The halogen present was identified by TLC in the following manner. Silica gel plates were freed of interfering material by a full-length sham chromatographic development in 1-propanol–concentrated ammonium hydroxide [25:11 (v/v)]. The plates were air-dried prior to application of the test samples. Standards of NH_4Br (10 mg/mL) and NH_4Cl (10 mg/mL) were compared to the hydrobromide of **2** (20 mg/mL) using the propanol-ammonia development. Plates were dried with a stream of warm air and then placed in an oven at 120 °C for 5 min. The plates were sprayed with silver nitrate (1% in 95% acetone–water), dried, and placed under an ultraviolet (254 nm) lamp for 10 min. The halides appeared as dark spots against a white background. R_f values: NH_4Br, 0.54; NH_4Cl, 0.50; hydrobromide of **2**, 0.54. The Pauly-positive component of **2** hydrobromide was located at R_f 0.39.

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References and Notes

(1) M. Berman and C. Dohlman, *Arch. Ophtalmol.*, **35**, 95 (1975).
(2) P. A. McCroskery, J. F. Richards, and E. D. Harris, Jr., *Biochem. J.*, **152**, 131 (1975).
(3) E. D. Harris, C. S. Faulkner, II, and F. E. Brown, *Clin. Orthop. Relat. Res.*, **No. 110** (July–Aug), 303 (1975).
(4) D. L. Head and J. A. Yankeelov, Jr., *Int. J. Pept. Protein Res.*, 8, 155 (1976).
(5) E. Harper and S. Seifter, *Isr. J. Chem.*, **12**, 515 (1974).
(6) S. Seifter and E. Harper, *Methods Enzymol.*, 19, 613 (1970).
(7) J. A. Yankeelov, Jr., W. B. Wacker, and M. M. Schweri, *Biochim. Biophys. Acta*, **482**, 159 (1977).
(8) The use of the terms "compound **2**" or "mercaptoimidazolylpropionic acid" in the text infers the hydrobromide of this substance.
(9) J. A. Yankeelov, Jr., and C. J. Jolley, *Biochemistry*, 11, 159 (1972).
(10) D. J. Martin and C. C. Greco, *J. Org. Chem.*, **33**, 1275 (1968).
(11) J.-C. Janson, *J. Chromatogr.*, **28**, 12 (1967).
(12) D. Eaker and J. Porath, *Sep. Sci.*, **2**, 507 (1967).
(13) G. Ingram, "Methods of Organic Elemental Microanalysis," Reinhold, New York, N.Y., 1962, p 204.
(14) J. A. Yankeelov, Jr., and H. A. Parish, Jr., unpublished observations.
(15) K.-F. Fok, D. J. Carothers, and J. A. Yankeelov, Jr., *Fed. Proc., Fed. Am. Soc. Exp. Biol.*, **37**, 1586 (1978).
(16) J. A. Yankeelov, Jr., K.-F. Fok, and D. J. Carothers, *J. Org. Chem.*, **43**, 1623 (1978).
(17) Z. Reyes and R. M. Silverstein, *J. Am. Chem. Soc.*, **80**, 6367 (1958).
(18) F. Duus, *Tetrahedron*, **30**, 3753 (1974).
(19) R. J. Abraham and H. J. Bernstein, *Can. J. Chem.*, **39**, 216 (1961).
(20) W. B. Jennings, *Chem. Rev.*, **75**, 307 (1975).
(21) R. B. Martin and R. Mathur, *J. Am. Chem. Soc.*, **87**, 1065 (1965).
(22) K. G. R. Pachler, *Spectrochim. Acta*, 19, 2085 (1963).
(23) R. J. Weinkam and E. C. Jorgensen, *J. Am. Chem. Soc.*, **95**, 6084 (1973).
(24) J. L. Seltzer, J. J. Jeffrey, and A. Z. Eisen, *Biochim. Biophys. Acta*, **485**, 179 (1977).
(25) T. Kada, K. Tutikawa, and Y. Sadaie, *Mutat. Res.*, 16, 165 (1972).
(26) J. D. Elmore, J. L. Wong, A. D. Laumbach, and U. N. Streips, *Biochim. Biophys. Acta*, **442**, 405 (1976).
(27) J. McCann, V. Simmon, D. Streitwieser, and B. N. Ames, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 3190 (1975).
(28) I. Smith and M. Birchenough in "Chromatographic and Electrophoretic Techniques", Vol. I, I. Smith, Ed., Interscience, New York, N.Y., 1960, p 212.
(29) H. Maeda, C. B. Glaser, and J. Meienhofer, *Biochem. Biophys. Res. Commun.*, **39**, 1211 (1970).
(30) J. M. Stewart and J. D. Young, "Solid Phase Peptide Synthesis", W. H. Freeman, San Francisco, Calif., 1969, p 56.
(31) G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).
(32) Y. Nagai, C. M. Lapiere, and J. Gross, *Biochemistry*, **5**, 3123 (1966).
(33) T. Sakai and J. Gross, *Biochemistry*, **6**, 518 (1967).

