dihydroxy-5-bromopyrimidine-6-carboxaldehyde (VII-19).—To a solution of 16 g (86 mmoles) of VI-22 in 200 ml of HOAc 6.7 ml (20.8 g, 0.13 mole) of Br_2 in 15 ml of HOAc was added dropwise with stirring at the room temperature. After adding Br_2, the mixture was heated at 40° for 5 min. The white product that started to precipitate was stirred for 1 hr at room temperature. After cooling at 0° for 1 hr, the precipitate was filtered and washed with cold H_2O. The product was recrystallized from EtOH; yield, 12 g (63.5%) of white crystals, mp 268–270° dec. Table VII lists the compounds prepared in this manner.

α-(Dimethoxyacetyl)-*p*-chlorophenylacetonitrile was prepared by known procedure[12] from *p*-chlorophenylacetonitrile and methyl dimethoxyacetate; yield, 55.5% of white crystals, mp 100–101°. *Anal.* ($C_{12}H_{12}NO_3Cl$) C, H.

2,4-Diamino-6-(dimethoxymethyl)-5-(4-chlorophenyl)pyrimidine (VI-21).—To a solution of 6.4 g (25 mmoles) of α-(dimethoxyacetyl)-*p*-chlorophenylacetonitrile in Et_2O (50 ml), CH_2N_2 [from 5 g of nitrosomethylurea in ether (100 ml) and 40% KOH (30 ml)] was added. N_2 gas was evolved at once. After standing overnight the ether and excess CH_2N_2 evaporated. The residue was dissolved in 25 ml of EtOH and a solution of guanidine [from 2.38 g (25 mmoles) of guanidine hydrochloride and 0.575 g (25 g-atoms) of Na in 30 ml of EtOH] was added. The solution was refluxed with stirring for 3 hr. The EtOH was removed by a flash evaporator. The residue was then dissolved in 30 ml of HOAc, decolorized with charcoal, and neutralized with 4 *N* NaOH resulting in a precipitate, which was filtered and washed with cold H_2O. The product was recrystallized from EtOH; yield, 4 g (55%) of white crystals, mp 261–263°.

2-Acetamido-6-dimethoxymethyl-5-(4-aminobenzyl)-4-pyrimidol (VI-20).—A stirred suspension of 5 g (13.8 mmoles) of 2-acetanido-6-dimethoxymethyl-5-(4-nitrobenzyl)-4-pyrimidol (from acetylation of VI-13 by Ac_2O), 0.25 g of 10% Pd–C, and 50 ml of MeOH was hydrogenated at 20–25° by using a Parr catalytic hydrogenation apparatus. The theoretical amount of H_2 was absorbed after 30 min. The suspension was filtered, and the filtrate was evaporated *in vacuo* to leave 4.5 g (98%) of white crystals. The residue was recrystallized (EtOAc); yield, 3.7 g (81%) of white crystals, mp 164–165°.

2-Bis(hydroxymethyl)amino-6-(dimethoxymethyl)-5-(4-chlorobenzyl)-4-pyrimidol (VI-19) was prepared by a known procedure[30] from VI-12 by refluxing with 37% formalin; yield 28% of white crystals, mp 70–75°.

4-(2,4-Dihydroxy-5-bromo-6-pyrimidylmethylidene)-2-phenyl-5-oxazolone (VIII-4).—A mixture of 2.19 g (10 mmoles) of VII-19, 1.92 g (10.7 mmoles) of hippuric acid, and 0.8 g of NaOAc in 40 ml of Ac_2O was heated with stirring at 90–95° for 2 hr. After cooling to 20°, the excess Ac_2O was decomposed by the addition of 80 ml of H_2O with shaking. The mixture was cooled at 0° for 2 hr. The azlactone was filtered and washed with cold EtOH. The product was recrystallized from 5% NaOH followed by neutralizing with 10% HCl; yield, 2.4 g (66.3%) of yellow crystals, mp 352–353° dec. Table VIII lists the compounds prepared by a similar procedure.

