(2). To a solution of methyl 2-*O*-methyl-α-D-arabinofuranoside (148 mg, 0.84 mmol) in dry pyridine (5 ml) at 5° was added dropwise 0.54 ml (4 mmol) of benzoyl chloride. After stirring at 5° for 1 hr, the solution was allowed to stand at room temperature overnight. After the dropwise addition of water (5 ml), it was evaporated to near dryness *in vacuo*, and the residue was dissolved in chloroform (25 ml), which was then washed with 1 *N* sodium bicarbonate (6 × 10 ml) and water (10 ml) before drying over $MgSO_4$. The chloroform was evaporated to a small volume, which was absorbed on a column of silica gel (175 g, 140–200 mesh). Elution of the column with chloroform gave an oil: yield, 228 mg (70%); mass spectrum 355 $(M - OCH_3)^+$; pmr ($CDCl_3$) δ 3.44 and 3.48 (2 s, 2 OMe), 3.95 (d, H_2), 4.6 (m, H_4 and $2H_5$), 5.08 (s, H_1), 5.4 (m, H_3), 7.5 and 8.1 (2 m, phenyl). The spectrum was assigned by use of a shift reagent [Eu(fod)$_3$-d_{30}] to eliminate the accidental degeneracy of the protons at C_5 and H_4. After this, spin–spin coupling was demonstrated between H_4 and H_3 and between H_3 and H_2.

Methyl 3,5-Di-*O*-(*p*-chlorobenzoyl)-2-*O*-methyl-α-D-arabinofuranoside (3). This comopund was prepared from 1 (5.85 g, 328 mmol) and *p*-chlorobenzoyl chloride (9.2 ml, 128.7 mmol) as described above for 2. Purification on a silica gel column gave 14.17 g of an oil (95%): mass spectrum 423 $(M - OMe)^+$, 409 $(M - CH_2OCH_3)^+$, 394 (M − 1 − MeOCHO), 285 $(423 - ClC_6H_4CO + H)^+$, 238 $(294 - ClC_6H_4CO_2H)^+$, 139 $(ClC_6H_4CO)^+$.

3,5-Di-*O*-(*p*-chlorobenzoyl)-2-*O*-methyl-D-arabinose (4). A solution of methyl 3,5-di-*O*-(*p*-chlorobenzoyl)-2-*O*-methyl-α-D-arabinofuranoside (13.67 g, 30 mmol) in a mixture of 41 ml of 6 *N* HCl and 275 ml of glacial acetic acid was heated in a boiling water bath for 2 hr, cooled, and poured onto 1250 g of ice. The resulting mixture was extracted with CH_2Cl_2 (5 × 250 ml), and the combined extracts were washed in 1 *N* bicarbonate (3 × 200 ml) and water (2 × 100 ml) and dried over $MgSO_4$ before evaporation to dryness. The residue was purified by chromatography on a silica gel column (*vide supra*) using benzene–ethyl acetate (9:1) as eluent. The product was crystallized from ether by the addition of hexane: yield, 2.91 g (22%); pmr ($CDCl_3$) δ 3.2 (d, $J_{OH,H(1')} = 4$ Hz, OH), 3.45 (s, OMe), 3.98 (m, H_2), 4.6 (m, H_4 and $2H_5$), 5.35 (m, H_3), 5.55 (d, $J_{12} = 4$ Hz, H_1), 7.4 and 8.0 (2 m, phenyl). Addition of D_2O caused the disappearance of the doublet at 3.2 and collapse of the doublet at 5.55 to a singlet.

1-[3,5-Di-*O*-(*p*-chlorobenzoyl)-2-*O*-methyl-β-D-arabinofuranosyl]-4-methoxypyrimidin-2(1*H*)-one (6). A solution of 3,5-di-*O*-(*p*-chlorobenzoyl)-2-*O*-methyl-D-arabinose (500 mg, 1.13 mmol) in 35 ml of $CHCl_2$ saturated with HCl gas over $MgSO_4$ (5 g) was stirred for 18 hr before it was filtered and evaporated to dryness: yield of chloro sugar 5, 492 mg (95%); pmr ($CDCl_3$) δ 3.50 (s, OMe), 4.3 (m, H_2), 4.7–4.9 (m, H_4 and $2H_5$), 5.35 (d, H_3), 6.3 (s, H_1), 7.4 and 8.1 (2 m, phenyl).

