layer was washed with water and evaporated; recrystallization of the solid residue out of methanol–water gave crude diacetate, 153 mg, mp 169–180°. Two recrystallizations to constant specific activity using acetone–water gave feathery needles: mp 185–186° (lit.[2] mp 187–188°); λ_{max} 2.79 (OH), 5.75, 5.83 μ (C=O), 4368 × 10^6 cpm/mole.

A solution of 67 mg of the diacetate in 0.87 ml of methanol and 0.01 ml of concentrated HCl was stirred for 30 hr at room temperature. Water was added and the turbid mixture was cooled and filtered. A benzene solution of the crude 7-monoacetate was allowed to evaporate overnight, leaving fine needles, mp 177–178.5°. It was recrystallized to constant specific activity by two recrystallizations in benzene–petroleum ether; the pure product (29 mg) showed mp 179–179.5° (lit.[5] mp 178–179°); λ_{max} 2.83 (OH), 5.79 μ (C=O), 29 × 10^6 cpm/mole.

Synthesis and Hydrolysis of Methyl Cholate 3-Acetate 7-(1-C^{14} Acetate).—Using the labeled acetic anhydride mentioned above, methyl cholate 3-acetate in benzene and pyridine was converted to the diacetate and recrystallized to constant specific activity; mp 186–188°, 4298 × 10^6 cpm/mole. This was hydrolyzed in methanolic HCl to the 7-monoacetate and recrystallized to constant specific activity, mp 177–179°, 4291 × 10^6 cpm/mole.

Registry No.—3-Labeled diacetate, 7432-66-8; methyl cholate, 1448-36-8; 7-labeled diacetate, 7432-68-0; 7-labeled acetate, 7432-69-1; 3-labeled acetate, 7430-18-4.

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A Convenient Synthesis of Arabinosylcytosine (Cytosine Arabinoside)[1,2]

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In the original preparation of arabinosylcytosine (1-β-D-arabinofuranosyl cytosine),[3] polyphosphoric acid was used to convert 2′(3′)-cytidylic acid to a phosphorylated 2,2′-anhydrocytidine and the latter was subsequently hydrolyzed and dephosphorylated to give the cytidine epimer. Ion-exchange fractionation at the nucleotide stage was required to separate arabinosylcytosine 3′,5′-diphosphate from cytidine derivatives. An alternative multistep synthesis was later described by Evans, *et al.*,[4] involving the conversion of uridine to 1-β-D-arabinofuranosyluracil *via* the 2,2′-anhydride[5] with eventual conversion to the corresponding cytosine derivative by thiation and ammonation.[6] Shen, *et al.*,[7] have described a more practical synthesis which utilizes the readily available 2,3,5,-tri-O-benzyl-D-arabinosyl chloride of Glaudemans and Fletcher.[8] Since completion of the present study several new approaches to synthesis have been reported.[9,10]

The availability of this nucleoside analog has made possible certain metabolic experiments[11] as well as tests to establish its chemotherapeutic value as an antitumor and antiviral agent.[12–14] The initial success of such tests, particularly those directed toward DNA-containing viruses, has led to the demand for large quantities of the arabinosyl derivative for clinical testing. A procedure based on a new method of nucleoside fractionation[15] which can be conveniently applied to the preparation of gram or kilogram quantities is herein described.

Experimental Section

Cytidine (5 g) or the molar equivalent of 2′(3′)-cytidylic acid is suspended in 100 g of polyphosphoric acid in a stoppered flask[16] and the suspension is heated at 80° for 30 hr.[17] The dark brown, homogeneous mass is dissolved in 200 ml of water and the solution is heated at 100° for 60 min to break pyrophosphate bonds. The solution is brought to pH 9 by the slow addition of 10% lithium hydroxide resulting in hydrolysis of the 2,2′-anhydro intermediate and giving, as the major product, the 3′,5′-diphosphate of arabinosylcytosine.

