The Adamantyl Group in Medicinal Agents. III. Nucleoside 5′-Adamantoates. The Adamantoyl Function as a Protecting Group[1]

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The preparation and biological evaluation are described for the 5′-adamantoates of the deoxyribonucleosides, thymidine, 2′-deoxy-5-fluorouridine, and deoxyadenosine, and of the ribonucleosides, adenosine, inosine, 6-thioinosine, and 6-azauridine. The adamantoylation of the deoxyribonucleosides has been found to proceed preferentially to give the 5′-esters in which the adamantoyl function can serve as a base-sensitive protecting group in nucleoside synthesis. The adamantoate of 6-thioinosine suppresses antibody formation in the mouse following challenge by sheep erythrocytes. The adamantoate of adenosine inhibits adenosine diphosphate induced platelet aggregation *in vitro*. The antitumor activity of the adamantoates of several nucleoside analogs is recorded. The possible mode of action of adamantane-containing agents is described in terms of a precise binding of the adamantane moiety to complementary, "hydrophobic" regions in the receptor site protein molecule.

In paper II of this series[2] we reported the synthesis and evaluation of nortestosterone 17β-adamantoate, a steroid ester possessing significant duration of anabolic action with minimal androgenic activity. Apparently, esterification with adamantoic acid does not merely serve to prolong or intensify the intrinsic activity of nortestosterone, but also effects a favorable separation of the nitrogen-retaining capacity from the undesirable hormonal activity.

Observation of this improvement in activity prompted an extension of this investigation to other classes of biologically active compounds. In view of published information on increased efficacy following acylation of certain growth-inhibitory nucleosides,[3-6] we chose to study the influence of adamantoylation on the biological activity of selected purine and pyrimidine nucleosides and their structural analogs.

Two ancillary aims could be served by such a study. First, with regard to the chemical aspects of adamantoylation, it seemed worthwhile to investigate if esterification with the bulky, symmetrical adamantoic acid might proceed preferentially, as does tritylation, to yield a nucleoside derivative with a blocking group at the 5′-hydroxyl function.

Secondly, a study of the biological activity of nucleoside adamantoates might serve to provide a partial answer to the question raised in paper I of this series[7] concerning the relative importance of three factors presumably contributing to the favorable activity of certain adamantane-containing agents. These three factors are the absorption/distribution characteristics of these agents, their metabolic disposition in the animal host, and the precision with which they fit at the receptor site. An indication that the last factor is of major importance may be seen in the pattern of structure–activity relationships now apparent among these compounds.

Among the compounds evaluated thus far, a surprising specificity attending the influence of the adamantane group has been noted. Seemingly minor structural changes in the adamantyl moiety, such as positional isomerism[7] or substitution by one or two methyl groups,[2,7] drastically reduce or completely eliminate activity of the hypoglycemic N-tolylsulfonyl-N′-adamantylureas[7] as well as of the anabolic nortestosterone esters[2] studied. Quite possibly, the mode of action of these agents may involve a precise and specific binding of the adamantane moiety of intact agent to receptor site. This contention derives support from observations on some of the nucleoside adamantoates recorded in this paper.

Pharmacological and chemotherapeutic studies with acyl derivatives of 6-azauridine,[3] 6-thioinosine,[4] 6-thioguanosine,[5] and other structural analogs of nucleosides[6] have been reported in recent years. Increased lipid solubility and stabilization toward metabolic degradation of the acylated nucleosides have been mentioned[3-5] as important alterations contributing to their improved chemotherapeutic efficacy.

The present study of nucleoside 5′-adamantoates was undertaken to ascertain if the introduction of the adamantane group might render these esters superior to the parent nucleoside as well as to the previously reported acylated derivatives. The 5′-adamantoates are soluble in several organic solvents, but the free 3′-hydroxyl or 2′,3′-glycol function constitutes a locus of polar nature which presumably imparts to these esters a certain desirable degree of hydrophilicity not present in the corresponding triacetylated derivatives.

To provide further information on the effects of methyl substitutions in the adamantane moiety on biological activity, a number of 3-methyl-, 3,5-dimethyl-, and 3,5,7-trimethyladamantoates were prepared for comparison with the unsubstituted 5′-esters.

Chemistry.—The preparation of nucleoside 5′-adamantoates proceeded satisfactorily by direct, preferential acylation of the deoxyribonucleosides, thymidine, 2′-deoxy-5-fluorouridine, and deoxyadenosine, with 1 equiv of the adamantoyl chloride in pyridine solution. The esters obtained are generally crystalline solids,

(1) Presented in part at the 10th National Medicinal Chemistry Symposium, American Chemical Society, Bloomington, Ind., June 28, 1966.

(2) R. T. Rapala, R. J. Kraay, and K. Gerzon, *J. Med. Chem.*, **8**, 580 (1965).

(3) (a) R. E. Handschumacher, P. Calabresi, A. D. Welch, V. Bono, H. Fallon, and E. Frei, II, *Cancer Chemotherapy Rept.*, **21**, 1 (1962); (b) W. A. Creasey, M. E. Fink, R. E. Handschumacher, and P. Calabresi, *Cancer Res.*, **23**, 444 (1963); (c) P. Calabresi and R. W. Turner, *Ann. Internal Med.*, **64**, 352 (1966).

(4) L. R. Lewis, R. K. Robins, and C. C. Cheng, *J. Med. Chem.*, **7**, 200 (1964).

(5) G. A. LePage and I. G. Junga, *Cancer Res.*, **25**, 46 (1965).

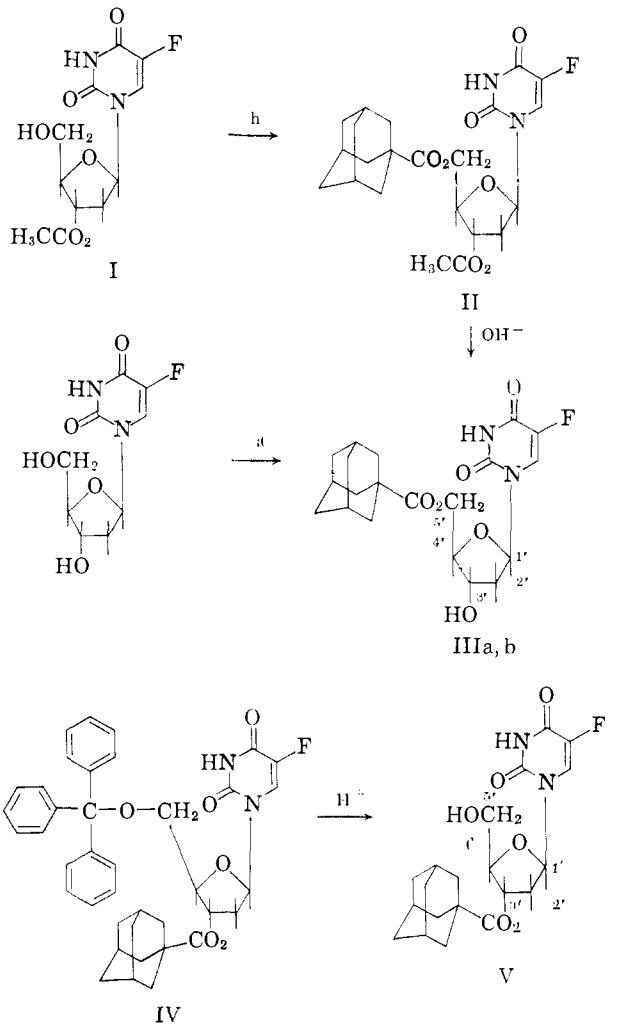
(6) K. L. Mukherjee and C. Heidelberger, *ibid.*, **22**, 815 (1962).

(7) K. Gerzon, E. V. Krumkalns, R. L. Brindle, F. J. Marshall, and M. Root, *J. Med. Chem.*, **6**, 760 (1963).

soluble in ethyl acetate. Evidence for their formulation as 5′-adamantoates was obtained for the esters of thymidine and 2′-deoxy-5-fluorouridine. Coupling of 2-cyanoethyl phosphate[8,9] to the 3′-hydroxyl group of thymidine 5′-adamantoate with dicyclohexylcarbodiimide produced 5′-O-adamantoylthymidine-3′-(2-cyanoethyl hydrogen phosphate). Removal of the cyanoethyl and adamantoyl groups from this phosphodiester yielded thymidine 3′-phosphate.[9]

The product of the direct adamantoylation of 2′-deoxy-5-fluorouridine was characterized as the 5′-adamantoate (IIIa) by comparison with the 5′-adamantoate (IIIb) obtained from 3′-O-acetyl-2′-deoxy-5-fluorouridine[10] (I) by adamantoylation and subsequent preferential deacetylation of the intermediate 3′-O-acetyl-5′-O-adamantoyl-2′-deoxy-5-fluorouridine (II) with dilute base (Chart I). The 3′-adamantoate (V) was prepared, for purposes of comparison, from 2′-deoxy-5-fluoro-5′-O-trityluridine[10] by adamantoylation, followed by treatment of the fully protected intermediate (IV) with aqueous acetic acid to remove the trityl group.

CHART I



That the 3′-adamantoate V differed from the 5′-adamantoate III was shown by comparison of their respective melting points, their chromatographic behavior, and their spectral properties. The nmr spectra of the 3′- (V) and 5′-adamantoate (IIIa) are in agreement with these structures; the pertinent data for the sugar protons are listed in Table I.

TABLE I
CHEMICAL SHIFTS OF SUGAR PROTONS IN 2′-DEOXY-5-FLUOROURIDINE ADAMANTOATES[a]

Proton	III	V
H(1′)	6.29 (t)	6.35 (t)
H(2′)	2.40 (m)	2.33 (m)
H(3′)		5.33 (m)
H(4′)	4.30[b]	4.00 (m)
H(5′)		3.82 (m)
H(5′-OH)		4.96[c]
H(3′-OH)	3.58[c]	

[a] Chemical shifts are expressed in δ values; t denotes a triplet, m denotes a multiplet. [b] Four-proton multiplet centered at δ 4.3. [c] Exchangeable upon addition of D_2O.

Published information[11] reveals that the C(2′), C(3′), C(4′), and C(5′) protons of ribonucleosides or their 5′-derivatives all occur at higher field than δ 5.0 and that the introduction of an electron-withdrawing substituent, *e.g.*, an acyl group, at the 2′ or 3′ position leads to substantial deshielding of the H(2′) or H(3′), respectively. This downfield shift has been found to be of the order of 1–1.2 ppm. In the spectrum of the 3′-adamantoate (V), this downfield shift for the H(3′) is clearly in evidence (see Table I), while for the 5′-adamantoate (III) this proton H(3′) is contained in the multiplet centered at δ 4.3.

