

(84%) of very light tan crystals: mp 200–201° (tle in 1:1 EtOAc–petroleum ether). *Anal.* (C₁₄H₁₁FN₂O₄S) 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 BzO₂, and 200 ml of CCl₄ was refluxed with stirring for 25 hr, then cooled in ice. The pptd succinimide was removed by filtration and washed with CCl₄. 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 K₂CO₃, and 200 ml of DMF. The mixture was stirred at room temp for 26 hr and then added to 1500 ml of H₂O. The pptd product was collected on a filter and washed with a large vol of H₂O, then with petroleum ether. Recrystn from DMF–H₂O afforded 40.0 g (62%) of light tan needles: mp 207–208° (tle in 1:1 EtOAc–petroleum ether). *Anal.* (C₁₅H₁₃ClNO₅) 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 K₂CO₃, and 50 ml of DMF was stirred at 75–80° for 45 min, then cooled, and added to 750 ml of H₂O. The product was collected on a filter, washed with H₂O, and recrystd from MeOH to give 10.8 g (78%) of light yellow crystals: mp 72–73° (tle in C₆H₆). *Anal.* (C₁₀H₁₀ClNO₄S) 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 H₂O. The oil, which sepd, crystd readily upon scratching. The crude solid was dissolved as completely as possible in 100 ml of 5% NaHCO₃. The soln was filtered, washed with three 100-ml portions of CHCl₃, and finally acidified with 5% HCl. The product was collected on a filter and washed with H₂O. Recrystallization from C₆H₆ yielded 3.60 g (64%) of light yellow crystals: mp 117–118° (tle in MeOH). *Anal.* (C₉H₇ClNO₄S·0.25C₆H₆) 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 PtO₂, and 100 ml of EtOH was shaken with H₂ 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 EtSO₃H, 260 mg (3.1 mmoles) of cyanoguanidine, and 30 ml of Me₂CO. The mixture was refluxed with stirring for 24 hr, then cooled, and filtered. The crude product was washed with Me₂CO and recrystd twice from *i*-PrOH–H₂O to give 586 mg (35%) of white crystals: mp 194–196° dec (tle in *i*-PrOH). *Anal.* (C₂₄H₂₇FN₆O₇S₂) C, H, F.

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

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

Synthesis and Biological Activity of Some 5-(1-Adamantyl)pyrimidines. 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 choriocarcinoma² 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 reductase⁶ 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 BF₃.

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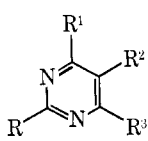
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(7) The term "hydroxypyrimidine" will be used throughout this paper for convenience even though it is realized that these compounds actually exist in the lactam, rather than the lactim, form.

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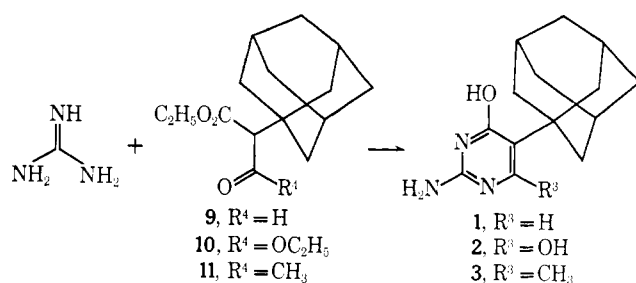
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TABLE I

No.				
	R	R ¹	R ²	R ³
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 ₂ H ₅	OH

^a Adamantyl (C₁₀H₁₅). ^b D. J. Brown and N. W. Jacobsen, *J. Chem. Soc.*, 3172 (1962). ^c A. V. Merkat, *Ber.*, **52**, 869 (1919).

SCHEME I



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 NH₃-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 μ M concentration in Eagle's medium¹⁰ 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 be-

TABLE II

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

Cell line	—50% inhibitory concentration, μ M—	
	2-Amino-4- hydroxy-5-ada- mantyl-6-methyl- pyrimidine	2-Amino-4- hydroxy-5-ada- mantylpyrimidine
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.

tween TMP synthetase content¹¹ 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).