To the filtrate remaining after removal of lithium phosphate is added 20 g of magnesium chloride, 20 ml of 30% ammonium chloride, and concentrated ammonium hydroxide to pH 9.5. The magnesium ammonium phosphate is removed by filtration, 20 mg of alkaline phosphatase[18] is added to the filtrate, and the solution is incubated under toluene at 37° for 24 hr. Paper chromatography in isopropyl alcohol–ammonia–water (7:1:2) can be used to demonstrate complete conversion of nucleoside mono-, di-, and triphosphates to free nucleosides. The magnesium ammonium phosphate is then removed and the filtrate (*ca.* 1500 ml) is concentrated to 200 ml to remove excess ammonia. After dilution to 400 ml with water, the solution is placed on a 4.7 × 15 cm column of Dowex 50-8x (H^+, 50–100 mesh). Elution with water (1500 ml) yields the mineral acids derived from the anions of the salts plus uracil nucleosides (60 mg) resulting from slight deamination of the corresponding cytosine compounds. Cytidine and arabinosylcytosine are then displaced with 1 *N* ammonium hydroxide (1000 ml). The fractions containing the pentofuranosyl derivatives of cytosine are evaporated to dryness to remove ammonia, taken up in 35 ml of 30% methanol, and applied to a 3.6 × 13 cm column of Dowex 1-2x (OH^-, 200–400 mesh) previously equilibrated with 30% methanol. Upon elution with 30% methanol, cytidine emerges and can be recovered in chromatographically pure form by evaporation of solvent (recovery 423 mg).

After 1500 ml of aqueous methanol has passed through the column, it is stripped with 0.1 *M* ammonium bicarbonate. The arabinosylcytosine appears coincident with the bicarbonate front and is completely eluted in a volume of *ca.* 200 ml. Repeated flash evaporation of the aqueous solution decomposes the

(1) This work was supported by a grant (GB-882) from the National Science Foundation.

(2) In keeping with the "Rules of Carbohydrate Nomenclature" [*Chem. Eng. News*, **31** (17), 1776 (1953)], the term "arabinosylcytosine" has been used throughout this manuscript instead of the commonly applied "cytosine arabinoside." The latter term has been retained in the title for the convenience of "keyword index" users.

(3) E. R. Walwick, W. K. Roberts, and C. A. Dekker, *Proc. Chem. Soc.*, 84 (1959). In this paper the title compound was designated 3-β-D-arabofuranosylcytosine to satisfy British nomenclature requirements.

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(8) C. P. J. Glaudemans and H. G. Fletcher, *ibid.*, **28**, 3004 (1963).

(9) J. J. Fox and I. Wempen, *Tetrahedron Letters*, No. 11, 643 (1965); J. J. Fox, N. Miller, and I. Wempen, *J. Med. Chem.*, **9**, 101 (1966).

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(11) L. I. Pizer and S. S. Cohen, *J. Biol. Chem.*, **235**, 2387 (1960).

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(14) D. A. Buthala, *Proc. Soc. Exptl. Biol. Med.*, **115**, 69 (1964).

(15) C. A. Dekker, *J. Am. Chem. Soc.*, **87**, 4027 (1965).

(16) Prewarming the polyphosphoric acid reduces its viscosity and facilitates handling.

(17) The progress of the reaction can be estimated by dilution of a small aliquot of the reaction mixture with cold 0.1 *N* hydrochloric acid and examination of the OD_{280}/OD_{250} ratio. When the value has decreased to *ca.* 0.7, extensive formation of the 2,2′-anhydride intermediate has occurred.

