analogs—a direct effect and an indirect effect mediated through inhibition of messenger RNA synthesis.

General Discussion

The experimental results presented in this paper show that the derivatives of 5-fluoroorotic aldehyde have biochemical inhibitory properties which are quite different from FU in Ehrlich ascites carcinoma cells.

Compound VI inhibits incorporation of orotic acid into RNA by blocking the conversion of orotic acid to OMP in contrast to the weak inhibitory effect of FU on incorporation of orotic acid into RNA, but the inhibition of DNA synthesis was greater with FU than VI. On the basis of the results obtained with the structural analogs of VI it is concluded that, in addition to the 6-carboxaldehyde, the 2-ethylmercapto group and the 5-fluoro group of VI are important for the inhibition of orotic acid incorporation. Compound VII shows even stronger inhibition of incorporation of orotic acid into RNA and DNA, and again the primary site of inhibition seems to be the conversion of orotic acid to OMP. The unsubstituted phenyl group on the azlactone ring and the intact hydrogen atom at C-5 of VII are necessary for the inhibition. Although VI and VII are much inferior to AzUR as inhibitors of orotic acid metabolism, they are much superior to AzU.

The analogs having a 5-fluoro and 6-carboxaldehyde group, III–VI, inhibit incorporation of formate-^{14}C into purines of RNA and thymine of DNA in contrast to the lack of inhibition of purine biosynthesis by FU. The structural requirements for the inhibition of purine biosynthesis are a C-6 carboxaldehyde and a C-5 fluorine. The inhibition seems to occur at a step preceding IMP. Compound III (and possibly IV–VI) do not inhibit N^{10}-formyl-THFA synthetase and cyclohydrolase activity.

The incorporation of formate-^{14}C and thymidine-^{3}H into DNA-thymine is inhibited by the 5-fluoroorotic aldehydes III–VI, but FU inhibits formate incorporation only. By comparison of III with FU, the aldehyde group of III seems to play an important role in the inhibition of thymidine incorporation into DNA-thymine. This inhibition is obtained at a concentration somewhat higher than that used for the experiments with orotic acid-^{14}C and uracil-^{14}C. The double-isotope labeling experiments with ^{14}C- and ^{3}H-thymidine have shown[46] that the kinase reaction for conversion of TdR to TMP and the DNA polymerase reaction are the primary sites of inhibition by these analogs. The introduction of an aldehyde group into position C-6 of FU, however, seems to reduce markedly the activity as an inhibitor of thymidylate synthetase.

Protein biosynthesis also is affected by the derivatives of 5-fluoroorotic aldehydes as shown by inhibition of formate-^{14}C, glycine-1-^{14}C, and phenylalanine-1-^{14}C into proteins. By contrast, FU showed no inhibitory activity for protein synthesis at the concentrations tested. The inhibition of RNA–purine biosynthesis by these analogs seems not to be responsible for the inhibition of protein synthesis since the restoration of purine synthesis by the addition of AICAr did not reverse the inhibition of phenylalanine incorporation into proteins. However, Caffery and Irvin[47] have made observations which support the conclusion that the inhibition of protein synthesis by VI may occur at the messenger RNA level: VI inhibits the hydrocortisone induction but not the substrate induction of tryptophan pyrrolase in liver of the adrenalectomized mouse.

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Synthesis and Biological Activity of Some 8-Aza-9-cyclopentylpurines[1,2]

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The preparation of a number of 6-substituted 8-aza-9-cyclopentylpurines is described. Using ethyl malonate-1-^{14}C, a mixture of 4-^{14}C- and 6-^{14}C-labeled 6-chloro-8-aza-9-cyclopentylpurine has been synthesized. The results of the biological evaluation of these compounds as antineoplastic agents and as inhibitors of T2osr bacteriophage induced lysis of *Escherichia coli* B are reported.

