(84%) of very light tan crystals: mp 200–201° (tlc in 1:1 EtOAc–petroleum ether). *Anal.* ($C_{14}H_{11}FN_2O_6S$) C, H, N.

Methyl α-(2-Chloro-4-nitrophenoxy)-*p*-toluate (24a) (Method E).—A mixture of 30.0 g (200 mmoles) of methyl *p*-toluate, 35.6 g (200 mmoles) of NBS, 300 mg of BzO_2, and 200 ml of CCl_4 was refluxed with stirring for 25 hr, then cooled in ice. The pptd succinimide was removed by filtration and washed with CCl_4. The combined filtrate and washings were spin-evapd under vacuum. To the residue were added 34.8 g (200 mmoles) of 2-chloro-4-nitrophenol, 27.6 g (200 mmoles) of K_2CO_3, and 200 ml of DMF. The mixture was stirred at room temp for 26 hr and then added to 1500 ml of H_2O. The pptd product was collected on a filter and washed with a large vol of H_2O, then with petroleum ether. Recrystn from DMF–H_2O afforded 40.0 g (62%) of light tan needles: mp 207–208° (tlc in 1:1 EtOAc–petroleum ether). *Anal.* ($C_{15}H_{12}ClNO_5$) C, H, N.

Ethyl 2-[(2-Chloro-4-nitrophenyl)thio]acetate (29a) (Method F).—A mixture of 9.60 g (50 mmoles) of 3,4-dichloronitrobenzene, 6.0 g (50 mmoles) of ethyl 2-mercaptoacetate, 6.9 g (50 mmoles) of K_2CO_3, and 50 ml of DMF was stirred at 75–80° for 45 min, then cooled, and added to 750 ml of H_2O. The product was collected on a filter, washed with H_2O, and recrystd from MeOH to give 10.8 g (78%) of light yellow crystals: mp 72–73° (tlc in C_6H_6). *Anal.* ($C_{10}H_{10}ClNO_4S$) C, H, N.

3-[(2-Chloro-4-nitrophenyl)thio]propionic Acid (30c) (Method G).—A stirred mixture of 5.50 g (20 mmoles) of **29c**, 100 ml of 6 *N* HCl, and 50 ml of dioxane was refluxed for 75 min, then cooled, and added to 500 ml of H_2O. The oil which sepd crystd readily upon scratching. The crude solid was dissolved as completely as possible in 100 ml of 5% $NaHCO_3$. The soln was filtered, washed with three 100-ml portions of $CHCl_3$, and finally acidified with 5% HCl. The product was collected on a filter and washed with H_2O. Recrystallization from C_6H_6 yielded 3.60 g (64%) of light yellow crystals: mp 117–118° (tlc in MeOH). *Anal.* ($C_9H_8ClNO_4S \cdot 0.25C_6H_6$) C, H, N.

***N*-[*m*-(4,6-Diamino-1,2-dihydro-2,2-dimethyl-*s*-triazin-1-yl)phenoxyacetyl]sulfanilyl Fluoride Ethanesulfonate (6) (Method H).**—A mixture of 1.06 g (3.0 mmoles) of **20e**, 100 mg of PtO_2, and 100 ml of EtOH was shaken with H_2 at 1–3 atm until the reaction was complete (21 hr). THF was added to dissolve some precipitated product and the filtered soln was evapd *in vacuo*. To the residue were added 335 mg (3.05 mmoles) of $EtSO_2H$, 260 mg (3.1 mmoles) of cyanoguanidine, and 30 ml of Me_2CO. The mixture was refluxed with stirring for 24 hr, then cooled, and filtered. The crude product was washed with Me_2CO and recrystd twice from *i*-PrOH–H_2O to give 586 mg (35%) of white crystals: mp 194–196° dec (tlc in *i*-PrOH). *Anal.* ($C_{21}H_{25}FN_6O_7S_2$) C, H, F.

Method I was the same as method H except that Raney Ni was used as catalyst.

Method J was the same as method H except that 10% Pd-C was used as catalyst.