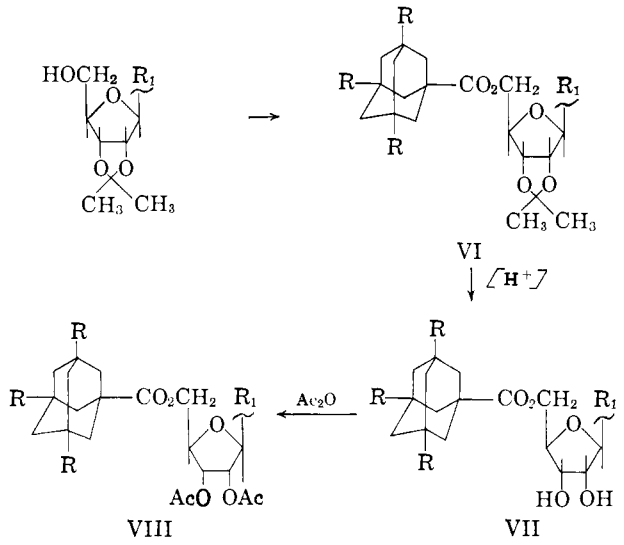
The above transformations demonstrate double utility of the adamantoyl group as a temporary blocking function in nucleoside synthesis. Under acidic conditions, removal of the trityl group in IV is effected with retention of the 3′-adamantoyl group, while mildly alkaline conditions (0.025 *N* NaOH) permit the preferential removal of the 3′-acetate in II with retention of the adamantoyl group on the primary 5′-hydroxyl function; the 5′-adamantoate is readily cleaved by stronger base (0.25 *N*). Again, the preparation of thymidine 3′-phosphate exemplifies a shortened synthesis (*cf.* ref 9) of a deoxynucleotide which obviates the need for acidic conditions during isolation of the product.

The preferential acylation of the primary 5′-hydroxyl function in deoxynucleosides with adamantoyl chloride coupled with the use of the adamantoyl function as a protecting group in nucleoside synthesis suggests the general utility of this function as a base-sensitive counterpart of the trityl group in syntheses involving nucleosides and carbohydrates.[12] The utility of the adamantyloxycarbonyl function as a protecting group in peptide synthesis has recently been described.[13]

For the preparation of the 5′-adamantoates of the ribonucleosides, 6-azauridine, 6-thioinosine, adenosine, and inosine, the respective 2′,3′-isopropylidene derivatives were acylated with adamantane- or methyl-substituted adamantoyl chlorides (Chart II). The

(8) E. Cherbuliez, G. Cordahi, and J. Rabinowitz, *Helv. Chim. Acta*, **43**, 863 (1960).
(9) G. M. Tener, *J. Am. Chem. Soc.*, **83**, 159 (1961).
(10) H. J. Thomas and J. A. Montgomery, *J. Med. Chem.*, **5**, 24 (1962).
(11) (a) R. P. M. Frognaeot, B. E. Griffin, C. B. Reese, J. E. Sulston, and D. R. Trentham, *Tetrahedron*, **22**, 705 (1966); (b) L. D. Hall, *Advan. Carbohydrate Chem.*, **19**, 51 (1964).
(12) C. B. Reese and J. E. Sulston [*Progr. Chem. Soc.*, 214 (1964)] have mentioned the pivaloyl group for similar purposes.
(13) W. L. Haas, E. V. Krumkalns, and K. Gerzon, *J. Am. Chem. Soc.*, **88**, 6988 (1966).

CHART II



R = CH_3
R_1 = 6-azauracil, 6-mercaptopurine, adenine, hypoxanthine

resulting fully protected nucleosides (VI, R = H or CH_3) were hydrolyzed with aqueous acetic acid or methanolic HCl on the steam bath to give the monoesters (VII, R = H or CH_3). Selected 5′-adamantoyl-2′,3′-diacetyl ribonucleosides (VIII) were prepared by treatment of the esters (VII) with excess acetic anhydride in pyridine solution.

The direct adamantoylation of ribonucleosides to give preferentially 5′-O-adamantoates is at present under investigation, and results will be reported at a later date.

Experimental Section

Melting points are uncorrected. Ultraviolet spectra were determined in ethanol solution with a Cary Model 15 spectrophotometer. Unless otherwise indicated, infrared spectra were determined in $CHCl_3$ solution with a Perkin-Elmer Model 21 spectrophotometer. Nmr spectra were obtained on an A-60 Varian spectrometer using 5% solutions in $CDCl_3$ with TMS as internal standard. Exchangeable protons were removed by washing such solutions with D_2O. Electrometric titrations were carried out in 66% DMF solution. Paper chromatographic behavior was evaluated using Schleicher and Schuell (orange ribbon) paper no. 589 with a 2-propanol–water–NH_3 mixture (7:2:1) by the descending method. Thin layer chromatographic (tlc) behavior was assessed using plates with silica gel with phosphor (from Research Specialties Co.). R_f values refer to materials which show a single absorbing spot and are expressed (rR_f) relative to the parent nucleoside. Solvents for tlc, unless otherwise stated, were ethyl acetate for the deoxyribonucleosides and their derivatives and an ethyl acetate–ethanol mixture (3:1) for the ribonucleoside compounds. For the 6-thioinosine derivatives, plates of silica gel G (E. Merck Co., Darmstadt) were used, and visualization was aided by exposure to iodine vapors.

Materials.—The natural nucleosides and nucleotides were obtained from California Biochemical Corp. Isopropylideneinosine and adenosine were supplied by International Chemical and Nuclear Corp.; 6-thioinosine by Burroughs Wellcome Co.; 6-azauridine by Eli Lilly and Co., Ltd., Bromborough, England; and 2′-deoxy-5-fluorouridine (5-FUdR) by Hoffmann-La Roche, Inc.

Adamantoyl Chlorides.—Adamantoyl-,[14] 3-methyladamantoyl-,[2] and 3,5-dimethyladamantoyl chloride[2] have been previously reported. The acid chloride of 1,3,5-trimethyladamantoic acid[15] (obtained from 1,3,5-trimethyladamantane[15,16]) was prepared by the dropwise addition of oxalyl chloride (5 ml) to a solution of the acid (5 g) in anhydrous benzene (10 ml). After the solution was heated under reflux for 1 hr, the solvent was removed under reduced pressure and the residue was taken up in anhydrous benzene (10 ml). Removal of the solvent followed by repetition of this operation gave 3,5,7-trimethyladamantoyl chloride, which was taken up in anhydrous benzene and used without further purification in acylation reactions. Purity of the product was judged by inspection of the strong C=O absorption band at 1785 cm^{-1} coupled with the absence or low intensity of the band at 1725 cm^{-1} (CO_2H).

Deoxyribonucleoside Adamantoates.—5′-O-Adamantoyl-2′-deoxy-5-fluorouridine (III). A. By Direct Adamantoylation.—A solution of adamantoyl chloride (0.81 g, 4.07 mmoles) in anhydrous benzene (10 ml) was added dropwise over a 20-min period to a stirred solution of 2′-deoxy-5-fluorouridine (1.0 g, 4.07 mmoles) in anhydrous pyridine (10 ml). The temperature of the reaction was maintained at 0–5° during the addition and at room temperature for 16 hr afterward. Water (2 ml) was added; the solution was stirred for 30 min, and the solvents were removed under reduced pressure at 45–50°. The residue was dissolved in ethyl acetate (50 ml); the organic layer was separated and washed first with 1 *N* HCl solution and then with two portions of water (25 ml). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a residue (rR_f 2.7), which was taken up in benzene (50 ml). Upon cooling, 637 mg of crystalline ester (IIIa) was obtained: mp 116–117°; λ_{max} 268 mμ ($\epsilon \times 10^{-3}$ 8.48); titration, initial pH 5.4, mol wt 421 (calcd 408); pK_a = 8.8.

Anal. Calcd for $C_{20}H_{25}FN_2O_6$: C, 58.81; H, 6.17; N, 6.86. Found: C, 58.51; H, 6.29; N, 6.89.

B. From 3′-O-Acetyl-2′-deoxy-5-fluorouridine (I).—Adamantoyl chloride (200 mg, 1.0 mmole) was added in one portion to a stirred solution of the 3′-acetate (I)[10] (270 mg, 0.94 mmole) in anhydrous pyridine (25 ml). After 16 hr, water (5 ml) was added, and the mixture was stirred for 30 min. The mixture was concentrated under reduced pressure, and the residue was dissolved in $CHCl_3$ (75 ml). The organic layer was washed with water (two portions of 50 ml each), dried (Na_2SO_4), and concentrated under reduced pressure. Trituration of the residue with petroleum ether (bp 60–80°) (100 ml) and recrystallization of the solids obtained from an ether–cyclohexane mixture gave 280 mg of 3′-acetyl-5′-adamantoyl-2′-deoxy-5-fluorouridine (II), mp 62–65° (rR_f 3.9), λ_{max} 266 mμ ($\epsilon \times 10^{-3}$ 8.0).

Anal. Calcd for $C_{22}H_{27}FN_2O_7$: N, 6.22. Found: N, 6.19.

Preferential hydrolysis of the 3′-acetyl group in II was studied by dissolving 10-mg samples in 10 ml of 0.005 *N*, 0.025 *N*, 0.05 *N*, 0.125 *N*, 0.25 *N*, and 0.5 *N* NaOH solutions, respectively, in aqueous dioxane (1:1). At intervals aliquots were removed, treated with Dowex Resin 50W-X4, 100–200 mesh (NH_4^+), and applied to tlc plates. While 0.005 *N* base was ineffective and 0.25 *N* base removed both the adamantoyl- and the acetyl group within 20 min to give 2′-deoxy-5-fluorouridine, 0.025 *N* NaOH solution converted II into the 5′-adamantoate (IIIb) to the extent of approximately 80–90%.

By tlc the 5′-adamantoate IIIb, rR_f 2.7, obtained from II (route b), was indistinguishable from the product IIIa (rR_f 2.7) obtained by direct adamantoylation (route a) but differed from the 3′-adamantoate (V, rR_f 4.0, see below) and also differed from the starting material, 3′-O-acetyl-2′-deoxy-5-fluorouridine (I, rR_f 2.8). Because of the small amounts of material available, no attempt was made to isolate the product of hydrolysis (IIIb).

3′-O-Adamantoyl-2′-deoxy-5-fluorouridine (V).—Adamantoyl chloride (0.8 g, 4.0 mmoles) was added to a stirred solution of 2′-deoxy-5-fluoro-5′-O-trityluridine[10] (1.0 g, 2.05 mmoles) in anhydrous pyridine (25 ml), and this reaction mixture was heated at 50° for 30 min. After 16 hr, water (5 ml) was added; the mixture was stirred for 30 min, and was then concentrated under reduced pressure. The residue was triturated with water (10 ml), and dissolved in ethyl acetate (50 ml). The organic layer was washed with water (50 ml), dried (Na_2SO_4), and concentrated under reduced pressure. The residue (rR_f 4.7) was dissolved in anhydrous benzene (15 ml), filtered, and carefully concentrated under reduced pressure to approximately 5 ml. Slow crystallization ensued to give 3′-O-adamantoyl-2′-deoxy-5-

(14) H. Stetter, M. Schwarz, and A. Hirshorn, *Chem. Ber.*, **92**, 1629 (1959).