It has been shown that 2′-deoxyadenosine inhibits the steroid-induced synthesis of Δ^5-3-keto steroid isomerase in *Pseudomonas testosteroni*, whereas adenosine was completely inactive.[3] A subsequent study of structure–activity relationships, in which analogs of 2′-deoxyadenosine were tested as inhibitors of the formation of induced enzyme, indicated that inhibitory activity was enhanced when (a) the 6-amino group of adenine was substituted by chlorine, and (b) the 8-carbon in the purine ring was replaced by nitrogen. The presence of a 2′-hydroxy substituent on the pentose moiety or a deviation from the natural β,D configuration of 2′-deoxyribose caused the loss of activity as an inhibitor of the synthesis of induced enzyme, whereas analogs deficient in 2′-hydroxy-containing substituents, such as 2′,3′,5′-trideoxyadenosine and 9-cyclopentyladenine, retained activity. These observations led to the design and synthesis of 6-chloro-8-aza-9-cyclopentylpurine (3-cyclopentyl-7-chloro-*v*-triazolo[4,5-*d*]pyrimidine, **1**). This compound, as predicted from structure–activity considerations, proved to

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(2) Presented in part before the Division of Medicinal Chemistry at the 154th National Meeting of the American Chemical Society, Chicago, Ill., Sept 1967.

(3) A. D. Welch, *Proc. Natl. Acad. Sci. U. S.*, **54**, 1359 (1965).

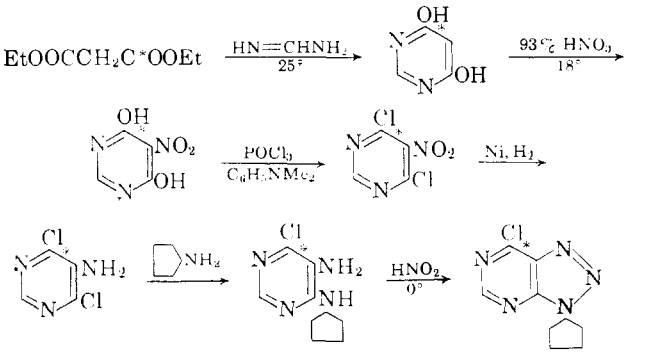
be the most potent inhibitor tested of the formation of induced enzyme.[4]

Investigation of the metabolic effects produced by **1** indicated that, at low levels, marked inhibition of the synthesis of both DNA and protein occurred; under these conditions, the formation of RNA was relatively insensitive to this agent.[5] The cyclopentyl moiety of **1**, though lacking in stereospecificity, would appear to be the analogous counterpart of the 2′-deoxyribosyl group in purine 2′-deoxyribonucleosides, especially in those situations where direct phosphorylation of the pentose ring is not a prerequisite for inhibitory activity. To facilitate studies of the effect of the 6-substituent on biological activity, a number of 6-substituted derivatives of **1**, as well as its ^{14}C-labeled form, were synthesized.

4-Chloro-5-amino-6-cyclopentylaminopyrimidine[6] reacted with nitrous acid to give **1** in excellent yield. The 6-chloro substituent was readily displaced by a variety of nucleophilic reagents to yield the 6-amino, 6-N,N-dimethylamino, 6-hydrazino, 6-N-hydroxylamino, 6-mercapto, 6-hydroxy, 6-fluoro, 6-bromo, and 6-iodo derivatives of **1** using established procedures. The parent compound, 8-aza-9-cyclopentylpurine, was obtained in good yield by the catalytic reduction of **1**. Methylation of the 6-mercapto derivative gave the 6-methylmercapto-substituted compound.

A mixture of 4-^{14}C and 6-^{14}C-labeled **1** was prepared from ethyl malonate-1-^{14}C in a six-step synthesis as shown in Scheme I. The procedures[7-9] for the synthesis of the pyrimidine intermediates were methods of choice for millimolar-scale preparations.

SCHEME I



(and the 6-^{14}C isomer) (and the 4-^{14}C isomer)

Biological Activity. The compounds were tested as inhibitors of the T2osr bacteriophage induced lysis of *Escherichia coli* B, a process involving the induction of several enzymes. At the concentrations employed in this study, the 6-halo and the 6-mercapto derivatives were found to be most active (Table I). The 6-fluoro compound could not be tested because of its extreme hygroscopicity which results in the formation of the analogous hydroxy compound. The mechanism by which these agents inhibit the lysis of *E. coli* induced by the bacteriophage particles is currently under investigation.