Nucleoside 5′-Monophosphate Analogues. Synthesis of 5′-Sulfamino-5′-deoxynucleosides

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The synthesis of two new nucleotide analogues is described. 5′-Sulfamino-5′-deoxyadenosine (**1**) was prepared by reaction of 5′-amino-5′-deoxyadenosine with $(CH_3)_3N{\cdot}SO_3$, and 5′-sulfamino-5′-deoxythymidine (**2**) was prepared from 5′-amino-5′-deoxythymidine by a similar reaction. The 5′-sulfamino nucleosides are shown to be quite stable to hydrolysis in acidic or basic aqueous solution. Tests show that these compounds do not inhibit the growth of *Escherichia coli* or L1210 cells at concentrations $<10^{-4}$ M. At 10^{-4} M compound **2** was found to give 70% inhibition of the replication of herpes simplex virus (type 1) with no effect on host cell growth (CV-1 monkey line).

In recent years there has been a great deal of interest in the preparation of analogues of nucleoside 5′-monophosphates. Much of this interest is a result of the knowledge that natural nucleosides require phosphorylation at their 5′ position before they can be utilized for many metabolic functions. Similarly, many therapeutically useful nucleoside analogues, such as arabinosylcytosine and 5-fluoro-2′-deoxyuridine, must be phosphorylated in vivo before they exhibit biological activity.[1] The analogues of nucleoside 5′-monophosphates not only provide potential new drugs but also they are important tools in the study of enzyme mechanisms and biochemical pathways.[2]

We wish to report the synthesis of the first two compounds (**1** and **2**) of a new class of nucleoside 5′-monophosphate analogues, the 5′-sulfamino-5′-deoxynucleosides. These analogues are of special interest for several reasons. The sulfamino group is very stable in aqueous solution and is not expected to cleave easily under physiological con-

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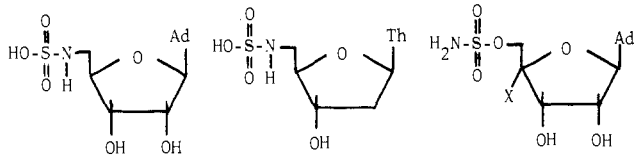

1 2 3a, X=fluorine 3b, X=hydrogen

ditions. The sulfamino group has approximately the same charge and size as the monophosphate group, and therefore the 5′-sulfamino-5′-deoxynucleosides may compete with natural nucleotides for enzyme binding sites. The 5′-sulfamino-5′-deoxynucleosides are also analogues of the naturally occurring antibiotic nucleocidin[3] (**3a**) and of the 5′-*O*-sulfamoylnucleosides[4] (**3b**) which have been found to inhibit the growth of bacteria and L1210 leukemia cells.[5]

5′-Sulfamino-5′-deoxyadenosine (**1**) was prepared from 5′-amino-5′-deoxyadenosine[6] by reaction with 1.5 equiv of $(CH_3)_3N{\cdot}SO_3$ in methanol at 50 °C. Analysis by high-pressure liquid chromatography (LC) showed 80% conversion to product in 90 min with no increase in the desired material with longer reaction times. The product, compound **1**, was purified by ion-exchange chromatography and isolated as the sodium salt in 79% yield after lyophilization. The lyophilized material was crystallized from aqueous ethanol. 5′-Sulfamino-5′-deoxythymidine (**2**) was prepared by reacting 5′-amino-5′-deoxythymidine[7] with 1.2 equiv of $(CH_3)_3N{\cdot}SO_3$ in refluxing methanol. Analysis by LC showed 83% conversion of the amino nucleoside to product after 2.5 h. The product, the sodium salt of compound **2**, was isolated in 83% yield after chromatography and lyophilization. The lyophilized material was crystallized from aqueous ethanol. This reaction gave essentially the same yield of **2** when water (at 55 or 100 °C) was used as the solvent instead of methanol. The structures of compounds **1** and **2** were determined by elemental analysis, electrophoresis, and spectroscopic analysis.