(15) H. Koch and J. Franken, *ibid.*, **96**, 213 (1963).

(16) A. Schneider, R. W. Warren, and E. J. Janoski, *J. Org. Chem.*, **31**, 1617 (1966); *J. Am. Chem. Soc.*, **86**, 5365 (1964).

fluoro-5′-O-trityluridine (IV): 360 mg; mp 167°; λ_{max} 265 mμ (ε × 10^{-3} 6.8); titration, initial pH 6.3, mol wt 725 (calcd 650.72); pK_a = 8.9.

Anal. Calcd for $C_{39}H_{33}FN_2O_6$: C, 71.98; H, 6.04; N, 4.31. Found: C, 71.32; H, 6.66; N, 4.10.

A second crop of product, 50–100 mg, rR_f 4.7, was obtained and used without purification for pilot hydrolysis studies.

Removal of the trityl group was effected by dissolving the fully protected nucleoside (IV, 300 mg, 0.46 mmole) in 80% aqueous acetic acid (50 ml). The solution was heated under reflux for 20 min, allowed to cool, and then concentrated under reduced pressure at 45–50°. The residue (rR_f 4.0) was crystallized from benzene–CH_2Cl_2 to give the 3′-O-adamantoate (V): 170 mg; mp 209–210°; λ_{max} 268 mμ (ε × 10^{-3} 9.0); titration, initial pH 6.90, mol wt 408 (calcd 408); pK_a = 9.20; rR_f 4.0.

Anal. Calcd for $C_{20}H_{25}FN_2O_6$: C, 58.81; H, 6.17; N, 6.86. Found: C, 58.74; H, 6.10; N, 6.81.

The R_f values relative to 2′-deoxy-5-fluorouridine (5-FudR) for the products (IIIa and b and V) and intermediate derivatives (I, II, and IV) are summarized in Table II.

TABLE II[a]

R_f VALUES RELATIVE TO 2′-DEOXY-5-FLUOROURIDINE FOR 3′- AND 5′-ADAMANTOATES AND INTERMEDIATE PRODUCTS

Compd	rR_f
2′-Deoxy-5-fluorouridine	1.0
5′-O-Adamantoyl-5-FUdR (IIIa)	2.7
5′-O-Adamantoyl-5-FUdR (IIIb)	2.7
3′-O-Adamantoyl-5-FUdR (V)	4.0
3′-O-Acetyl-5-FUdR (I)	2.8
3′-O-Acetyl-5′-O-adamantoyl-5-FUdR (II)	3.9
5′-O-Trityl-5-FUdR	3.0
3′-O-Adamantoyl-5′-O-trityl-5-FUdR (IV)	4.7

[a] For experimental conditions, see introduction to Experimental Section.

5′-O-Adamantoylthymidine.[17,18]—Adamantoyl chloride [from 300 mg (1.65 mmoles) of adamantoic acid] was added to a solution of thymidine (250 mg, 1.0 mmole) in anhydrous pyridine (3 ml). After 20 hr, water (0.5 ml) was added, and the mixture was allowed to stand for 2 hr. Pyridine was removed under reduced pressure, and the residue was partitioned between $CHCl_3$ (25 ml) and 5% $NaHCO_3$ solution. The $CHCl_3$ solution was washed with water (10 ml) and dried (Na_2SO_4). The $CHCl_3$ was removed, and the resulting oil was chromatographed with the aid of 6.5 g of silicic acid (Davison, 100–200 mesh). Elution with benzene gave a small amount of adamantoic anhydride, and subsequent elution with ether–benzene (1:1) gave first a glass (49 mg) judged to be the 3′,5′-diadamantoate from its infrared spectrum (no OH bands), its ultraviolet spectrum (calcd mol wt 638; theory for diester 568, for monoadamantoate 406), and its tlc behavior (rR_f 5.5). Continued elution with ether–benzene (1:1) gave the 5′-adamantoate (330 mg) in crystalline form (rR_f 4.4). One recrystallization gave 230 mg of the analytically pure substance: mp 190–191°; λ_{max} 266 mμ (ε × 10^{-3} 10.35); ν_{max}^{Nujol} 1728 (ester C=O), 1666 (imide NH) cm^{-1}.

Anal. Calcd for $C_{21}H_{28}N_2O_6$: C, 62.34; H, 7.05; N, 6.92. Found: C, 62.42; H, 7.11; N, 7.21.

Thymidine 3′-Phosphate.[18]—5′-O-Adamantoylthymidine (300 mg, 0.75 mmole) was dissolved in anhydrous pyridine and added to a solution of cyanoethyl phosphate[9] in anhydrous pyridine (from 500 mg of the Ba salt). The solution was dried by threefold concentration *in vacuo* and finally brought to a volume of 5 ml. Dicyclohexylcarbodiimide (650 mg) was added, and the solution was allowed to stand for 24 hr at room temperature. Water (0.5 ml) was added. After standing at room temperature for 15 min, the crystals of dicyclohexylurea were filtered off. The solvent was evaporated under reduced pressure, the residue was dissolved in water (5 ml), and the solution was extracted with three successive volumes of 25 ml of ether. The aqueous solution containing 5′-O-adamantoylthymidine-3′-(2-cyanoethyl hydrogen phosphate) was made alkaline with 5 ml of 2 *N* LiOH solution and heated for 1 hr at 100° to remove the cyanoethyl and the adamantoyl group. The basic hydrolysate was acidified to pH 2 by batchwise treatment with Dowex 50 resin (H⁺ form) and extracted with ether to remove adamantanecarboxylic acid and polymeric material. Neutralization of the solution with saturated $Ba(OH)_2$ solution, followed by the addition of 2 vol of ethanol, yielded the Ba salt. The salt was filtered and washed successively with aqueous ethanol, ethanol, and finally with ether. The dried salt weighed 105 mg (theory, 350 mg).

Sensitivity toward Venom Diesterase.[18,19]—Solutions containing 10 optical density units of thymidine 5′- and 3′-phosphate, respectively, in 1.0 ml of 0.05 *M* Tris buffer (pH 8.2–9.0) were incubated with 0.4 ml of crude snake venom diesterase (Ross-Allen's Reptile Farm, Silver Springs, Fla.) at 37°. Aliquots were taken out at 10, 30, and 60 min and spotted on paper. The 5′-phosphate was completely cleaved to yield thymidine within 10 min while the 3′-phosphate remained resistant for the whole hour. The 5′-nucleotidase present in the crude venom enzyme preparation is known to cleave 5′-nucleotides but not 3′-nucleotides. The resistance of the phosphate prepared from the thymidine ester thus demonstrates this to be a 5′-adamantoate.

5′-O-Adamantoyl-2′-deoxyadenosine.—To a cooled (0–5°), stirred solution of 2′-deoxyadenosine (1.0 g, 4.0 mmoles) in anhydrous pyridine (25 ml) was added over a period of 15 min a solution of adamantoyl chloride (0.99 g, 5.0 mmoles) in anhydrous benzene (10 ml). After 16 hr, tlc showed two spots, both with wavelength maxima of 258 mμ. No starting material was present. Water (5 ml) was introduced, and the mixture was stirred for 30 min. The mixture was concentrated under reduced pressure, and the residue was dissolved in ethyl acetate (100 ml). The organic layer was washed with water (50 ml), with 5% $NaHCO_3$ solution (50 ml), and finally with water (two 50-ml portions). The solution was dried (5 g of Na_2SO_4) and concentrated. The residue was crystallized from a benzene–CH_2Cl_2 mixture. The crystals were filtered and dried in the air. Thin layer chromatography (silica gel G, Eastman Chromatogram Sheet, K301R, ethyl acetate–ethanol (3:1)) now showed a single spot (rR_f 1.8); the filtrate contained the faster running material (rR_f 2.2), presumably the by-product 3′,5′-diadamantoate. The crystalline adamantoate weighed 0.913 g; mp 182–183°; λ_{max} mμ (ε × 10^{-3}) 208 mμ (ε × 10^{-3} 19.8), 259 mμ (ε × 10^{-3} 13.8); $\bar{\nu}_{max}^{Nujol}$ 3122 (OH, H-bonded), 1700 (C=O ester, H-bonded) cm^{-1}; titration, initial pH 7.25, mol wt 420 (calcd 413.466); pK_a ~ 3.1.

Anal. Calcd for $C_{21}H_{27}N_5O_4$: C, 61.00; H, 6.58; N, 16.94. Found: C, 61.23; H, 6.56; N, 16.75.

Ribonucleoside Adamantoates. 5′-O-Adamantoyl-6-azauridine. 2′,3′-O-Isopropylidene-6-azauridine.[20]—6-Azauridine (6.0 g, Diera Products Ltd.) was suspended in anhydrous acetone (240 ml), and to the stirred suspension 70% $HClO_4$ solution[21] (0.48 ml) was added. After 30 min, a homogeneous solution resulted. The reaction was allowed to proceed for a total of 2 hr, at the end of which time complete conversion had taken place. Concentrated NH_4OH (1.2 ml) was added and the solvent was evaporated *in vacuo* leaving a white froth. This was dissolved in 15 ml of acetone and cyclohexane was added until incipient crystallization. After storage at 5° overnight, the isopropylidene derivative (5.4 g, 78%) was collected. The material showed a single spot on paper chromatography (R_f 0.73) and melted at 139–140° (lit.[20] mp 139–141°), rR_f 4.0.

5′-O-Adamantoyl-2′,3′-O-isopropylidene-6-azauridine.—To a solution of 2′,3′-O-isopropylidene-6-azauridine (10 g, 0.035 mole) in anhydrous pyridine (100 ml), adamantoyl chloride (12.25 g, 0.07 mole) was added, and the resulting solution was stirred at room temperature for 1 hr. At this time the acylation was complete as judged by tlc (rR_f 6.5). Water (10 ml) was introduced into the reaction flask. The mixture was stirred for 30 min and then concentrated under reduced pressure to give an oily residue. The residue was dissolved in acetone (50 ml) and dripped into 500 ml of chipped ice and water. The solids that formed were collected, dissolved in acetone (50 ml), and dripped into 500 ml of iced water, collected again, and then dissolved in ether (300 ml). The ethereal solution was washed with cold 1 *N* H_2SO_4 (25 ml), and then with water (150 ml),

(17) The help of Dr. Alex L. Nussbaum of the Biochemistry Department, School of Medicine, Stanford University, in carrying out this experiment is gratefully acknowledged.

(18) The experiment was carried out by one of us (K. G.) while a guest at the Syntex Research Institute for Molecular Biology, Palo Alto, Calif.

(19) The guidance of Dr. W. E. Razzell in the course of this experiment is thankfully remembered.

(20) S. Gurdek and J. Smrt, *Collection Czech. Chem. Commun.*, **28**, 1301 (1963).

(21) J. Zemlicka, J. G. Moffatt, K. Gerzon, O. Kau, and W. E. Grigsby, *J. Med. Chem.*, **8**, 375 (1965).

treated with 5 g of activated charcoal, dried (Na_2SO_4, 20 g), and finally concentrated under reduced pressure. A yellowish solid (22 g) was recovered. (This crude material, rR_f 6.5, was used in the next reaction step without further purification.)