TABLE I

THE EFFECT OF 6-SUBSTITUTED 8-AZA-9-CYCLOPENTYLPURINES UPON T2OSR BACTERIOPHAGE INDUCED LYSIS OF *E. coli* B[a]

8-Aza-9-cyclopentylpurine	Concn, m*M*	% inhibition of a decrease in absorbancy (660 mμ)
6-Chloro	0.3	100
	0.1	20
6-Bromo	0.3	100
	0.1	40
6-Iodo	0.3	100
	0.1	35
6-Amino	0.3	0
6-Hydrazino	0.3	0
6-Hydroxylamino	0.3	10
6-Dimethylamino	0.3	0
6-Mercapto	0.3	100
	0.1	20
6-Methylthio	0.3	0
6-Hydrogen	0.3	0
6-Hydroxy	0.3	0

[a] *E. coli* B cells were maintained on C medium–agar plates and grown at 37° in C medium [D. B. Cowie, G. N. Cohen, E. T. Bolton, and H. de Robichon-Szulmajster, *Biochim. Biophys. Acta*, **34**, 39 (1959)], except that 0.2% glucose was used instead of maltose. The test compounds, dissolved in DMSO, were added to the bacterial suspension simultaneously with the bacteriophage (T2osr) at a ratio of two bacteriophage particles/bacterium. Lysis of the cells was followed by measuring the decrease in absorbancy of the bacterial suspension using a modified procedure of A. H. Doermann [*J. Bacteriol.*, **55**, 257 (1948)].

The 6-halo derivatives were also assayed for their potential as anticancer agents according to previously described methods.[10] The effect of these compounds on the survival time of mice bearing L1210 lymphoma cells is shown in Table II. A slight but nevertheless

TABLE II

EFFECT OF 6-HALO-8-AZA-9-CYCLOPENTYLPURINES ON THE SURVIVAL TIME OF MICE-BEARING L1210 LYMPHOMA CELLS

Compd	Daily dosage, (mg/kg)[a]	Av Δ wt, %[b]	Av survival time, days ± SE	No. of mice
None	...	+12.2	8.0 ± 0.2	25
6-Chloro-8-aza-9-cyclopentylpurine	2.5	+10.0	8.0 ± 0.3	10
	5	+11.3	8.0 ± 0.3	10
	10	+7.2	8.7 ± 0.2	10
	15	0	7.8 ± 0.2	10
	20	+0.2	8.2 ± 0.3	10
	40	−0.2	8.6 ± 0.2	5
6-Bromo-8-aza-9-cyclopentylpurine	5	+15.5	9.0 ± 0.4	10
	10	+16.3	9.2 ± 0.4	10
	20	+4.4	8.2 ± 0.7	10
	40	...	4.2 ± 0.6	5
6-Iodo-8-aza-9-cyclopentylpurine	5	+17.6	9.3 ± 0.2	10
	10	+11.4	9.1 ± 0.4	10
	20	+10.5	8.7 ± 0.3	10
	40	+1.2	6.8 ± 1.6	5

[a] Administered as a suspension intraperitoneally once daily for 6 consecutive days, beginning 24 hr after tumor implantation. [b] Average weight change from onset to termination of drug treatment.

(4) M. S. Zedeck, A. C. Sartorelli, P. K. Chang, K. Raška, Jr., R. K. Robins, and A. D. Welch, *Mol. Pharmacol.*, **3**, 386 (1967).

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(9) R. K. Robins, K. L. Dille, and B. E. Christensen, *J. Org. Chem.*, **19**, 930 (1954).

(10) A. C. Sartorelli and B. A. Booth, *Cancer Res.*, **27**, 1614 (1967).

significant prolongation of the life span of the tumor-bearing mice was produced by these agents. Weight loss, which is indicative of host toxicity, did not accompany the weak antineoplastic activity that was demonstrated by these compounds. In an analogous experiment, the 6-mercapto derivative was found to be inactive against this neoplasm.

