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5'-*O*-Methyl Derivatives of 1-β-D-Arabinofuranosylcytosine and 1-β-D-Arabinofuranosyluracil

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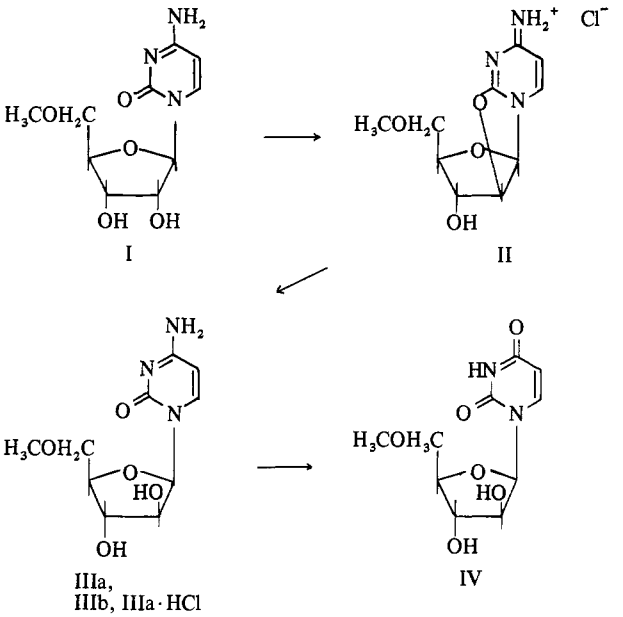
The potential utility as antimetabolites of analogs of 1-β-D-arabinofuranosylcytosine (ara-C) containing alkylated sugar hydroxyls has been discussed elsewhere,[1] and is further underlined by the recent demonstration of improved immunosuppressive and antileukemic activities exhibited by some 5'-acyl esters of ara-C.[2] We have undertaken the preparation of all possible *O'*-alkyl derivatives of ara-C, based on the observation that 1-substituted cytosine nucleosides undergo little or no ring N_3 alkylation in alkaline medium, rendering possible the preparation of the various *O'*-alkyl (methyl and ethyl) derivatives of cytidine and, by subsequent deamination, of uridine.[3,4]

The 2'-*O*-methyl and 3'-*O*-methyl derivatives of ara-U were reported by Codington, *et al.*,[5] but the methylation procedure employed was such that the products were also methylated on the ring N_3. For reasons discussed elsewhere,[1] such analogs are of limited interest as antimetabolites.

It occurred to us that an unambiguous synthesis of 5'-*O*-methyl-ara-C (III) might be feasible *via* the recently reported 5'-*O*-methylcytidine (I),[3,4] using the procedure of Kanai, *et al.*,[6] for the conversion of cytidine to ara-C *via* the 2,2'-anhydride. It was found that the conversion of I to II proceeded in almost 50% yield. The high alkaline lability of II ($t_{1/2}$ for hydrolysis at pH 10–11 about 1–2 min at room temp, the reaction being followed by the shift in λ_{max} from 262 to 271 nm and the decrease in optical density at 232 nm) suggested the use of milder conditions for this reaction. In fact, it was confirmed that hydrolysis in aqueous triethylamine simplified subsequent isolation of IIIa, which was recovered from II in 85% yield, and converted to the HCl salt.

Attempts to extend this procedure to the preparation of 3'-*O*-methyl-ara-C *via* 3'-*O*-methylcytidine were unsuccessful. A similar failure was encountered in attempts to prepare ara-C 3'-phosphate *via* cytidine 3'-phosphate. These findings suggest that the presence of a *cis*-glycol group is a prerequisite for the inversion at $C_{2'}$. Formation of an intermediate between chlorophosphoric acid and the *cis*-glycol grouping would then be followed by nucleophilic attack of the O_2 on $C_{2'}$, analogous to the mechanism proposed by Fox, *et al.*,[7] for the reaction of thiocarbonyldiimidazole with the *cis*-glycol grouping of 5-fluorouridine.

Conversion of III to 5'-*O*-methyl-ara-U (IV) profited from the observation of Notari, *et al.*,[8] on the high rate of deamination of ara-C relative to cytidine in acetate buffers and ascribed to intramolecular participation of the 2'-hydroxyl in ara-C. We have confirmed this observation and have found that, for preparative purposes, it is simpler to conduct the deamination reaction in 1 *M* AcOH on a water bath overnight, conversion of ara-C to ara-U under these conditions being quantitative. Application of this procedure to 5'-*O*-methyl-ara-C gave 5'-*O*-methyl-ara-U quantitatively.



It has been shown by Dr. M. Swierkowski that 5'-*O*-methyl-ara-C is fairly resistant to enzymatic deamination, using a highly active mouse kidney cytidine deaminase preparation which readily deaminated ara-C (*cf.* ref 1). This may account for the improved therapeutic activity noted by Gish, *et al.*,[2] for some 5'-acyl esters of ara-C.