5′-O-Adamantoyl-6-azauridine.—Crude 5′-O-adamantoyl-2′,3′-O-isopropylidene-6-azauridine (22 g) was added to 800 ml of acetic acid, and the mixture was stirred until a homogeneous solution resulted. Water (200 ml) was added, and the solution was heated under reflux for 30 min. The reaction mixture was cooled in an ice bath, and the solution was concentrated under reduced pressure at approximately 45–50°. The residue was dissolved in ethyl acetate (300 ml). The organic solution was washed with water (two 200-ml portions), dried (Na_2SO_4 20 g), and concentrated under reduced pressure to approximately 30 ml. Upon cooling in an ice bath, crystallization occurred. The crystals (rR_f 3.7) were filtered, washed with 10 ml of cold CH_2Cl_2, and dried under reduced pressure; yield 12 g; mp 179–180°; λ_{max} 264 mμ ($\epsilon \times 10^{-3}$ 5.85); titration, initial pH 5.40, mol wt 427 (calcd 407.4); $pK_a = 7.70$.

Periodate titrations[22] revealed the presence of 98.2, 98.5, and 100.1% of 1 mole equiv of α-glycol.

Anal. Calcd for $C_{19}H_{23}N_3O_7$: C, 56.01; H, 6.19; N, 10.31. Found: C, 55.95; H, 6.34; N, 10.06.

5′-O-(3″,5″,7″-Trimethyladamantoyl)-6-azauridine was prepared by the above procedure using 2.05 g (7.2 mmoles) of 2′,3′-O-isopropylidene-6-azauridine and 3.47 g (14.4 mmoles) of 3,5,7-trimethyladamantoyl chloride. The intermediate 2′,3′-O-isopropylidene-5′-O-(3″,5″,7″-trimethyladamantoyl)-6-azauridine (rR_f 7.0) was allowed to crystallize from a mixture of ether and petroleum ether (1:1); yield 2.85 g; mp 147–148°; titration, initial pH 5.4, mol wt 491 (calcd 490.554); $pK_a = 7.55$.

Anal. Calcd for $C_{25}H_{35}N_3O_7$: C, 61.33; H, 7.21; N, 8.58. Found: C, 61.39; H, 7.39; N, 8.44.

Heating of the above intermediate in methanol (300 ml) and 100 ml of 0.5 *N* HCl for 1 hr effected the removal of the isopropylidene group. The crystalline 5′-O-(3″,5″,7″-trimethyladamantoyl)-6-azauridine (rR_f 4.1) was obtained from a mixture of ether and petroleum ether (1:1); yield 1.91 g; mp 122–124°; λ_{max} 263 mμ ($\epsilon \times 10^{-3}$ 6.0), in 0.1 *N* NaOH solution 256 mμ ($\epsilon \times 10^{-3}$ 7.27); titration, initial pH 5.6, mol wt 458 (calcd 449.492); $pK_a = 7.8$.

Anal. Calcd for $C_{22}H_{31}N_3O_7$: C, 58.78; H, 6.95; N, 9.35. Found: C, 58.76; H, 7.18; N, 9.69.

5′-O-Adamantoyl-6-thioinosine.—To a solution of 2′,3′-O-isopropylidene-6-thioinosine[21] (690 mg, 2.14 mmoles) in anhydrous pyridine (20 ml) there was added 1.106 g (5.56 mmoles) of adamantoyl chloride, and the reaction mixture was stirred for 16 hr at room temperature. Water (5 ml) was introduced, and the mixture was stirred for 30 min and then concentrated under reduced pressure at approximately 60°. The residue was dissolved in 100 ml of hot methanol. After the solution was allowed to cool slowly to room temperature, crystals of 5′-O-adamantoyl-2′,3′-O-isopropylidene-6-thioinosine (rR_f 3.2), free of starting material (rR_f 2.6), formed; yield 957 mg; mp 214° dec–221°; λ_{max} 324 mμ ($\epsilon \times 10^{-3}$ 20.8); $\bar{\nu}_{max}$ 1740 (C=O ester) cm^{-1}; titration, initial pH 3.9, mol wt 478 (calcd 487.6); $pK_a = 8.55$.

Anal. Calcd for $C_{24}H_{30}N_4O_5S$: C, 59.12; H, 6.20; N, 11.49. Found: C, 59.74; H, 6.58; N, 10.79.

Removal of the isopropylidene group was effected by dissolving the fully protected nucleoside (448 mg, 1.0 mmole) in a mixture of methanol (300 ml) and 0.3 *N* HCl (175 ml) and heating the solution at 75–85° for 45 min. The solution was cooled and the pH was adjusted to 8.5 with concentrated NH_4OH. The mixture was concentrated under reduced pressure at approximately 45–50°. Solids precipitated as the volume of solution diminished. The solids were collected and dried in the air. Thin layer chromatography revealed two spots, the slower corresponding to 6-mercaptopurine (rR_f 2.1) and the faster to the desired 5′-adamantoate (rR_f 2.5). Recrystallization from hot methanol gave the pure ester; first crop yield 122 mg, second crop 50 mg; mp 226–227°; λ_{max} 224 mμ ($\epsilon \times 10^{-3}$ 7.55), 324 mμ ($\epsilon \times 10^{-3}$ 20.9); $\bar{\nu}_{max}$ 3500 (OH), 1746 (C=O ester) cm^{-1}; titration, initial pH 6.35, mol wt 454 (calcd 446); $pK_a = 8.75$.

Anal. Calcd for $C_{24}H_{26}N_4O_5S$: C, 56.48; H, 5.87; N, 12.55. Found: C, 56.59; H, 6.08; N, 12.48.

5′-O-Adamantoyladenosine.—To a cooled (0–5°), stirred solution of isopropylideneadenosine (5.0 g, 16.3 mmoles) in anhydrous pyridine (25 ml) was added dropwise a solution of adamantoyl chloride (3.26 g, 16.3 mmoles) in anhydrous benzene. The reaction was left at room temperature for 16 hr. Water (10 ml) was introduced and the mixture was stirred for 30 min. The mixture was concentrated under reduced pressure, and the resultant residue was dissolved in 100 ml of ethyl acetate and 100 ml of water. The organic solution was separated from the aqueous layer, then washed with 10% $NaHCO_3$ (two 100-ml portions), water (three 100-ml portions), and finally dried (Na_2SO_4, 5 g). The organic solution was concentrated under reduced pressure and the residue (rR_f 3.1) was allowed to crystallize from ether; yield 4.92 g; mp 159–160°; λ_{max} 258 mμ ($\epsilon \times 10^{-3}$ 14.1); $\bar{\nu}_{max}$ 1724 (C=O ester) cm^{-1}.

Anal. Calcd for $C_{24}H_{31}N_5O_5$: C, 61.39; H, 6.66; N, 14.92. Found: C, 61.23; H, 6.78; N, 14.79.

Two grams (4.47 mmoles) of the protected nucleoside was dissolved in 200 ml methanol and 50 ml of 0.5 *N* HCl solution. The solution was heated under reflux for 1.25 hr. The solution was cooled and was made slightly basic with dilute NH_4OH. The methanol was removed under reduced pressure, and the resulting emulsion was extracted with ethyl acetate (100 ml). The aqueous layer was again extracted with ethyl acetate (50 ml), and the combined ethyl acetate solutions were washed with 100 ml of water, dried (Na_2SO_4, 5 g), and concentrated. The residue (rR_f 2.2) was dissolved in warm CH_2Cl_2 and on cooling precipitated to yield 1.1 g of the desired 5′-O-adamantoate which sintered at 120° and melted completely at 140°; λ_{max} 258 mμ ($\epsilon \times 10^{-3}$ 14.3); $\bar{\nu}_{max}$ 1720 (ester C=O) cm^{-1}.

Anal. Calcd for $C_{21}H_{27}N_5O_5$: C, 58.73; H, 6.34; N, 16.31. Found: C, 58.80; H, 6.42; N, 16.29.

5′-O-(3″-Methyladamantoyl)adenosine.—The procedure for the reaction of 3-methyladamantoyl chloride (1.87 g, 8.8 mmoles) with isopropylideneadenosine (2.0 g, 6.5 mmoles) and the isolation of the product were the same as described above for the unsubstituted adamantoic acid. The residue obtained was allowed to crystallize from ether, yielding 2.5 g of 5′-O-(3″-methyladamantoyl)-2′,3′-O-isopropylideneadenosine (rR_f 3.2), mp 151–153°, λ_{max} 259 mμ ($\epsilon \times 10^{-3}$ 13.4), $\bar{\nu}_{max}$ 1725 (adamantoyl C=O) cm^{-1}.

Anal. Calcd for $C_{25}H_{33}N_5O_5$: C, 62.09; H, 6.88; N, 14.48. Found: C, 62.82; H, 7.38; N, 13.03.

Heating of 2′,3′-O-isopropylidene-5′-O-(3″-methyladamantoyl)adenosine (2 g, 4.13 mmoles) in 200 ml of methanol and 50 ml of 0.5 *N* HCl effected the removal of the isopropylidene group. 5′-O-(3″-Methyladamantoyl)adenosine (rR_f 2.2) was obtained in crystalline form from a methanol–ethyl acetate mixture (1:2); yield 1.3 g; mp 194–195°; λ_{max} 259 mμ ($\epsilon \times 10^{-3}$ 14.3).

Anal. Calcd for $C_{22}H_{29}N_5O_5$: C, 59.58; H, 6.49; N, 15.79. Found: C, 59.48; H, 6.86; N, 15.87.

5′-O-(3″,5″-Dimethyladamantoyl)adenosine.—The procedure for the reaction of 3,5-dimethyladamantoyl chloride (0.92 g, 4.06 mmoles) with isopropylideneadenosine (1.0 g, 3.25 mmoles) was the same as that described above. The residue obtained (rR_f 3.3), consisting of 5′-O-(3″,5″-dimethyladamantoyl)-2′,3′-O-isopropylideneadenosine, was heated under reflux in 160 ml methanol and 40 ml of 0.5 *N* HCl to effect the removal of the isopropylidene group. The product (rR_f 2.3) was obtained in crystalline form from ethyl acetate; yield 637 mg; mp 158–159°; λ_{max} 260 mμ ($\epsilon \times 10^{-3}$ 13.6); $\bar{\nu}_{max}^{mull}$ 1730 (C=O ester) cm^{-1}; titration, initial pH 8.4, a molecular weight could not be calculated; $pK_a = 3.1$.

Anal. Calcd for $C_{23}H_{31}N_5O_5$: C, 60.38; H, 6.83; N, 15.31. Found: C, 60.23; H, 6.85; N, 15.05.