Synthesis and Biological Activity of Some 5-(1-Adamantyl)pyrimidines. I[1]

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Preparation of several 2-amino-4-hydroxy-5-(1-adamantyl)pyrimidines (**1–4**) and 5-(1-adamantylamino)uracil (**5**) is described. 2-Amino-4-hydroxy-5-(1-adamantyl)pyrimidine (**1**) and 2-amino-4-hydroxy-5-(1-adamantyl)-6-methylpyrimidine (**3**) were found to be moderately inhibitory to several lines of mouse sarcoma 180 cells (S-180) and to mouse mammary adenocarcinoma (TA3) in culture. Neither of these pyrimidines inhibited the enzyme folate reductase.

Diaminopteridines and pyrimidines play an important role as chemotherapeutic agents. Methotrexate is widely used in the treatment of acute childhood leukemia and choriocarcinoma[2] while pyrimethamine is effective in the treatment of malaria.[3] The chemotherapeutic activity of these drugs is due to the inhibition of the enzyme dihydrofolate reductase (also known as folate reductase and tetrahydrofolate dehydrogenase, EC 1.5.1.3).[4,5] Whereas methotrexate, one of the most potent inhibitors of this class of compounds, is distinguished by its lack of species specificity, diaminopyrimidines with 5-phenyl substituents exhibit highly specific inhibitory effects for dihydrofolate reductases from different species. Thus, for instance, pyrimethamine is 4000 and 50,000 times more inhibitory for plasmodial dihydrofolate reductase[6] than for the corresponding enzymes from human tissue or *Escherichia coli*, respectively.[5] On the other hand, trimethoprim (2,4-diamino-5-trimethoxyphenylpyrimidine) is 60,000 times more inhibitory for *E. coli* dihydrofolate reductase than for that of human origin.[5]

It was of interest to investigate the biochemical and biological activity of pyrimidines having in position 5 a highly lipophilic and bulky adamantyl group. The ultimate aim of this work was to prepare 2,4-diaminopyrimidines substituted with adamantane and its derivatives in position 5. However, preparation of such compounds was much more difficult than that of corresponding 2-amino-4-hydroxypyrimidines.[7] While the work on 2,4-diaminopyrimidines continues, a series of 2-amino-4-hydroxypyrimidines was prepared and tested for their biological activity.[8]

Synthesis.—Pyrimidines **1–3** (Table I) were prepared by condensing the appropriate β-carbonyl ester derivatives (**9–11**) with guanidine (Scheme I). The ester derivatives were synthesized by adaptation and modification of the procedures of Lunn, *et al.*,[9] who reported the preparation of ethyl (1-adamantyl)malonate (**10**) by condensation of ethyl malonate with 1-adamantanol as catalyzed by BF_3.

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(3) G. H. Hitchings, *ibid.*, **1**, 570 (1960).

(4) S. F. Zakrzewski, *J. Biol. Chem.*, **238**, 1485 (1963).

(5) J. J. Burchall and G. H. Hitchings, *Mol. Pharmacol.*, **1**, 126 (1965).

(6) R. Ferone, J. J. Burchall, and G. H. Hitchings, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, **27**, 390 (1968).

(7) The term "hydroxypyrimidine" will be used throughout this paper for convenience even though it is realized that these compounds actually exist in the lactam, rather than the lactim, form.

(8) Recently, preparation of some 6-(1-adamantyl)pyrimidines as potential antiviral agents has been reported. M. Kuchar, J. Strof, and J. Vacek, *Coll. Czech. Chem. Commun.*, **34**, 2278 (1969).

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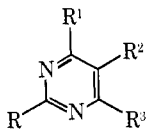
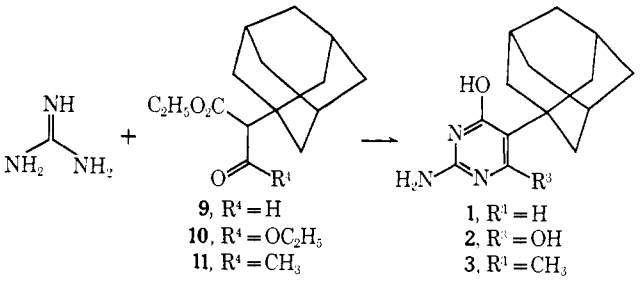
TABLE I