(18) Worthington Biochemical Corp. preparation PC-P 639.

ammonium bicarbonate, leaving crystalline arabinosyl derivative of >95% purity as established by chromatography (yield 2440 mg, 53.4%, based on unrecovered cytidine). The pure compound can be obtained by recrystallization from alcohol and alternatively, the hemisulfate can be prepared by addition of a slight excess of sulfuric acid to a 10% aqueous solution followed by precipitation with alcohol: for the hemisulfate, $[\alpha]^{25}D$ +126° (0.5%, H_2O), $\lambda_{max}^{pH\ 1}$ 280 mμ (ε 13,400) and $\lambda_{max}^{pH\ 13}$ 273.5 mμ (shoulder) (ε 10,000 and 8400); for the free nucleoside, mp 212–213°, with authentic sample mmp 211–212°, $[\alpha]^{25}D$ +151° (0.5%, H_2O). *Anal.* Calcd for $C_9H_{13}N_3O_5$: C, 44.45; H, 5.39; N, 17.28. Found: C, 44.49; H, 5.53; N, 17.18. In addition, the R_f values in several solvents and the electrophoretic mobility in saturated borax are identical with those of authentic material.

Registry No.—Arabinosylcytosine, 147-94-4; arabinosylcytosine hemisulfate, 7771-30-4.

Nucleosides. XI. 2′,3′-Dideoxycytidine

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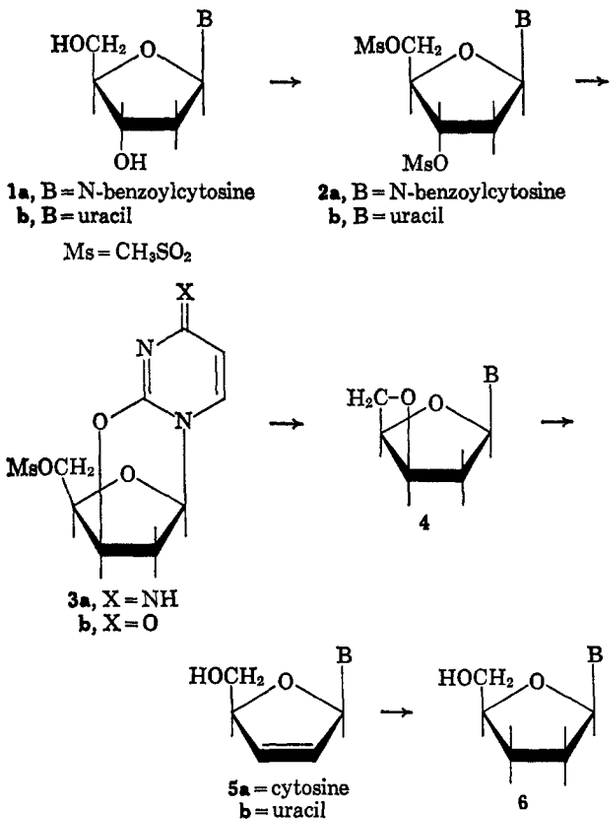
Recent reports[1] from this laboratory described the direct introduction of 2′,3′ unsaturation into the carbohydrate moieties of several pyrimidine and purine nucleosides *via* novel base-catalyzed elimination reactions.[2] A rationale for the synthesis of olefinic nucleosides was provided in an earlier communication.[1] The elucidation of the structure of the antibiotic blasticidin S, which has been shown to be a derivative of the 2′,3′-unsaturated nucleoside, cytosinine,[3] has attracted additional attention to this class of compounds. Apart from these considerations, the 2′,3′-olefinic derivatives afford a direct approach to 2′,3′-dideoxynucleosides of which 2′,3′-dideoxyadenosine and 3′-deoxythymidine are of particular interest as possible chain terminators of deoxyribonucleic acid (DNA) biosynthesis.[4] 2′,3′-Dideoxycytidine (**6a**) would be of interest for similar reasons[5] and the present communication describes the application of methods developed in our previous studies to the synthesis of **6a** *via* the corresponding 2′,3′-unsaturated derivative (**5a**). (See Scheme I.)