A mixture of this residue and 2,4-dimethoxypyrimidine (441 mg, 3 mmol) was heated at 80° for 8 hr. The blocked nucleoside was purified by chromatography on a dry silica gel column developed with cyclohexane–ethyl acetate (4:1). Extraction of the principal band with ethyl acetate gave 318 mg of an oil (58%): mass spectrum 548 $(M)^+$, 516 $(M - CH_3OH)^+$, 423 $(s)^+$, 127 $(b + 2H)^+$, 126 $(b + H)^+$; pmr ($CDCl_3$) δ 4.00 (s, 4 OMe), 3.35 (s, 2′ OMe), 4.3 (d, $J_{2'3'} = 4$ Hz, $H_{2'}$), 4.55 (m, $H_{4'}$), 4.7 (m, $2H_{5'}$), 5.45 (m, $H_{3'}$), 5.9 (d, $J_{56} = 7$ Hz, H_5), 6.38 (d, $J_{1'2'} = 3.5$ Hz, H_1), 7.85 (d, $J_{56} = 7$ Hz, H_6), 7.5 and 8.1 (2 m, phenyl). A double resonance experiment irradiating $H_{1'}$ gave a 15% NOE on the signal from $H_{2'}$, indicating the cis arrangement. The upfield shift of the OMe signal (0.15 ppm) is due to shielding by the pyrimidine ring in keeping with the cis assignment. Assignments of $H_{2'}$, $C_{4'}$, and C_5 were verified by spin decoupling.

1-(2-*O*-Methyl-β-D-arabinofuranosyl)cytosine (7). A solution of 1-[3,5-di-*O*-(*p*-chlorobenzoyl)-2-*O*-methyl-β-D-arabinofuranosyl]-4-methoxypyrimidin-2(1*H*)-one (760 mg, 1.38 mmol) in 50 ml of methanol saturated at 0° with ammonia was heated at 100° for 18 hr before it was evaporated to dryness. The residue was extracted with water, which was washed with $CHCl_3$ before evaporation to dryness: yield of crude solid, 365 mg. The picrate was prepared in water: yield, 505 mg (74%). *Anal.* ($C_{10}H_{15}N_3O_5 \cdot C_6H_3N_3O_7 \cdot \frac{1}{3}H_2O$) C, H, N.

The picrate was converted to the free nucleoside in water with Dowex 1-X8 (CO_3^{2-}): yield, 242 mg (92%); uv λ_{max} in nm ($\epsilon \times 10^{-3}$) (0.1 *N* HCl) 279 (15.6), (pH 7) 230 (sh), 270 (9.33), (0.1 *N* NaOH) 230 (sh), 272 (9.69); pmr (DMSO-d_6) δ 3.18 (s, OMe), 3.5 (m, $H_{4'}$ and $H_{5'}$), 3.75 (m, $H_{2'}$), 4.0 (t, $H_{3'}$), 4.8–5.7 (OH), 5.7 (d, $J_{56} = 7$ Hz, H_5), 6.15 (d, $J_{1'2'} = 4$ Hz, $H_{1'}$), 7.1 (br s, NH_2), 7.55 (d, $J_{56} = 7$ Hz, H_6). *Anal.* ($C_{10}H_{15}N_3O_5 \cdot 0.2H_2O$) C, H, N.

Acknowledgment. The authors are indebted to Dr. W. C. Coburn, Jr., and members of the Molecular Spectroscopy Section of Southern Research Institute for spectral and microanalytical data and to Mrs. Martha Thorpe and Mr. Marion Kirk for their help in the interpretation of some of the data.