No.	R	R¹	R²	R³
1	NH_2	OH	Ad[a]	H
2	NH_2	OH	Ad	OH
3	NH_2	OH	Ad	CH_3
4	NH_2	OH	Ad	Cl
5	OH	OH	NHAd	H
6	NH_2	NH_2	Br	NH_2
7[b]	NH_2	NH_2	CH_3	OH
8[c]	NH_2	OH	C_2H_5	OH

[a] Adamantyl ($C_{10}H_{15}$). [b] D. J. Brown and N. W Jacobsen, *J. Chem. Soc.*, 3172 (1962). [c] A. V. Merkatz, *Ber.*, **52**, 869 (1919).

SCHEME I



9, $R^4 = H$
10, $R^4 = OC_2H_5$
11, $R^4 = CH_3$

1, $R^1 = H$
2, $R^1 = OH$
3, $R^1 = CH_3$

Compound **4** was prepared from **2** by replacing OH with Cl. Dichlorination could not be accomplished even under strenuous conditions. Attempts to aminate the chloro compound **4** to the biologically interesting 2,4-diamino-5-(1-adamantyl)-6-hydroxypyrimidine in NH_3–MeOH (150°) resulted in recovery of starting material while at higher temperature (200°), a mixture of unidentified products was obtained.

Compound **5** was made by a simple condensation of **1**-adamantylamine with 5-bromouracil. A similar approach to the corresponding 2,4,6-triamino-5-amino-(1-adamantyl)pyrimidine was attempted but only the starting materials, **6** and 1-adamantylamine, were isolated from the reaction mixture.

Compounds **7** and **8** were prepared according to published procedures and were used as model compounds for biological testing and for chemical and physical properties.

Biological Data.—The growth inhibitory effect of **1**–**7** listed in Table I was tested in mammalian cell cultures *in vitro*. Of these, **2**, **5**, **6**, and **7** had no effect and **4** only a slight effect on the growth of mouse sarcoma 180 cells (S-180) at 100 μM concentration in Eagle's medium[10] when the controls grew 8- to 16-fold. Two of the compounds, **1** and **3**, were moderately inhibitory as shown in Table II. It is interesting to note that **1** was significantly more potent against sublines of S-180 cells resistant to amethopterin (AH/67, AT/174, and AT/3000). However, a clear relationship between the degree of resistance to amethopterin (increased folate reductase content) and the sensitivity to **1** is missing. Likewise, there seems to be a lack of correlation between TMP synthetase content[11] of the various sublines (high in AH cells and very low in AT cells) and their sensitivity to **1**. Mouse mammary adenocarcinoma cells (TA3) were significantly more sensitive than S-180 cells to compound **3**. This cell line has been found to be more sensitive than S-180 to several other unrelated antimetabolites such as amethopterin, 6-mercaptopurine, and vincristine (unpublished data).

TABLE II

GROWTH INHIBITORY EFFECT OF TWO 5-ADAMANTYLPYRIMIDINES ON MAMMALIAN CELLS *in Vitro*

Cell line	50% inhibitory concentration, μM: 2-Amino-4-hydroxy-5-adamantyl-6-methylpyrimidine	2-Amino-4-hydroxy-5-adamantylpyrimidine
S-180[a]		
Parent	46	110
AH/67		40
AT/174	48	60
AT/3000		45
TA3[b]	5.0	30 (no effect)

[a] The sublines of S-180 cells sensitive (parent) and resistant (AH/67, AT/174 and AT/3000) to amethopterin have been described elsewhere.[11] The letters, A, H, and T indicate amethopterin, hypoxanthine, and thymidine, respectively, which were present in the medium during the development and maintenance of the sublines. The numbers indicate the degree of resistance. [b] TA3 is a mouse mammary adenocarcinoma cell originating from ascites form grown in female A/Ha mice and generously supplied by Dr. T. Hauschka of this Institute.

Enzymatic Studies.—The two compounds, **1** and **3**, that were active as growth inhibitors of S-180 cells were tested as inhibitors of the enzyme folate reductase which was partially purified from the subline AT/3000 of S-180 cells.[12] As may be expected for 2-amino-4-hydroxypyrimidines, there was no inhibition of the reduction of folic acid at pH 5.3 and at the inhibitor concentration of 3.8×10^{-4} M. The enzymatic site of action of these two analogs remains to be determined in future studies.