Experimental Section

Melting points, uncorrected, were measured on a Boetius hot stage. Thin-layer chromatography made use of Merck HF_{254} silica gel, and paper chromatography was on Whatman No. 1. The following solvent systems were used with paper chromatography:[9] (A) *n*-BuOH saturated with saturated aqueous H_3BO_3; (B) isopropyl alcohol–1% H_3BO_3–NH_4OH (*d* 0.88), 7:2:1, v/v, using paper previously saturated with 1% H_3BO_3; (C) *n*-BuOH–AcOH–H_2O, 5:2:3, v/v; (D) isopropyl alcohol–NH_4OH (*d* 0.88)–H_2O, 7:1:2, v/v) (E) *n*-BuOH–H_2O, 84:16, v/v. Uv absorption spectra were run on a Zeiss (Jean) VSU-2P and on a Perkin-Elmer Model 450 recording instrument.

2,2'-Anhydro-5'-*O*-methylcytidine (II). To a suspension of 580 mg (2 mmoles) of 5'-*O*-methylcytidine hydrochloride (I),[3,4] in 70 ml of EtOAc, was added 6 ml of partially hydrolyzed phosphorus oxychloride ($POCl_3/H_2O$ = 1, mole/mole), the mixture was heated at the boiling point for 2.5 hr and then added to 250 ml of water (with ice), and the whole was stirred for 1 hr to completely hydrolyze the $POCl_3$. EtOAc was then removed under reduced pressure, and the aqueous phase deposited on a 43 × 2.6 cm column of Dowex 50W (H^+) 200–400 mesh. The column was washed with water until the effluent was neutral, and the product then eluted with 1.5 l. of 1 *M* pyridine–HCOOH buffer (pH 4). The eluate was brought to dryness, water was added to the residue, and it was again evaporated to dryness. This was repeated several times to remove traces of pyridine. The resulting glassy solid was dissolved in 20 ml of water and 1 *M* HCl added to give a strong acid reaction. The solution was brought to dryness, and the residue evaporated several times with water to remove excess HCl and evapd from 80% EtOH to give small, colorless needles, which, on recrystallization from 96% EtOH, gave 310 mg (48%) of the HCl salt of 2,2'-anhydro-5'-*O*-methylcytidine: mp 254–256°, dec at 257°; uv_{max} (pH 2–7) 231 nm (ε 9500), 263 (10,300). The product was chromatographically homogeneous in solvents A, C, and E (R_f 0.14, 0.65, 0.23) and gave a negative reaction with periodate.

1-(5-*O*-Methyl-β-D-arabinofuranosyl)cytosine (IIIa). A solution of 350 mg (1.27 mmoles) of II in 10 ml of 10% aqueous triethylamine was left overnight at room temp. The solution was brought to dryness, dissolved in 250 ml of water, and deposited on a 32 × 2.2 cm column of Dowex 50W (H^+), 200–400 mesh, which was washed with water until the effluent was neutral. The product was

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then eluted with 250 ml of 1 *N* NaOH, and the eluate deposited on an 18 × 3 cm column of Dowex 50W (NH_4^+), which was washed with water until the effluent was neutral. The aqueous eluate was concentrated under reduced pressure to remove ammonia (until pH was 7–8) and then passed through a 20 × 1.4 cm column of Dowex 1-X8 (borate form), which was washed with water until all uv-absorbing material was removed. The combined eluates were concentrated to an oil which, following several evaporations from anhydrous EtOH, yielded microscopic, colorless needles of IIIa: 300 mg (85%), mp 190–192°; uv_{max} (pH 2) 280 nm (ε 13,000), uv_{max} (pH 12) 271 nm (9900); chromatographically homogeneous in solvents A–E (R_f 0.26, 0.70, 0.57, 0.59, 0.28) as well as on silica gel with $CHCl_3$–MeOH (5:1). *Anal.* ($C_{10}H_{15}N_3O_5$) C, H, N.

HCl Salt of IIIa (IIIb). A solution of 40 mg (0.16 mmole) of IIIa in 2 ml of water was acidified with dilute HCl and brought to dryness, and the residue dried several times from anhydrous EtOH. Crystallization in the cold (−10°) from anhydrous EtOH–Me_2CO yielded colorless, hygroscopic prisms of IIIb: 37.5 mg (94%); mp 151–154°.

The 100-MHz nmr spectrum of IIIb, in D_2O solution, presented a unique methyl singlet at 3.45 ppm (relative to internal DSS) and, as would be expected, the protons at the 5′ position exhibited enhanced shielding, δ (H-5′, 5″) 3.77 ppm, as compared to 3.90 for the parent ara-C.