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, BF₃ was passed through the mixture at a rate rapid enough to keep the temperature between 7 and 13°. Addition of BF₃ was continued 20 min after fumes were detected at the mouth of the drying tube. Maintenance of proper temperature and saturation with BF₃ are imperative. After stirring at ambient temperatures for 1.5

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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 Et₂O. The ether layers were washed twice with cold H₂O and dried (MgSO₄) in a refrigerator for 30 min. After filtration, Et₂O 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 CHCl₃-CCl₄ (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 CHCl₃-CCl₄ (2:1); nmr in CDCl₃ 9.78 d (HCO); 4.16 q (CH₂CH₃); 2.70 d [COCH(C₁₀H₁₅)CO]; 2.30–1.50 m (C₁₀H₁₅); 1.25 t (CH₂CH₃). *Anal.* (C₁₅-H₂₂O₃) 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. Na₂CO₃ 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 H₂O 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 H₂O; mp >350°; tlc with MeOH, R_f 0.76; λ_{max} 228, 289 mμ (ε₂₈₉ 9.8 × 10³); λ_{min} 251 mμ. *Anal.* (C₁₄H₁₉N₃O) C, H, N, calcd, 17.13; found, 16.45.

2-Amino-4,6-dihydroxy-5-(1-adamantyl)pyrimidine (2).—Ethyl (1-adamantyl)malonate⁹ (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 (Me₂CO, 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.* (C₁₄H₁₉N₃O₂) 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. BF₃ 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 Et₂O, the combined extracts were washed with cold H₂O and dried (MgSO₄). 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 CHCl₃ to give a water-white oil (3.4 g, 44%). Tlc with CHCl₃ revealed a single spot when developed in I₂ at R_f 0.85, nmr in CCl₄ confirmed the assigned structure: 4.12 q (CH₂CH₃), 3.13 s [COCH(C₁₀H₁₅)CO], 2.20 s (CH₃CO), 2.00–1.40 m (C₁₀H₁₅), 1.22 t (CH₂CH₃). No enol form of this compound was detected. *Anal.* (C₁₆H₂₄O₃) 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 H₂O. 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.* (C₁₅H₂₁N₃O·H₂O)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 POCl₃ (8 ml) containing PCl₅ (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.* (C₁₄H₁₈ClN₃O) Cl.

5-(1-Adamantylamino)uracil (5).—5-Bromouracil¹⁴ (15.9 g, 83.3 mmoles) and 1-adamantylamine (49.6 g, 0.328 mole) were mixed with 360 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 Et₂O. The yield of the fine white crystalline product was 6.8 g, ε_{max}^{H₂O} 5100 (292 mμ), λ_{min}^{H₂O} 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.* (C₁₄H₁₉N₃O₂) C, H, N.

2,4,6-Triamino-5-bromopyrimidine (6).—2,4,6-Triamino-pyrimidine (5.00 g, 40.0 mmoles) was dissolved in the smallest possible amount of H₂O. Br₂ (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 (H₂O) and a small amount of Me₂CO; yield 8.20 g (100%); mp 200–202°; R_f 0.60; λ_{max}^{H₂O} 275 mμ; λ_{min}^{H₂O} 255 mμ. Analytical sample was recrystd from 95% EtOH. *Anal.* (C₄H₆BrN₃) Br.

2,4-Diamino-6-hydroxy-5-methylpyrimidine (7).—2,4-Diamino-6-hydroxy-5-methylpyrimidine was prepared according to the procedure of Brown¹⁵ 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 Merckat.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 Na₂CO₃ 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 H₂O and the resultant basic soln was acidified with AcOH. The product was collected and washed with H₂O and Me₂CO (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 HCO₂Et (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 Et₂O (100 ml) until H₂ 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 Et₂O and dried *in vacuo* at 50° over NaOH to give 11.4 g (58%) of a very powdery product.

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(13) The adamantylpyrimidine 3 may seem unique in this series because of its H₂O content. We will describe other adamantylpyrimidines of this nature at a later date. Kuchar, *et al.* (ref 8), have presented a similar situation without comment.

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