Experimental Section

All melting points were taken on a Fisher-Johns apparatus and are uncorrected. Nmr spectra were run with TMS as an internal standard on a Varian A-60A instrument. Chemical shifts are in parts per million (δ); spectral designations are as follows: s (singlet), d (doublet), t (triplet), q (quartet), and m (multiplet). Elemental analyses were performed by G. I. Robertson, Florham Park, N. J. Those analyses in which the results are within 0.4% of the calculated values are denoted by the symbols for these elements. Tlc was carried out on Brinkman silica gel (F-254) plates on aluminum with abs EtOH as the eluent unless stated otherwise. Ir spectra confirmed the assigned structure of all compounds discussed. Uv spectra were obtained on a Cary 14 spectrophotometer and were run in abs EtOH unless stated otherwise. No attempt was made to optimize yields in the reactions described below.

Ethyl Formyl(1-adamantyl)acetate (9).—Pentane (60 ml), 1-adamantanol (2.5 g, 16.4 mmoles), and Na ethyl formylacetate (2.5 g, 18.1 mmoles) were mixed. While cooling, BF_3 was passed through the mixture at a rate rapid enough to keep the temperature between 7 and 13°. Addition of BF_3 was continued 20 min after fumes were detected at the mouth of the drying tube. Maintenance of proper temperature and saturation with BF_3 are *imperative*. After stirring at ambient temperatures for 1.5

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(12) S. F. Zakrzewski, M. T. Hakala, and C. A. Nichol, *Mol. Pharmacol.*, **2**, 423 (1966).

hr, 10 ml of cold KOH (50%) was added cautiously while the temperature of the mixture was kept below 5°. The reaction mixture was extracted several times with cold Et_2O. The ether layers were washed twice with cold H_2O and dried ($MgSO_4$) in a refrigerator for 30 min. After filtration, Et_2O was removed *in vacuo* to give a mixture of an oil and a solid. Refluxing with heptane (75 ml, 1.5 hr) and removal of the solvent *in vacuo* was performed twice to give ultimately 2.48 g (68%) of oil. The product was dissolved in a small amount of $CHCl_3$-CCl_4 (1:3) and added to a silica gel column and the column was washed with a total of 800 ml of this solvent. The desired product was finally eluted from the column with $CHCl_3$-CCl_4 (2:1): nmr in $CDCl_3$ 9.78 d (**HCO**); 4.16 q (CH_2CH_3); 2.70 d [$COCH(C_{10}H_{15})CO$]; 2.30–1.50 m ($C_{10}H_{15}$); 1.25 t (CH_2CH_3). *Anal.* ($C_{13}H_{22}O_3$) C, H.

2-Amino-4-hydroxy-5-(1-adamantyl)pyrimidine (1).—Free guanidine was generated from guanidine carbonate (0.77 g, 4.28 mmoles) by neutralization with NaOEt (0.246 g, 0.0107 g-atoms of Na) in 30 ml of abs EtOH. Na_2CO_3 formed after 0.5 hr was washed with a small amount of abs EtOH and the wash added to the original filtrate. The combined filtrate and washings were mixed with a soln of ethyl formyl(1-adamantyl)acetate (**9**) (2.13 g, 8.54 mmoles) in 20 ml of EtOH and refluxed for 18 hr. After cooling in the refrigerator for 1 hr, addition of H_2O to the reaction mixture precipitated the desired product (1.36 g, 63%), which was 80% pure. The crude material was purified by dissolving in 2 *M* NaOH, treating with charcoal, and precipitating by neutralization with HCl. The analytical sample was obtained by dissolving in EtOH, treatment with charcoal, and precipitation by H_2O; mp >350°; tlc with MeOH, R_f 0.76; λ_{max} 228, 289 mμ (ϵ_{289} 9.8 × 10^3); λ_{min} 251 mμ. *Anal.* ($C_{14}H_{19}N_3O$) C, H, N, calcd, 17.13; found, 16.45.