Experimental Section[11]

6-Chloro-8-aza-9-cyclopentylpurine (1).—To a suspension of 4-chloro-5-amino-6-cyclopentylaminopyrimidine[6] (1.13 g, 5.3 mmoles) in 2 *N* AcOH (12.5 ml) was added a solution of $NaNO_2$ (5%, 20 ml) dropwise at 0° with stirring over a period of 20 min. The mixture was stirred for an additional 30 min during which time it achieved room temperature. The solid (1.12 g, 93%) which separated was collected by filtration and washed with cold H_2O. The analytical sample was recrystallized (H_2O), mp 66–69°. *Anal.* ($C_9H_{10}ClN_5$) C, H, Cl, N.

6-Amino-8-aza-9-cyclopentylpurine.—A solution of **1** (0.45 g, 2 mmoles) in 6 *N* NH_4OH (15 ml) was heated on a steam bath for 40 min. The 6-amino derivative (0.36 g, 86%) separated as a white solid after standing overnight at 0°. The analytical sample was recrystallized (C_6H_6), mp 216°. *Anal.* ($C_9H_{12}N_6$) C, H, N.

6-N,N-Dimethylamino-8-aza-9-cyclopentylpurine.—A suspension of **1** (0.45 g, 2 mmoles) in 25% aqueous $NHMe_2$ (15 ml) was stirred at room temperature for 1 hr. The mixture, after dilution with H_2O (15 ml), was extracted three times (30-ml portions) with petroleum ether (bp 35–52°). The combined extracts were concentrated *in vacuo* to give the dimethylamino derivative (0.37 g, 79%). The analytical sample was recrystallized (H_2O), mp 34–37°. *Anal.* ($C_{11}H_{16}N_6$) C, H, N.

6-Hydrazino-8-aza-9-cyclopentylpurine.—A mixture of **1** (0.45 g, 2 mmoles) and $N_2H_4 \cdot H_2O$ (2 ml) was stirred at room temperature for 2 hr. The mixture was treated with H_2O and the aqueous layer was extracted with $CHCl_3$. The hydrazino derivative precipitated after the removal of the organic solvent *in vacuo* at 40°. The analytical sample was recrystallized (H_2O), mp 154–159°. *Anal.* ($C_9H_{13}N_7$) C, H, N.

6-N-Hydroxylamino-8-aza-9-cyclopentylpurine.—To a solution of $NH_2OH \cdot HCl$ (0.35 g, 5 mmoles) in boiling EtOH (10 ml) was added a solution of KOH (0.28 g, 5 mmoles) in EtOH (3 ml). The precipitated KCl was removed by filtration and **1** (0.23 g, 1 mmole) was added to the solution. The mixture was refluxed for 30 min with stirring and then concentrated *in vacuo* at 40°. The residue was recrystallized from EtOH to yield the 6-*N*-hydroxylamino derivative (0.17 g, 75%). The analytical sample was recrystallized (EtOH), mp 172–176°. *Anal.* ($C_9H_{12}N_6O$) C, H, N.

6-Mercapto-8-aza-9-cyclopentylpurine.—A mixture of **1** (0.45 g, 2 mmoles), thiourea (0.15 g, 2 mmoles), and absolute EtOH (5 ml) was refluxed for 10 min. The solvent was removed *in vacuo*. The residue was dissolved in 2 *N* NaOH (5 ml) and the solution was adjusted to pH 5 with AcOH. The mercapto compound separated as a yellow crystalline solid (0.41 g, 93%). The analytical sample was prepared by dissolving the crude product in 0.2 *N* NaOH, treating the solution with Norit and adjusting to pH 5 with AcOH; mp 158°. *Anal.* ($C_9H_{11}N_5S$) C, H, N, S.