5′-O-(3″,5″,7″-Trimethyladamantoyl)adenosine.—The procedure for the reaction of 3,5,7-trimethyladamantoyl chloride (0.786 g, 3.26 mmoles) with isopropylideneadenosine (1.0 g, 3.26 mmoles) was the same as that described above for the unsubstituted adamantoic acid. The crude residue obtained (rR_f 3.4) was allowed to crystallize from an ether–petroleum ether solvent mixture; yield 1.2 g; mp 156–162; λ_{max} 258 mμ ($\epsilon \times 10^{-3}$ 15.18); $\bar{\nu}_{max}$ 1724 (C=O ester) cm^{-1}.

Anal. Calcd for $C_{27}H_{37}N_5O_5$: C, 63.38; H, 7.29; N, 13.69. Found: C, 63.31; H, 7.52; N, 13.50.

One gram (1.92 mmoles) of 5′-O-(3″,5″,7″-trimethyladamantoyl)-2′,3′-O-isopropylideneadenosine was hydrolyzed and the product was isolated as described above. The residue (rR_f 2.5) was allowed to crystallize from ethyl acetate solution; yield of 5′-trimethyladamantoyladenosine was 0.607 g; mp 179–180°; λ_{max} 259 mμ ($\epsilon \times 10^{-3}$ 15.2); $\bar{\nu}_{max}$ 1724 (C=O ester), absence of peak at 1162 (O ether peak for isopropylidene) cm^{-1}.

(22) E. L. Jackson, *Org. Reactions*, **2**, 341 (1944).

Anal. Calcd for $C_{24}H_{33}N_5O_5$: C, 61.13; H, 7.05; N, 14.85. Found: C, 60.89; H, 7.46; N, 14.43.

5′-O-Adamantoyl-2′,3′-O-diacetyladenosine.—To a stirred solution of 5′-O-adamantoyladenosine (0.52 g, 1.21 mmoles) in anhydrous pyridine (5 ml) there was added in one batch 2 ml of acetic anhydride. The reaction mixture was maintained below 30° for 4 hr. Ethanol (5 ml) was introduced, the mixture was stirred for 30 min, and the solution was concentrated under reduced pressure at 45–50°. The residue was dissolved in ethyl acetate (100 ml), the organic layer was washed with cold, dilute $NaHCO_3$ solution (50 ml) and then with water (three 100-ml portions). The solution was dried (Na_2SO_4, 5 g) and concentrated. The residue obtained was dissolved in ether (25 ml) and the addition of hexane (10 ml) caused the precipitation of amorphous solids. The solids were filtered and dissolved in warm ethyl acetate. Upon cooling, the solution deposited the crystalline triester (rR_f 3.0); yield 196 mg; mp 236–237°; λ_{max} 259 mμ ($\epsilon \times 10^{-3}$ 13.7); $\bar{\nu}_{max}^{mull}$ 1758 (acetyl C=O), 1735 (adamantoyl C=O) cm^{-1}.

Anal. Calcd for $C_{25}H_{31}N_5O_7$: N, 13.82. Found: N, 13.82.

The low solubility (less than 0.01 mg/ml) of this ester precluded its assay *in vitro* as an inhibitor of ADP-induced platelet aggregation.

5′-O-Benzoyladenosine.—The procedure for the reaction of benzoyl chloride (0.36 ml, 3.26 mmoles) with isopropylideneadenosine (1.0 g, 3.26 mmoles), as well as the isolation of the product, was the same as that described above. The residue obtained was dissolved in methanol (75 ml) and 25 ml of 0.5 *N* HCl solution. The solution was heated under reflux for 45 min to remove the isopropylidene grouping and cooled, and the methanol was removed under reduced pressure. The remaining aqueous solution (pH 1) was titrated to pH 6 with a 5% $NaHCO_3$ solution. The precipitated ester (rR_f 2.2) was washed with water (two 50-ml portions) and allowed to crystallize from aqueous ethanol to yield 486 mg of the benzoate: mp 166–170°; λ_{max} 231 mμ ($\epsilon \times 10^{-3}$ 14.85), 258 mμ ($\epsilon \times 10^{-3}$ 14.65); $\bar{\nu}_{max}$ 1730 (ester C=O) cm^{-1}.

Anal. Calcd for $C_{17}H_{17}N_5O_5$: C, 54.98; H, 4.61; N, 18.86. Found: C, 55.04; H, 5.15; N, 19.13.

The R_f values relative to adenosine for the various adenosine adamantoates and intermediate compounds are listed in Table III.

TABLE III[a]
R_f VALUES RELATIVE TO ADENOSINE (rR_f) FOR ADENOSINE ADAMANTOATES AND INTERMEDIATES

Adenosine derivative[b]	rR_f[a]
Adenosine	1.0
2′,3′-Isopropylidene-	2.0
5′-Adamantoyl-2′,3′-isopropylidene-	3.1
Monomethyladamantoylisopropylidene-	3.2
Dimethyladamantoylisopropylidene-	3.3
Trimethyladamantoylisopropylidene-	3.4
5′-Adamantoyl-	2.2
Monomethyladamantoyl-	2.2
Dimethyladamantoyl-	2.3
Trimethyladamantoyl-	2.5
5′-O-Adamantoyl-2′,3′-O-diacetyl-	3.0
5′-Benzoyl-	2.2

[a] For experimental conditions see introductory paragraph of Experimental Section. [b] Mono-, di-, and trimethyladamantoyl derivatives refer to the 3″,3″,5″-, and 3″,5″,7″-substituted esters described in the text.

5′-O-Adamantoylinosine.—The procedure for the reaction of 2′,3′-isopropylideneinosine (1.0 g, 3.25 mmoles) and adamantoyl chloride (1.28 g, 6.5 mmoles) and the isolation of the intermediate, fully protected nucleoside, were the same as described above for the adenosine esters. The crude residue obtained was dissolved in a mixture of 300 ml of methanol and 100 ml of 0.5 *N* HCl solution. The solution was heated under reflux for 45 min, then cooled to room temperature, and the pH was adjusted to 7.0 with cold dilute NH_4OH solution. It was then heated under reflux with activated charcoal for 5 min and filtered. The filtrate was concentrated, whereupon solids precipitated. The solids were allowed to crystallize from ethyl acetate–methanol (1:1); yield of product 300 mg; mp 202–205°; λ_{max} 200 mμ ($\epsilon \times 10^{-3}$ 19.6), 245 mμ ($\epsilon \times 10^{-3}$ 11.25), in 0.1 *N* NaOH solution 253 mμ ($\epsilon \times 10^{-3}$ 13.25); $\bar{\nu}_{max}$ 1710 (C=O ester) cm^{-1}; titration, initial pH 7.0, mol wt 428 (calcd 430.45); pK_a = 9.85.

Anal. Calcd for $C_{21}H_{26}N_4O_6$: C, 58.59; H, 6.09; N, 13.02. Found: C, 58.53; H, 6.33; N, 12.44.

A second crop yielded an additional 153 mg of material.

Biological Evaluation

The search for biological assays capable of a quantitative, comparative evaluation of the various nucleoside adamantoates led to three promising systems. First, 5′-O-adamantoyl-6-thioinosine was found to suppress the formation of antibodies in mice following the injection of sheep red blood cells.[23] Subsequently, selected experimental murine tumor systems[24] were found to be sensitive to treatment with adamantoates of nucleoside analogs; the leukemia L1210 responded favorably to the 5′-adamantoate of 6-thioinosine, and the Taper hepatoma[25] to the 5′-adamantoate of 6-azauridine.

Useful as these two *in vivo* systems may be for the study of structure–activity relationships and for the understanding of the mode of action of the various adamantoates, progress toward such understanding is, of necessity, slow because of the complex nature and the long duration of such assays. Therefore, suitable *in vitro* assays appeared desirable because such systems, especially those of short duration, might eliminate the factors of drug absorption, distribution, and metabolism, which do complicate the interpretation of results obtained in the animal host. *In vitro* cytotoxicity studies using the L5178Y mammalian cell culture system,[26] as well as *in vitro* antiviral assays[27] with the adamantoates of 6-azauridine, 6-thioinosine, and 2′-deoxy-5-fluorouridine, indicated these esters to possess activities of the same magnitude as those of the parent nucleoside analog. However, these assays cannot be expected to provide distinctive evidence of superiority of the adamantoates; because of the conditions and the duration of such assays (about 4 days) the possibility of ester cleavage to give the biologically active parent nucleoside cannot be excluded.

Finally, the ADP-induced aggregation of platelets in rabbit platelet rich plasma[28] and its inhibition by adenosine species[29] furnished an attractive *in vitro* assay of short duration (about 2–5 min) in which the activities of adenosine 5′-adamantoate and its methylated congeners could be compared with a satisfactory degree of quantitation.

Biological Methods and Results

Immune Suppression.—The procedure for the determination of immune suppression (antoimmune screen), adapted[30] from the

(23) H. C. Nathan, S. Bieber, G. B. Elion, and G. H. Hitchings, *Proc. Soc. Exptl. Biol. Med.*, **107**, 796 (1961).

(24) (a) I. S. Johnson, H. F. Wright, G. H. Svoboda, and J. Vlantis, *Cancer Res.*, **20**, 1016 (1960); (b) H. E. Skipper and L. H. Seidel, *Cancer Chemotherapy Rept.*, **17**, 1 (1962).

(25) H. S. Taper, G. W. Woolley, M. N. Teller, and M. P. Laride, *Cancer Res.*, **26**, 143 (1966).

(26) E. E. Haley, G. A. Fischer, and A. D. Welch, *Ibid.*, **21**, 532 (1961); G. A. Fischer, *Ann. N. Y. Acad. Sci.*, **76**, 543 (1958).

(27) P. Sninnoff, *Appl. Microbiol.*, **9**, 66 (1961).

(28) J. F. Mustard, B. Hegardt, H. C. Rowsell, and R. L. MacMillan, *J. Lab. Clin. Med.*, **64**, 548 (1964).

(29) G. V. R. Born, R. J. Haslam, and M. Goldman, *Nature*, **205**, 678 (1965).

(30) The authors are greatly indebted to Dr. R. L. Stone and Mr. G. N. Wolfe, Biological Research Division, The Lilly Research Laboratories, for these assays. The details of this modified method will be published separately.

method described by Nathan, *et al.*,[23] involved the intraperitoneal injection of groups of five mice (Swiss, 20 g av wt) with standardized suspensions of sheep erythrocytes. Test compounds are injected by the same route 48 hr before and 48 hr after the red cell injections. Eight days after the red cell antigen injections, the mice are bled and the sera from each group of five mice are pooled. Antibody determinations are made on the serum pools by hemagglutination pattern procedure, and comparisons are made between treated and control animals.

The test results for the 6-thioinosine species, additional nucleoside adamantoates, 6-mercaptopurine, and Imuran[31] are listed in Table IV.