1-(5-*O*-Methyl-β-D-arabinofuranosyl)uracil (IV). A solution of 100 mg (0.39 mmole) of IIIa in 5 ml of 1 *M* AcOH was heated on a water bath. The course of deamination was followed spectrally[8] and by tlc with $CHCl_3$–MeOH (85:15, v/v). When starting product had virtually disappeared (10 hr), the solution was brought to dryness, the residue dissolved in 5 ml of water and to this was added 0.5 ml of Dowex 50W (H^+). After 15 min, the resin was filtered off and washed with water, the combined filtrates were brought to dryness, and the residue was dried from anhydrous EtOH. The compound was chromatographically homogeneous in solvent systems A–E (R_f 0.38, 0.67, 0.64, 0.50, 0.44), but its high solubility made crystallization difficult. Slow evaporation of a solution in anhydrous EtOH–Me_2CO finally yielded 15 mg of colorless, hygroscopic needles: mp 98–100°; uv_{max} (pH 2) 262 nm (ε 10,400), uv_{max} (pH 12) 262 (8100).

Acknowledgment. We are indebted to Mr. M. Remin for the nmr spectrum. This investigation was supported by the Polish Academy of Sciences (Project 09.3.1), and also profited from the support of The Wellcome Trust, the World Health Organization, and the Agricultural Research Service, U. S. Department of Agriculture.

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Cotinine *N*-Oxide, a New Metabolite of Nicotine

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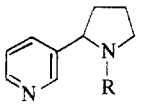
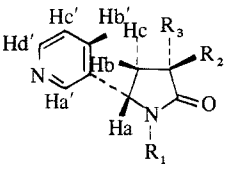
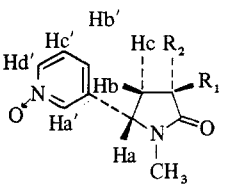
The metabolism of the tobacco alkaloid nicotine (**1**) has been studied in a variety of species,[1] including man.[2] In addition to the principle metabolites nornicotine (**2**) and cotinine (**3a**), a number of minor constituents have been reported including *N*-demethylcotinine (**4**), hydroxycotinine (**5**), and a relatively polar molecule, which in the literature has been designated compound Y.[3] During our investigations on the metabolism of cotinine in the monkey[4] we have isolated a compound with the reported chromatographic characteristics of compound Y. On the basis of spectroscopic evidence and a chemical synthesis, compound Y has been shown to be cotinine *N*-oxide (**6a**).



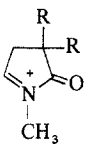
1, R = CH_3
2, R = H



3a, R_1 = CH_3; R_2 = Hd; R_3 = He
3b, R_1 = CH_3; R_2 = R_3 = D
4, R_1 = R_2 = H
5, R_1 = CH_3; R_2 = OH; R_3 = H



6a, R_1 = Hd; R_2 = He
6b, R_1 = R_2 = D



7a, R = H
7b, R = D

The basic fraction isolated from the urine of a 4-kg male rhesus monkey treated by intravenous infusion with *S*-cotinine showed 5 major fluorescent spots on silica gel tlc. The slowest moving spot (R_f 0.28, relative to cotinine) corresponded to literature R_f values reported for compound Y chromatographed in the same solvent system.[3] When further purification was attempted by glpc, the retention time was found to be the same as that of synthetic *S*-cotinine. Furthermore, the material collected off the glpc when submitted to high-resolution mass spectral analysis gave a parent ion with an empirical formula $C_{10}H_{12}N_2O$, isomeric with cotinine, and a mass fragmentation pattern corresponding to that of cotinine.[5] However, mass spectral analysis of compound Y purified by preparative tlc followed by alumina column chromatography gave an empirical formula $C_{10}H_{12}N_2O_2$ for the parent ion, suggesting that Y was suffering loss of oxygen when subjected to glpc. *trans*-3-Hydroxycotinine (**5**), a reported cotinine metabolite,[4] is isomeric with compound Y. However, the R_f value relative to cotinine for compound **5** is 0.60 compared to 0.28 for Y. Additionally, the mass fragmentation pattern of hydroxycotinine[4] is clearly different from metabolite Y. The spectrum of compound Y is dominated by a single fragment at *m/e* 98 which also occurs in the mass spectrum of cotinine where it has been attributed to species **7a**.[5] In independent studies on the metabolism of the dideuterated cotinine derivative **3b**, the dideuterated compound corresponding to compound Y was obtained and displayed a base peak in the mass spectra at *m/e* 100, consistent with species **7b**. The mass spectral evidence therefore suggests that the conversion of cotinine to metabolite Y involves oxidation of the pyridyl moiety. While bacteria are reported to oxidize the pyridyl group of nicotine at the C-2′ and C-6′ positions,[6] the reported oxidations by mammals of nicotine and cotinine involve alterations only of the 5-membered ring. However, mammalian systems have been reported to oxidize a number of pyridine-containing compounds to the corresponding pyridine *N*-oxides,[7] suggesting that the structure of compound Y is cotinine *N*-oxide (**6a**).