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The 2′-*O*-Methyl Ether of 1-β-D-Arabinofuranosylcytosine†

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Received October 11, 1973

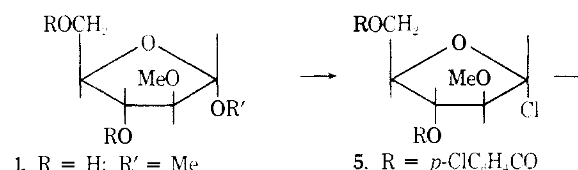
1-β-D-Arabinofuranosylcytosine (ara-C)[1] and its acyl derivatives[2,3] are effective anticancer agents in animals, and ara-C has found clinical utility.[4] More recently, *O*-2,2′-cyclocytidine has also shown activity and has been shown to be resistant to deamination.[5] Ara-C owes its biologic activity largely to its interference with DNA synthesis, after its conversion to the triphosphate.[6] Its substrate and inhibitor properties result from its resemblance to 2′-deoxycytidine, indicating that the hydroxyl group at $C_{2'}$ cis to the pyrimidine does not interfere with the binding of this compound to the active sites of the enzymes that normally metabolize 2′-deoxycytidine.[7] Although it would seem logical that other substituents at $C_{2'}$ cis to the cytosine moiety would also be tolerated by these enzyme active sites, only one such structure, 2′-deoxy-2′-fluoro-β-D-arabinofuranosylcytosine, has been evaluated for anticancer activity, and it was found to be active.[7]

To examine the effect of modification of ara-C at the 2′ carbon on biologic activity, we selected the 2′-*O*-methyl ester **7** for synthesis. This compound was produced in 3% yield, along with six other products, by the dimethyl sulfate–aqueous base methylation of ara-C.[8] Since this hardly seemed a practical approach for the preparation of enough material for biologic evaluation, another procedure was sought. Methylation of 3′,5′-di-*O*-dibutyryl-ara-C[3] by diazomethane in dimethoxyethane seemed promising, since ribonucleosides can be selectively alkylated at the 2′-hydroxyl in this manner.[9] In the present case, no reaction occurred in 24 hr. The addition of boron trifluoride etherate caused reaction to occur, but the product isolated in 25% yield and identified by spectral data was a mixture of two O-methylated nucleosides of uracil.

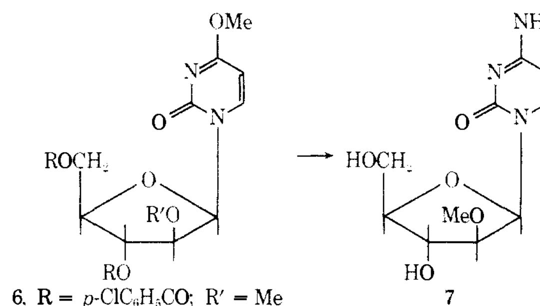
†This work was supported by funds from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Contract No. NIH-NCI-C-73-3712.

The failure of these methylation procedures led us to seek another approach. Austin, *et al.*,[10] found that, although nucleophilic attack on methyl 2,3-anhydro-β-D-ribofuranoside occurs predominantly at C-3, sodium methoxide attacks the α anomer exclusively at C-2. We have confirmed these results, obtaining a good yield of methyl 2-*O*-methyl-α-D-arabinofuranoside (**1**) by this reaction with no chromatographic or pmr spectra evidence for the formation of the 3-*O*-methyl xylo isomer.‡ Treatment of the resulting methyl 2-*O*-methyl-α-D-arabinofuranoside (**1**) with either benzoyl or *p*-chlorobenzoyl chloride in pyridine gave the 3,5-dibenzoylated sugars **2** and **3**. Hydrolysis of **3** gave 3,5-di-*O*-(*p*-chlorobenzoyl)-2-*O*-methyl-D-arabinose (**4**), which was chlorinated with hydrogen chloride in methylene chloride. Heating a neat mixture of the chloro sugar **5** and 3,4-dimethoxypyrimidine gave a single nucleoside, identified by a nuclear Overhauser pmr experiment as the β or cis anomer **6** in good yield. The *p*-chlorobenzoyl groups were removed with methanolic ammonia, which also replaced the 4-methoxy group to give the desired 1-(2-*O*-methyl-β-D-arabinofuranosyl)cytosine (**7**) (Scheme I). Unfortunately, this nucleoside failed to show cytotoxicity to H.Ep.-2 cells in culture or to inhibit the L1210 leukemia *in vivo*. The reasons for this failure are not yet known.

Scheme I



1. R = H; R′ = Me
2. R = C_6H_5CO; R′ = Me
3. R = p-ClC_6H_4CO; R′ = Me
4. R = p-ClC_6H_4CO; R′ = H
5. R = p-ClC_6H_4CO



6. R = p-ClC_6H_5CO; R′ = Me

Experimental Section§

Methyl 2′-*O*-Methyl-α-D-arabinofuranoside (**1**). A solution of methyl 2,3-anhydro-α-D-ribofuranoside (146 mg, 1 mmol) and sodium methoxide (1.35 g, 25 mmol) in 5 ml of methanol was refluxed for 18 hr before it was neutralized and evaporated to dryness. The residue was extracted with acetonitrile (3 × 25 ml), and the extracts were evaporated to dryness: yield of oil, 173 mg (97%); mass spectrum 147 $(M - OCH_3)^+$; pmr ($CDCl_3$) δ 3.39 and 3.42 (2 s, 2 OMe), 3.4–4.1 (H_2, H_3, H_4, $2H_5$), 2.8–5.4 (very broad, OH), 4.9 (d, J_{12} = 1–2 Hz, H_1).

Methyl 3,5-Di-*O*-benzoyl-2-*O*-methyl-α-D-arabinofuranoside

‡Earlier work in these laboratories[11] revealed that, contrary to literature,[12,13] ammonia attacks this epoxide at C_2 and C_3 giving approximately equal amounts of the arabino and xylo isomers.

§Melting points were determined with a Mel-Temp apparatus and are not corrected. The pmr spectra were determined in the solvent indicated (Me_4Si) with a Varian XL-100-15 spectrometer, and the correct integrals were obtained for the assignments indicated; chemical shifts quoted for multiplets were measured from the approximate centers. The mass spectra were determined with a Hitachi Perkin-Elmer RMU-6D-3 spectrometer. Chromatographic analyses were carried out on tlc plates of silica gel H (Brinkmann). The spots were detected by uv light after spraying with Ultraphor (WT, highly concentrated) and by charring after spraying with aqueous ammonium sulfate.

(**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 (ε × 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.

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Antiviral Agents. 1-Aralkyloxyadenosines†

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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.