TABLE IV
SUPPRESSION OF ANTIBODY FORMATION IN MICE AFTER SHEEP ERYTHROCYTE INJECTION BY NUCLEOSIDE ADAMANTOATES

Nucleoside derivative	End point activity,[a] mg/kg × 2	Activity
6-Mercaptopurine	≥150[b]	+ (?)
Imuran[31]	300[c]	+
6-Thioinosine	>200	−
2′,3′-Isopropylidene-	>300	−
2′,3′,5′-Triacetyl-[4]	>400	−
5′-O-Adamantoyl-	200[d]	+
5′-O-Adamantoyladenosine	>300	−
5′-O-Adamantoylinosine	>300	−
6-Azauridine	>800	−
5′-O-Adamantoyl-	>800	−
2′,3′,5′-O-Triacetyl-[3]	>800	−

[a] Fourfold or greater suppression of antibody titer. Dosage by routint prc- and postantigen intraperitoneal administration of compounds except where otherwise indicated. [b] Active at 150 mg/kg level in one out of three tests; toxic at 200 mg/kg × 2. [c] Active at 300 mg/kg × 2 or at 300 mg/kg × 1 (postantigen). [d] Very active; antibody virtually undetectable at 300 mg/kg × 2 or 300 mg/kg × 1 (postantigen). Expressed on a molar basis, the activity of 5′-O-adamantoyl-6-thioinosine (mol wt 429) is about 2.5 times that of Imuran (mol wt 261). Assays performed by Dr. R. L. Stone and Mr. R. N. Wolfe, The Lilly Research Laboratories.[23,30]

Antitumor Testing.—The procedures for antitumor testing in this laboratory have been previously described.[24a] The L1210 leukemia is a well-known acute lymphocytic leukemia maintained in DBA/2 mice; the Taper hepatoma is a newly described murine hepatoma.[25] Preliminary antitumor data[32a] are reported in Table V for the 6-azauridine group of compounds against the solid form of the Taper hepatoma, in Table VI for the 6-thioinosine group aginst leukemia L1210, and in Table VII against the ascites form of the Taper hepatoma. Activities of 2′-deoxy-5-fluorouridine esters[32b] aga nst Adenocarcinoma 755 are listed in Table VIII.

Cytotoxicity Studies.—Cytotoxicity determinations[33] were performed with the lymphoblastic leukemia L5178Y cell[26] by the methods described previously.[33,34] The cytotoxicity of a representative group of nucleoside 5′-adamantoates and additional reference compounds is presented in Table IX in terms of the dose inhibiting 50% of control growth (ID_{50}).

In Vitro **Antiviral Studies.**—The activity of a limited number of adamantoates in suppressing growth of vaccinia virus was

TABLE V
ANTITUMOR ACTIVITY OF 6-AZAURIDINE 5′-ADAMANTOATES IN THE TAPER HEPATOMA SYSTEM[a]

Compd (dosage[b])	Av wt change, g, T/C	Tumor size, mm², T/C	% activity[c]
6-Azauridine			
(500)	−3.8/3.2 (I)	7.4/18.2	60[9]
(500)	−2.4/1.9 (II)	5.9/16.8	65[8]
(500)	0.0/3.2 (III)	8.0/13.9	43[10]
Triacetate[3]			
(500)	−3.9/3.2 (I)	8.1/18.2	56[9]
(500)	−1.1/1.9 (II)	7.5/16.8	56[9]
Adamantoate			
(500)	−1.2/3.2 (I)	6.0/18.2	67[10]
(500)	−2.7/1.9 (II)	6.2/16.8	63[4]
Trimethyladamantoate			
(300)	−1.0/1.9 (II)	9.6/16.8	42[0]
(400)	−4.8/3.2 (III)	9.4/13.9	32[6]

[a] The Taper hepatoma[25] was received in the ascitic form from Sloan-Kettering Laboratories through the courtesy of Dr. M. N. Teller. It was converted to the solid form used in these experiments by subcutaneous injection of the ascitic cells and serial passage of the resulting solid tumor by trocar implantation. Assays using the C_3H mouse were performed by Dr. I. S. Johnson, Mr. G. A. Poore, and associates, The Lilly Research Laboratories.[32a] The results of two separate assays I and II performed within 3 weeks of each other are given; experiment III was performed approximatey 2 months later. [b] Daily intraperitoneal dose in milligrams per kilogram given for 10 days commencing on the day after tumor implantation. [c] The superscript denotes the number of animals surviving out of a group of 10. Activity of adamantoate (mol wt 407) on a molar basis is approximately 1.5 times that of the parent nucleoside (mol wt 242).

TABLE VI
ANTITUMOR ACTIVITY OF 6-THIOINOSINE 5′-ADAMANTOATE IN THE LEUKEMIA L1210 SYSTEM[a]

Compd (dosage[b])	Av wt change, g, T/C	Av life span, days, T/C	% activity
6-Mercaptopurine			
(30)	0.0/4.0	16.0/10.4	54
(30)	−1.7/3.5	13.4/10.3	30
6-Thioinosine			
(30)	2.0/4.0	11.6/10.4	..
(30)	0.5/3.5	10.8/10.3	..
Triacetate[4]			
(45)	1.5/4.0	11.4/10.4	..
(45)	3.0/3.5	11.3/10.3	..
Adamantoate			
(30)	0.0/4.0	15.4/10.4	48
(30)	−1.7/3.5	13.4/10.3	30

[a] Assays were performed by Dr. I. S. Johnson and Mr. G. A. Poore, The Lilly Research Laboratories.[32a] The strain of the acute lymphocytic leukemia L1210 was obtained from Dr. Dorris J. Hutchinson of the Walker Laboratories of the Sloan-Kettering Institute and was maintained in these laboratories in DBA/2 mice. [b] Daily intraperitoneal dose in milligrams per kilogram given for 10 days commencing the day after inoculation.

TABLE VII
ANTITUMOR ACTIVITY OF 6-THIOINOSINE 5′-ADAMANTOATE IN TAPER HEPATOMA ASCITES SYSTEM[a]

Compd (dosage)[b]	Av wt change, g, T/C	Av life span, days, T/C	% activity
6-Mercaptopurine (30)	6.0/8.6	16.9/14.8	15
6-Thioinosine (30)	5.0/8.6	14.6/14.8	..
Adamantoate (30)	6.1/8.6	24.5/14.8	66

[a] Assays using the C_3H mouse were performed by Dr. I. S. Johnson and Mr. G. A. Poore, The Lilly Research Laboratories.[32a] The results are given for a single experiment. [b] Daily intraperitoneal dose in milligrams per kilogram given for 10 days commencing the day after tumor inoculation.

(31) 6-[1-Methyl-4-nitro-5-imidazolyl)thio]purine: D. A. Clarke, G. B. Elion, G. H. Hitchings, and C. C. Stock, *Cancer Res.* **18**, 445 (1958); *Cancer Chemotherapy Rept.*, **14**, 93 (1961).

(32) (a) The authors gratefully acknowledge the courtesy of Dr. I. S. Johnson and Mr. G. A. Poore, Biological Research Division, The Lilly Research Laboratories, in obtaining the test results with the 6-azauridine and 6-thioinosine esters. (b) They are indebted to Dr. C. Heidelberger, McArdle Institute, Madison, Wis., for the results with the esters of 2′-deoxy-5-fluorouridine.

(33) K. Gerzon, I. S. Johnson, G. B. Boder, J. C. Cline, P. J. Simpson, C. Speth, N. J. Leonard, and R. A. Laursen, *Biochim. Biophys. Acta*, **119**, 445 (1966). The authors gratefully acknowledge the help of Dr. I. S. Johnson and Miss Carla Speth (Mrs. G. Boder), Biological Research Division, The Lilly Research Laboratories, in obtaining the cytotoxicity data.

(34) C. Waymouth, *J. Natl. Cancer Inst.*, **17**, 305 (1956).

TABLE VIII
ANTITUMOR ACTIVITY OF 2′-DEOXY-5-FLUOROURIDINE 5′-ADAMANTOATE IN THE ADENOCARCINOMA 755 SYSTEM[a]

Compd (dosage)[b]	Wt change, g, Day 0	Wt change, g, Day 15	Tumor vol, mm³, Day 11	Tumor vol, mm³, Day 15	% activity, Day 11	% activity, Day 15
2′-Deoxy-5-fluorouridine (40.0)	−2.3	+0.3	450	6187	82	32
3′,5′-Diacetate[c] (52.5)	−0.3	+3.3	1950	8699	22	5
5′-Adamantoate[c] (66.5)	−2.2	+1.1	287	4825	89	47
Control	−1.1	+1.1	2511	9120	..	..

[a] Assays performed through the courtesy of Dr. C. Heidelberger at the McArdle Institute, Madison, Wisconsin.[32b] The compounds were administered by stomach tube in propylene glycol and the controls also received this solvent. [b] Daily dose in milligrams per kilogram given for 7 days commencing the day after tumor transplantation. [c] Two mice dead on day 11.

TABLE IX
CYTOTOXICITY OF NUCLEOSIDE 5′-ADAMANTOATES IN THE L5178Y TISSUE CULTURE CELL SYSTEM[a]

Compound (mol wt)	50% inhibitory dose[b], μg/ml	50% inhibitory dose[b], μmoles/l.
Thymidine (242)	44.0	1.8×10^2
Adamantoate (404)	41.0	1.0×10^2
Deoxyadenosine (251)	58.0	2.3×10^2
Adamantoate (413)	56.0	1.4×10^2
6-Azauridine (245)	0.42	1.7
Triacetate[3] (471)	[c]	
Adamantoate (407)	0.65	1.6
Trimethyladamantoate[c] (449)	67.0	1.5×10^2
6-Thioinosine (284)	4.0	14.0
Triacetate (410)	[c]	
Adamantoate (446)	8.0	18.0
2′-Deoxy-5-fluorouridine (246)	2.5×10^{-4}	1.0×10^{-3}
Adamantoate (408)	4.4×10^{-4}	1.1×10^{-3}

[a] These studies were carried out by Miss Carla Speth (Mrs. C. Boder), The Lilly Research Laboratories. Compounds were tested as described previously;[33] cells were grown for 4 days at 37°, harvested by centrifugation, and resuspended in 1.0 ml of growth medium, and the total cell volume was determined by the hematocrit method.[34] The 50% inhibitory dose is that concentration of the compound in the medium which inhibits cell growth to 50% of the controls. [b] A single experiment indicates an activity of the same magnitude as that of the adamantoate. [c] No satisfactory test obtained.

evaluated by the methods of Siminoff[35] and of Grady, *et al.*,[36] as modified in this laboratory,[36] using a human amnion cell line growing in tissue culture. At a concentration of 62.5 μg/ml applied to the paper pad, 5′-adamantoyl-2′-deoxy-5-fluorouridine (mol wt 408) produced cell protection in a 20-mm zone, while a 40-mm zone resulted from the parent nucleoside (mol wt 246) itself at that concentration. In either case, no toxicity to the cells in the culture was noted. The cells in the activity zone appeared normal with some indication of decreased metabolism.[36] Thymidine 5′-adamantoate did not inhibit the virus, nor did it show toxicity toward the cells.