6-Methylmercapto-8-aza-9-cyclopentylpurine.—To a solution of 6-mercapto-8-aza-9-cyclopentylpurine (0.44 g, 2 mmoles) in MeOH (10 ml) was added K_2CO_3 (0.3 g, 2 mmoles), followed by MeI (0.31 g, 2 mmoles). The mixture was refluxed with stirring for 1 hr. The filtrate was concentrated to dryness *in vacuo* at 40°. The resultant residue was dissolved in H_2O (10 ml) and the solution was adjusted to pH 5 with AcOH to yield the methylated derivative (0.45 g, 92%). The analytical sample was recrystallized (H_2O), mp 70–72°. *Anal.* ($C_{10}H_{13}N_5S$) C, H, N, S.

6-Hydroxy-8-aza-9-cyclopentylpurine.—A solution of 1 (0.45 g, 2 mmoles) in 2 *N* AcOH (10 ml) was refluxed for 30 min. The 6-hydroxy derivative (0.33 g, 80%) separated on cooling to 0°. The analytical sample, after treatment with Norit, was recrystallized (10% AcOH), mp 209–211°. *Anal.* ($C_9H_{11}N_5O$) C, H, N.

6-Fluoro-8-aza-9-cyclopentylpurine.—A mixture of **1** (1.12 g, 5 mmoles), AgF (6 g), and C_6H_5Me (20 ml) was refluxed for 90 min. After cooling, the silver salts were removed by centrifugation and the clear solution was concentrated *in vacuo* at 40°. The oily residue (0.98 g, 94%) was distilled *in vacuo* to yield the analytically pure 6-fluoro derivative, bp 100–112° (0.3 mm). *Anal.* ($C_9H_{10}FN_5$) C, H, F, N.

6-Bromo-8-aza-9-cyclopentylpurine.—A mixture of **1** (2 g, 9 mmoles), $BaBr_2$ (10 g), and dry $MeOC_2H_4OMe$ (250 ml) was refluxed with stirring for 3 days. The barium salts were removed by filtration and were washed with $MeOC_2H_4OMe$. The filtrates were combined and concentrated *in vacuo* at 25° to yield the crude bromo derivative (2.27 g, 94%). Concentration of its EtOAc solution, which was extracted with H_2O and treated with Norit, gave the pure product. The Norit treatment was repeated to give the analytical sample, mp 69–74°. *Anal.* ($C_9H_{10}BrN_5$) C, H, Br, N.

6-Iodo-8-aza-9-cyclopentylpurine.—A mixture of **1** (0.23 g, 1 mmole), NaI (1 g), and dry $MeOC_2H_4OMe$ (20 ml) was refluxed with stirring for 3 days. The sodium salts were removed by filtration and the filtrate was concentrated *in vacuo* at 40°. The salts and the residue were repeatedly extracted with petroleum ether (bp 35–52°) and the combined extracts were concentrated *in vacuo* at 40° to yield the crude iodo derivative (0.19 g, 61%). The analytical sample was recrystallized (aqueous MeOH), mp 73–75°. *Anal.* ($C_9H_{10}IN_5$) C, H, I, N.

8-Aza-9-cyclopentylpurine.—A solution of **1** (0.9 g, 4 mmoles) in 50% EtOH (50 ml) was hydrogenated over 10% Pd–C (0.5 g) in the presence of MgO (0.5 g) until the theoretical amount of H_2 had been absorbed. The excess MgO and catalyst were removed by filtration and washed with EtOH. To the combined filtrates was added a 5% Na_2CO_3 solution (12.3 ml) and the mixture was evaporated to dryness *in vacuo*. The residue was dissolved in H_2O and the solution was extracted with $CHCl_3$. The combined extracts were dried ($MgSO_4$) and evaporated *in vacuo* at 25° to yield the crude product as an oil (0.56 g, 74%). Recrystallization from petroleum ether (bp 37–48°) rendered it solid. The analytical sample was recrystallized from the same solvent, mp 43–46°. *Anal.* ($C_9H_{11}N_5$) C, H, N.

(11) Melting points were determined in a capillary tube in a copper block and are corrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.