2-Amino-4,6-dihydroxy-5-(1-adamantyl)pyrimidine (2).—Ethyl (1-adamantyl)malonate[9] (**10**) (1.00 g, 3.38 mmoles), guanidine carbonate (0.61 g, 3.39 mmoles), and abs EtOH (8 ml) were refluxed for 24 hr. The reaction mixture thickened considerably during this time. After cooling, the product was collected, washed (Me_2CO, abs EtOH), and suction-dried for 8 hr. The yield was 0.67 g (76%). The analytical sample was recrystallized by dissolving in NaOH, filtering, and precipitating by acidification with HCl: mp >350°; λ_{max} 254 mμ, λ_{min} 231 mμ. *Anal.* ($C_{14}H_{19}N_3O_2$) C, H.

Ethyl Aceto(1-adamantyl)acetate (11).—1-Adamantanol (5.00 g, 33.0 mmoles) and ethyl acetoacetate (4.70 g, 36.2 mmoles) were stirred in about 60 ml of pentane. BF_3 was passed through the mixture while maintaining the temperature below 10°. In less than 5 min the insoluble solid had turned into a syrup. The reaction mixture was then stirred at room temperature for 1 hr, and neutralized with 15 ml of 50% KOH while cooled below 5°. After extraction with cold Et_2O, the combined extracts were washed with cold H_2O and dried ($MgSO_4$). After removal of the solvent, the residue was dissolved in isooctane and refluxed for 1.5 hr. The solvent was removed *in vacuo* to give a thick white oil (5.1 g); an nmr spectrum showed this material to be a mixture of product and starting material (70:30). The oil was eluted from a silica gel column with $CHCl_3$ to give a water-white oil (3.4 g, 44%). Tlc with $CHCl_3$ revealed a single spot when developed in I_2 at R_f 0.85; nmr in CCl_4 confirmed the assigned structure: 4.12 q (CH_2CH_3), 3.13 s [$COCH(C_{10}H_{15})CO$], 2.20 s (CH_3CO), 2.00–1.40 m ($C_{10}H_{15}$), 1.22 t (CH_2CH_3). No enol form of this compound was detected. *Anal.* ($C_{16}H_{24}O_3$) C, H.

2-Amino-4-hydroxy-5-(1-adamantyl)-6-methylpyrimidine (3).—Ethyl aceto(1-adamantyl)acetate (**11**) (4.00 g, 15.3 mmoles) and guanidine·HCl (1.44 g, 15.1 mmoles) were mixed in 50 ml of abs EtOH. A white solid formed immediately upon addition of a soln of NaOEt prepared by dissolving NaH (1.44 g of a 50% dispersion, 30.0 mmoles) in abs EtOH (30 ml). After refluxing for 60 hr, the white solid was dissolved by addition of 1 *M* NaOH, then pptd by addition of H_2O. The insol product was collected (2.16 g, 55%) (mp 290–300°) and recrystallized (EtOH). The analytical sample was further purified by double elution from a silica gel column with abs EtOH; λ_{max} 230, 292.5 mμ, λ_{min} 254 mμ; mp >300°; R_f 0.55. *Anal.* ($C_{15}H_{21}N_3O \cdot H_2O$)[13] C, H.

2-Amino-4-hydroxy-6-chloro-5-(1-adamantyl)pyrimidine (4).—2-Amino-4,6-dihydroxy-5-(1-adamantyl)pyrimidine (**2**) (0.50 g, 1.92 mmoles) was refluxed in $POCl_3$ (8 ml) containing PCl_5 (0.50 g, 2.41 mmoles) for about 12 hr. After cooling, the red soln was poured on ice and stirred. The solid which formed was collected and weighed 0.41 g (76%): mp 187° (with effervescence); λ_{max} 235, 293 mμ. *Anal.* ($C_{14}H_{18}ClN_3O$) Cl.