References

(1) J. S. Evans, E. A. Musser, G. D. Mangel, K. R. Forsblad, and J. H. Hunter, *Proc. Soc. Exp. Biol. Med.*, **106**, 350 (1961).
(2) G. L. Neil, P. F. Wiley, R. C. Manak, and T. E. Moxley, *Cancer Res.*, **30**, 1047 (1970).
(3) J. A. Montgomery and H. J. Thomas, *J. Med. Chem.*, **15**, 116 (1972).
(4) R. D. Casey and R. R. Ellison, *Clin. Res.*, **13**, 337 (1965).
(5) A. Hochi, *et al.*, *Gann*, **62**, 145 (1971).
(6) S. S. Cohen, *Progr. Nucl. Acid Res.*, **5**, 1 (1966); W. H. Prusoff and B. Goz, *Fed. Proc., Fed. Amer. Soc. Exp. Biol.*, 1679 (1973).
(7) J. A. Wright, D. P. Wilson, and J. J. Fox, *J. Med. Chem.*, **13**, 269 (1970).
(8) E. Darzynkiewicz, J. T. Kusmierek, and D. Shugar, *Biochem. Biophys. Res. Commun.*, **46**, 1734 (1972).
(9) T. A. Khwaja and R. K. Robins, *J. Amer. Chem. Soc.*, **88**, 3640 (1966).
(10) P. W. Austin, J. G. Buchanan, and S. M. Oakes, *Chem. Commun.*, 466 (1965).
(11) J. A. Montgomery, M. C. Thorpe, S. D. Clayton, and H. J. Thomas, in press.
(12) C. D. Anderson, L. Goodman, and B. R. Baker, *J. Amer. Chem. Soc.*, **80**, 5247 (1958).
(13) R. E. Schaub and M. J. Weiss, *J. Amer. Chem. Soc.*, **80**, 4683 (1958).

Antiviral Agents. 1-Aralkyloxyadenosines†

W. M. Shannon, A. Shortnacy, G. Arnett, and J. A. Montgomery*

Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205. *Received October 26, 1973*

Among a number of widely varied purine nucleoside structures tested for antiviral activity, adenosine 1-oxide[1] appeared to be one of the most interesting, having moderate activity against rhinovirus 1A, an RNA-containing virus, and significant activity against vaccinia virus and herpes simplex virus, two DNA-containing viruses. 1-Methyladenosine and 2-methyladenosine were also active in these systems, but the adenosine 1-oxide isomer, *N*-hydroxyadenosine,[2] was inactive. The 2′-deoxyadenosine 1-oxide was active against the DNA-containing viruses only.

As a follow-up to this lead, 1-benzyloxyadenosine hydrobromide[3] was evaluated and found to have a degree of activity as great as the 1-oxide, although requiring a 100-fold increase in concentration to achieve this effect. Several 1-(substituted benzyloxy)adenosine hydrobromides were then prepared for testing. These compounds appeared to be even less stable than 1-benzyloxyadenosine hydrobromide,[3] and only one, the 1-(3-methylbenzyloxy)-adenosine hydrobromide, could be obtained analytically pure. Attempts to recrystallize these salts were frustrated by regeneration of adenosine 1-oxide by attack of the bromide ion on the benzyl–oxygen linkage. In order to circumvent this problem, we investigated conversion of 1-benzyloxyadenosine hydrobromide to other salts with less

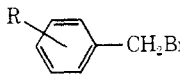
†This work was supported by funds provided by Southern Research Institute.

Table I. *In Vitro* Antiviral Activity of Various Adenosine Analogs and 1-Aralkyloxyadenosines

Compound	Virus rating (VR)[a]						
	DNA viruses			RNA viruses			
	Herpes simplex virus	Vaccinia virus	Adeno-virus	Rhino-virus	Influenza virus	Para-influenza virus	MED_{50},[b] μg/ml
Adenosine 1-oxide	0.9 1.2	1.6 1.1	ND[c]	0.5 0.6	ND	0	3.2
1-Methyladenosine	0.9 0.7	0.8 0.8	ND	0.8 1.2	ND	0	100
2-Methyladenosine	0.7 0.8	1.0 0.8	ND	1.0 0.8	ND	0	100
N-Hydroxyadenosine	0.1	0	ND	0.3	ND	0	
2′-Deoxyadenosine 1-oxide	1.2 1.2	1.4 1.1	ND	0.2	ND	ND	10
1-Benzyloxyadenosine	1.5 1.1	1.9	ND	0.7 0.8	ND	ND	320
1-(2-Methylbenzyloxy)adenosine fluoborate	2.4 1.5	1.5	0	0	0	ND	320
1-(3-Methylbenzyloxy)adenosine hydrobromide	2.1 2.2	1.9	0	0	0.2	ND	100
1-(4-Methylbenzyloxy)adenosine hydrobromide	1.6	1.6	ND	0.8	0.1	ND	100
1-(3-Fluorobenzyloxy)adenosine fluoborate	1.6 0.9	2.1	0	0	0.3	ND	320
1-(4-Fluorobenzyloxy)adenosine fluoborate	2.6	2.7	ND	0.8	0	ND	100
1-(4-Nitrobenzyloxy)adenosine fluoborate	0	0	0	0	0	0	

