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

John A. Montgomery* and Anne G. Laseter

Department of Organic Chemistry, Kettering-Meyer Laboratory, Southern Research Institute, Birmingham, Alabama 35205.
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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.

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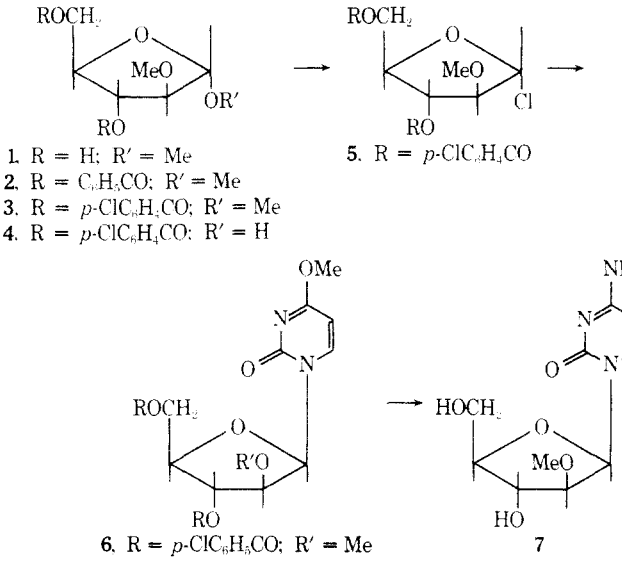
The failure of these methylation procedures led us to seek another approach. Austin, *et al.*,[10] found that, although nucleophilic attack on methyl 2,3-anhydro-β-D-ribofuranoside occurs predominantly at C-3, sodium methoxide attacks the α anomer exclusively at C-2. We have confirmed these results, 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_4CO; R′ = Me
7

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 ($\epsilon \times 10^{-3}$) (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.

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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

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