It has been demonstrated[1b,c] that the decyclization of the oxetane ring in, for example, 1-(2-deoxy-3,5-epoxy-β-D-*threo*-pentofuranosyl)uracil (**4b**) is readily effected by potassium *t*-butoxide (*t*-BuOK) in dimethyl sulfoxide (DMSO), affording 1-(2,3-dideoxy-2-ene-β-D-*glycero*-pentofuranosyl)uracil (**5b**, 2′,3′-dideoxy-2′-uridinene[6]) in good yield. The requisite 3′,5′-oxetane derivatives are readily obtained by the action of aqueous sodium hydroxide on 3′,5′-di-O-methylsulfonate esters of pyrimidine 2′-deoxynucleosides (**2**).[1c,7]

SCHEME I



The reaction of N-benzoyl-2′-deoxycytidine (**1a**)[5a] with 2 equiv of methanesulfonyl chloride gave the 3′,5′-di-O-mesyl derivative (**2a**) in near quantitative yield. The latter, on treatment with excess aqueous sodium hydroxide, yielded a crystalline product (58% yield) with properties consistent with the 3′,5′-epoxy nucleoside (**4a**). The chemical shifts of the carbohydrate protons in **4a** are readily assigned by analogy with the nmr spectra of other members of this series of nucleosides (Table I). Moreover, the specific rotation of the product is in accord both in sign and magnitude with optical rotatory values of all the 3′,5′-epoxynucleosides prepared to date (*cf.* Table I) in this laboratory.

It is now firmly established[1c,7] that the conversion of, for example, 3′,5′-di-O-mesyl-2′-deoxyuridine (**2b**) to **4b** involves the intermediate formation of the 2,3′-anhydronucleoside (**3b**). Rupture of the anhydro bond occurs at C-2 of the aglycon and intramolecular displacement of the 5′-O-mesyloxy function follows to effect formation of the oxetane ring. On this basis, then, it seems reasonable to conclude that the conversion of **2a** to **4a** involves the corresponding intermediate, 5′-O-mesyl-2,3′-anhydro-2′-deoxycytidine (**3a**). This conclusion is noteworthy in view of the fact that,

(1) (a) J. P. Horwitz, J. Chua, I. L. Klundt, M. A. Da Rooge, and M. Noel, *J. Am. Chem. Soc.*, **86**, 1896 (1964); (b) J. P. Horwitz, J. Chua, M. A. Da Rooge, and M. Noel, *Tetrahedron Letters*, 2725 (1964); (c) J. P. Horwitz, J. Chua, M. A. Da Rooge, M. Noel, and I. L. Klundt, *J. Org. Chem.*, **31**, 205 (1966); (d) J. P. Horwitz, J. Chua, and M. Noel, *Tetrahedron Letters*, 1343 (1966).

(2) A synthesis of 2′,3′-dideoxy-2′,3′-didehydroadenosine, which is essentially the same as that described in **1d**, has also been reported by J. R. McCarthy, Jr., M. J. Robins, L. B. Townsend, and R. K. Robins, *J. Am. Chem. Soc.*, **88**, 1549 (1966).

(3) For key references to this subject, see H. Yonehara and N. Otake, *Tetrahedron Letters*, 3785 (1966).

(4) (a) M. J. Robins and R. K. Robins, *J. Am. Chem. Soc.*, **86**, 3585 (1964); (b) M. J. Robins, J. R. McCarthy, Jr., and R. K. Robins, *Biochemistry*, **5**, 224 (1966).

(5) (a) An unsuccessful attempt to prepare **6a** by an alternate route has been described by E. Benz, N. F. Elmore, and L. Goldman, *J. Org. Chem.*, **30**, 3067 (1965). (b) After the completion of the present study, one of us (J. P. H.) learned (personal communication) that **6a** had been prepared by an alternate though undisclosed route, by Dr. James H. Hunter of The Upjohn Co.

(6) See ref 1c for the basis of this nomenclature.

(7) J. P. Horwitz, J. Chua, J. A. Urbanski, and M. Noel, *J. Org. Chem.*, **28**, 942 (1963).