[a]Virus rating (VR): a measurement of antiviral activity, based on the *in vitro* inhibition of virus-induced cytopathogenic effects (CPE), determined by a modification of the method of Ehrlich, *et al.*[10] A VR ≥ 1.0 indicates definite (+) antiviral activity; a VR of 0.5–0.9 indicates marginal to moderate (±) activity, and a VR < 0.5 indicates no apparent antiviral activity (−). [b]Minimum effective dose required for 50% inhibition of virus-induced cytopathogenic effects. [c]ND: not done.

Table II. Preparation of 1-Aralkyloxyadenosines



	Hydrobromide		Fluoborate			
	Mp, °C	Yield, %	Mp, °C	Yield, %	Hydration[a]	Analyses[e]
H	149[b]	90	110–113	46	Anhydrous	C, H, N
o-Me		91	105–110	34	$^1/_2H_2O$	C, H, N
m-Me	144[b]	70				C, H, N[c]
p-Me		~100[d]				
m-F	150[b]	91	96–98	33	$^1/_2H_2O$	C, H, N
p-F		68	150–152	43	$^5/_8H_2O$	C, H, N
p-NO_2	118	~100[d]	115–135	50	$^5/_6H_2O$	C, H, N

[a]The presence of water was verified by the pmr spectra. [b]Decomposition. [c]Hydrobromide. [d]Triturated with ether, not crystallized from methanol-ethyl acetate. [e]Analyses agree within 0.4% of calculated values.

nucleophilic counterions. An aqueous solution of the hydrobromide salt was treated with the silver salts of sulfuric, perchloric, citric, and fluoboric acids. Of the salts thus formed, the fluoborates, which could not be recrystallized from water without detectable decomposition, were the most satisfactory, even though all but one was hydrated and some of them were hygroscopic.

Biological Evaluation. The 1-aralkyloxyadenosines described in this report were examined for *in vitro* antiviral activity against a number of representative DNA-containing and RNA-containing animal viruses by means of quantitative assays of their ability to inhibit virus-induced cytopathogenic effects (CPE) in infected cell cultures. The viruses employed in these studies were: herpes simplex virus type 1 (strain HF), vaccinia virus (strain Lederle chorioallantoic), human adenovirus type 2 (strain Adenoid 6), human rhinovirus type 1A (strain 2060), parainfluenza virus type 3 (strain Huebner C243), and human influenza virus type A_0/PR8/34. The herpes simplex virus (HSV), vaccinia virus (VV), adenovirus (Ad), rhinovirus (RV), and parainfluenza virus (PIV) were propagated and assayed in continuous-passage human carcinoma of the nasopharynx (KB) cells[4] grown in 32-oz glass prescription bottles and in vinyl plastic titration panels[5] as monolayer cultures using Eagle's basel medium (BME),[6] supplemented with 10% fetal calf serum and antibiotics, as growth medium. The PR8 influenza virus was propagated and assayed in continuous-passage Madin–Darby canine kidney (MDCK) cells[7,8] grown as monolayer cultures in reinforced Eagle's medium[9] supplemented with 2% fetal calf serum.

Growth medium was decanted from the wells of plastic titration panels containing replicate, preformed cell monolayers. To each of the triplicate test wells was added 0.5 ml of medium containing a given concentration of test compound and 0.5 ml of virus suspension. Virus was diluted in medium to yield a final virus concentration of 32 cell culture infectious dose, 50% ($CCID_{50}$) units per panel well. Five to eight drug concentrations were tested in each experiment and these were selected to range, in half-log dilutions, from cytotoxic to noncytotoxic concentrations. Cytotoxicity controls, virus controls, and cell controls were included in each experiment. The panels were sealed and incubated at 33° for the antirhinovirus tests and at 37° for the other antiviral assays. At 4 days after virus inoculation, the monolayers were examined microscopically