Both chemical and spectral evidence supports the presence of a 5′-*N*-sulfate, and not an *O*-sulfate, in compounds **1** and **2**. In the NMR spectra, the signal for the 5′ protons in compound **1** is shifted downfield by 0.35 ppm relative to the corresponding signal of 5′-amino-5′-deoxyadenosine and the signal for the 5′ protons in **2** is shifted 0.23 ppm downfield relative to the corresponding signal of 5′-amino-5′-deoxythymidine. This downfield shift is predicted on N-sulfation of the 5′-amino group from model studies.[8] No change in the chemical shift was observed for the signal of the 2′ or 3′ protons in the NMR spectra of compounds **1** and **2** relative to the corresponding amino nucleosides. A downfield shift of these signals would have been observed if O-sulfation had occurred.[8] Compounds **1** and **2** also show hydrolytic stability that is characteristic of the sulfamino group.

Compounds **1** and **2** were found to be very stable to hydrolysis in either acidic or basic solutions. After treatment with 0.1 M NaOH at 23 °C for 24 h, no cleavage of the sulfamino group in either compound could be detected. Upon treatment in 0.1 M HCl at 23 °C for 24 h, compound **1** showed 0.75% hydrolysis of the 5′-sulfamino group to give the amino nucleoside and 16% hydrolysis of the glycosidic linkage to give adenine. Under these same acidic conditions, compound **2** showed only 2.4% hydrolysis of the 5′-sulfamino group to give the amino nucleoside and only a trace of glycosidic bond cleavage.

Compounds **1** and **2** were tested for biological activity against *Streptococcus faecalis*, *Escherichia coli* K-12, and *E. coli* B bacterial organisms and L1210 culture cells.[5,9] Compounds **1** and **2** showed less than 10% inhibition at 1×10^{-3} M concentration in bacterial systems. These compounds also have no inhibition activity in the L1210 cell culture system, and at 1×10^{-4} M concentration both compounds show a slight stimulation of cell growth.

Compounds **1** and **2** tested for inhibition of viral replication with herpes simplex virus (type 1) in the CV-1 cell line.[5,10] Compound **1** showed no inhibition of viral replication and a modest stimulation of cell growth at 10^{-4} M. Compound **2** showed a 70% inhibition of viral replication at 1×10^{-4} M (35% inhibition at 1×10^{-5} M) and had little, if any, effect on cell growth.

Experimental Section

Melting points were determined on a Thomas-Hoover or a Fisher-Johns apparatus and are uncorrected. UV spectra were recorded on a Cary 14 spectrophotometer. A Perkin-Elmer 621 spectrophotometer was used to record IR spectra, and a Varian A-60 spectrometer was used to record proton NMR spectra. Optical rotations were measured on a Perkin-Elmer 141 polarimeter at 25 °C. Solvents were evaporated in a rotatory evaporator under reduced pressure with a bath temperature <35 °C. Elemental analyses were done by Galbraith Laboratories, Inc., Knoxville, Tenn.

Paper chromatography utilized the descending technique on Whatman 3MM paper with a solvent of isopropyl alcohol–concentrated NH_4OH–H_2O (7:1:2, v/v/v). Electrophoresis was performed at a constant 500 V on Whatman 3MM paper with a phosphate buffer of pH 7.2 (5.4 g of $NaH_2PO_4{\cdot}H_2O$ and 15.8 g of Na_2HPO_4 in 3.0 L of H_2O). Thymidine 5′-monophosphate was used as the reference standard for electrophoresis. Ion-exchange chromatography used Dowex 50W-X8 resin which had been rinsed with alternate washings of 2 M NaOH in 50% aqueous methanol and 2 M HCl to remove resin impurities. High-pressure liquid chromatography was carried out using a Chromatec 1200 chromatograph with a UV (254 nm) monitor, a reverse phase column ($^3/_8$ in. × 50 cm),[11] and an isocratic solvent of 0.5 M NH_4OAc (pH 7.1) at a flow of 1.6 mL/min.