5-(1-Adamantylamino)uracil (5).—5-Bromouracil[14] (15.9 g, 83.3 mmoles) and 1-adamantylamine (49.6 g, 0.328 mole) were mixed with 300 ml of pyridine and refluxed for 48 hr. During this time most of the solid passed into soln. The suspension was filtered hot and the filtrate cooled to give a solid which was collected and washed with pyridine and Et_2O. The yield of the fine white crystalline product was 6.8 g, $\epsilon^{H_2O}_{max}$ 5100 (292 mμ), $\lambda^{H_2O}_{min}$ 259 mμ at pH 7. The analytical sample was further purified by dissolving 5 *M* NaOH and pptg by neutralization with 2 *M* HCl, mp >350°. *Anal.* ($C_{14}H_{19}N_3O_2$) C, H, N.

2,4,6-Triamino-5-bromopyrimidine (6). 2,4,6-Triaminopyrimidine (5.00 g, 40.0 mmoles) was dissolved in the smallest possible amount of H_2O. Br_2 (2.0 ml, 39 mmoles) was added. A brown solid formed which dissolved when the reaction mixture was heated on a steam bath. After cooling 1.5 hr in the refrigerator, a small amount of a brown solid had separated. The filtrate was neutralized with 1 *N* NaOH to produce a fluffy, white solid which was washed (H_2O) and a small amount of Me_2CO: yield 8.20 g (100%); mp 200–202°; R_f 0.60; $\lambda^{H_2O}_{max}$ 275 mμ; $\lambda^{H_2O}_{min}$ 255 mμ. Analytical sample was recryst'd from 95% EtOH. *Anal.* ($C_4H_6BrN_5$) Br.

2,4-Diamino-6-hydroxy-5-methylpyrimidine (7).—2,4-Diamino-6-hydroxy-5-methylpyrimidine was prepared according to the procedure of Brown[15] by alkylation of 2,4-diamino-6-hydroxypyrimidine: mp 320–324° (lit. 308–310°); λ_{max} 270, 237 (lit. λ_{max} 270, 237) 0.1 *N* NaOH.

2-Amino-4,6-dihydroxy-5-ethylpyrimidine (8).—This compound was prepared according to a modified procedure of Merkatz.[16] Free guanidine was generated from its carbonate (1.0 g, 5.6 mmoles) by neutralization with a soln of NaOEt prepared by dissolving NaH (0.75 g of 50% oil dispersion, 15.6 mmoles) in abs EtOH (8 ml). After stirring for 30 min, the insol Na_2CO_3 was separated and the filtrate mixed with a solution of diethyl ethylmalonate (1.0 g, 5.3 mmoles) and abs EtOH (5 ml). The reaction mixture was stirred at room temperature for 15 min and then refluxed for 8 hr. The white solid that formed was collected and dissolved in H_2O and the resultant basic soln was acidified with AcOH. The product was collected and washed with H_2O and Me_2CO (λ_{max} 268 mμ). Recrystallization was accomplished by dissolving in NaOH and precipitating with glacial AcOH to give 0.45 g of 55%; charred at 340° (lit. mp not reported.)

Sodium Ethyl Formylacetate.[17]—A soln of HCO_2Et (13.5 ml, 0.166 mole) and EtOAc (13.0 ml, 0.142 mole) was dropped slowly with stirring into a mixture of NaH (6.85 g of 50% oil dispersion, 0.143 mole) in abs Et_2O (100 ml) until H_2 evolution was detected. Ester addition was resumed after 15 to 20 min. The gray suspension gradually became yellow over the period of 2 hr. The yellow solid was collected and washed several times with Et_2O and dried *in vacuo* at 50° over NaOH to give 11.4 g (58%) of a very powdery product.

Acknowledgment.—The authors wish to gratefully acknowledge the capable assistance of Mrs. Jadwiga Drobniak, Miss Aurelie Mulhern, and Miss Dorris Sugg.

(13) The adamantylpyrimidine **3** may seem unique in this series because of its H_2O content. We will describe other adamantylpyrimidines of this nature at a later date. Kuebar, *et al.* (ref 8), have presented a similar situation without comment.

(14) S. Y. Wang, *J. Org. Chem.*, **24**, 11 (1959).

(15) D. J. Brown and N. W. Jacobsen, *J. Chem. Soc.*, 3172 (1962).

(16) A. V. Merkatz, *Ber.*, **52**, 869 (1919).

(17) This procedure is a modification of that described by S. Gabriel, *ibid.*, **37**, 3638 (1904).