for virus-induced cytopathogenic effects (CPE) and the amount of reduction in CPE caused by the test compound was determined. A virus rating (VR) was calculated for each compound tested against each virus by use of a modification of the method of Ehrlich, *et al.*,[10] which has been described previously.[11] The results are shown in Table I. As can be seen, the 1-(methylbenzyloxy)adenosines exhibited significant antiviral activity against HSV and VV *in vitro* with VR's ranging from 1.5 to 2.4 and, except for the 2-methylbenzyloxy compound, at one-third the molar concentration of the 1-benzyloxyadenosine. The 1-(fluorobenzyloxy)adenosines likewise demonstrated definite *in vitro* activity against these two DNA viruses, but 1-(*p*-nitrobenzyloxy)adenosine was inactive. Thus, it would appear, on the basis of this limited data, that groups capable of donating electrons to the benzene ring by resonance may enhance antiviral activity, whereas groups that withdraw electrons destroy activity. These interesting preliminary findings are being pursued. No significant activity was noted with any of these compounds against Ad, PIV, or influenza virus. Moderate antiviral activity was observed against RV with 1-(4-methylbenzyloxy)adenosine and 1-(4-fluorobenzyloxy)adenosine.

Experimental Section

Melting points were determined with a Mel-Temp apparatus and are not corrected. Uv were determined in aqueous solution with a Cary Model 14 spectrophotometer. The pmr spectra were determined in DMSO-d_6 (TMS) with a Varian XL-100 spectrometer. All compounds were tlc homogeneous on silica gel plates developed in $CHCl_3$–MeOH (3:1).

1-(Aralkyloxy)adenosine Fluoborates. A solution of adenosine 1-oxide[1] and the aralkyl bromide (4 mol/mol of adenosine 1-oxide) in DMA (20 ml/mol of adenosine 1-oxide) was stirred 3–5 days at ambient temperature. Evaporation of the DMA *in vacuo* gave a residue which was triturated with ether before it was dissolved in methanol. The methanol solution was filtered, diluted with ethyl acetate, and chilled overnight. The solid that crystallized was dissolved in water, and the solution was filtered before it was treated with 1 equiv of silver fluoborate. After removal of the silver bromide, the solution was treated with KI to precipitate silver iodide, which was removed by filtration before the solution was concentrated to a small volume *in vacuo* and refrigerated overnight. The crude fluoborate salt was recrystallized from water. The ultraviolet spectra of all these compounds were essentially identical with that of 1-benzyloxyadenosine,[3] except that of the 1-(4-nitrobenzyloxy)adenosine, which exhibited hyperchromicity. Details for individual compounds are given in Table II.

Acknowledgment. The authors are indebted to Dr. W. C. Coburn, Jr., and members of the Molecular Spectroscopy Section of Southern Research Institute for spectral and microanalytical data.

References

(1) M. A. Stevens, D. I. Magrath, H. W. Smith, and G. B. Brown, *J. Amer. Chem. Soc.*, **80**, 2755 (1958).
(2) A. Giner-Sorolla, L. Medick, and A. Bendich, *J. Med. Chem.*, **9**, 143 (1966).
(3) J. A. Montgomery and H. J. Thomas, *J. Med. Chem.*, **15**, 182 (1972).
(4) H. Eagle, *Proc. Soc. Exp. Biol. Med.*, **89**, 362 (1955).
(5) W. A. Rightsel, P. Schultz, D. Muething, and I. W. McLean, Jr., *J. Immunol.*, **76**, 464 (1956).
(6) H. Eagle, *Science*, **130**, 432 (1959).
(7) C. R. Gaush, W. L. Hard, and T. F. Smith, *Proc. Soc. Exp. Biol. Med.*, **122**, 931 (1966).
(8) C. R. Gaush and T. F. Smith, *Appl. Microbiol.*, **16**, 588 (1968).
(9) R. Bablanian, H. J. Eggers, and I. Tamm, *Virology*, **26**, 100 (1965).
(10) J. Ehrlich, B. J. Sloan, F. A. Miller, and H. Machamer, *Ann. N. Y. Acad. Sci.*, **130**, 5 (1965).
(11) R. W. Sidwell, G. Arnett, G. J. Dixon, and F. M. Schabel, Jr., *Proc. Soc. Exp. Biol. Med.*, **131**, 1226 (1969).