5′-*N*-Sulfamino-5′-deoxyadenosine (1). 5′-Amino-5′-deoxyadenosine[6] (0.20 g, 0.55 mmol) was dissolved in 50 mL of anhydrous methanol at 50 °C with stirring and then treated with $(CH_3)_3N{\cdot}SO_3$ (0.12 g, 0.83 mmol). The solution was analyzed at 30-min intervals by LC (retention time of 5′-amino-5′-deoxyadenosine, 9.5 min; retention time of 1, 21.5 min). After 90 min, analysis showed 80% conversion to product. The solvent was then evaporated under reduced pressure and the resulting residue was dissolved in water. The aqueous solution was passed through a column of ion-exchange resin (11 mL) in the H^+ form and then through a second column containing ion-exchange resin (11 mL) in the Na^+ form. The aqueous eluate was lyophilized, and 0.22 g (79%) of **1** was isolated as the sodium salt. This material was homogeneous on paper chromatography (R_f 0.33) and on paper electrophoresis (R_m 0.56 relative to pT). The lyophilized powder was crystallized from aqueous ethanol to give the analytically pure sodium salt of **1**: mp 230–235 °C dec; λ_{max} (H_2O) 259 nm (ϵ 1.38×10^4); λ_{min} 226 nm (ϵ 2.3×10^3); IR (KBr) 1415 cm^{-1} (SO_2 ν_{asym}); NMR (D_2O, δ DSS) δ 3.35 (br d, 2 H, 5′ protons); $[\alpha]^{25}_D$ −43° (*c* 0.5, H_2O). Anal. ($C_{10}H_{13}N_6O_6SNa$) C, H, N, S.

5′-*N*-Sulfamino-5′-deoxythymidine (2). 5′-Amino-5′-deoxythymidine[7,12] (1.33 g, 5.49 mmol) was treated with $(CH_3)_3N{\cdot}SO_3$ (0.93 g, 6.6 mmol) in refluxing methanol. After 1 h, LC analysis showed 83% conversion of the amino nucleoside into **2**. After a total of 2.5 h, analysis showed no further conversion to **2**, and the reaction was worked up as described above to give 1.49 g (83%) of the sodium salt of **2**. This compound was homogeneous on paper chromatography (R_f 0.44) and on electrophoresis (R_m 0.62 relative to pT). An analytical sample was obtained by crystallization from aqueous ethanol: mp 197–200 °C dec; λ_{max} (H_2O) 267 nm (ϵ 9.2×10^3); λ_{min} 233 nm (ϵ 2.1×10^3); IR (KBr) 1194 cm^{-1} (SO_2 ν_{sym}); NMR (D_2O, δ DSS) δ 3.30 (complex d, 2 H, 5′ protons); $[\alpha]^{25}_D$ +41° (c 0.14, H_2O). Anal. ($C_{10}H_{14}N_3O_7SNa$) C, H, N, S.

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References and Notes

(1) A. Bloch in "Medicinal Chemistry. A Series of Monographs. Volume IV. Drug Design", E. J. Ariens, Ed., Academic Press, New York, N.Y., 1973, p 345.
(2) F. Cramer, *Acc. Chem. Res.*, **2**, 338 (1969).
(3) For a review on nucleocidin see R. J. Suhadolnik, "Nucleoside Antibiotics", Wiley-Interscience, New York, N.Y., 1970, pp 246–255.
(4) (a) D. A. Shuman, R. K. Robins, and M. J. Robins, *J. Am. Chem. Soc.*, 91, 3391 (1969); (b) D. A. Shuman, M. J. Robins, and R. K. Robins, *ibid.*, **92**, 3434 (1970).
(5) A. Bloch and C. Coutsogeorgopoulos, *Biochemistry*, **10**, 4394 (1971).
(6) B. Jastorff and H. Hettler, *Chem. Ber.*, **102**, 4119 (1969).
(7) J. P. Horwitz, A. J. Tomson, J. A. Urbanski, and J. Chua, *J. Org. Chem.*, **27**, 3045 (1962).
(8) Y. Inove and K. Nagasawa, *J. Org. Chem.*, **38**, 1810 (1973).
(9) We are grateful to Dr. Alexander Bloch and Dr. Robert G. Hughes, Jr., Roswell Park Memorial Institute, Buffalo, N.Y., for carrying out these tests.
(10) R. G. Hughes, Jr., and W. H. Munyon, *J. Virol.*, **16**, 275 (1975).
(11) HPLC analysis was performed using a column prepared according to R. A. Holton, D. M. Spatz, E. E. van Tamelen, and W. Wierenga, *Biochem. Biophys. Res. Commun.*, **58**, 605 (1974).
(12) W. S. Mungall, G. L. Greene, G. A. Heavner, and R. L. Letsinger, *J. Org. Chem.*, **40**, 1659 (1975).