Platelet Aggregation Studies.[37]—The method for studying ADP-induced platelet aggregation[37] was essentially that described by Mustard, *et al.*[38] The evaluation of the relative potency of the various inhibitors was carried out and expressed relative to adenosine as described by Born.[38] Rabbit platelet rich plasma (PRP) without any added anticoagulant was used exclusively. The PRP was prepared by differential centrifugation and was kept in an ice bath until used. One milliliter of PRP was added to 0.3 ml of imidazole buffer (pH 7.4) containing the proper concentration of drug and incubated for 3 min at 37° prior to the addition of a suitable standard dose of ADP varying from 2.1×10^{-7} to 8.4×10^{-7} *M* (final concentration) (0.1 ml), giving a final total volume of 1.4 ml for the reaction mixture. Aggregation of platelets was measured in terms of the decrease in optical density of the PRP, and inhibitory potency was expressed as the extent of the diminution of this parameter of aggregation. The particular value of the dose of ADP used in each individual experiment is selected from each sample of PRP to give an aggregation response of the proper magnitude for inhibition studies.

Relative potency was calculated as follows. For each individual sample of PRP, adenosine was tested along with one or more nucleoside derivatives. A dose–response curve was plotted on semilog paper for each substance (molar concentration on the log scale, per cent inhibition on the normal scale). Assigning a potency of 100 to adenosine, the relative potency for each substance was then calculated in terms of that of adenosine. The inhibitory activities of adenosine adamantoates and additional nucleosides are listed in Table X.

TABLE X
INHIBITION OF ADP-INDUCED PLATELET AGGREGATION BY ADENOSINE 5′-ADAMANTOATES[a]

Nucleoside	$ID_{50} \times 10^7$ [b], Expt 1st	$ID_{50} \times 10^7$ [b], Expt 2nd	Rel potency[c], Expt 1st	Rel potency[c], Expt 2nd
Adenosine	2.7	27	100	100
Adamantoate	2.4	37	112	73
Methyladamantoate	...[d]	28	...[d]	96
Dimethyladamantoate	93	95	3	28
Trimethyladamantoate	130[e] (30)[f]	3×10^3 (4)[g]	1	NA[h]
Benzoate	83[e] (30)	NT[g]	1	NT[g]
5′-Adamantoate				
Deoxyadenosine	990[e] (18)		1	NT
Inosine	380[e] (13)		1	NT
6-Azauridine	0[e] (0)		NA	NT
6-Thioinosine	65[e] (0)		NA	NT

[a] Assays were performed by Dr. R. G. Herrmann and Messrs. J. D. Franks and C. M. Sage, The Lilly Research Laboratories.[37] Except where indicated otherwise the molar concentration given is the dose which elicits a 50% inhibition of the aggregation induced by ADP at the levels of 2.1×10^{-7} to 8.4×10^{-7} *M*. In some experiments (see *d*) the lack of solubility of the tested agent or the failure to obtain a proper dose–response curve precluded the attainment of a 50% inhibition level. The data given are for two separate experiments done on different days several weeks apart. The dose of ADP given in each experiment was different; namely 2.1×10^{-7} and 8.4×10^{-7} *M*, respectively, leading to different values for the adenosine control of 2.7 and 27×10^{-7} *M* for 50% inhibition. [b] Activity of agents listed relative to adenosine.[29] It is obvious from the large variation (tenfold) in the 50% inhibition value for adenosine that the figures given for the relative potency should be taken as an approximation, indicating an order of magnitude rather than an accurate quantitation. [c] Compound not available at first test. [d] Insufficient solubility prevented testing at higher levels. [e] Figure in bracket is the observed per cent inhibition at the concentration indicated. [f] Not active. [g] Not tested.

Adenosine Deaminase Activity.—Adenosine deaminase assays[38] were performed to ascertain whether adenosine 5′-adamantoate inhibits the enzymatic deamination of adenosine or is itself

(35) J. E. Grady, W. L. Loomis, and C. G. Smith, *Proc. Soc. Exptl. Biol. Med.*, **103**, 727 (1960).

(36) D. C. DeLong, W. S. Boniece, J. C. Cline, and I. S. Johnson, *Ann. N. Y. Acad. Sci.*, **130**, 440 (1965). The authors acknowledge with thanks the help of Mr. J. C. Cline, Biological Research Division, The Lilly Laboratories, in obtaining the antiviral data.

(37) The authors are grateful to Dr. R. G. Herrmann and Messrs. J. D. Franks and C. M. Sage, Pharmacological Research Division, The Lilly Research Laboratories, for the platelet aggregation results. A manuscript describing the details of this method and additional observations is in preparation and will be submitted for publication.

(38) The authors are grateful to Dr. R. W. Fuller, Pharmacological Research Division, The Lilly Research Laboratories, for the results of the adenosine deaminase studies.

deaminated by this enzyme. The method of Kaplan[39] was used to measure the deamination of adenosine as the substrate at 6.6×10^{-5} *M* by following the disappearance of absorption at 265 mμ and the appearance of absorption at 240 mμ (formation of inosine). Adenosine deaminase (0.1 μg) was obtained from Boehringer Co. and used without prior purification at 30° over a period of 30 min. Adenosine adamantoate was dissolved in 10% ethanol and used at a final concentration of 1×10^{-4} *M* with a final concentration of ethanol of 2 vol. %. This concentration of ethanol produced an enzyme inhibition of less than 5%. The enzyme at pH 6.5 deaminated 11.8 mμmoles of adenosine/min and at pH 7.4 deaminated 9.4 mμmoles/min.

Under the conditions of these experiments, adenosine 5′-adamantoate does not inhibit the deamination of adenosine and is itself not deaminated by adenosine deaminase.

Discussion[40]

In the course of our earlier studies of hypoglycemic N′-adamantylsulfonylureas[7] and anabolic nortestosterone 17β-adamantoates,[2] two general features were noted: (a) in selected cases the introduction of the adamantane moiety produced agents with outstanding biological activity superior in a quantitative or qualitative sense to those containing more conventional hydrocarbon groups, and (b) the favorable influence of the adamantyl group on the efficacy of these agents was apparently governed by a surprisingly high degree of structural specificity. Among the results presently reported for the activity of nucleoside adamantoates in various other biological assay systems the same two features are again discernible.

Thus the former feature is seen in the pronounced ability of 6-thioinosine 5′-adamantoate to suppress antibody formation following challenge with sheep erythrocytes while 6-thioinosine or its triacetate are inactive under the same experimental conditions (Table IV). It is also seen in the remarkable ability of adenosine 5′-adamantoate at the low concentration of 10^{-7} *M* to inhibit ADP-induced platelet aggregation (Table X) and finally, albeit to a lesser degree, in the antitumor activity of the adamantoates of 6-thioinosine, 6-azauridine, and 2′-deoxy-5-fluorouridine in the L1210 leukemia, the Taper hepatoma, and the Adenocarcinoma 755 systems, respectively.

The latter feature, that of structural specificity, is clearly in evidence among the four adenosine adamantoates (Table X): the monomethyladamantoate retains the activity of the adamantoate, and the substitution of two methyl groups results in appreciable loss, of three methyl groups in total loss of activity. Also, the inactivity of the 3″,5″,7″-trimethyladamantoyl-6-azauridine in the Taper hepatoma system (Table V) and the negligible cytotoxicity of this ester toward the L5178Y cell culture (Table IX) are additional, if less complete, examples demonstrating this trend of structure–activity relationship.

As a corollary to these principal features, it appears that in several instances the biological effects of the adamantoates are an expression of the intact ester, rather than the result of hydrolysis of the ester to give the parent nucleoside. Action of the intact agent has previously been proposed for the nortestosterone adamantoate,[2] and such action is self-evident for the sulfonylureas.[7] Indications of *in toto* action of a nucleoside adamantoate is most convincingly seen in the brief (2–3 min) *in vitro* assay demonstrating the inhibition (prevention) of platelet aggregation by 5′-adamantoyladenosine. Also, the cytotoxicity of 5′-adamantoyl-6-azauridine toward the L5178Y cell culture and, in contrast, the lack of such activity in the trimethyl-substituted ester (see Table IX) is compatible with this notion. Whether *in vivo* activity of the adamantoate of 6-thioinosine in suppressing antibody formation, for example, could possibly be an expression of action *in toto* remains a question for future investigation.

Biological Activities.—The activities listed (Methods and Results) are of a preliminary, exploratory nature and serve two purposes: to uncover new leads or to improve existing agents in various areas of chemotherapy, and to gain an understanding into the nature of the contribution of the adamantane group to these activities. Though emphasis in this report is laid, by title, on aspects of adamantane pharmacodynamics, brief comments on the chemotherapeutic aspects are in order. Because of limitations in supply and because of the ease in interpretation and comparison of the test results obtained, the intraperitoneal route of administration was used with a single exception (Table VIII) for the *in vivo* studies. Studies employing the oral route are currently in progress and will be reported at a later date. Extension of the present investigation to adamantoates of additional nucleoside analogs, 5-iodo-2′-deoxyuridine,[41] S-methyl-6-thioinosine,[42] and others, is in progress.

The ability of 5′-adamantoyl-6-thioinosine to suppress antibody formation (Table IV) and the inability of the esters of adenosine, inosine, and 6-azauridine to do so points to a specific dependence of this action on the thioinosine fragment. The degree of dependence of this activity on the adamantane moiety has not been ascertained at this time because of the unavailability of the required compounds; the trimethyladamantoate, for example, though obtained quite pure, has thus far failed to crystallize.

A comparison of the activities of the 6-azauridine species against the Taper hepatoma (Table V) does not reveal superiority of the adamantoate when administered by the intraperitoneal route. Extensive studies with the triacetate[3] have demonstrated its superiority over the parent nucleoside when evaluated by the oral route; final judgment of the adamantoate awaits studies by this route. The lack of demonstrated activity of the trimethyladamantoate is noted. Thus far, this is the only instance of an *in vivo* assay of a trimethyladamantoyl nucleoside.

The activity of 6-thioinosine adamantoate (mol wt 429) against the L1210 leukemia (Table VI) on a molecular basis is between 2.5 and 3.0 times that of 6-mercaptopurine (mol wt 152). The activity profile for the four compounds reported resembles that of these same agents in the autoimmune assay (Table IV).

The activity of the 6-thioinosine adamantoate against the ascites form of the Taper hepatoma (Table

(39) N. O. Kaplan, *Methods Enzymol.*, **2**, 473 (1955).

(40) Acknowledgments for the assistance and cooperation received from colleagues and associates of the Lilly Laboratories and from others in the performance of the varied assays are found in the legends to the individual tables and in the footnotes cited (30, 32, 33, and 36–38).

(41) H. E. Kaufman, *Proc. Soc. Exptl. Biol. Med.*, **109**, 251 (1962).

(42) (a) F. M. Schabel, Jr., J. A. Montgomery, H. E. Skipper, W. R. Laster, Jr., and J. R. Thomson, *Cancer Res.*, **21**, 690 (1961); (b) J. A. Montgomery, T. P. Johnston, A. Gallagher, C. R. Stringfellow, and F. M. Schabel, Jr., *J. Med. Pharm. Chem.*, **3**, 265 (1961).