Isosteres of Natural Phosphates. 2. Synthesis of the Monosodium Salt of 4-Hydroxy-3-oxobutyl-1-phosphonic Acid, an Isostere of Dihydroxyacetone Phosphate

Stephen L. Goldstein, Diza Braksmayer, Burton E. Tropp, and Robert Engel*

Department of Chemistry, Queens College of the City University of New York, Flushing, New York 11367. *Received August 13, 1973*

In this laboratory's continuing examination of the effect of phosphonic acid isosteres (substituting CH_2 for O in the ester linkage of naturally occurring phosphates) on phospholipid synthesis in *Escherichia coli*, the synthesis of 4-hydroxy-3-oxobutyl-1-phosphonic acid (**3**), the analog of dihydroxyacetone phosphate, was undertaken. Concurrently, a route for the synthesis of 3,4-dihydroxybutyl-1-phosphonic acid (**8**) was sought which would be readily applicable to the incorporation of a radioactive label (tritium) into the molecule. These two routes are described below.

The monosodium salt of 4-hydroxy-3-oxobutyl-1-phosphonic acid (**4**) serves as a substrate for rabbit muscle L-glycerol 3-phosphate:NAD oxidoreductase and for the biosynthetic L-glycerol 3-phosphate dehydrogenase from *E. coli* which was initially described by Kito and Pizer.[1,†] The herein described compound is of use in determining the mode of action of 3,4-dihydroxybutyl-1-phosphonic acid which affects the synthesis of phospholipids, primary precursors for which are the natural phosphates, glycerol 3-phosphate and dihydroxyacetone phosphate. These isosteres may thereby serve as metabolic regulators and probes of biochemical mechanisms. The overall synthetic scheme is outlined in Scheme I.

Experimental Section

Acetoxymethyl Vinyl Ketone (1). The procedure of Hennon and Kupiecki[2] was followed. 2-Butyne-1,4-diol (1 mol) was treated with acetic anhydride (2.5 mol). The resulting solution was treated with mercuric oxide and sulfuric acid and the product isolated by distillation. The yield of **1** was 90 g (78%). This material was prepared immediately prior to use.

Diethyl 1-Acetoxy-2-ethoxybut-2-enyl-4-phosphonate (2a). To a cooled (0°) solution of 90 g (0.78 mol) of the ketone **1** and 130 g (2.8 mol) of absolute ethanol was added dropwise 133 g (0.80 mol) of triethyl phosphite.[3] The solution was stirred for 1 hr at 0° and overnight at room temperature. Upon fractional distillation there was obtained a colorless liquid, bp 145° (0.3 mm). The nmr and ir spectra of this material indicated that although it consisted primarily of **2a**, significant amounts of the isomeric enol ether **2b** and the ketal **2c** were also present. Further attempts at separation were unsuccessful. This mixture was considered suitable for the next stage in the synthesis. The yield, based on **2a**, was 140 g (70%).

Monosodium 4-Hydroxy-3-oxobutyl-1-phosphonate (4). A solution of 10 g (0.034 mol based on **2a**) of the mixture **2a–c**, 30 ml of concentrated HCl, and 70 ml of water was refluxed for 22 hr. The volatile components were removed under reduced pressure and the pale yellow oil was dissolved in water, treated with activated charcoal, and filtered. The water was evaporated and the ir and nmr spectra of the resulting oil were in accord with the proposed structure **3** for the phosphonic acid. The special data obtained for **3** are as follows: ir (film) 2.95, 3.60, 5.85, 7.17, 8.20, 9.45, 10.16, 10.22, 12.75, 14.13 μ; nmr (TFA) δ 2.22–3.16 (m, 4 H, PCH_2CH_2), 4.62 (s, 2 H, CH_2O).

This material was dissolved in water and titrated with 1 *N* NaOH to pH 4.0. The water was evaporated to yield a colorless semisolid to which was added 100 ml of absolute methanol. The mixture was sitrred until the product crystallized. The resulting white powder was isolated by filtration and the filtrate was concentrated to about 10 ml. The solution was added to 100 ml of absolute ethanol and the resulting second crop of white powder was isolated by filtration. The solids were combined and washed with

† P. J. Cheng, R. Engel, and B. E. Tropp, unpublished results of this laboratory.