Synthesis of Branched-Chain Apiosylpyrimidines and Their Inhibition of Lymphocyte Proliferation

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Branched-chain nucleosides containing β-D-apio-L-furanose were synthesized by condensation of the bis(trimethylsilyl) derivative of uracil (**11**), thymine (**12**), 5-bromouracil (**13**), and 5-iodouracil (**14**) with the protected 1-*O*-acetylapiose in the presence of a Friedel–Crafts catalyst. D-Apio-L-furanosyluracil (**11**) and D-apio-L-furanosylthymine (**12**) show immunosuppressive activity for rat T-lymphocytes stimulated to grow by phytohemagglutinin but exhibit no inhibitory activity against herpes simplex virus. Compounds **11**–**14** did not inhibit herpes simplex virus replication, while low inhibition was obtained with the one nucleoside containing D-apio-D-furanose, D-apio-D-furanosyladenine. D-Apio-L-furanosyl-5-bromouracil (**13**), D-apio-L-furanosyl-5-iodouracil (**14**), bromouridine, and iodouridine suppressed growth of human lymphoblasts significantly more than the nonhalogenated apiosylpyrimidines.

Purine and pyrimidine nucleosides containing the sugar analogue arabinose have been reported to elicit a variety of biological effects, including antiviral and antilymphocyte activities. At the clinical level the suppression of herpes simplex and of lymphocyte replication has been realized.[1,2] In order to ascertain whether the introduction of sugar analogues other than arabinose likewise might lead to therapeutically useful agents, the synthesis and biological evaluation of D-apio-L-furanosylpyrimidines appeared worthy of investigation.

The chemistry and biochemistry of the branched-chain, naturally occurring sugar D-apiose (3-*C*-hydroxymethylaldotetrose) have recently been reviewed.[3] The presence of asymmetric centers at carbon 1, 2, and 3 results in eight possible apiose isomers. Apiosyl nucleosides with the L configuration at carbon atom 2 would have the stereochemistry which provides antiherpes and immunosuppressive activity of 1-β-D-arabinofuranosylcytosine (*ara*-C) and 9-β-D-arabinofuranosyladenine (*ara*-A).[1,2] The relationship of structure to function such as inhibition of viral growth in L-apiosyl nucleosides could not be attributed a priori to the L configuration at carbon 2. Structure–function relationships would be complicated by any effects on their biological activity of the hydroxymethyl group at carbon atom 3 and the D or L stereochemistry of the hydroxyl group on carbon atom 3. On the other hand, D-apiosyl nucleosides would differ structurally from ribosyl nucleosides only in the location and stereochemistry at carbon atom 3 of the hydroxymethyl group. Hence, any biological activity could be attributed to the constituents on carbon atom 3, their stereochemical location, and any effects on the overall configuration of the nucleoside. Other than slight bacteriostatic effects on the growth of the two bacteria tested, *Escherichia coli* and *Staphylococcus aureus*, by 9-[3-*C*-(hydroxymethyl)-β-L-threofuranosyl]adenine, D-Api-L-A, nothing is known of the effects of D-Api-L-A or D-Api-D-A, 9-[3-*C*-(hydroxymethyl)-β-D-erythrofuranosyl]adenine, on the replication of viruses, bacteria, lymphocytes, or other mammalian cells (Figure 1).[3,4] Therefore, we have synthesized several D-apiosyl nucleosides as potential inhibitors of lymphocyte proliferation or growth of herpes simplex virus, bacteria, and fungi.

Chemistry. Silylation of the corresponding pyrimidine base was performed by refluxing the base with hexamethyldisilazane (HMDS).[5,6] Excess hexamethyldisilazane was removed by vacuum distillation and the residue was used for condensation. 1,2-Di-*O*-acetyl-3,3′-di-*O*-benzyl-D-apio-L-furanose (**5**) and 1,2-di-*O*-acetyl-3,3′-*O*-carbonyl-D-apio-L-furanose (**6**) were prepared by published procedures.[7,8] Condensation of bis(trimethylsilyl)pyrimidine with **5** or **6** in the presence of stannic chloride according to the procedure of Niedballa and Borbruggen[9] gave blocked nucleosides. Condensation was monitored by TLC in ethyl acetate and showed the absence of anomeric nucleosides in each case. Removal of the blocking groups gave free nucleosides **11**–**14** (see Figure 2). Assignment of the anomeric configuration was made by NMR, mainly on the basis of the $J_{1'2'}$ coupling constant. The small coupling constant ($J_{1'2'}$) of about 2.0 Hz was indicative of a β-nucleoside,[10] which would be expected to result from 2-*O*-acyl group participation.[11]

Biological Activity. Lymphocyte viability was measured by trypan blue dye exclusion after 5 days of incu-

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