VII) represents a single preliminary experiment which thus far has not been reproduced successfully.

The activity of the adamantoate of 2′-deoxy-5-fluorouridine in the Adenocarcinoma 755 system (Table VIII), though of the same order of activity as that of the parent nucleoside or its diacetate, is nevertheless of interest as a first demonstration of efficacy of a nucleoside adamantoate by absorption from the gastrointestinal tract.

The cytotoxicity of the adamantoates of nucleosides and nucleoside analogs (Table IX), with the exception of the trimethyladamantoate of 6-azauridine, is of the same order as that of the parent compound. Naturally, the activities observed may be due to chemical or enzymatic hydrolysis of the ester to give the parent nucleoside; however, the negligible activity of the 6-azauridine trimethyladamantoate renders this simplified explanation questionable. Whether these adamantoates exert their action *in toto* or by prior hydrolysis obviously remains a problem for future investigation. The same problem is encountered in the interpretation of the *in vitro* antiviral activity (vaccinia) of the adamantoate of 2′-deoxy-5-fluorouridine.

The individual ability of the adenosine esters to inhibit ADP-induced platelet aggregation (Table X) apparently depends on the integrity of both the adamantane and the adenosine moiety. Monomethylation is permissible, but other variations lead to loss of activity. The inactivity of the 5′-benzoate is worthy of note. That the activity of these esters is an expression of the intact agent appears plausible in view of the short duration (2–3 min) of the *in vitro* assay and the striking decrease of activity following progressive methylation of the adamantane fragment. The alternate view, which cannot be ruled out rigorously, holds that activity of the adamantoate and 3-methyladamantoate may be the result of rapid enzymatic hydrolysis to yield adenosine and that the dimethyl- and trimethyladamantoate may be poorly hydrolyzed or not at all. Additional unpublished experiments[37] render this alternate view quite unlikely: virtually immediate (10–15 sec) *reversal* of platelet aggregation is observed upon the addition of adenosine adamantoate to the assay system 30 sec *after* its induction is initiated by ADP.

The observed resistance of adenosine 5′-adamantoate to the action of adenosine deaminase raises the hope, as yet unfulfilled,[37] of demonstrating activity *in vivo*.[42c] Inasmuch as certain antitumor agents, *e.g.*, arabinosyladenine, suffer inactivation *in vivo* by enzymatic deamination,[43] the attempt to block such metabolic degradation by means of adamantoylation becomes an inviting proposition.

Finally, dependence of the respective biological activities on integrity of the nucleoside fragment is summarized in Table XI.

TABLE XI

Adamantoate	Antitumor activity	Suppression of antibody formation	Inhibition of platelet aggregation
6-Thioinosine	+	+	+
6-Azauridine	±	−	−
Adenosine	−	−	+
Inosine	−	−	

Mode of Action.—In comparing the activity profile of adamantane agents *in vivo* with the *in vitro* profile of the adenosine adamantoates, an extensive similarity is apparent: the adamantane-containing compounds show outstanding activity while structural alterations (methylation) result in a parallel decrease of activity in both situations. For the favorable influence of the adamantane moiety on agents assayed *in vivo*, three possible contributing factors have been listed (see above), namely, the absorption and distribution characteristics of these agents, their metabolic disposition in the animal host, and the precision of agent–receptor site fit. Naturally, for the adenosine adamantoates which inhibit platelet aggregation *in vitro*, the principal factor contributing to their high efficacy must be the precision with which the adamantane moiety matches a complementary region of the receptor site molecule.

By analogy, we consider it quite plausible that the *principal factor contributing to the high efficacy of the adamantane agents in vivo also is the precision of agent–receptor site fit*, rather than aspects of absorption, distribution, or metabolism of the agent.

The question concerning the molecular basis of the precise binding of the adamantane moiety presents itself. Gill has reviewed various types of bonding that operate between drugs and receptor site molecules,[44] and has evaluated the relative importance of their contributions in terms of the free energy of binding. The data reported in the present paper, when seen in the light of Gill's study,[44] suggest that the adamantane–receptor site fit may involve strong hydrophobic bonding[45] between the rigid, strainless adamantane moiety and regions of lipoid nature in the receptor site protein molecule. (The entropy requirements of spatial reorientation of less ordered hydrocarbon groups for hydrophobic bonding[45] is obviated in the case of the adamantane group wherein the atoms are favorably predisposed toward such bonding.) Lipoid (nonpolar) regions in proteins consisting of aggregates of hydrocarbon side chains of amino acids have been documented in the case of the myoglobin molecule.[44,46] Clusters of aliphatic amino acid side chains, arranged in a conformation of least strain, conceivably could delineate structural regions where hydrophobic bonding of adamantane is thermodynamically favorable.

An indication of binding of adamantane-containing agents to protein was observed in early explorations of the usefulness of adamantane in medicinals. Adamantoylpenicillin (N-adamantoyl-6-aminopenicillanic acid) was found[47] to possess approximately 25% of the activity of penicillin G against *S. aureus*. In the presence of serum this activity was reduced to a negligible level, presumably because of binding to serum protein. Physicochemical studies aimed at evaluating

(42c) NOTE ADDED IN PROOF.—Further repeated attempts to demonstrate *in vivo* activity have been consistently unsuccessful.

(43) A. J. Bloch and C. A. LePage, *Cancer Res.*, **24**, 312 (1964).

(44) E. W. Gill, *Progr. Med. Chem.*, **4**, 30 (1965).

(45) W. Kauzmann, *Advan. Protein Chem.*, **14**, 33 (1959).

(46) (a) J. C. Kendrew, H. C. Watson, B. E. Strandberg, R. E. Dickerson, D. C. Phillips, and V. C. Shore, *Nature*, **190**, 666 (1961); (b) M. F. Perutz, J. C. Kendrew, and H. C. Watson, *J. Mol. Biol.*, **13**, 669 (1965).

(47) Unpublished experiments by Dr. C. W. Godzeski, The Lilly Research Laboratories, Indianapolis, Ind.

the extent of binding to protein of the four adenosine adamantoates have been initiated in our laboratories.[48]

In conclusion, the nucleoside adamantoates as well as the adamantane-containing agents earlier described may derive their high efficacy, at least in part, through a process of precise and specific binding of the adamantane moiety to complementary, lipoid regions of the protein receptor site molecule.

Naturally, binding to protein of adamantane-containing agents acting *in vivo* may also affect their absorption and their distribution (transport) in the animal host. The effect of protein binding on these processes may be responsible for the quantitative differences seen between the *in vivo* activities, on the one hand, of the monomethylated analogs relative to the adamantane agent in the sulfonylurea series[7] (about 25%) and the nortestosterone esters[2] (about 15–20%), and on the other hand, the activity of adenosine monomethyladamantoate relative to the adamantoate (about 130%) in inhibiting platelet aggregation *in vitro* (Table X).

The study of a different class of adamantane-containing agents which display a structure–activity relationship opposite to that reported in this paper and which presumably do not benefit from binding to protein will be the subject of a forthcoming publication.

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(48) Preliminary results indicate the possibility that the effect of methyl substitution on partition values parallel the test results obtained in the platelet aggregation studies (Table X). The help of Mr. M. M. Marsh and associates, Analytical Research and Development Division, The Lilly Research Laboratories, in obtaining these results is gratefully acknowledged.

Totally Synthetic Steroid Hormones. XIII.[1] The Chemical Resolution of Some Racemic Estrane, 13β-Ethylgonane, and 13β-*n*-Propylgonane Derivatives and the Preparation of Some Estrane and 13β-Ethylgonane Derivatives of Unnatural Configuration

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The following six racemic steroids have been resolved into optical antipodes by salt formation between the corresponding hemisuccinate esters and various optically active bases: 3-methoxyestra-1,3,5(10),8,14-pentaen-17β-ol, 3-methoxyestra-1,3,5(10),8-tetraen-17β-ol, 3-methoxyestra-1,3,5(10)-trien-17β-ol, 13β-ethyl-3-methoxygona-1,3,5(10)-trien-17β-ol, 13β-ethyl-3-methoxygona-1,3,5(10),8-tetraen-17β-ol, and 3-methoxy-13β-*n*-propylgona-1,3,5(10)-trien-17β-ol. Racemic 3-benzyloxy-13β-ethylgona-1,3,5(10)-trien-17β-ol has been chemically resolved by separation of its diastereoisomeric (−)-menthoxyacetates. Chemical transformations of several of the steroidal enantiomorphs are reported. Results of biological testing are given for some of the steroids of unnatural configuration.

Previous parts of this series have reported efficient, stereoselective total syntheses of various racemic estrone, estrane (19-norandrostane), and 13β-polycarbonalkylgonane derivatives.[3,4] These researches have already provided a possible basis for the commercial production of estrone, and, therefore, of those of its derivatives which are medicinally important as estrogenic, progestational, anabolic, and antifertility agents, and have also led to a variety of biologically interesting racemic 13β-polycarbonalkylgona-1,3,5(10)-trienes and gon-4-en-3-ones of potential or actual clinical utility.[4b]

This paper describes resolution procedures of the purely chemical type which lead from our previously described racemates[3-6] to enantiomorphic steroids in the estrane, 13β-ethylgonane, and 13β-*n*-propylgonane series. Such studies were undertaken to furnish the final links in our totally synthetic chain to *d*-estrone,[7] to permit a more detailed examination of the properties of the biologically

(1) Part XII: G. A. Hughes and H. Smith, *Steroids*, **8**, 547 (1966).

(2) Postal address, P. O. Box 8299, Philadelphia, Pa. 19101.

(3) G. A. Hughes and H. Smith, *Chem. Ind.* (London), 1022 (1960); G. H. Douglas, J. M. H. Graves, D. Hartley, G. A. Hughes, B. J. McLoughlin, J. Siddall, and H. Smith, *J. Chem. Soc.*, 5072 (1963).

(4) (a) H. Smith, *et al.*, *Experientia*, **19**, 394 (1963); (b) *J. Chem. Soc.*, 4472 (1964).

(5) G. A. Hughes and H. Smith, German Patents 1,213,404, 1,213,405, and 1,214,679 (Feb 19, 1960, June 22, 1961, and Feb 29, 1961, respectively).

(6) G. A. Hughes and H. Smith, Japanese Patent 40-20700 (May 16, 1962).

(7) We denote the absolute configuration of enantiomorphic steroids according to a convention proposed by L. F. Fieser and M. Fieser, "Steroids," Reinhold Publishing Corp., New York, N. Y., 1959, p 336, by which estra- and gona-1,3,5(10)-trienes are defined as *d* steroids if they have the same configuration as cholesterol at C-13 and as *l* steroids if they have the opposite configuration at that center. The graphic formulas correspond to enantiomorphs of the *d* series but are used to denote the structure and absolute configuration of any steroid by use of the appropriate prefix *d*, *l*, or *dl*. The prefixes (+) and (−) are used, where necessary, to denote dextro- or levorotatory compounds, respectively.