Discussion

Difficulty in the hydrolysis of the acetal group of 5-benzylpyrimidines was due to insolubility of the ace-

(30) K. Miura, M. Ikeda, T. Oohashi, Y. Igarashi, and I. Okada, *Chem. Pharm. Bull.* (Tokyo), **13**, 529 (1965).

tals in the acid solution. Addition of dioxane made this hydrolysis reaction proceed smoothly. However, slow hydrolysis of VI-4 and VI-8 may be due to steric hindrance by the chlorine atom *ortho* in the benzyl group. On the other hand, the 5-bromopyrimidine-6-carboxaldehydes were easily obtained upon bromination with Br_2 at 30–40°.

The results of the screening tests *vs.* the Ehrlich ascites carcinoma (Table I) indicate that several of the 5-benzyl-(VII-3 and VII-6) and 5-bromopyrimidine-6-carboxaldehydes (VII-19) inhibit this carcinoma to approximately the same extent as the most effective of the 5-fluoropyrimidine-6-carboxaldehydes previously reported,[31] and they are only slightly less active than 5-fluorouracil (FU). In fact compound VII-3 was practically identical in activity with FU in this test system.

There is good correlation between the *in vivo* inhibitory activities (Table I) and the results of the *in vitro* tests for inhibition of nucleic acid and protein synthesis (Tables II–IV).

On the other hand, there is no correlation between the *in vivo* tests and the results of the inhibition of the folate reductases (Table V). The new compounds reported here exhibit only weak antifol activity in comparison with accepted antifols, and it seems probable that the *in vivo* inhibitory activity of these compounds does not involve inhibition of the folate reductases.

The 5-benzyl- and 5-bromopyrimidine-6-carboxaldehydes exhibit significant activity in the inhibition of incorporation of formate into RNA and DNA (Table IV). As a group they are only slightly less active than FU in inhibiting incorporation of formate into DNA of the carcinoma cells and VII-3 is practically identical in activity with FU. On the other hand, these compounds are much more active than FU in inhibiting incorporation of formate into RNA. In a previous study[5] we have reported that various 5-fluoropyrimidine-6-carboxaldehydes inhibit the incorporation of formate into the purine nucleotides, and this results in inhibition of incorporation of formate into RNA. It seems probable that the 5-substituted pyrimidine-6-carboxaldehydes reported here also inhibit synthesis of purine nucleotides. The exact enzymatic site of this inhibition must be determined, but the present work seems to eliminate the folate reductases, and the previous study eliminated the N^{10}-formyltetrahydrofolate synthetase as sites of inhibition. Similarly, these enzymatic sites cannot account for the inhibition of incorporation of formate into DNA-thymine by these 5-substituted pyrimidine-6-carboxaldehydes.

Flaks and Cohen[32] have reported that the inhibition of DNA synthesis by FU is attributable to the *in vivo* conversion of FU to fluorodeoxyuridylic acid (FdUMP) which is a strong inhibitor of thymidylate synthetase. It seems probable that the 5-substituted pyrimidine-6-carboxaldehydes also may be converted to more active forms (such as the ribo- or deoxyribonucleotides), but further work will be necessary to test this possibility.

Although the 5-substituted pyrimidine-6-carboxaldehydes also inhibit incorporation of orotic acid into RNA and thymidine into DNA (Table III), these effects seem less important from a quantitative standpoint than the inhibition of formate incorporation into RNA and DNA. The inhibition of protein synthesis (Table II) may be attributable principally to the inhibition of RNA synthesis.

The fact that the 5-substituted pyrimidine-6-carboxaldehydes inhibit RNA and protein synthesis as well as DNA synthesis may make them of interest in multiple-drug chemotherapy of malignant growth in combination with FU and other accepted drugs. Their ability to inhibit RNA and protein synthesis may make them of interest as possible immunosuppresants.

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